(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 July 2009 (02.07.2009)

PCT

(10) International Publication Number
WO 2009/082624 A2

(51) International Patent Classification:
C07K 16/00 (2006.01)

(21) International Application Number:
PCT/US2008/086292

(22) International Filing Date:
10 December 2008 (10.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/012,767	10 December 2007 (10.12.2007)	US
61/019,518	7 January 2008 (07.01.2008)	US
61/039,022	24 March 2008 (24.03.2008)	US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK,

[Continued on next page]

(54) Title: ANTAGONISTS OF IL-17A, IL-17F, AND IL-23 AND METHODS OF USING THE SAME

(57) Abstract: The present invention relates to blocking, inhibiting, reducing, antagonizing or neutralizing the activity of IL-17, IL-23p19 or both IL-17 and IL-23p19. IL-17 and IL-23 are cytokines that are involved in inflammatory processes and human disease. In particular, antibodies and antibody fragments that have superior neutralizing potency for the IL-17A/F heterodimer, and bispecific molecules that combine this superior anti-IL-17A antibody with anti-IL-23A antibodies are disclosed.

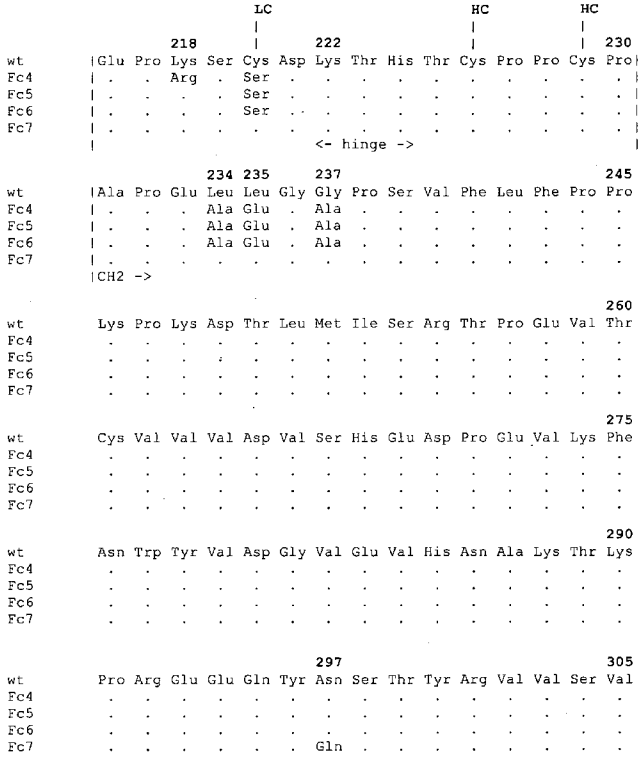


Fig. 1A

WO 2009/082624 A2

LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *without international search report and to be republished upon receipt of that report*

— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

ANTAGONISTS OF IL-17A, IL-17F, AND IL-23 AND METHODS OF USING THE SAME

FIELD OF THE INVENTION

[1] The present invention relates generally to the identification and isolation of antagonists to IL-17 and IL-23 p19, including bispecific antibodies, and methods of using the same.

BACKGROUND OF THE INVENTION

[2] The immune system protects individuals from infective agents (e.g. viruses, bacteria, and multi-cellular organisms), as well as from cancer and neoplasms. The immune system includes many lymphoid and myeloid cell types such as neutrophils, monocytes, macrophages, dendritic cells (DCs), eosinophils, T cells, and B cells. These cells are capable of producing signaling proteins known as cytokines. Cytokines are soluble, small proteins that mediate a variety of biological effects, including the induction of immune cell proliferation, development, differentiation, and/or migration, as well as the regulation of the growth and differentiation of many cell types (see, for example, Arai *et al., Annu. Rev. Biochem. 59*:783 (1990); Mosmann, *Curr. Opin. Immunol. 3*:311 (1991); Paul and Seder, *Cell 76*:241 (1994)). Cytokine-induced immune functions can also include an inflammatory response, characterized by a systemic or local accumulation of immune cells. Although they do have host-protective effects, these immune responses can produce pathological consequences when the response involves excessive and/or chronic inflammation, as in autoimmune disorders (such as multiple sclerosis) and cancer/neoplastic diseases (Oppenheim and Feldmann (eds.) *Cytokine Reference,* Academic Press, San Diego, CA (2001); von Andrian and Mackay *New Engl. J. Med. 343:* 1020 (2000); Davidson and Diamond, *New Engl. J. Med. 345*:340 (2001); Lu *et al, Mol. Cancer Res.* 4:221(2006); Dalgleish and O'Byrne, *Cancer Treat Res. 130*:1 (2006)).

[3] Proteins that constitute the cytokine group include interleukins, interferons, colony stimulating factors, tumor necrosis factors, and other regulatory molecules. For example, human interleukin-17A (also known as "IL-17") is a cytokine which stimulates, for example, the expression of interleukin-6 (IL-6), intracellular adhesion molecule 1 (ICAM-1), interleukin-8 (IL-8), granulocyte macrophage colony-stimulating factor (GM-CSF), and prostaglandin E2, and plays a role in the preferential maturation of CD34+ hematopoietic precursors into neutrophils (Yao *et al., J. Immunol. 155*:5483 (1995); Fossiez *et al., J. Exp. Med. 183*:2593 (1996)). As another example, human interleukin-23 (also known as "IL-23") is a cytokine which has been reported to promote the proliferation of T cells, in particular memory T cells and can contribute to the differentiation and/or maintenance of Th17 cells.

[4] Accordingly, the demonstrated *in vivo* activities of cytokines and their receptors illustrate the clinical potential of, and need for, other cytokines, cytokine receptors, cytokine agonists, and cytokine antagonists. For example, demonstrated *in vivo* activities of the pro-inflammatory cytokine family illustrates the enormous clinical potential of, and need for antagonists of pro-inflammatory molecules such as IL-17A and IL-23.

DESCRIPTION OF THE FIGURES

[5] Figures 1A-1C illustrate the amino acid sequences of certain immunoglobulin Fc polypeptides. Amino acid sequence numbers are based on the EU index (Kabat et al., Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, Bethesda, 1991). The illustrated sequences include a wild-type human sequence ("wt"; SEQ ID NO:2241) and five variant sequences, designated Fc-488 (SEQ ID NO:2242), Fc4 (SEQ ID NO:2243), Fc5 (SEQ ID NO:11 and SEQ ID NO: 333), Fc6 (SEQ ID NO:2244), and Fc7 (SEQ ID NO:2245). The Cys residues normally involved in disulfide bonding to the light chain constant region (LC) and heavy chain constant region (HC) are indicated. A "." indicates identity to wild-type at that position. *** indicates the stop codon; the C-terminal Lys residue has been removed from Fc6. Boundaries of the hinge, CH2, and CH3 domains are shown.

[6] Figure 2 depicts tetravalent, bispecific Fc fusion and Mab formats having Fv regions with specificity for two different targets (referred to herein as targets X and Y). Fv domains against target X are indicated by a striped fill, Fv domains against target Y are indicated by a gray fill, and the Ig constant domains are indicated by stippled fill. Figure 2A shows a tandem single chain Fv Fc fusion (tascFv-Fc); Figure 2B shows a bi-single chain Fv Fc fusion (biscFv-Fc); and Figure 2C shows a whole monoclonal antibody with a single chain Fv (scFv) fused to the carboxyl terminus (BiAb).

[7] Figure 3 shows a SEC/MALS analysis of an anti-IL-17/anti-IL-23 tascFv-Fc molecule.

[8] Figure 4a and 4b show that treatment with suboptimal concentrations of anti-mouse IL-17A with anti-IL-23p19 mAbs significantly reduced disease scores in the PLP EAE model of relapsing-remitting MS, compared to mice treated with vehicle (PBS). Importantly, the mAb combination completely prevented disease relapse.

[9] Figure 5 shows that treatment with suboptimal concentrations of anti-mouse IL-17A and anti-IL-23p19 mAbs from day -5 of the oxazalone colitis (OXC) model to day 1, resulted in a significant reduction in disease score (weight loss, stool consistency and blood in stool) vs. oxazalone-mice treated with PBS. [mean + SEM]. The mAb combination also alleviated colitis-mediated colon shortening observed at day 2 of the model compared to OXC mide treated with PBS [mean $\pm$ SEM].

[10] Figure 6 shows that hIL-23 induces a dose-dependent increase in pSTAT3 activity which is neutralized with bsAbs, IL-23p19 scFv, and to a lesser extent, soluble IL-23R. There is no neutralization with the IL-17A scFv alone, and no neutralization against hIL-12 or murine ligands. BsAbs were able to neutralize cynomolgus IL-23-mediated activity to a similar extent as the scFv.

[11] Figure 7 shows that all three bispecific formats of anti-IL-23p19/anti-IL-17A bsAbs neutralize hIL-23-mediated STAT3 phosphorylation in human PHA T cell blasts in the presence of high concentrations of hIL-17A.

[12] Figure 8 shows that all three bispecific formats of anti-IL-23p19/anti-IL-17A bsAbs neutralize hIL-23-mediated production of mIL-17A in murine splenocytes. hIL-23 induces a dose-dependent increase in mIL-17A production in primary murine splenocytes which is neutralized with bsAbs, IL-23p19 scFv, and to a lesser extent, soluble IL-23R. Similar trends were observed for neutralizing hIL-23 mediated production of IL-22 and IFN-g.

[13] Figure 9 shows that all three bispecific formats of anti-IL-23p19/anti-IL-17A bsAbs neutralize cynomolgus IL-23-mediated production of IL-17A in cyno splenocytes. Cyno IL-23 induces a dose-dependent increase in IL-17A production in primary cyno splenocytes which is neutralized with bsAbs.

[14] Figure 10 shows that anti-IL-23p19/anti-IL-17A biscFv-Fc neutralizes hIL-23-mediated production of IL-17A and IL-17F in human NK cells. hIL-23 induces IL-17A, IL-17F and IL-17A/F production in primary hu NK cells which is neutralized with bsAbs and IL23p19 scFv, and to a lesser extent, IL-17A scFv.

[15] Figure 11 shows that all three bispecific formats of anti-IL-23p19/anti-IL-17A bsAbs neutralize hIL-17A-mediated NFkB signaling. hIL-17A induces a dose-dependent increase in luciferase in NIH3T3 cells transfected with an NFkB promotor which is neutralized with bsAbs, IL-17A scFv, and to a lesser extent, soluble IL-17RA. There is no neutralization with the IL-23p19 scFv alone, and no neutralization of the bsAbs against hIL-17F or murine ligands. BsAbs were able to neutralize cynomolgus IL-17A-mediated activity to a similar extent.

[16] Figure 12 shows that all three bispecific formats of anti-IL-23p19/anti-IL-17A bsAbs neutralize hIL-17A/F heterodimer-mediated NFkB signaling. hIL-17A/F induces a dose-dependent increase in luciferase in NIH3T3 cells transfected with an NFkB promotor which is neutralized with bsAbs and the IL-17A scFv. There is no neutralization with a sol IL-17RA or IL-23p19 scFv.

[17] Figure 13 show that all three bispecific formats of anti-IL-23p19/anti-IL-17A bsAbs neutralize hIL-17A-mediated NFkB signaling in the presence of high concentrations of hIL-23.

[18] Figure 14 shows that all three bispecific formats of anti-IL-23p19/anti-IL-17A bsAbs neutralize hIL-17A-mediated G-CSF production in primary human epithelial cells. hIL-17A induces a dose-dependent increase in G-CSF production in small airway epithelial cells (24 h) which is neutralized with bsAbs, IL-17A scFv, and to a lesser extent, soluble IL-17RA. There is no

neutralization with IL-23p19 scFv alone, and no neutralization of bsAbs against hIL-17F or murine ligands.

[19] Figure 15 shows that all three bispecific formats of anti-IL-23p19/anti-IL-17A bsAbs neutralize hIL-17A-mediated G-CSF production in human glial cells. hIL-17A induces a dose-dependent increase in G-CSF production in a glial cell line (U87MG) (24 h) which is neutralized with bsAbs, and to a lesser extent, IL-17A scFv and a soluble IL-17RA (right panel). There is no neutralization with the IL-23p19 scFv alone, and no neutralization of the bsAbs against hIL-17F or murine ligands.

[20] Figure 16 shows that bsAbs are able to simultaneously co-bind targets without any effect on binding ability. Using surface plasmon resonance (Biacore), each bsAb was captured onto a CM4 chip via an anti-human IgG Fc-g specific Ab. Saturating concentrations of hIL-17A (100 nM) and hIL-23 (250 nM) were allowed to bind in series to each bsAb.

DETAILED DESCRIPTION OF THE INVENTION

[21] In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

[22] As used herein, the term "antibody" or "antibody peptide(s)" refers to an antibody, or a binding fragment thereof that competes with the antibody for specific binding to its target and includes chimeric, humanized, fully human, and bispecific antibodies as well as diabodies, linear antibodies, multivalent or multispecific hybrid antibodies (as described above and in detail in: Lanzavecchia *et al., Eur. J. Immunol. 17*, 105 (1987)) and in single chains (e.g., Huston *et al., Proc. Natl. Acad. Sci. U.S.A., 85,* 5879-5883 (1988) and Bird *et al., Science, 242*, 423-426 (1988), which are incorporated herein by reference). (See, generally, Hood *et al.*, "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, *Nature, 323,* 15-16 (1986), which are incorporated herein by reference).

[23] Antibody fragments can be produced by recombinant DNA techniques, expression, synthesis, or by enzymatic or chemical cleavage of intact antibodies. Bispecific antibodies may be produced by a variety of methods including, but not limited to, fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann (1990), *Clin. Exp. Immunol. 79*:315-321; Kostelny *et al.* (1992), *J. Immunol. 148:*1547-1553.

[24] The present invention addresses these needs by providing antagonists to pro-inflammatory cytokines IL-17A (SEQ ID NOs:1 and 2) and IL-23p19 (SEQ ID NOs:3 and 4). Additionally, the antagonists to IL-17A can bind the IL-17A/F heterodimer. IL-17A and IL-17F bind to the IL-17 receptors (i.e., IL-17R (SEQ ID NO: 8) and IL-17RC (SEQ ID NO: 10)). IL-23p19 forms a heterodimer with IL-23p40 (SEQ ID NO: 7) and binds to IL-23 receptor (IL-23R (SEQ ID NO: 9)). IL-23p19 has been given the HUGO name “IL-23A”.

[25] Antibody fragments include, but are not limited to, Fab, Fab', $F(ab)_2$, $F(ab')_2$, Fv, single-chain antibodies, and minimal binding unites comprising a LCDR1, LCDR2, and LCDR3 of a light chain variable region and a HCDR1, HCDR2, and HCDR3 of a heavy chain variable region .

[26] The term "isolated antibody" as used herein refers to an antibody or portion thereof that has been identified and separated and/or recovered from other antibodies or portions thereof.

[27] A "variant" anti-IL-17A antibody, anti-IL-23p19 antibody, or anti-IL-17A/anti-IL-23p19 antibody refers herein to a molecule which differs in amino acid sequence from a "parent" antibody amino acid sequence by virtue of addition, deletion and/or substitution of one or more amino acid residue(s) in the parent antibody sequence. In an embodiment, the variant comprises one or more amino acid substitution(s) in one or more hypervariable and/or framework region(s) of the parent antibody. For example, the variant may comprise at least one, e.g. from about one to about ten, and preferably from about two to about five, substitutions in one or more hypervariable and/or framework regions of the parent antibody. Ordinarily, the variant will have an amino acid sequence having at least 75% amino acid sequence identity with the parent antibody heavy or light chain variable domain sequences, at least 80%, at least 85%, at least 90%, and at least 95%. Identity or homology with respect to this sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the parent antibody residues, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the antibody sequence shall be construed as affecting sequence identity or homology. The variant retains the ability to bind human and or cynomologous IL-17A, human and or cynomologous IL-17F and/or human and or cynomologous IL-23p19 and may have properties which are superior to those of the parent antibody. For example, the variant may have a stronger binding affinity, enhanced ability to inhibit IL-17A, IL-17F and/or IL-23-induced inflammation. To analyze such properties, one should compare a Fab or scFv form of the variant to a Fab or scFv form of the parent antibody or a full length form of the variant to a full length form of the parent antibody

[28] The term "parent antibody" as used herein refers to an antibody which is encoded by an amino acid sequence used for the preparation of the variant.

[29] The term "agonist" refers to any compound including a protein, polypeptide, peptide, antibody, antibody fragment, large molecule, or small molecule (less than 10 kD), that increases the activity, activation or function of another molecule.

[30] The term "bind(ing) of a polypeptide of the invention to a ligand" includes, but is not limited to, the binding of a ligand polypeptide of the present invention to a receptor; the binding of a receptor polypeptide of the present invention to a ligand; the binding of an antibody of the present invention to an antigen or epitope; the binding of an antigen or epitope of the present invention to an antibody; the binding of an antibody of the present invention to an anti-idiotypic antibody; the binding

of an anti-idiotypic antibody of the present invention to a ligand; the binding of an anti-idiotypic antibody of the present invention to a receptor; the binding of an anti-anti-idiotypic antibody of the present invention to a ligand, receptor or antibody, etc.

[31] The "valency" of an antibody or fragment or portion thereof is the number of different molecules that it can combine with.

[32] The "specificity" of an antibody or fragment or portion thereof is its ability to distinguish between different antigens.

[33] A "cross reactive" antibody is an antibody that can bind to an antigen other than the antigen that was used as an immunogen to raise the antibody.

[34] A "bivalent antibody" other than a "multispecific" or "multifunctional" antibody, in certain embodiments, is understood to comprise binding sites having identical antigenic specificity.

[35] A "bispecific" or "bifunctional" antibody is a hybrid antibody having two different heavy/light chain pairs and two different binding sites.

[36] The term "chimeric antibody" or "chimeric antibodies" refers to antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to human constant segments, such as gamma 1 and gamma 3. A typical therapeutic chimeric antibody is thus a hybrid protein comprising the variable or antigen-binding domain from a mouse antibody and the constant domain from a human antibody, although other mammalian species may be used. Specifically, a chimeric antibody is produced by recombinant DNA technology in which all or part of the hinge and constant regions of an immunoglobulin light chain, heavy chain, or both, have been substituted for the corresponding regions from another animal's immunoglobulin light chain or heavy chain. In this way, the antigen-binding portion of the parent monoclonal antibody is grafted onto the backbone of another species' antibody. One approach, described in EP 0239400 to Winter *et al.* describes the substitution of one species' complementarity determining regions (CDRs) for those of another species, such as substituting the CDRs from human heavy and light chain immunoglobulin variable region domains with CDRs from mouse variable region domains. These altered antibodies may subsequently be combined with human immunoglobulin constant regions to form antibodies that are human except for the substituted murine CDRs which are specific for the antigen. Methods for grafting CDR regions of antibodies may be found, for example in Riechmann *et al.* (1988) *Nature 332*:323-327 and Verhoeyen *et al.* (1988) *Science 239*:1534-1536.

[37] As used herein, the term "epitope" refers to a site on an antigen recognized by an antibody or an antigen receptor. A T-cell epitope is a short peptide derived from a protein antigen. It binds to an MHC molecule and is recognized by a particular T cell. B-cell epitopes are antigenic determinants recognized by B cells. Antigenic epitopes need not necessarily be immunogenic. Such

epitopes can be linear in nature or can be a discontinuous epitope. Thus, as used herein, the term "conformational epitope" refers to a discontinuous epitope formed by a spatial relationship between amino acids of an antigen other than an unbroken series of amino acids.

[38] The term "epitope tagged" when used herein refers to the anti-IL-17A and/or anti- IL-23p19 antibody fused to an "epitope tag". The epitope tag polypeptide has enough residues to provide an epitope to which an antibody can bind, yet is short enough such that it does not interfere with activity of antibodies of the present invention. Suitable tag polypeptides generally have at least 6 amino acid residues and usually between about 8-50 amino acid residues (preferably between about 9-30 residues). Examples include the flu HA tag polypeptide and its antibody 12CA5 (Field *et al. Mol. Cell. Biol.* 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al., Mol. Cell. Biol. 5*(12):3610-3616(1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky *et al., Protein Engineering 3(*6):547-553(1990)). In certain embodiments, the epitope tag is a "salvage receptor binding epitope". As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

[39] "Avidity" is the functional affinity of multiple antigen molecules binding to multivalent binding molecules such as antibodies, or the binding molecules of the present invention. Avidity strengthens binding to antigens with repeating identical epitopes. The more antigen-binding sites an individual antibody molecule has, the higher its avidity for antigen.

[40] The term "fragment" as used herein refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of a IL-17 or IL-23p19 polypeptide or an antibody that immunospecifically binds to a either IL-17 or IL-23p19 or both IL-17 and IL-23p19 polypeptide.

[41] As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. One form of immunoglobulin constitutes the basic structural unit of an antibody. This form is a tetramer and consists of two identical pairs of immunoglobulin chains, each pair having one light and one heavy chain. In each

pair, the light and heavy chain variable regions are together responsible for binding to an antigen, and the constant regions are responsible for the antibody effector functions.

[42] Full-length immunoglobulin "light chains" (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH--terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes (about 330 amino acids). Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. (See generally, Fundamental Immunology (Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989), Ch. 7 (incorporated by reference in its entirety for all purposes).

[43] An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions. Thus, the term "hypervariable region" refers to the amino acid residues of an antibody which are responsible for antigen binding. The hypervariable region comprises amino acid residues from a "Complementarity Determining Region" or "CDR" (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" in the heavy chain variable domain; Chothia and Lesk, 1987, *J. Mol. Biol.* 196: 901-917) (both of which are incorporated herein by reference). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. Thus, a "human framework region" is a framework region that is substantially identical (about 85% or more, usually 90-95% or more) to the framework region of a naturally occurring human immunoglobulin. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDR's. The CDR's are primarily responsible for binding to an epitope of an antigen.

[44] The term "humanized" immunoglobulin refers to an immunoglobulin comprising a human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and the human immunoglobulin providing the framework is called the "acceptor". Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, i.e., at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a

humanized antibody would not encompass a typical chimeric antibody as defined above, e.g., because the entire variable region of a chimeric antibody is non-human.

[45] As used herein, the term "human antibody" includes and antibody that has an amino acid sequence of a human immunoglobulin and includes antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described, for example, by Kucherlapati *et al.* in U.S. Patent No. 5,939,598.

[46] As used herein, the terms "single-chain Fv," "single-chain antibodies," "Fv" or "scFv" refer to antibody fragments that comprises the variable regions from both the heavy and light chains, but lacks the constant regions or may contain only a portion of the constant region, but within a single polypeptide chain. Generally, a single-chain antibody further comprises a polypeptide linker between the VH and VL domains which enables it to form the desired structure which would allow for antigen binding. Single chain antibodies are discussed in detail by Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994). Various methods of generating single chain antibodies are known, including those described in U.S. Pat. Nos. 4,694,778 and 5,260,203; International Patent Application Publication No. WO 88/01649; Bird (1988) *Science 242*:423-442; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA 85:*5879-5883; Ward *et al.* (1989) *Nature 334*:54454; Skerra *et al.* (1988) *Science 242*:1038-1041, the disclosures of which are incorporated by reference for any purpose. In specific embodiments, single-chain antibodies can also be bispecific or multispecific and/or humanized.

[47] A "Fab fragment" is comprised of one light chain and the C_{H1} and variable regions of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule.

[48] A "Fab' fragment" contains one light chain and one heavy chain that contains more of the constant region, between the C_{H1} and C_{H2}domains, such that an interchain disulfide bond can be formed between two heavy chains to form a $F(ab')_2$ molecule.

[49] A "$F(ab')_2$fragment" contains two light chains and two heavy chains containing a portion of the constant region between the C_{H1} and C_{H1}domains, such that an interchain disulfide bond is formed between two heavy chains.

[50] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al., Proc. Natl. Acad. Sci. USA 90*:6444-6448 (1993).

[51] The term "linear antibodies" refers to the antibodies described in Zapata *et al. Protein Eng. 8(*10):1057-1062 (1995). Briefly, these antibodies comprise a pair of tandem Fd segments (V_H-C_{H1}-V_H-C_{H1}) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[52] The term "immunologically functional immunoglobulin fragment" as used herein refers to a polypeptide fragment that contains at least the variable domains of the immunoglobulin heavy and light chains. An immunologically functional immunoglobulin fragment of the invention is capable of binding to a ligand, preventing binding of the ligand to its receptor, interrupting the biological response resulting from ligand binding to the receptor, or any combination thereof. Preferably, an immunologically functional immunoglobulin fragment of the invention binds specifically to either IL-17A, IL-17A/F or IL-23p19 or to both IL-17A and IL-23p19.

[53] The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[54] As used herein, “nucleic acid” or “nucleic acid molecule” refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (*e.g.,* α-enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term “nucleic acid molecule” also includes so-called “peptide nucleic acids,” which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

[55] The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence.

[56] The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (*i.e.,* GAU and GAC triplets each encode Asp).

[57] The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

[58] An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species.

[59] A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

[60] "Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

[61] "Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

[62] A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee *et al.*, *Mol. Endocrinol. 7*:551 (1993)), cyclic AMP response elements (CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol. 1*:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as

CRE/ATF (O'Reilly *et al., J. Biol. Chem. 267*:19938 (1992)), AP2 (Ye *et al., J. Biol. Chem. 269*:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr. 3*:253 (1993)) and octamer factors (see, in general, Watson *et al.,* eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J. 303*:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

[63] A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

[64] A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner.

[65] An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

[66] "Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (*i.e.,* endogenous DNA) so long as that host DNA is combined with non-host DNA (*i.e.,* exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

[67] A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

[68] A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid

backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

[69] A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

[70] A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, that has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

[71] An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

[72] A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example of a recombinant host is a cell that produces an antagonist of the present invention from an expression vector. In contrast, such an antagonist can be produced by a cell that is a "natural source" of said antagonist, and that lacks an expression vector.

[73] "Integrative transformants" are recombinant host cells, in which heterologous DNA has become integrated into the genomic DNA of the cells.

[74] A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a fusion protein can comprise at least part of a an anti-IL17A antibody or an anti_IL-23p19 antibody polypeptide fused with a polypeptide that binds an affinity matrix. Such a fusion protein provides a means to isolate large quantities of said antibody using affinity chromatography.

[75] The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. Receptors can be membrane bound, cytosolic or nuclear; monomeric (*e.g.,* thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (*e.g.,* PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction.

In certain membrane-bound receptors, the extracellular ligand-binding domain and the intracellular effector domain are located in separate polypeptides that comprise the complete functional receptor.

[76] In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

[77] The term "secretory signal sequence" denotes a DNA sequence that encodes a peptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

[78] An "isolated polypeptide" is a polypeptide that is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, *i.e.,* at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, such as 96%, 97%, or 98% or more pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

[79] The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

[80] The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

[81] As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, co-stimulatory molecules, hematopoietic factors, and the like, and synthetic analogs of these molecules.

[82] The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and

avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than 10^9 M^{-1}.

[83] As used herein, a "therapeutic agent" is a molecule or atom which is conjugated to an antibody moiety to produce a conjugate which is useful for therapy. Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

[84] A "detectable label" is a molecule or atom which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

[85] The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson *et al., EMBO J. 4*:1075 (1985); Nilsson *et al., Methods Enzymol. 198*:3 (1991)), glutathione S transferase (Smith and Johnson, *Gene 67*:31 (1988)), Glu-Glu affinity tag (Grussenmeyer *et al., Proc. Natl. Acad. Sci. USA 82*:7952 (1985)), substance P, FLAG peptide (Hopp *et al., Biotechnology 6*:1204 (1988)), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford *et al., Protein Expression and Purification 2*:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (*e.g.,* Pharmacia Biotech, Piscataway, NJ).

[86] A "target polypeptide" or a "target peptide" is an amino acid sequence that comprises at least one epitope, and include IL-17A, IL-17F and IL-23p19.

[87] Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

[88] IL-17A has been identified as a cellular ortholog of a protein encoded by the T lymphotropic Herpes virus Saimiri (HSV) [see, Rouvier *et al., J. Immunol., 150*(12): 5445-5456 (1993); Yao *et al., J. Immunol., 122*(12):5483-5486 (1995) and Yao *et al., Immunity, 3*(6):811-821 (1995)]. Subsequent characterization has shown that this protein is a potent cytokine that acts to induce proinflammatory responses in a wide variety of peripheral tissues. IL-17A is a disulfide-linked homodimeric cytokine of about 32 kDa which is synthesized and secreted primarily by CD4+activated memory T cells (reviewed in Fossiez *et al., Int. Rev. Immunol., 16*: 541-551 [1998]). Specifically, IL-17 is synthesized as a precursor polypeptide of 155 amino acids with an N-terminal signal sequence of

19-23 residues and is secreted as a disulfide-linked homodimeric glycoprotein. IL-17 is disclosed in WO9518826 (1995), WO9715320 (1997) and WO9704097 (1997), as well as US Patent No. 6,063,372. Recently, IL-17A has also been shown to form a heterodimer with IL-17F in activated human CD4+ T Cells. See Wright, J. F., et al., J. Biol. Chem.,. 282: 13447-13455, 2007

[89] IL-17A is a cytokine which stimulates the expression of IL-6, ICAM-1, IL-8, GM-CSF, and prostaglandin E2, among others, and plays a role in the preferential maturation of CD34+ hematopoietic precursors into neutrophils (Yao *et al., J. Immunol. 155*:5483 (1995); Fossiez *et al., J. Exp. Med. 183*:2593 (1996)). IL-17A is also believed to play a key role in certain other autoimmune disorders such as multiple sclerosis (Matusevicius *et al., Mult. Scler. 5*:101 (1999); Park *et al, Nat Immunol. 6:*1133 (2005)). IL-17A has further been shown, by intracellular signalling, to stimulate Ca^{2+} influx and a reduction in [cAMP], in human macrophages (Jovanovic *et al, J. Immunol.* 160:3513 (1998)). Fibroblasts treated with IL-17A induce the activation of NF-kappa.B, (Yao et al., Immunity, 3:811 (1995), Jovanovic et al., supra), while macrophages treated with it activate NF-kappaB and mitogen-activated protein kinases (Shalom-Barek *et al, J. Biol. Chem.* 273:27467 (1998)).

[90] IL-23 is a heterodimeric cytokine composed of a unique subunit, p19 (herein referred to interchangeably as "IL23p19"), and the p40 subunit, which is shared with interleukin-12 (IL-12) (Oppmann, *Immunity 13*:715 (2000)). IL-23 has been found to stimulate the production and/or maintenence of IL-17 A and F from activated CD4+ T cells in what has now been termed as a "new" T-helper (Th) subset, designated Th17. A review of IL-23 cytokine and receptor biology is reviewed in Holscher, *Curr. Opin. Invest. Drugs 6*:489 (2005) and Langrish *et al. Immunol Rev. 202*:96 (2004). Similar to Th1 and Th2 lineages, Th17 cells have most likely evolved to provide adaptive immunity to specific classes of pathogens, such as extracellular bacteria. However, inappropriate Th17 responses have been strongly implicated in a growing list of autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and psoriasis.

[91] In fact, both IL-17A and IL-23 have also been reported to play important roles in many autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, Crohn's disease, and psoriasis. Both IL-23 and IL-17A are overexpressed in the central nervous system of humans with multiple sclerosis and in mice undergoing an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). The overexpression is observed in mice when the EAE is induced by either myelin oligodendrocyte glycoprotein (MOG) 35-55 peptide- or proteolipid peptide (PLP). Furthermore, neutralization of either IL-23p19 or IL-17 results in amelioration of EAE symptoms in mice (Park et al, *Immunol. 6:*1133 (2005); Chen *et al, J Clin Invest. 116*:1317 (2006)).

[92] Many immune related diseases are known and have been extensively studied. Such diseases include immune-mediated inflammatory diseases (such as rheumatoid arthritis, multiple sclerosis, demyelinating diseases, autoimmune ocular diseases, uveitis; scleritis, immune mediated

renal disease, hepatobiliary diseases, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), psoriasis, and asthma), non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, etc. These diseases can be treated by the anti-IL-17A and anti-IL-23p19 antibodies, either singly or in combination as described herein. In one embodiment, the administration can be as a single entity that binds, interferes, blocks, reduces and/or neutralizes the interaction of IL-17A with its cognate receptor(s) and that binds, interferes, blocks, reduces and/or neutralizes the interaction of IL-23p19 with its cognate receptor(s).

[93] It has also been demonstrated that IL-17 and Th17 cells can be produced from IL-23-independent sources, and the *in vivo* development of an IL-17 effector response has been shown to be IL-23-independent (Mangan *et al*, *Nature 441*:231 (2006)). Neutralization of IL-23 would theoretically eliminate existing IL-17 producing cells, but would not completely prevent the development of new Th17 cells. Recently, IL-1alpha, IL-1beta, and IL-6 have been demonstrated by us and others to be important for the differentiation of IL-23-mediated human Th17 cells (see Example 21 herein; see alos Acosta-Rodriguez et al, Nature Med 8:942; 2007). Thus, there is the need for a treatment regimen that provides for combination therapy of an anti-IL-17A and an anti-IL-23p19 antibodies. Such therapy can include combination treatment wherein the antibodies are administered to the patient as separate entities, either sequentially or concurrently, or wherein the antibodies are administered to the patient as a single bispecific entity, such as the bispecific anti-IL-17A/anti-Il-23p19 antibodies described herein.

[94] The present invention concerns the inhibition of both of these proinflammatory cytokines, IL-17 and IL-23p19. The invention provides anti-IL-17A antibodies that have been identified from a phage display library based on their ability to bind and neutralize IL-17A. These antibodies are futher described thoughout the present application. These antibodies are useful to inhibit the symptoms and biological activities that manifest as autoimmune and inflammatory disorders and are associated with IL-17A/IL-17R receptor interactions. Within an embodiment, the invention provides anti-IL-17A antibodies that bind the IL-17A/F heterodimer.

[95] The invention also provides anti-IL-23p19 antibodies that have been identified from a phage display library based on their ability to bind and neutralize IL-23p19. These antibodies are futher described thoughout the present application. These antibodies are useful to inhibit the symptoms and biological activities that manifest as autoimmune and inflammatory disorders and are associated with IL-23p19/IL-23 receptor interactions.

[96] The invention also provides anti-IL-17A/anti-IL-23p19 antibodies that are single entites that are bispecific for IL-17A and IL-23p19. Within an embodiment, the single entity can also neutralize IL-17F. These antibodies are futher described thoughout the present application. These antibodies are useful to inhibit the symptoms and biological activities that manifest as autoimmune

and inflammatory disorders and are associated with IL-17A/IL-17R and/or IL-23p19/IL-23R receptor interactions.

[97] The invention also provides methods of treating autoimmune and inflammatory disorders associated with IL-17A/IL-17R and/or IL-23p19/IL-23R receptor interactions. In an aspect the disorder symptoms and characteristics are reduced, diminished or ameliorated. In additional aspects the disorder symptoms and characteristics are prevented. In one embodiment, the administration of the anti-IL-17A and anti-IL-23p19 antibodies prevent relapse of multiple sclerosis.

[98] The present invention is based in part on the identification of anti-IL-17A antibodies that have superior neutralizing potency for the IL-17A/F heterodimer, and on the creation of a bispecific molecules that combine this superior anti-IL-17A antibody with newly identified or previously described anti-IL-23A antibodies to antagonize, reduce, inhibit or neutralized the effects of IL-17A and IL-17F. A limited number of anti-IL-17A and anti-IL-23A antibodies, including antibody fragments and scFvs have been previously described in co-owned, co-pending U.S. Patent Application Serial Number 11/762,738, filed June 13, 2007, which is incorporated herein by reference for the sequences of the anti-IL-17A and anti-IL-23A antibodies and the discussion of making the antibodies and assays for measuring the activity of the antibodies. However, none of the anti-IL-17A antibodies described in U.S. Patent Application Serial Number 11/762,738 are capable of binding the IL-17A/F heterodimer. It has been shown that a majority of the naturally occurring IL-17A forms a heterdimer with IL-17F. See Wright et al., J Biol Chem., 4: 282(18):13447-55. (2007). Thus, an antibody that binds IL-17A and is capable of neutralizing the IL-17A/F heterodimer will be more efficacious in treating diseases, not only diseases resulting from over-expression of the IL-17A/A homodimer, but also diseases resulting from over-expression of the IL-17A/F heterodimer. Furthermore, a bispecific antibody with one neutralizing antibody that binds and neutralizes the IL-17A/A homodimer and the IL-17A/F heterdimer coupled with a second neutralizing antibody that binds and neutralizes IL-23A will provide a superior therapeutic molecule to treat diseases associated with inflammation. Such a molecule will neutralize existing IL-17A/A homodimer and existing IL-17A/F heteodimer, via the neutralizing anibody that binds the IL-17A/A homodimer and existing IL-17A/F heteodimer, as well it will reduce, limit, or inhibit additional production of the IL-17A/A homodimer and the IL-17A/F heterodimer via the second neutralizing anibody that binds and neutralizes IL-23, which has been shown to be a survival factor for IL-A and IL-17F production. The invention provides these superior molecules for treatment of disease. In certain embodiments, the anti-IL-17A neutralizing antibodies provided herein, either as stand-alone antibodies, or as a component of bispecific molecules, are more potent than the soluble IL-17 receptor, and can bind to IL-17A (either as a homodimer or as a heterodimer with IL-17F) at less than 20 nM, less than 15 nM, less than 10nM, less than 5 nM, less than 4 nM, less than 3 nM, less than 2 nM, or at less than 1 nM.

[99] More specifically, the present invention concerns the inhibition or neutralization of both IL-17A, IL-17A/F, and IL-23p19 with a single antagonistic molecule or neutralizing entity. The benefit of combining these IL-17A and IL-23p19 antagonists in a treatment regimen for autoimmune and inflammatory disorders was initially described in The anti-IL17A and anti-IL-23p19 antibodies described herein have the additional technical effect of improved binding to their respective ligand. For example, as will be evident from the teachings herein, some of the anti-IL-17A and anti-IL-23p19 antibodies described herein have a slower off-rate from binding or they are better neutralizers in the bioassays described further in the specification and examples.

[100] The antagonistic molecule or neutralizing entity inhibits the activity of both IL-17 and IL-23p19, and thus, inhibits the production, maintenance, and activity of new and existing IL-17 and IL-17-producing T cells (Th17). Th17 cells include IL-17A and IL-17F. The invention further concerns the use of IL-17 and IL-23p19 antagonists or neutralizing entities in the treatment of inflammatory diseases characterized by the presence of elevated levels of IL- 17 and/or IL-23. The invention also concerns the use of IL-17 and IL-23p19 antagonists in the treatment of cancers characterized by the presence of elevated levels of IL- 17 and/or IL-23.

[101] Accordingly, the present invention is directed to antagonizing both IL-17 and IL-23p19, either singly or together. Antagonists of the present invention, block, inhibit, reduce, antagonize or neutralize the activity of IL-17, IL-23, IL-23p19 or both IL-17A and IL-23p19, will have advantages over therapies that target only one of these two cytokines. The invention further provides uses therefore in inflammatory disease and cancer, as well as related compositions and methods.

A) Overview

[102] Immune related diseases can be treated by suppressing the immune response. Using the antagonists of the present invention (i.e. anti-IL-17 and/or anti-IL-23p19 antibodies) that inhibit molecules having immune stimulatory activity are beneficial in the treatment of immune-mediated and inflammatory diseases. Molecules which inhibit the immune response can be utilized (proteins directly or via the use of antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

[103] Antagonists to IL-17 and IL-23 activity, such as the antagonists of the present invention (i.e. anti-IL-17 antibodies, anti-IL-23p19 antibodies, and/or anti-IL-17A/anti-IL-23p19 bispecific antibodies), are useful in therapeutic treatment of inflammatory diseases, particularly as antagonists to both IL-17 and IL-23p19, either singly or together in the treatment of autoimmune and inflammatory diseases . These antagonists are capable of binding, blocking, inhibiting, reducing, antagonizing or neutralizing IL-17 and IL-23p19 (either individually or together) in the treatment of multiple sclerosis, rheumatoid arthritis, psoriasis, atopic and contact dermatitis, colitis, endotoxemia,

arthritis, psoriatic arthritis, autoimmune ocular diseases (uveitis, scleritis), adult respiratory disease (ARD), demyelinating diseases, septic shock, multiple organ failure, inflammatory lung injury such as asthma, chronic obstructive pulmonary disease (COPD), airway hyper-responsiveness, chronic bronchitis, allergic asthma, eczema, irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) such as ulcerative colitis and Crohn's disease, diabetes, *Helicobacter pylori* infection, intraabdominal adhesions and/or abscesses as results of peritoneal inflammation (i.e. from infection, injury, etc.), systemic lupus erythematosus (SLE), systemic sclerosis, nephrotic syndrome, organ allograft rejection, graft vs. host disease (GVHD), kidney, lung, heart, etc. transplant rejection, streptococcal cell wall (SCW)-induced arthritis, osteoarthritis, gingivitis/periodontitis, herpetic stromal keratitis, restenosis, Kawasaki disease, and cancers/neoplastic diseases that are characterized by IL-17 and/or IL-23 expression, including but not limited to prostate, renal, colon, ovarian and cervical cancer, and leukemias (Tartour et al, *Cancer Res. 59*:3698 (1999); Kato *et al, Biochem. Biophys. Re.s Commun. 282*:735 (2001); Steiner *et al, Prostate. 56*:171 (2003); Langowski *et al, Nature 442*: 461, 2006). Accordingly, antagonists of the present invention (i.e. antibodies or binding peptides that bind IL-17 and IL-23 either singly or together) are also useful to prepare medicines and medicaments for the treatment of these diseases. The present invention provides isolated polypeptides that bind IL-17A (e.g., human IL-17A polypeptide sequence as shown in SEQ ID NO:2) and that bind the IL-17A/F heterodimer. The present invention also provides isolated polypeptides as disclosed above that bind IL-23p19 (e.g., human IL-23 polypeptide sequence as shown in SEQ ID NO:4).

[104] It has been recently reported (Chang and Dong. Cell Res. 17:435 (2007); Wright et al. J Biol Chem. 282:13447 (2007)) and shown by us (see EXAMPLE 34) that IL-17F and IL-17A can form homodimeric as well as heterodimeric proteins when expressed in recombinant cell systems. We and Wright et al. have demonstrated that all three forms of the recombinant proteins (AA, FF, and AF) have in vitro functional activity, such as induction of NFkB signaling pathway and cytokine/chemokine induction (see EXAMPLE 11; also Chang and Dong. Cell Res. 17:435 (2007); Wright et al. J Biol Chem. 282:13447 (2007)). Data in these reports indicate that IL-17A (defined throughout as the IL-17AA homodimer), is the most potent in terms of activity, followed by IL-17AF, and then IL-17F (defined throughout as the IL-17FF homodimer). It has also been shown that activated human CD4+ T cells produce the IL17AF heterodimer in addition to IL-17A and IL-17F (Wright et al. .J Biol Chem. 282:13447 (2007)). Moreover, we have demonstrated that rhIL-23 can induce the production of all three forms of these proteins (IL-17A, IL-17F, and IL-17AF) in murine and cynomolgus splenocytes (see EXAMPLES 23 and 24). Therefore, since IL-17AF is produced by bioassay/culture systems in which IL-17A is also produced, and IL-17AF induces the activity and production of inflammatory cytokines and chemokines that play a role in the diseases mentioned herein, it is important to be able to neutralize the activity of this biologically relevant molecule, IL-17AF, in the treatment of diseases for which IL-23 and/or IL-17 play a role.

[105] The present invention also provides isolated polypeptides and epitopes comprising at least 15 contiguous amino acid residues of an amino acid sequence of SEQ ID NO:2 or 4. Illustrative polypeptides include polypeptides that either comprise, or consist of SEQ ID NO:2 or 4, an antigenic epitope thereof. Moreover, the present invention also provides isolated polypeptides as disclosed above that bind to, block, inhibit, reduce, antagonize or neutralize the activity of IL-17 or IL-23.

[106] The term "antagonist" refers to any compound including a protein, polypeptide, peptide, antibody, antibody fragment, large molecule, or small molecule (less than 10 kD), that decreases the activity, activation or function of another molecule. IL-17A antagonists bind IL-17A such that the interaction of IL-17A protein with its cognate receptor(s) is blocked, inhibited, reduced or neutralized. Other effects of the IL-17A antagonists described herein are the reduction in inflammation or secretion of inflammatory cytokines. IL-23p19 antagonists bind IL-23p19 such that the interaction of IL-23p19 protein with its cognate receptor(s) is blocked, inhibited, reduced or neutralized. Other effects of the IL-23p19 antagonists described herein are the reduction in inflammation or secretion of inflammatory cytokines. IL-17F antagonists bind IL-17F such that the interaction of IL-17F protein with its cognate receptor(s) is blocked, inhibited, reduced or neutralized. Other effects of the IL-17F antagonists described herein are the reduction in inflammation or secretion of inflammatory cytokines.

[107] Antagonists to IL-17A provided herein include binding proteins and antibodies, and any fragments or permutations thereof that bind to IL-17A (herein referred to as "IL-17A antagonists" "anti-IL-17A antibodies" and "anti-IL-17A binding entities"). Within an embodiment, the anti-IL-17A antibodies and anti-IL17A binding entities bind the IL17A/F heterodimer. Within an embodiment, the anti-IL-17A antibodies comprise the complementarity determining regions (CDRs) of the light and heavy chain variable regions of the antibody fragments described herein. Within an embodiment, the anti-IL-17A antibodies comprise the light and heavy chain variable regions of the antibody fragments described herein.

[108] Antagonists to IL-23p19 provided herein include binding proteins and antibodies, and any fragments or permutations thereof that bind to IL-23p19 (herein referred to as "IL-23p19 antagonists", "anti-IL-23p19 antibodies" and "anti-IL-23p19 binding entities"). Within an embodiment, the anti-IL-23p19 antibodies comprise the complementarity determining regions (CDRs) of the light and heavy chain variable regions of the antibody fragments described herein. Within an embodiment, the anti-IL-23p19 antibodies comprise the light and heavy chain variable regions of the antibody fragments described herein.

[109] Antagonists to IL-17A and IL-23p19 provided herein include binding proteins and antibodies, and any fragments or permutations thereof that bind to IL-17A and IL-23p19 (herein referred to as "IL-17A/IL-23p19 antagonists", "anti-IL-17A/anti-IL-23p19 antibodies" and "anti-IL-17A/anti-IL-23p19 binding entities"). Within an embodiment, the anti-IL-17A antibodies and anti-

IL17A binding entities bind the IL17A/F heterodimer. Within an embodiment, the anti-IL-17A/anti-IL-23p19 antibodies comprise the complementarity determining regions (CDRs) of the light and heavy chain variable regions of the anti-IL-17A and anti-IL-23p19 antibody fragments described herein. Within an embodiment, the anti-IL-17A and anti-IL-23p19 antibodies comprise the light and heavy chain variable regions of the antibody fragments described herein.

[110] Thus, the present invention provides antibodies and antibody fragments that specifically or exclusively bind with IL-17A (including the IL17A/F heterodimer) and/or IL-23p19. Exemplary antibodies include neutralizing antibodies, polyclonal antibodies, murine monoclonal antibodies, chimeric antibodies, humanized antibodies derived from murine monoclonal antibodies, human monoclonal antibodies and antibodies that comprise at least the CDRs of the light and heavy chain variable regions described herein. Illustrative antibody fragments include $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv, minimal recognition units, and monomers or dimers of antibody heavy or light chains or mixtures thereof. Neutralizing antibodies preferably bind IL-17A, IL-17F, or IL-23p19 such that the interaction of the ligands with their respective receptors (i.e. IL-17RA or IL-17RC; IL-12b1 and IL-23R for IL-23) is blocked, inhibited, reduced, antagonized or neutralized. That is, the neutralizing IL-17A, IL-17F, and IL-23p19 antibodies of the present invention can either either bind, block, inhibit, reduce, antagonize or neutralize each of IL-17A, IL-17F or IL-23p19 singly, or bind, block, inhibit, reduce, antagonize or neutralize IL-17A, IL-17F, and IL-23p19 in combination therapy or as a single binding entity. The present invention further includes compositions comprising a carrier and a peptide, polypeptide, or antibody described herein.

[111] The antibodies of the invention may include intact immunoglobulins of any isotype including types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). The antibodies can include an Fc(5) (SEQ ID NO: 11 or SEQ ID NO: 333). The light chains of the immunoglobulin may be kappa or lambda..

[112] The present invention also contemplates anti-idiotype antibodies, or anti-idiotype antibody fragments, that specifically bind an antibody or antibody fragment that specifically binds a polypeptide comprising the amino acid sequence of SEQ ID NOs:2, 4, or 6 or any fragment thereof. An exemplary anti-idiotype antibody binds with an antibody that specifically binds a polypeptide consisting of SEQ ID NOs:2 4, or 6.

[113] The present invention also provides fusion proteins, comprising an antagonist of the present invention and an immunoglobulin moiety. In such fusion proteins, the immunoglobulin moiety may be an immunoglobulin heavy chain constant region, such as a human F_c fragment. The present invention further includes isolated nucleic acid molecules that encode such fusion proteins. In one embodiment the Fc is Fc(5).

[114] In an aspect, the present invention provides bispecific antibodies or binding proteins that bind both IL-17A and IL-23p19. In an embodiment, the bispecific antibodies or binding proteins

bind the IL17A/F heterodimer. Bispecific antibodies (BsAbs) are antibodies that can specifically bind to two different antigens, such as IL-17A and IL-23p19, and are provided as a single molecular entity comprising a first antibody portion that binds IL-17A and a second antibody portion that binds IL-23p19. In one embodiment, the first antibody portion binds the IL17A/F heterodimer. Antibodies having higher valencies (i.e., the ability to bind to more than two antigens) can also be prepared; they are referred to as multivalent antibodies. The bispecific antibodies provided herein can be bispecific and bivalent, trispecific and bivalent, bispecific and trivalent, bispecific and tetravalent, or multispecific and multivalent. Bispecific antibodies of the invention, which bind IL-17A and IL-23p19 (as well as bind the IL17A/F heterodimer), are referred to herein as "bispecific anti-IL-17A/anti-IL-23p19 antibodies".

[115] Different configurations of the bispecfic antibodies comprise tandem scFv molecules (herein referred to as "tascFv"), and scFv molecules that are not tandem (herein referred to as "biscFv" and "BiAb").

[116] In certain embodiments, two or more different entities of a bispecific binding composition are linked via linker to form a multimer (e.g., a dimer). For example, in the case of a bispecific binding composition comprising a fusion of at least two polypeptide components (e.g., an anti-IL-17A antibody and another polypeptide component; or an anti-IL-23A antibody and another polypeptide component), a peptide linker sequence may be employed to separate, for example, the polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Fusion proteins can also be expressed as recombinant proteins in an expression system by standard techniques. Suitable linkers are further described herein, infra.

[117] A linker can be naturally-occurring, synthetic, or a combination of both. For example, a synthetic linker can be a randomized linker, e.g., both in sequence and size. In one aspect, the randomized linker can comprise a fully randomized sequence, or optionally, the randomized linker can be based on natural linker sequences. The linker can comprise, for example, a non-polypeptide moiety (e.g., a polynucleotide), a polypeptide, or the like.

[118] A linker can be rigid, or alternatively, flexible, or a combination of both. Linker flexibility can be a function of the composition of both the linker and the subunits that the linker interacts with. The linker joins two selected binding entitities (e.g., two separate polypeptides or proteins, such as two different antibodies) and maintains the entities as separate and discrete. The linker can allow the separate, discrete domains to cooperate yet maintain separate properties such as multiple separate binding sites for the same target in a multimer or, for example, multiple separate binding sites for different targets in a multimer. In some cases, a disulfide bridge exists between two linked binding entities or between a linker and a binding entity.

[119] Choosing a suitable linker for a specific case where two or more binding entities are to be connected may depend on a variety of parameters including, e.g., the nature of the binding entities, the structure and nature of the target to which the bispecific composition should bind, and/or the stability of the linker (e.g., peptide linker) towards proteolysis and oxidation.

[120] Particularly suitable linker polypeptides predominantly include amino acid residues selected from Glycine (Gly), Serine (Ser), Alanine (Ala), and Threonine (Thr). For example, the peptide linker may contain at least 75% (calculated on the basis of the total number of residues present in the peptide linker), such as at least 80%, at least 85%, or at least 90% of amino acid residues selected from Gly, Ser, Ala, and Thr. The peptide linker may also consist of Gly, Ser, Ala and/or Thr residues only. The linker polypeptide should have a length that is adequate to link two binding entities in such a way that they assume the correct conformation relative to one another so that they retain the desired activity, such as binding to a target molecule as well as other activities that may be associated with such target binding (e.g., agonistic or antagonistic activity for a given biomolecule).

[121] A suitable length for this purpose is, e.g., a length of at least one and typically fewer than about 50 amino acid residues, such as 2-25 amino acid residues, 5-20 amino acid residues, 5-15 amino acid residues, 8-12 amino acid residues or 11 residues. Other suitable polypeptide linker sizes may include, e.g., from about 2 to about 15 amino acids, from about 3 to about 15, from about 4 to about 12, about 10, about 8, or about 6 amino acids. The amino acid residues selected for inclusion in the linker polypeptide should exhibit properties that do not interfere significantly with the activity or function of the polypeptide multimer. Thus, the peptide linker should, on the whole, not exhibit a charge that would be inconsistent with the activity or function of the multimer, or interfere with internal folding, or form bonds or other interactions with amino acid residues in one or more of the domains that would seriously impede the binding of the multimer to the target in question.

[122] The use of naturally occurring as well as artificial peptide linkers to connect polypeptides into novel linked fusion polypeptides is well-known in the art. (See, e.g., Hallewell et al., J. Biol. Chem. 264, 5260-5268, 1989; Alfthan et al., Protein Eng. 8, 725-731, 1995; Robinson and Sauer, Biochemistry 35, 109-116, 1996; Khandekar et al., J. Biol. Chem. 272, 32190-32197, 1997; Fares et al., Endocrinology 139, 2459-2464, 1998; Smallshaw et al., Protein Eng. 12, 623-630, 1999; U.S. Patent No. 5,856,456.)

[123] One example where the use of peptide linkers is widespread is for production of single-chain antibodies where the variable regions of a light chain (VL) and a heavy chain (VH) are joined through an artificial linker, and a large number of publications exist within this particular field. A widely used peptide linker is a 15mer consisting of three repeats of a Gly-Gly-Gly-Gly-Ser amino acid sequence ((Gly4Ser)3) (SEQ ID NO:2240). Other linkers have been used, and phage display technology, as well as selective infective phage technology, has been used to diversify and select

appropriate linker sequences (Tang et al., J. Biol. Chem. 271, 15682-15686, 1996; Hennecke et al., Protein Eng. 11, 405-410, 1998). Peptide linkers have been used to connect individual chains in hetero- and homo-dimeric proteins such as the T-cell receptor, the lambda Cro repressor, the P22 phage Arc repressor, IL-12, TSH, FSH, IL-5, and interferon-γ. Peptide linkers have also been used to create fusion polypeptides. Various linkers have been used, and, in the case of the Arc repressor, phage display has been used to optimize the linker length and composition for increased stability of the single-chain protein (see Robinson and Sauer, Proc. Natl. Acad. Sci. USA 95, 5929-5934, 1998).

[124] Still another way of obtaining a suitable linker is by optimizing a simple linker (e.g., (Gly4Ser)n) through random mutagenesis.

[125] As discussed above, it is generally preferred that the peptide linker possess at least some flexibility. Accordingly, in some variations, the peptide linker contains 1-25 glycine residues, 5-20 glycine residues, 5-15 glycine residues, or 8-12 glycine residues. Particularly suitable peptide linkers typically contain at least 50% glycine residues, such as at least 75% glycine residues. In some embodiments, a peptide linker comprises glycine residues only. In certain variations, the peptide linker comprises other residues in addition to the glycine. Preferred residues in addition to glycine include Ser, Ala, and Thr, particularly Ser. In some embodiments the peptide linker comprises other residues selected from Val, Leu, Ile, Met, Phe, Trp, Pro, Cys, Tyr, Asn, Gln, Lys, Arg, His, Asp, and Glu.

[126] In some cases, it may be desirable or necessary to provide some rigidity into the peptide linker. This may be accomplished by including proline residues in the amino acid sequence of the peptide linker. Thus, in another embodiment, a peptide linker comprises at least one proline residue in the amino acid sequence of the peptide linker. For example, a peptide linker can have an amino acid sequence wherein at least 25% (e.g., at least 50% or at least 75%) of the amino acid residues are proline residues. In one particular embodiment of the invention, the peptide linker comprises proline residues only.

[127] In some embodiments, a peptide linker is modified in such a way that an amino acid residue comprising an attachment group for a non-polypeptide moiety is introduced. Examples of such amino acid residues may be a cysteine or a lysine residue (to which the non-polypeptide moiety is then subsequently attached). Another alternative is to include an amino acid sequence having an in vivo N-glycosylation site (thereby attaching a sugar moiety (in vivo) to the peptide linker). An additional option is to genetically incorporate non-natural amino acids using evolved tRNAs and tRNA synthetases (see, e.g., U.S. Patent Application Publication 2003/0082575) into a polypeptide binding entity or peptide linker. For example, insertion of keto-tyrosine allows for site-specific coupling to an expressed polypeptide.

[128] In certain variations, a peptide linker comprises at least one cysteine residue, such as one cysteine residue. For example, in some embodiments, a peptide linker comprises at least one cysteine residue and amino acid residues selected from the group consisting of Gly, Ser, Ala, and Thr. One example of a specific peptide linker includes a peptide linker having the amino acid sequence Glyx-Xaa-Glyy-Xaa-Glyz (SEQ ID NO:336), wherein each Xaa is independently selected from Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile), Methionine (Met), Phenylalanine (Phe), Tryptophan (Trp), Proline (Pro), Glycine (Gly), Serine (Ser), Threonine (Thr), Cysteine (Cys), Tyrosine (Tyr), Asparagine (Asn), Glutamine (Gln), Lysine (Lys), Arginine (Arg), Histidine (His), Aspartate (Asp), and Glutamate (Glu), and wherein x, y, and z are each integers in the range from 1-5. In some such embodiments, a peptide linker comprises glycine residues and cysteine residues, such as glycine residues and cysteine residues only. Typically, only one cysteine residue will be included per peptide linker. See, for example, the peptide linker of SEQ ID NO:337.

[129] As previously noted, in certain embodiments, a bispecific binding composition comprises an anti-IL-17A antibody and an anti-IL-23A antibody. In some such embodiments, the anti-IL-17A and anti-IL-23A antibodies are covalently linked (e.g., via a peptide linker) to form a bispecific antibody. In some variations, the bispecific antibody comprises an immunoglobulin heavy chain constant region such as, for example, an Fc fragment. Particularly suitable Fc fragments include, for example, Fc fragments comprising an Fc region modified to reduce or eliminate one or more effector functions (e.g., Fc5, having the amino acid sequence shown in SEQ ID NO:11 or SEQ ID NO: 333).

[130] For example, in some embodiments, a bispecific antibody that neutralizes both IL-17A and IL-23A in accordance with the present invention comprises an antigen-binding region of an anti-IL-17A antibody as described herein and an antigen-binding region of an anti-IL-23A antibody as described herein. In certain embodiments, a bispecific antibody comprises a first antigen-binding region having the CDRs of an anti-IL-17A antibody listed in Table 1 and a second antigen-binding region having the CDRs of an anti-IL-23A antibody listed in Table 2. In particular variations, a bispecific antibody comprises a first antigen-binding region having the VL and VH domains of an anti-IL-17A antibody listed in Table 1 and a second antigen-binding region having the VL and VH domains of an anti-IL-23A antibody listed in Table 2. In specific variations, the CDRs or the VL and VH domains of the first antigen-binding region are those of an anti-IL-17A antibody selected from the clones listed in Table 5 (clones c87, c200, c209, c632, c389, and c631); and the CDRs or the VL and VH domains of the second antigen-binding region are those of an anti-IL-23A antibody selected from the clones listed in Table 6 (clones c29, c305, c361, c490, and c472). In certain preferred embodiments, a bispecific antibody in accordance with the present invention is a tandem single chain Fv (tascFv), bispecific single chain Fv (biscFv), or a bispecific antibody (biAb).

[131] For the tascFv molecule, two scFv molecules are constructed such that one scFv is amino terminal to the other one in a tandem configuration. This can be done in each orientation. Tandem scFv molecules can be prepared with a linker between the scFv entites. In some some embodiments, the linker is a a Gly-Ser linker comprising a series of glycine and serine residues, and optionally including additional amino acids. In other embodiments, the linker is a lambda stump or a CH1 stump, both of which are derived from the native sequence just after the V region in the Fab, or a Gly-Ser tether. The tascFv can be further constructed as fusion protein to contain a Fc component ("tascFv Fc"). In some such embodiments, such an Fc fragment comprises an Fc region modified to reduce or eliminate one or more effector functions (e.g., Fc5, having the amino acid sequence shown in SEQ ID NO:11 or SEQ ID NO: 333). Thus an anti-IL-17A binding entitiy can be the scFv entity that is either proximal or distal to a Fc component. Likewise, an anti-IL-23 binding entity can be the scFv entity that is either proximal or distal to a Fc component. In certain embodiments, when the anti-IL-17A and anti-IL-23A antibodies described herein are produced in a tandem format the potency of the anti-IL-17A antibody is higher when it is the antibody furthest away from the Fc fragment than when it is the antibody closest to the Fc fragment.

[132] The biscFv molecule is not a tandem configuration. Rather, it has a scFv at the N terminus and another at the C terminus of an Fc ("biscFv Fc"). These molecules can be made with the N terminal scFv directly fused to the Fc hinge and with either a short or a long linker at the C terminus connecting to the second scFv. These linkers are Gly-Ser. In some embodiments, the Fc fragment comprises an Fc region modified to reduce or eliminate one or more effector functions (e.g., Fc5, having the amino acid sequence shown in SEQ ID NO:11 or SEQ ID NO: 333). Thus, an anti-IL-17A binding entity can be the scFv entity that is either at the N terminus or at the C terminus to a Fc component. Likewisie, an anti-IL-23 bidning entity can be the scFv entity that is either at the N terminus or at the C terminus to a Fc component.

[133] The Biab molecule is also not a tandem format. It comprises a whole monoclonal antibody with a scFv fused to the C terminus of the heavy chain. These molecules can be made, for example, by converting one scFv back to a light chain (kappa or lambda) and a gamma1 heavy chain with the second scFv connected by either a short or long Gly-Ser linker. Thus an anti-IL-17A binding entity can be either scFv that is converted back to a light chain (kappa or lambda) and a gamma1 heavy chain or the second scFv fused to the C terminus. Likewise, an anti-IL-23 binding entity can be either scFv that is converted back to a light chain (kappa or lambda) and a gamma1 heavy chain or the second scFv fused to the C terminus. Also, a Fab (either anti-IL-17A Fab or anti-IL-23 binding Fab) can be fused to the Fc portion rather than converting a scFv back to a light chain (kappa or lambda) and a gamma1 heavy chain. These molecules can be made with a whole anti-IL-17A monoclonal antibody fused to an anti-IL-23A scFv or, alternatively, with a whole anti-IL-23A monoclonal antibody fused to an anti-IL-17A scFv. In some particular embodiments, a biAb in accordance with

the present invention comprises a whole anti-IL-17A monoclonal antibody (IgG1) with the C-terminal end of the heavy chain fused to an anti-IL-23A scFv comprising an amino acid sequence selected from the group consisting of c305.1 scFv (SEQ ID NO: 2165), c631.1VH - VL scFv of SEQ ID NOs: 2167 and 2166; c632.1VH - VL scFv of SEQ ID NOs: 2168 and 2169; c472.2 VL - VH of SEQ ID NOs: 2170 and 2171; c87 scFv of SEQ ID NOs: 798 and 799; c200 scFv of SEQ ID NOs: 842 and 843; c209 scFv of SEQ ID NOs: 852 and 853; c389 scFv of SEQ ID NOs: 932 and 933; c29 scFv of SEQ ID NOs: 1647 and 1648; c361 scFv of SEQ ID NOs: 1934 and 1935; and c490 scFv of SEQ ID NOs: 2014 and 2015. In some specific variations, a biAb in accordance with the present invention comprises a light chain polypeptide as shown in Table 12, herein, and a heavy chain with C-Terminus scFv as shown in Table 13, herein.

[134] Additional bispecific molecules are known in the art and include single variable domain antibodies, camelid antibodies, variable domains fused to human serum albumin (See Muller, D, et al., J. Biol. Chem. 282 (Issue 17):12650-12660, 2007), and dual variable domain immunoglobin molecules (See WIPO patent publication number WO/2007/024715, published March 1, 2007, by Wu, Chengbin, et al.).

[135] Bispecific and multi-valent antibodies to IL-17A and IL-23p19 are taught throught this application and are described in Example 6.

[136] The bispecific antibody can be a monoclonal antibody (MAb) with respect to each target. In particular embodiments, the antibody is chimeric, or humanized, or fully human. Fully human antibodies may be generated by procedures that involve immunizing transgenic mice, wherein human immunoglobulin genes have been introduced into the mice, as discussed below. Illustrative antibodies are taught throughout the specification and claims. See, for example, the antibodies in Example 8.

[137] In another embodiment, the anti-IL-17A, anti-IL-23p19 and anti-IL-17A/anti-IL-23p19 antibodies are linked to one or more non-proteinaceous polymers selected from the group consisting of polyethylene glycol, polypropylene glycol, and polyoxyalkylene, or to a cytotoxic agent or enzyme, or to a radioisotope, fluorescent compound or chemiluminescent compound.

[138] Typical methods of the invention include methods to treat pathological conditions or diseases in mammals associated with or resulting from increased or enhanced IL-17 and/ or IL-23 expression and/or activity. In the methods of treatment, the antibodies of the present invention may be administered which preferably block or reduce the respective receptor binding or activation to their receptor(s). Optionally, the antibodies employed in the methods will be capable of blocking or neutralizing the activity of both IL-17A and IL-23p19, e.g., a dual antagonist which blocks or neutralizes activity of IL-17A, IL-17F, or IL-23. The methods contemplate the use of a single bispecific binding peptide or antibody or a combination of two or more antibodies (each of which specifically binds to either IL-17A or IL-23p19).

[139] The invention also provides compositions which include pharmaceutically acceptable carriers or diluents. Preferably, the compositions will include one or more anti-IL-17A or anti-IL-23p19 or anti-IL-17A/anti-IL-23p19antibodies in an amount which is therapeutically effective to treat a pathological condition or disease.

[140] In another embodiment, the invention concerns a composition of matter comprising anti-IL-17A antagonists, anti-IL-23p19antagonists, or anti-IL-17A/anti-IL-23p19 antagonistswhich binds both IL-17A and IL-23 in admixture with a carrier or excipient. In one aspect, the composition comprises a therapeutically effective amount of the anti-IL-17A antagonists, anti-IL-23p19antagonists, or anti-IL-17A/anti-IL-23p19 antagonists. The composition is useful for: (a) reducing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) inhibiting or reducing an immune response in a mammal in need thereof, (c) decreasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen, (d) inhibiting the activity of T-lymphocytes, (e) decreasing the vascular permeability, or (f) reducing systemic and/or local concentrations of IL-17AA, IL-17AF and/or IL-23.

[141] In another embodiment, the invention concerns a method of treating an immune related disorder in a mammal in need thereof, comprising administering to the mammal a therapeutically effective amount of an IL-17/IL-23 antagonist.

[142] In still another embodiment, the invention includes an isolated polynucleotide that encodes a polypeptides of the present invention, wherein said polypeptides are capable of binding to IL-17A, IL-17F, and/or IL-23p19.

[143] In still another embodiment, the invention inlcudes an isolated polypeptide of the present invention, wherein said polypeptide is capable of binding to IL-17A, IL-17F, and/or IL-23p19.

[144] In yet another embodiment, the invention includes a method for inhibiting IL-17A and IL-17F production and/or maintenance by treating the T cells with an antagonist of IL-23p19.

[145] In another aspect, the invention includes a method for the treatment of an inflammatory disease characterized by elevated expression of IL-17A, IL-17F and/or IL-23 in a mammalian subject, comprising administering to the subject an effective amount of anti-IL-17A antagonists, anti-IL-23p19antagonists, or anti-IL-17A/anti-IL-23p19 antagonists.

[146] Processes for producing the anti-IL-17A antagonists, anti-IL-23p19antagonists, or anti-IL-17A/anti-IL-23p19 antagonist are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of said antibody and recovering said antibody from the cell culture.

[147] In a further embodiment, the invention concerns an article of manufacture, comprising: (a) a composition of matter comprising an anti-IL-17A antibodies, anti-IL-23p19

antibodies, or anti-IL-17A/anti-IL-23p19 antibodiesy, or an antibody that specifically binds to IL-17A, IL-17F, and/or IL-23p19; (b) a container containing said composition; and (c) a label affixed to said container, or a package insert included in said container referring to the use of said nti-IL-17A antibodies, anti-IL-23p19 antibodies, or anti-IL-17A/anti-IL-23p19 antibodiesin the treatment of an immune related disease. The composition may comprise a therapeutically effective amount of the nti-IL-17A antibodies, anti-IL-23p19 antibodies, or anti-IL-17A/anti-IL-23p19 antibodies.

[148] In yet another embodiment, the present invention concerns a method of diagnosing an immune-related disease in a mammal, comprising detecting the level of expression of a gene encoding either or both IL-17 and/or IL-23 (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type or against a known normal standard, wherein a higher or lower expression level in the test sample as compared to the control sample indicates the presence of immune related disease in the mammal from which the test tissue cells were obtained.

[149] In another embodiment, the present invention concerns a method of diagnosing or monitoring an immune disease in a mammal, comprising (a) contacting an IL-17/IL-23 antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and either or both IL-17 and IL-23 in the test sample; wherein the formation of said complex is indicative of the presence or absence of said disease. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. The antibody can carry a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art.

[150] In another embodiment, the invention provides a method of diagnosing or monitoring an immune-related disease in a mammal which comprises detecting the presence or absence of both IL-17 and IL-23 in a test sample of tissue cells obtained from said mammal, wherein the presence or absence of both IL-17 and IL-23 in said test sample is indicative of the presence of an immune-related disease in said mammal.

[151] In another further embodiment, the invention provides a method of decreasing the activity of T-lymphocytes in a mammal comprising administering to said mammal anti-IL-17A antibodies, anti-IL-23p19 antibodies, or anti-IL-17A/anti-IL-23p19 antibodies, wherein the activity of T-lymphocytes in the mammal is decreased.

[152] In another embodiment, the invention provides a method of decreasing the proliferation of T-lymphocytes in a mammal comprising administering to said mammal (a) an anti-IL-17A antagonists, anti-IL-23p19antagonists, or anti-IL-17A/anti-IL-23p19 antagonists, such as an anti-IL-17A antibodies, anti-IL-23p19 antibodies, or anti-IL-17A/anti-IL-23p19 antibodies, wherein the proliferation of T-lymphocytes in the mammal is decreased.

[153] The invention also provides articles of manufacture and kits which include one or more anti-IL-17A antibodies, anti-IL-23p19 antibodies, or anti-IL-17A/anti-IL-23p19 antibodies.

[154] These and other aspects of the invention will become evident upon reference to the following detailed description. In addition, various references are identified below and are incorporated by reference in their entirety.

[155] The antibodies of the invention specifically bind to IL-17A, IL-17F and IL-23p19. In some embodiments, the antibodies of the invention specifically bind a monomeric form of both IL-17A and IL-23p19. In some embodiments, the antibodies of the invention bind a homodimeric form of either IL-17A or IL-23P19. In still other embodiments, the antibodies of the invention specifically bind a multimeric form of IL-17 or IL-23 (e.g., a heterodimeric form). For instance, IL-17A can form a heterodimer with any other member of the IL-17 family of ligands, such as IL-17B, IL-17C, or IL-17F. antibodies of the invention block the biological activities of IL-17A and IL-2p193, either singly or together.

[156] The antibodies of the invention include derivatives that are modified, e.g., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to its epitope. Examples of suitable derivatives include, but are not limited to fucosylated antibodies and fragments, glycosylated antibodies and fragments, acetylated antibodies and fragments, pegylated antibodies and fragments, phosphorylated antibodies and fragments, and amidated antibodies and fragments. The antibodies and derivatives thereof of the invention may themselves by derivatized by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other proteins, and the like. In some embodiments of the invention, at least one heavy chain of the antibody is fucosylated. In some embodiments, the fucosylation is N-linked. In some preferred embodiments, at least one heavy chain of the antibody comprises a fucosylated, N-linked oligosaccharide.

[157] The antibodies of the invention include variants having single or multiple amino acid substitutions, deletions, additions, or replacements that retain the biological properties (e.g., block the binding of IL-17A, IL-17F and/or IL-23p19 to their respective receptors, block the biological activity of IL-17A, IL-17F and/or IL-23p19, binding affinity) of the antibodies of the invention. The skilled person can produce variants having single or multiple amino acid substitutions, deletions, additions or replacements. These variants may include, inter alia: (a) variants in which one or more amino acid residues are substituted with conservative or nonconservative amino acids, (b) variants in which one or more amino acids are added to or deleted from the polypeptide, (c) variants in which one or more amino acids include a substituent group, and (d) variants in which the polypeptide is fused with another peptide or polypeptide such as a fusion partner, a protein tag or other chemical moiety, that may confer useful properties to the polypeptide, such as, for example, an epitope for an antibody, a polyhistidine sequence, a biotin moiety and the like.

[158] Within an aspect the invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a) a light chain variable region comprising: i) a LCDR1 consisting of the amino acid sequence of SEQ ID NO: 12; ii) a LCDR2 consisting of the amino acid sequence of SEQ ID NO: 13; and iii) a LCDR3 consisting of the amino acid sequence of SEQ ID NO: 14; and b) a heavy chain variable region comprising: i) a HCDR1 consisting of the amino acid sequence of SEQ ID NO: 15; ii) a HCDR2 consisting of the amino acid sequence of SEQ ID NO: 16; and iii) a HCDR3 consisting of the amino acid sequence selected from the group consisting of 1) the amino acid sequence of SEQ ID NO: 17; 2) the amino acid sequence of SEQ ID NO: 18; and 3) the amino acid sequence of SEQ ID NO:19. Within an embodiment, said antibody binds the heterodimer formed by IL-17A (SEQ ID NO: 2) and IL-17F (SEQ ID NO: 6). Within an embodiment, said antibody comprises a single chain Fv fragment (scFv). Within an embodiment, said antibody is a chimeric antibody. Within an embodiment, said antibody is a bispecific antibody that also binds IL-23A (SEQ ID NO: 4). Within another embodiment, LCDR1 has the amino acid sequence of SEQ ID NO: 30; LCDR2 has the amino acid sequence of SEQ ID NO: 26; LCDR3 has the amino acid sequence of SEQ ID NO: 31; HCDR1 has the amino acid sequence of SEQ ID NO: 34; HCDR2 has the amino acid sequence of SEQ ID NO: 35; and HCDR3 has the amino acid sequence of SEQ ID NO: 18.

[159] The invention provides an isolated antibody or combination of antibodies selected from the goup consisting of: a) the antibody having the ATCC Patent Deposit Designation PTA-8819; the antibody having the ATCC Patent Deposit Designation PTA-8821; the antibody having the ATCC Patent Deposit Designation PTA-8820; and the antibody having the ATCC Patent Deposit Designation -8818)

[160] Within an aspect, the invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a variable light domain comprising LCDRs LCDR1, LCDR2, and LCDR3 and a variable heavy domain comprising HCDRs HCDR1, HCDR2, and HCDR3, wherein the set of LCDRs has three, two, or one amino acid substitutions and the set of HCDRs has three or fewer amino acid substitutions in which LCDR1 has the amino acid sequence of SEQ ID NO: 30; LCDR2 has the amino acid sequence of SEQ ID NO: 26; LCDR3 has the amino acid sequence of SEQ ID NO: 31; HCDR1 has the amino acid sequence of SEQ ID NO: 34; HCDR2 has the amino acid sequence of SEQ ID NO: 35; and HCDR3 has the amino acid sequence of SEQ ID NO: 18. Within an embodiment, the antibody has two or one amino acid substitutions in the set of LCDRs. Within an embodiment, the antibody has two or one amino acid substitutions in the set of HCDRs.

[161] The invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a) a light chain variable region comprising: i) a LCDR1 consisting of the amino acid sequence of SEQ ID NO: 72; ii) a LCDR2 consisting of the amino acid sequence of SEQ ID NO: 73; and iii) a LCDR3 consisting of the amino acid sequence of SEQ ID NO: 74; and) a heavy chain

variable region comprising: i) a HCDR1 consisting of the amino acid sequence of SEQ ID NO: 75; ii) a HCDR2 consisting of the amino acid sequence of SEQ ID NO: 76; and iii) a HCDR3 consisting of the amino acid sequence selected from the group consisting of 1) the amino acid sequence of SEQ ID NO: 17; 2) the amino acid sequence of SEQ ID NO: 18; 3) the amino acid sequence of SEQ ID NO:19; 4) the amino acid sequence of SEQ ID NO: 77; 5) the amino acid sequence of SEQ ID NO: 78; and 6) the amino acid sequence of SEQ ID NO: 79. Within an embodiment, said HCDR3 amino acid consists of the amino acid sequence of SEQ ID NO: 19. Within an embodiment, said antibody binds the heterodimer formed by IL-17A (SEQ ID NO: 2) and IL-17F (SEQ ID NO: 6). Within another embodiment, said antibody comprises a single chain Fv fragment (scFv). Within another embodiment said antibody is a chimeric antibody. Within another embodiment, said antibody is a bispecific antibody that also binds IL-23A (SEQ ID NO: 4). Within another embodiment, LCDR1 has the amino acid sequence of SEQ ID NO: 25; LCDR2 has the amino acid sequence of SEQ ID NO: 26; LCDR3 has the amino acid sequence of SEQ ID NO: 27; HCDR1 has the amino acid sequence of SEQ ID NO: 28; HCDR2 has the amino acid sequence of SEQ ID NO: 29; and HCDR3 has the amino acid sequence of SEQ ID NO: 19.

[162] Within an aspect, the invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a variable light domain comprising LCDRs LCDR1, LCDR2, and LCDR3 and a variable heavy domain comprising HCDRs HCDR1, HCDR2, and HCDR3, wherein the set of LCDRs has three, two, or one amino acid substitutions and the set of HCDRs has three or fewer amino acid substitutions in which LCDR1 has the amino acid sequence of SEQ ID NO: 25; LCDR2 has the amino acid sequence of SEQ ID NO: 26; LCDR3 has the amino acid sequence of SEQ ID NO: 27; HCDR1 has the amino acid sequence of SEQ ID NO: 28; HCDR2 has the amino acid sequence of SEQ ID NO: 29; and HCDR3 has the amino acid sequence of SEQ ID NO: 19. Within an embodiment, the antibody has two or one amino acid substitutions in the set of LCDRs. Within another embodiment, the antibody has two or one amino acid substitutions in the set of HCDRs.

[163] Within an aspect, the invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a first polypeptide having at least 90% sequence identity to the variable light region of an antibody entity and comprising a second polypeptide having at least 90% sequence identity to the variable heavy region of said antibody entity, wherein the antibody entity comprises a variable light region and a variable heavy region selected from the group consisting of: a) the variable light region consisting of the amino acid sequence of SEQ ID NO: 932 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 933; b) the variable light region consisting of the amino acid sequence of SEQ ID NO: 1192 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 1193; and c) the variable light region consisting of the amino acid sequence of SEQ ID NO: 1194 and the variable heavy region consisting of the amino acid

sequence of SEQ ID NO: 1195. Within embodiments: the polypeptide has at least 95% sequence identity with the framework regions of the variable light region; the first polypeptide has at least 95% sequence identity with the variable light region; the first polypeptide has at least 96% sequence identity with the variable light region; the first polypeptide has at least 97% sequence identity with the variable light region; the first polypeptide has at least 98% sequence identity with the variable light region; the first polypeptide has at least 99% sequence identity with the variable light region; the second polypeptide has at least 95% sequence identity with the framework regions of the variable heavy region; the second polypeptide has at least 95% sequence identity with the variable heavy region; the second polypeptide has at least 96% sequence identity with the variable heavy region; the second polypeptide has at least 97% sequence identity with the variable heavy region; the second polypeptide has at least 98% sequence identity with the variable heavy region; or the first polypeptide has at least 99% sequence identity with the variable heavy region. Within an embodiment, said antibody binds the heterodimer formed by IL-17A (SEQ ID NO: 2) and IL-17F (SEQ ID NO: 6). Within an embodiment said antibody comprises a single chain Fv fragment (scFv). Within another embodiment said antibody is a chimeric antibody. Within another embodiment, said antibody is a bispecific antibody that also binds IL-23A (SEQ ID NO: 4). Within additional embodiments, the antibody comprises the variable light region of SEQ ID NO: 1904 and the variable heavy region of SEQ ID NO: 1905; or the antibody comprises the variable light region of SEQ ID NO: 1982 and the variable heavy region of SEQ ID NO: 1983. Within a further embodiment, said antibody neutralizes the activity of IL-17A (SEQ ID NO: 2).

[164] The invention provides. an n isolated antibody that specifically binds IL-23A (SEQ ID NO: 4) comprising a) a light chain variable region comprising: i) a LCDR1 consisting of the amino acid sequence of SEQ ID NO: 224; ii) a LCDR2 consisting of the amino acid sequence of SEQ ID NO: 225; and iii) a LCDR3 consisting of the amino acid sequence of SEQ ID NO: 226; and b) a heavy chain variable region comprising: i) a HCDR1 consisting of the amino acid sequence of SEQ ID NO: 227; ii) a HCDR2 consisting of the amino acid sequence of SEQ ID NO: 228; and iii) a HCDR3 consisting of the amino acid sequence of SEQ ID NO: 252. Within an embodiment, said antibody comprises a single chain Fv fragment (scFv). Within an embodiment, said antibody is a chimeric antibody. Within another embodiment, the antibody is a bispecific antibody that also binds IL-17A. Within another embodiment, LCDR1 has the amino acid sequence of SEQ ID NO: 2105; LCDR2 has the amino acid sequence of SEQ ID NO: 2106; LCDR3 has the amino acid sequence of SEQ ID NO: 2107; HCDR1 has the amino acid sequence of SEQ ID NO: 2108; HCDR2 has the amino acid sequence of SEQ ID NO: 2109; and HCDR3 has the amino acid sequence of SEQ ID NO: 2110.

[165] The invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a variable light domain comprising LCDRs LCDR1, LCDR2, and LCDR3 and a

variable heavy domain comprising HCDRs HCDR1, HCDR2, and HCDR3, wherein the set of LCDRs has three, two, or one amino acid substitutions and the set of HCDRs has three or fewer amino acid substitutions in which LCDR1 has the amino acid sequence of SEQ ID NO: 2105; LCDR2 has the amino acid sequence of SEQ ID NO: 2106; LCDR3 has the amino acid sequence of SEQ ID NO: 2107; HCDR1 has the amino acid sequence of SEQ ID NO: 2108; HCDR2 has the amino acid sequence of SEQ ID NO: 2109; and HCDR3 has the amino acid sequence of SEQ ID NO: 2110. Within an embodiment the antibody has two or one amino acid substitutions in the set of LCDRs. Within another embodiment, the antibody has two or one amino acid substitutions in the set of HCDRs.

[166] Within an aspect, the invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a first polypeptide having at least 90% sequence identity to the variable light region of an antibody entity and comprising a second polypeptide having at least 90% sequence identity to the variable heavy region of said antibody entity, wherein the antibody entity comprises a variable light region and a variable heavy region selected from the group consisting of: a) the variable light region consisting of the amino acid sequence of SEQ ID NO: 1934 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 1935; b) the variable light region consisting of the amino acid sequence of SEQ ID NO: 1982 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 1983; and c) the variable light region consisting of the amino acid sequence of SEQ ID NO: 2014 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 2015. Within embodiments: the first polypeptide has at least 95% sequence identity with the framework regions of the variable light region; the first polypeptide has at least 95% sequence identity with the variable light region; the first polypeptide has at least 96% sequence identity with the variable light region; the first polypeptide has at least 97% sequence identity with the variable light region; the first polypeptide has at least 98% sequence identity with the variable light region; the first polypeptide has at least 99% sequence identity with the variable light region; the second polypeptide has at least 95% sequence identity with the framework regions of the variable heavy region; the second polypeptide has at least 95% sequence identity with the variable heavy region; the second polypeptide has at least 96% sequence identity with the variable heavy region; the second polypeptide has at least 97% sequence identity with the variable heavy region; the second polypeptide has at least 98% sequence identity with the variable heavy region; or first polypeptide has at least 99% sequence identity with the variable heavy region. Within an embodiment, said antibody comprises a single chain Fv fragment (scFv). Within another embodiment, said antibody is a chimeric antibody. Within an another embodiment, said antibody is a bispecific antibody that also binds IL-17A (SEQ ID NO: 2). Within an another embodiment, the antibody comprises the variable light region of SEQ ID NO: 1192 and the variable heavy region of SEQ ID NO: 1193. Within an another embodiment, the bispecific antibody binds the heterodimer formed by IL-17A (SEQ ID NO: 2) with

IL-17F (SEQ ID NO: 6). Within another embodiment, the antibody comprises the variable light region of SEQ ID NO: 1194 and the variable heavy region of SEQ ID NO: 1195. Within a further embodiment, the bispecific antibody bind the heterodimer formed by IL-17A (SEQ ID NO: 2) with IL-17F (SEQ ID NO: 6) and said antibody neutralizes the activity of IL-23A (SEQ ID NO: 4).

[167] The invention provides an isolated bispecific antibody that specifically binds to IL-17A (SEQ ID NO: 2) and to IL-23A (SEQ ID NO: 6) comprising an anti-IL-17A antibody and an anti-IL-23A antibody, wherein the anti-IL-17A antibody comprises a first variable light region, which comprises a set of first LCDRs, and comprising a first variable heavy region, which comprises a set of first HCDRs , wherein said set of first LCDRs and said set of first HCDRs are selected from the group consisting of: a) a first LCDR1 consisting of the amino acid sequence of SEQ ID NO: 25, and a first LCDR2 consisting of the amino acid sequence of SEQ ID NO: 26, and a first LCDR3 consisting of the amino acid sequence of SEQ ID NO: 27, a first HCDR1 consisting of the amino acid sequence of SEQ ID NO: 28, a first HCDR2 consisting of the amino acid sequence of SEQ ID NO: 29, and a first HCDR3 consisting of the amino acid sequence of SEQ ID NO: 19; b) a first LCDR1 consisting of the amino acid sequence of SEQ ID NO: 30, and a first LCDR2 consisting of the amino acid sequence of SEQ ID NO: 26, and a first LCDR3 consisting of the amino acid sequence of SEQ ID NO: 31, a first HCDR1 consisting of the amino acid sequence of SEQ ID NO: 32, a first HCDR2 consisting of the amino acid sequence of SEQ ID NO: 33, and a first HCDR3 consisting of the amino acid sequence of SEQ ID NO: 18; and c) a first LCDR1 consisting of the amino acid sequence of SEQ ID NO: 30, and a first LCDR2 consisting of the amino acid sequence of SEQ ID NO: 26, and a first LCDR3 consisting of the amino acid sequence of SEQ ID NO: 31, a first HCDR1 consisting of the amino acid sequence of SEQ ID NO: 34, a first HCDR2 consisting of the amino acid sequence of SEQ ID NO: 35, and a first HCDR3 consisting of the amino acid sequence of SEQ ID NO: 18; and wherein the anti-IL-23A antibody comprises a second variable light region, which comprises a set of second LCDRs, and comprising a second variable heavy region, which comprises a set of second HCDRs , wherein said set of second LCDRs and said set of second HCDRs are selected from the group consisting of: a) a second LCDR1 consisting of the amino acid sequence of SEQ ID NO: 2086, and a second LCDR2 consisting of the amino acid sequence of SEQ ID NO: 2087, and a second LCDR3 consisting of the amino acid sequence of SEQ ID NO: 2088, a second HCDR1 consisting of the amino acid sequence of SEQ ID NO: 2089, a second HCDR2 consisting of the amino acid sequence of SEQ ID NO: 2092, and a second HCDR3 consisting of the amino acid sequence of SEQ ID NO: 2091; b) a second LCDR1 consisting of the amino acid sequence of SEQ ID NO: 2093, and a second LCDR2 consisting of the amino acid sequence of SEQ ID NO: 2094, and a second LCDR3 consisting of the amino acid sequence of SEQ ID NO: 2095, a second HCDR1 consisting of the amino acid sequence of SEQ ID NO: 2096, a second HCDR2 consisting of the amino acid sequence of SEQ ID NO: 2097, and a second HCDR3 consisting of the amino acid sequence of SEQ ID NO: 2098; c) a

second LCDR1 consisting of the amino acid sequence of SEQ ID NO: 2099, and a second LCDR2 consisting of the amino acid sequence of SEQ ID NO: 2100, and a second LCDR3 consisting of the amino acid sequence of SEQ ID NO: 2101, a second HCDR1 consisting of the amino acid sequence of SEQ ID NO: 2102, a second HCDR2 consisting of the amino acid sequence of SEQ ID NO: 2103, and a second HCDR3 consisting of the amino acid sequence of SEQ ID NO: 2104; and d) a second LCDR1 consisting of the amino acid sequence of SEQ ID NO: 2105, and a second LCDR2 consisting of the amino acid sequence of SEQ ID NO: 2106, and a second LCDR3 consisting of the amino acid sequence of SEQ ID NO: 2107, a second HCDR1 consisting of the amino acid sequence of SEQ ID NO: 2108, a second HCDR2 consisting of the amino acid sequence of SEQ ID NO: 2109, and a second HCDR3 consisting of the amino acid sequence of SEQ ID NO: 2110. Within an embodiment, the anti-IL-17A antibody and the anti-IL-23A antibody are covalently linked via a linker. Within another embodiment, the linker is a polypeptide linker. Within an embodiment, the anti-IL-17A and anti-IL-23A antibodies are single chain Fc fragments covalently linke d to form a tandem single chain Fv (tascFV) or a bispecific single chain Fv (biscFv). Within a further embodiment, the antibody comprises a pharmaceutically acceptable carrier. Within a different embodiment, the anti-IL-17A antibody binds the heterodimer formed by IL-17A (SEQ ID NO: 2) and IL-17F (SEQ ID NO: 6). Within an different embodiment, the anti-IL-17A antibody neutralizes the activity of IL-17A (SEQ ID NO: 2) and wherein the anti-IL-23A antibody neutralizes the activity of IL-23A (SEQ ID NO: 4). Within a different embodiment, said bispecific antibody comprises an immunoglobulin heavy chain constant region. Within a further embodiment, said immunoglobulin heavy chain constant region is an Fc fragment or said Fc fragment comprises a Fc region modified to reduce or eliminate one or more effector functions. Within an embodiment, the antibody comprises PEG.

[168] The invention provides a polynucleotide that encodes any of the antibodies and bispecific molecules described herein.

[169] The invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2), wherein the antibody binds at least one amino acid of SEQ ID NO: 2222. Within an embodiment, the antibody specifically binds at least one amino acid of SEQ ID NO:2221.

[170] The invention provides an isolated antibody that specifically binds IL-17A (SEQ ID NO: 2), wherein the antibody is capable of binding an immobilized peptide consisting of the amino acid sequence of TNTNPKRSSDYYNRSTSPW (SEQ ID NO: 2222). Within an embodiment, the antibody is additionally capable of binding an immobilized peptide consisting of the amino acid sequence of NLNIHNRNTNTNPKR, (SEQ ID NO: 2221).

[171] The invention provides an isolated antibody that specifically binds IL-23A (SEQ ID NO: 4), wherein the antibody binds at least one amino acid from the peptide sequence of LQRIHQGLIFYEKLLGSDIFTGE (SEQ ID NO: 2236), and at least one amino acid from the peptide

sequence of SLLPDSPVGQLHASLLGLSQLLQPEG (SEQ ID NO: 2237) , and at least one amino acid from the peptide sequence of WETQQIPSLSPSQPWQRLL (SEQ ID NO: 2238).

[172] The invention provides an isolated antibody that specifically binds IL-23A (SEQ ID NO: 4), wherein the antibody is capable of binding an immobilized peptide consisting of the amino acid sequence of LQRIHQGLIFYEKLLGSDIFTGE (SEQ ID NO: 2236) , and capable of binding an immobilized amino acid sequence of LLPDSPVGQLHASLLGLSQLLQPEG (SEQ ID NO: 2237), and capable of binding an immobilized peptide consisting of the amino acid sequence of WETQQIPSLSPSQPWQRLL (SEQ ID NO: 2238).

[173] The invention provides a method for inhibiting IL-17A production by T cells comprising treating said T cells with the anti-23A antibodies described herein. Within an embodiment, the invention provides a method for treating a disease characterized by elevated expression of IL-17A, IL-17F, or IL-23 in a mammalian subject, comprising administering to said subject one or more of the bispecific antibodies described herein.

[174] The invention provides a method for inhibiting or reducing relapse in multiple sclerosis or in inflammatory bowel disease in a mammalian subject, comprising administering to said subject one or more of the bispecific antibodies described herein.

[175] The invention provides a method for treating multiple sclerosis or in inflammatory bowel disease in a mammalian subject, comprising administering to said subject the one of more of the bispecific antibodies described herein.

[176] The invention provides a a method for inhibiting or reducing relapse in multiple sclerosis or in inflammatory bowel disease in a mammalian subject, comprising co-administering to said subject an anti-IL-17A antibody described herein with an anti-IL-23A antibody described herein. Within an embodiment the anti-IL-17A antibody and the anti-IL-23A antibody are administered in one entity.

[177] The invention provides an isolated bispecific antibody comprising the amino acid sequence selected from the group consisting of: a) the amino acid sequence of SEQ ID NO: 2160; b) the amino acid sequence of SEQ ID NO: 2151; c) the amino acid sequence of SEQ ID NO: 2152; d) the amino acid sequence of SEQ ID NO: 2138; and e) the amino acid sequence of SEQ ID NO: 2149.

[178] The invention also encompasses fully human antibodies and antibodies wherein the light chain variable region and a portion of the heavychain variable region are human and the remainder of the heavy chain variable region is expressed by synthetic DNA.

[179] Antibodies that bind to IL-17A and IL-23p19 have been identified by screening a phage display library. Within this group of antibodies, is a subgroup that also binds the IL17A/F heterodimer. Methods of screening by phage display are described in detail in standard reference texts, such as Babas, Phage Display: A Laboratory Manual Cold Spring Harbor Lab Press, 2001 and Lo, Benny K.C., A., Antibody Engineering, 2004. Such phage display libraries can be used to display

expressed proteins on the surface of a cell or other substance such that the complementary binding entity can be functionally isolated. In one such phage display library, genes encoding human antibody light chain variable regions and a portion of human heavy chain variable regions are combined with synthetic DNA, which are then displayed on phage and phagemid libraries as Fab antibody fragments (Dyax® Human Antibody Libraries, Dyax Corp., Cambridge, MA.). Thus, the variable light and heavy chain fragments of antibodies can be isolated in a Fab format. These variable regions can then be manipulated to generate antibodies, including antigen-binding fragments, such as scFvs, bispecific scFvs and multispecific, multifunctional antagonists to IL-17A, IL-17F, or IL-23p19.

[180] Using this technology the variable regions of Fabs and scFvs have been identified for their charactaristics of binding and or neutralizing either IL-17A, IL-17F, and/or IL-23p19 in the plate-based assays described in Examples 1 to 5 herein. These variable regions were manipulated to generate various binding entities, including scFvs that bind and/or neutralize IL-17A, IL-17F and/ or IL-23p19.

[181] Table 1 below shows a list of the Fabs or scFvs that bind IL-17A.

Table 1:

Cluster #	VL nucleotide SEQ ID NO:	VH nucleotide SEQ ID NO:	VL polypeptide SEQ ID NO:	VH polypeptide SEQ ID NO:	Light FR1 range	Light CDR1 range	Light FR2 range	Light CDR2 range	Light FR3 range	Light CDR3 range	Light FR4 range	Heavy FR1 range	Heavy CDR1 range	Heavy FR2 range	Heavy CDR2 range	Heavy FR3 range	Heavy CDR3 range	Heavy FR4 range
c83	385	386	790	791	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-116	117-127
c84	387	388	792	793	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-118	119-129
c85	389	390	794	795	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-67	68-99	100-115	116-126
c86	391	392	796	797	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c87	393	394	798	799	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c88	395	396	800	801	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c89	397	398	802	803	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c90	399	400	804	805	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c91	401	402	806	807	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-103	104-114
c92	403	404	808	809	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c93	405	406	810	811	1-23	24-39	40-54	55-61	62-93	94-103	104-113	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c94	407	408	812	813	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c95	409	410	814	815	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125

c96	411	412	816	817	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c97	413	414	818	819	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c98	415	416	820	821	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c99	417	418	822	823	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c100	419	420	824	825	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c157	421	422	826	827	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c168	423	424	828	829	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c191	425	426	830	831	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c192	427	428	832	833	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c193	429	430	834	835	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c195	431	432	836	837	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c196	433	434	838	839	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c199	435	436	840	841	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c200	437	438	842	843	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c202	439	440	844	845	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c203	441	442	846	847	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c204	443	444	848	849	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119

c207	445	446	850	851	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c209	447	448	852	853	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c210	449	450	854	855	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c212	451	452	856	857	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c213	453	454	858	859	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c214	455	456	860	861	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c215	457	458	862	863	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c218	459	460	864	865	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c219	461	462	866	867	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c220	463	464	868	869	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c221	465	466	870	871	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c222	467	468	872	873	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c223	469	470	874	875	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c224	471	472	876	877	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c225	473	474	878	879	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c226	475	476	880	881	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c227	477	478	882	883	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122

c229	479	480	884	885	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c230	481	482	886	887	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c231	483	484	888	889	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-27	28-32	33-46	47-63	64-95	96-103	104-114
c232	485	486	890	891	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c233	487	488	892	893	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c234	489	490	894	895	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c235	491	492	896	897	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c236	493	494	898	899	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c237	495	496	900	901	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c238	497	498	902	903	1-21	22-32	33-47	48-54	55-86	87-96	97-106	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c239	499	500	904	905	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c240	501	502	906	907	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c245	503	504	908	909	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c246	505	506	910	911	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c248	507	508	912	913	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c249	509	510	914	915	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-28	29-33	34-47	48-64	65-96	97-112	113-123
c250	511	512	916	917	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-112	113-123

c296	----	513	----	918	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-116	117-127
c297	514	----	1196	----	1-22	23-35	36-50	51-57	58-89	90-100	101-110	----	----	----	----	----	----	----
c380	----	515	----	919	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c381	----	516	----	920	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c382	517	518	921	922	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c383	519	520	923	924	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c384	----	521	----	925	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c385	1197	----	926	----	1-23	24-35	36-50	51-57	58-89	90-100	101-110	----	----	----	----	----	----	----
c386	522	523	927	928	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-115	116-126
c387	----	524	----	929	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c388	525	526	930	931	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c389	527	528	932	933	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c390	529	----	934	----	1-23	24-34	35-49	50-56	57-88	89-97	98-107	----	----	----	----	----	----	----
c391	530	531	935	936	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c392	532	533	937	938	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c393	----	534	----	939	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-116	117-127
c394	----	535	----	940	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-110	111-121

c396	536	----	941	----	1-23	24-35	36-50	51-57	58-89	90-98	99-108	----	----	----	----	----	----	----
c397	537	538	942	943	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c398	539	540	944	945	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c399	541	542	946	947	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c400	----	543	----	948	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c401	544	545	949	950	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c402	----	546	----	951	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c403	547	----	952	----	1-23	24-34	35-49	50-56	57-88	89-98	99-108	----	----	----	----	----	----	----
c404	548	549	953	954	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c405	550	551	955	956	1-23	24-34	35-49	50-56	57-88	89-99	100-109	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c406	552	553	957	958	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-64	65-96	97-109	110-120
c407	----	554	----	960	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-117	118-128
c408	555	556	961	962	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c409	557	558	963	964	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c410	559	560	965	966	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c411	561	562	967	968	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c412	563	564	969	970	1-23	24-39	40-54	55-61	62-93	94-103	104-113	1-30	31-35	36-49	50-66	67-98	99-107	108-145

c416	565	566	971	972	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c417	567	568	973	974	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c418	569	570	975	976	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c419	571	572	977	978	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c420	573	574	979	980	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-28	29-33	34-47	48-64	65-96	97-111	112-122
c421	575	576	981	982	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c422	577	578	983	984	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-29	30-34	35-48	49-65	66-97	98-113	114-124
c424	579	580	985	986	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c425	581	582	987	988	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c426	583	584	989	990	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c427	585	586	991	992	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c428	587	588	993	994	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c429	589	590	995	996	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-121	122-132
c430	591	592	997	998	1-22	23-35	36-50	51-57	58-89	90-101	102-111	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c431	593	594	999	1000	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c432	595	596	1001	1002	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c433	597	598	1003	1004	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-107	108-118

c434	599	600	1005	1006	1-22	23-33	34-48	49-55	56-87	88-98	99-108	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c435	601	602	1007	1008	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c436	603	604	1009	1010	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c437	605	606	1011	1012	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c438	607	608	1013	1014	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c440	609	610	1015	1016	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c441	611	612	1017	1018	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c442	613	614	1019	1020	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c443	615	616	1021	1022	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c444	617	618	1023	1024	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c445	619	620	1025	1026	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c446	621	622	1027	1028	1-22	23-30	31-45	46-52	53-84	85-95	96-105	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c447	623	624	1029	1030	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c448	625	626	1031	1032	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c449	627	628	1033	1034	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c450	629	630	1035	1036	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c451	631	632	1037	1038	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125

c452	633	634	1039	1040	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c453	635	636	1041	1042	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c454	637	638	1043	1044	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c455	639	640	1045	1046	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c456	641	642	1047	1048	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c457	643	644	1049	1050	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c458	645	646	1051	1052	1-22	23-36	37-51	52-58	59-90	91-100	101-110	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c459	647	648	1053	1054	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c460	649	650	1055	1056	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-120	121-131
c461	651	652	1057	1058	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c462	653	654	1059	1060	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c463	655	656	1061	1062	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c464	657	658	1063	1064	1-22	23-33	34-48	49-55	56-87	88-98	99-108	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c465	659	660	1065	1066	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c466	----	661	----	1067	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c513	662	663	1068	1069	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c514	664	665	1070	1071	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124

c515	666	667	1072	1073	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c516	668	669	1074	1075	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c517	670	671	1076	1077	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c518	672	673	1078	1079	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c519	674	675	1080	1081	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c520	676	677	1082	1083	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c521	678	679	1084	1085	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c522	680	681	1086	1087	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-118	119-129
c529	682	683	1088	1089	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c541	684	685	1090	1091	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c542	686	687	1092	1093	1-21	22-32	33-47	48-54	55-86	87-96	97-106	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c543	688	689	1094	1095	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c545	690	691	1096	1097	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c546	692	693	1098	1099	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c547	694	695	1100	1101	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c548	696	697	1102	1103	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c549	698	699	1104	1105	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119

c550	700	701	1106	1107	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c551	702	703	1108	1109	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c552	704	705	1110	1111	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c553	706	707	1112	1113	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c554	708	709	1114	1115	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c555	710	711	1116	1117	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c556	712	713	1118	1119	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c557	714	715	1120	1121	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c559	716	717	1122	1123	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c560	718	719	1124	1125	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c561	720	721	1126	1127	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c562	722	723	1128	1129	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c563	724	725	1130	1131	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c564	726	727	1132	1133	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c565	728	729	1134	1135	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c566	730	731	1136	1137	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c567	732	733	1138	1139	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-108	109-119

c570	734	735	1140	1141	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c571	736	737	1142	1143	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c572	738	739	1144	1145	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c573	740	741	1146	1147	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c574	742	743	1148	1149	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c575	744	745	1150	1151	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c576	746	747	1152	1153	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c577	748	749	1154	1155	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c578	750	751	1156	1157	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c579	752	753	1158	1159	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c580	754	755	1160	1161	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c581	756	757	1162	1163	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c582	758	759	1164	1165	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c583	760	761	1166	1167	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c584	762	763	1168	1169	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c585	764	765	1170	1171	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c586	766	767	1172	1173	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119

c587	768	769	1174	1175	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c588	770	771	1176	1177	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c589	772	773	1178	1179	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c590	774	775	1180	1181	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c592	776	777	1182	1183	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c593	778	779	1184	1185	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c594	780	781	1186	1187	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c595	782	783	1188	1189	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c596	784	785	1190	1191	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c631	786	787	1192	1193	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c632	788	789	1194	1195	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-112	113-123

[182] Table 2 below shows a list of the Fabs or scFvs that bind IL-23p19

Table 2:

Cluster #	VL nucleotide SEQ ID NO:	VH nucleotide SEQ ID NO:	VL polypeptide SEQ ID NO:	VH polypeptide SEQ ID NO:	Light FR1 range	Light CDR1 range	Light FR2 range	Light CDR2 range	Light FR3 range	Light CDR3 range	Light FR4 range	Heavy FR1 range	Heavy CDR1 range	Heavy FR2 range	Heavy CDR2 range	Heavy FR3 range	Heavy CDR3 range	Heavy FR4 range
c26	1198	1199	1641	1642	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c27	1200	1201	1643	1644	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c28	1202	1203	1645	1646	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c29	1204	1205	1647	1648	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c33	1206	1207	1649	1650	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c36	1208	1209	1651	1652	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c40	1210	1211	1653	1654	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-116	117-127
c41	1212	1213	1655	1656	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c43	1214	1215	1657	1658	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c101	1216	1217	1659	1660	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c102	1218	1219	1661	1662	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c103	1220	1221	1663	1664	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124

c110	1222	1223	1665	1666	1-22	23-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-119	120-130
c114	1224	1225	1667	1668	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c115	1226	1227	1669	1670	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c119	1228	1229	1671	1672	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c120	1230	1231	1673	1674	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c121	1232	1233	1675	1676	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c122	1234	1235	1677	1678	1-23	24-34	35-49	50-56	57-88	89-96	97-106	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c123	1236	1237	1679	1680	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c124	1238	1239	1681	1682	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c125	1240	1241	1683	1684	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c126	1242	1243	1685	1686	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c127	1244	1245	1687	1688	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c128	1246	1247	1689	1690	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c129	1248	1249	1691	1692	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c130	1250	1251	1693	1694	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c131	1252	1253	1695	1696	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c132	1254	1255	1697	1698	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-115	116-126

c134	1256	1257	1699	1700	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c135	1258	1259	1701	1702	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-116	117-127
c136	1260	1261	1703	1704	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c137	1262	1263	1705	1706	1-22	23-36	37-51	52-58	59-90	91-100	101-110	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c138	1264	1265	1707	1708	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c139	1266	1267	1709	1710	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c140	1268	1269	1711	1712	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c141	1270	1271	1713	1714	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c142	1272	1273	1715	1716	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c143	1274	1275	1717	1718	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c144	1276	1277	1719	1720	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c145	1278	1279	1721	1722	1-23	24-34	35-49	50-56	57-88	89-96	97-106	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c146	1280	1281	1723	1724	1-22	23-36	37-51	52-58	59-90	91-100	101-110	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c148	1282	1283	1725	1726	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c149	1284	1285	1727	1728	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c150	1286	1287	1729	1730	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c151	1288	1289	1731	1732	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119

c152	1290	1291	1733	75 1734	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-65	66-97	98-110	111-121
c153	1292	1293	1735	1736	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c154	1294	1295	1737	1738	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c155	1296	1297	1739	1740	1-23	24-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c156	1298	1299	1741	1742	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c157	1300	1301	1743	1744	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c158	1302	1303	1745	1746	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c159	1304	1305	1747	1748	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c160	1306	1307	1749	1750	1-23	24-34	35-49	50-56	57-88	89-96	97-106	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c161	1308	1309	1751	1752	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c162	1310	1311	1753	1754	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c163	1312	1313	1755	1756	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c164	1314	1315	1757	1758	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c165	1316	1317	1759	1760	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c166	1318	1319	1761	1762	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c167	1320	1321	1763	1764	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-103	104-114
c168	1322	1323	1765	1766	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119

c169	1324	1325	1767	1768	1-23	24-35	36-50	51-57	58-89	90-99	100-109	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c170	1326	1327	1769	1770	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c171	1328	1329	1771	1772	1-23	24-35	36-50	51-57	58-89	90-101	102-111	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c172	1330	1331	1773	1774	1-23	24-34	35-49	50-56	57-88	89-96	97-106	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c173	1332	1333	1775	1776	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c174	1334	1335	1777	1778	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c175	1336	1337	1779	1780	1-23	24-34	35-49	50-56	57-88	89-99	100-109	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c176	1338	1339	1781	1782	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c178	1340	1341	1783	1784	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c179	1342	1343	1785	1786	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c180	1344	1345	1787	1788	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c181	1346	1347	1789	1790	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c182	1348	1349	1791	1792	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c183	1350	1351	1793	1794	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c184	1352	1353	1795	1796	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-117	118-128
c185	1354	1355	1797	1798	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c186	1356	1357	1799	1800	1-23	24-35	36-50	51-57	58-89	90-99	100-109	1-30	31-35	36-49	50-66	67-98	99-107	108-118

c187	1358	1359	1801	1802	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c188	1360	1361	1803	1804	1-23	24-39	40-54	55-61	62-93	94-103	104-113	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c189	1362	1363	1805	1806	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c190	1364	1365	1807	1808	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c194	1366	1367	1809	1810	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c197	1368	1369	1811	1812	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c198	1370	1371	1813	1814	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c201	1372	1373	1815	1816	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c205	1374	1375	1817	1818	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c206	1376	1377	1819	1820	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c208	----	1378	----	1821	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c211	1379	1380	1822	1823	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c251	1381	1382	1824	1825	1-22	23-33	34-48	49-55	56-87	88-97	98-107	1-30	31-35	36-49	50-66	67-98	99-115	116-126
c252	1383	1384	1826	1827	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-120	121-131
c253	1385	1386	1828	1829	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-115	116-126
c254	1387	1388	1830	1831	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-115	116-126
c255	1389	1390	1832	1833	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-114	115-125

c256	1391	1392	1834	1835	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c257	1393	1394	1836	1837	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-118	119-129
c259	1395	1396	1838	1839	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c260	1397	1398	1840	1841	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c261	1399	1400	1842	1843	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c262	1401	1402	1844	1845	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c263	1403	1404	1846	1847	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c264	1405	1406	1848	1849	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c265	1407	1408	1850	1851	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c266	1409	1410	1852	1853	1-23	24-34	35-49	50-56	57-88	89-99	100-109	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c267	1411	1412	1854	1855	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c270	1413	1414	1856	1857	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c271	1415	1416	1858	1859	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c272	1417	1418	1860	1861	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c273	1419	1420	1862	1863	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c274	1421	1422	1864	1865	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c275	1423	1424	1866	1867	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-105	106-138

c276	1425	1426	1868	1869	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c277	1427	1428	1870	1871	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c278	1429	1430	1872	1873	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c279	1431	1432	1874	1875	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c280	1433	1434	1876	1877	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c281	1435	1436	1878	1879	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c282	1437	1438	1880	1881	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c283	1439	1440	1882	1883	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c284	1441	1442	1884	1885	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c285	1443	1444	1886	1887	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c287	1445	1446	1888	1889	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c288	1447	1448	1890	1891	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c289	1449	1450	1892	1893	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c290	1451	----	1894	----	1-23	24-34	35-49	50-56	57-88	89-97	98-107	----	----	----	----	----	----	----
c298	----	1452	----	1895	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-115	116-126
c299	1453	----	1896	----	1-23	24-34	35-49	50-56	57-88	89-97	98-107	----	----	----	----	----	----	----
c300	1454	----	1897	----	1-22	23-33	34-48	49-55	56-87	88-96	97-106	----	----	----	----	----	----	----

c301	2084	----	2085	----	1-23	24-34	35-49	50-56	57-88	89-97	98-107	----	----	----	----	----	----	----
c302	1455	1456	1898	1899	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c303	1457	1458	1900	1901	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c304	1459	1460	1902	1903	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c305	1461	1462	1904	1905	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c347	1463	1464	1906	1907	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c348	1465	1466	1908	1909	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c349	1467	1468	1910	1911	1-22	23-36	37-51	52-58	59-90	91-101	102-111	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c350	1469	1470	1912	1913	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c351	1471	1472	1914	1915	1-23	24-34	35-49	50-56	57-88	89-98	99-108	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c352	1473	1474	1916	1917	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c353	1475	1476	1918	1919	1-23	24-34	35-49	50-56	57-88	89-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c354	1477	1478	1920	1921	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c355	1479	1480	1922	1923	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c356	1481	1482	1924	1925	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-115	116-126
c357	1483	1484	1926	1927	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c358	1485	1486	1928	1929	1-23	24-34	35-49	50-56	57-88	89-93	94-103	1-30	31-35	36-49	50-66	67-98	99-108	109-119

c359	1487	1488	1930	1931	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c360	1489	1490	1932	1933	1-23	24-35	36-50	51-57	58-89	90-98	99-108	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c361	1491	1492	1934	1935	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c362	1493	1494	1936	1937	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c363	1495	1496	1938	1939	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c364	1497	1498	1940	1941	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c365	1499	1500	1942	1943	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c366	1501	1502	1944	1945	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-109	110-120
c367	1503	1504	1946	1947	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c368	1505	1506	1948	1949	1-23	24-34	35-49	50-56	57-88	89-96	97-106	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c369	1507	1508	1950	1951	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c370	1509	1510	1952	1953	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-111	112-122
c371	1511	1512	1954	1955	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c372	----	1513	----	1956	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-120	121-131
c373	1514	1515	1957	1958	1-23	24-34	35-49	50-56	57-88	89-96	97-106	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c374	1516	1517	1959	1960	1-22	23-36	37-51	52-58	59-90	91-100	101-110	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c375	1518	1519	1961	1962	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-109	110-120

c376	1520	1521	1963	1964	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c377	1522	1523	1965	1966	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c378	1524	1525	1967	1968	1-22	23-34	35-49	50-56	57-88	89-101	102-111	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c379	1526	1527	1969	1970	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c439	----	1528	----	1971	----	----	----	----	----	----	----	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c467	1529	1530	1972	1973	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c468	1531	1532	1974	1975	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c469	1533	1534	1976	1977	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c470	1535	1536	1978	1979	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c471	1537	1538	1980	1981	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c472	1539	1540	1982	1983	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c473	1541	1542	1984	1985	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c474	1543	1544	1986	1987	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c475	1545	1546	1988	1989	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c476	1547	1548	1990	1991	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c477	1549	1550	1992	1993	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c478	1551	1552	1994	1995	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118

c479	1553	1554	1996	1997	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c481	1555	1556	1998	1999	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c482	1557	1558	2000	2001	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c483	1559	1560	2002	2003	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c484	1561	1562	2004	2005	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c485	1563	1564	2006	2007	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c486	1565	1566	2008	2009	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c487	1567	1568	2010	2011	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c489	1569	1570	2012	2013	1-23	24-39	40-54	55-61	62-93	94-103	104-113	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c490	1571	1572	2014	2015	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c491	1573	1574	2016	2017	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c492	1575	1576	2018	2019	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c493	1577	1578	2020	2021	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c494	1579	1580	2022	2023	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c495	1581	1582	2024	2025	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c496	1583	1584	2026	2027	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c497	1585	1586	2028	2029	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118

c498	1587	1588	2030	2031	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c499	1589	1590	2032	2033	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c500	1591	1592	2034	2035	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c501	1593	1594	2036	2037	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-110	111-121
c502	1595	1596	2038	2039	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c503	1597	1598	2040	2041	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c504	1599	1600	2042	2043	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c505	1601	1602	2044	2045	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c506	1603	1604	2046	2047	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c507	1605	1606	2048	2049	1-23	24-39	40-54	55-61	62-93	94-101	102-111	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c508	1607	1608	2050	2051	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c509	1609	1610	2052	2053	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c510	1611	1612	2054	2055	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c511	1613	1614	2056	2057	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c512	1615	1616	2058	2059	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-106	107-117
c523	1617	1618	2060	2061	1-22	23-36	37-51	52-58	59-90	91-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c526	1619	1620	2062	2063	1-23	24-39	40-54	55-61	62-93	94-102	103-112	1-30	31-35	36-49	50-66	67-98	99-112	113-123

Table 1:

Cluster #	VL nucleotide SEQ ID NO:	VH nucleotide SEQ ID NO:	VL polypeptide SEQ ID NO:	VH polypeptide SEQ ID NO:	Light FR1 range	Light CDR1 range	Light FR2 range	Light CDR2 range	Light FR3 range	Light CDR3 range	Light FR4 range	Heavy FR1 range	Heavy CDR1 range	Heavy FR2 range	Heavy CDR2 range	Heavy FR3 range	Heavy CDR3 range	Heavy FR4 range
c83	385	386	790	791	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-116	117-127
c84	387	388	792	793	1-22	23-35	36-50	51-57	58-89	90-100	101-110	1-30	31-35	36-49	50-66	67-98	99-118	119-129
c85	389	390	794	795	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-67	68-99	100-115	116-126
c86	391	392	796	797	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-113	114-124
c87	393	394	798	799	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-108	109-119
c88	395	396	800	801	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c89	397	398	802	803	1-22	23-33	34-48	49-55	56-87	88-96	97-106	1-30	31-35	36-49	50-66	67-98	99-112	113-123
c90	399	400	804	805	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c91	401	402	806	807	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-103	104-114
c92	403	404	808	809	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-107	108-118
c93	405	406	810	811	1-23	24-39	40-54	55-61	62-93	94-103	104-113	1-30	31-35	36-49	50-66	67-98	99-105	106-116
c94	407	408	812	813	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125
c95	409	410	814	815	1-23	24-34	35-49	50-56	57-88	89-97	98-107	1-30	31-35	36-49	50-66	67-98	99-114	115-125

[183] Several of the anti-IL-17A and anti-IL23p19 antibodies have been deposited with the American Type Tissue Culture Collection (ATCC; Manassas VA) patent depository as original deposits under the terms of the Budapest Treaty on December 4, 2007 and were given the following ATCC Accession Numbers: clone c305 V_HV_L scFv-pARB013 in DH10B strain (MVE #158) was given ATCC Patent Deposit Designation PTA-8818; clone c472.2 V_LV_H scFv-pARB013 in DH10B strain (MVE # 263) was given ATCC Patent Deposit Designation PTA-8820; clone c631.1 V_HV_L scFv-pARB013 in zGold5 strain (MVE #228) was given ATCC Patent Deposit Designation PTA-8819; clone c632.1 V_LV_H scFv-pARB013 in DH10B strain (MVE #258) was given ATCC Patent Deposit Designation PTA-8821).

[184] The anti-IL-17A antibodies, anti-IL-23p19 antibodies, and anti-Il-17-A/anti-IL-23p19 antibodies, including scFv moleucles that bind IL-17A or IL-23p19 can be oriented with the variable light region either amino terminal to the variable heavy region or carboxylterminal to it. Additonally, scFvs can be prepared in a number of configurations, including tandem or non-tandem formats, such that each target, i.e, IL-17A and IL-23p19 can be bound by the variable regions or minimal CDR binding untis of its respective variable regions. Thus, the construct for a tandem scFV molecule can be prepared such that the variable light region and variable heavy region of the antibody or fragment thereof that targets IL-17A can be interspersed with the variable light and variable heavy regions of the antibody or fragment thereof that targets IL-23p19 as long as the variable regions are able to bind the targets. Tandem scFv molecules that bind both targets can be prepared with a linker between the scFv entites, including a Gly- Ser linker, which comprises a series of glycine and serine residues and can also include additional amino acids. Tandem and non-tandem bispecific scFv molecules with the variable regions of cluster numbers described in Tables 1 and 2 are further described herein.

[185] The antibodies and derivatives thereof of the invention have binding affinities that include a dissociation constant (K_d) of less than 1 X 10^{-2}. In some embodiments, the K_d is less than 1 X 10^{-3}. In other embodiments, the K_d is less than 1X 10^{-4}. In some embodiments, the K_d is less than 1X 10^{-5}. In still other embodiments, the K_d is less than 1X 10^{-6}. In other embodiments, the K_d is less than 1X 10^{-7}. In other embodiments, the K_d is less than 1X 10^{-8}. In other embodiments, the K_d is less than 1X 10^{-9}. In other embodiments, the K_d is less than 1X 10^{-10}. In still other embodiments, the K_d is less than 1X 10^{-11}. In some embodiments, the K_d is less than 1X 10^{-12}. In other embodiments, the K_d is less than 1X 10^{-13}. In other embodiments, the K_d is less than 1X 10^{-14}. In still other embodiments, the K_d is less than 1X 10^{-15}.

[186] The anti-IL-17Aantibodies, anti-IL23p19 antibodies, and anti-IL-17A/anti-IL-23p19 antibodies isolated and described herein have been grouped into families of consensus CDRs, which are shown in SEQ ID NOs: 12 to 173 and 384 for anti-IL-17A and SEQ ID NOs: 174 to 383 for anti-IL-23p19. Table 3, below correlates the SEQ ID NOs: with the anti-IL-17A consensus CDRs.

Table 3 SEQ ID NOs: for anti-IL-17A Consensus CDRs

CDR family	Clone#	Light Germlines	LCDR1	LCDR2	LCDR3	Heavy Germlines	HCDR1	HCDR2	HCDR3
A	c200, c209, c239, c631	VKI_L1, VKI_O12	12	13	14	VH3_3-23	15	16	17, 18, 19
B	c296, c380, c385, c391, c396, c402, c567	VKIII_A27	36	37	38	VH3_3-23	39	40	17, 41, 42, 43, 44
C	c403, c522	VKIII_L2	45	46	47	VH3_3-23	48	49	50
D	c405, c409	VKIII_L6	51	52	53	VH3_3-23	54	55	18, 56
E	c384, c514	VKII_A17	57	58	59	VH3_3-23	60	61	18, 62
F	c412, c436, c442, c93	VKII_A19	63	64	65	VH3_3-23	66	67	68, 69, 70, 71
G	c200, c204, c209, c223, c226, c234, c457, c460, c521, c541, c546, c547, c548, c549, c551, c552, c553, c554, c555, c556, c559, c560, c561, c562, c563, c564, c565, c566, c570, c571, c572, c573, c574, c576, c577, c578, c580, c584, c585, c586, c587, c588, c589, c590, c592, c593, c594, c595, c596, c632, c87	VKI_L1	72	73	74	VH3_3-23	75	76	17, 77, 78, 18, 79, 19
H	c100, c250, c404, c406, c408, c440, c516, c517, c545	VKI_L12	80	81	82	VH3_3-23	83	84	85, 86, 87, 88, 89, 90, 91
I	c407, c438, c92	VKI_L5	92	93	94	VH3_3-23	95	96	97, 98, 68
J	c397, c398	VKI_L8	100	101	102	VH3_3-23	103	104	105, 106
K	c168, c229, c382, c386, c389, c400, c410, c411, c416, c417, c418, c419, c421, c425, c428, c431, c435, c445, c447, c450, c453, c462, c463, c513, c515, c518, c519, c520, c529, c550, c582, c583, c631, c85, c88, c90, c94, c95	VKI_O12	107	108	109	VH3_3-23	110	111	112, 79, 113, 114, 18, 115, 116, 70, 117, 118
L	c191, c199, c202, c207, c212, c213, c214, c215, c222, c224, c230, c232, c233, c236, c246, c388, c390, c424, c543, c86, c91, c97, c98	VKI_O18	119	120	121	VH3_3-23	122	123	124, 125, 85, 126, 127, 18, 128, 129
M	c157, c297, c381, c383, c387, c429, c446, c451, c465, c84	VL1_1c	130	131	132	VH3_3-23	133	134	135, 136, 137, 138, 139, 140,

									141, 142
N	c392, c426, c427, c432, c433, c437, c444, c448, c449, c452, c454, c455, c459, c461, c466, c557, c575	VL1_1g	143	144	145	VH3_3-23	146	147	148, 140, 149, 150, 151, 152, 153, 154
O	c393, c464	VL3_3h	155	156	157	VH3_3-23	158	159	160, 161
P	c192, c193, c195, c196, c203, c210, c218, c219, c220, c221, c225, c227, c231, c235, c237, c238, c239, c240, c245, c248, c249, c420, c422, c441, c443, c542, c579, c581, c83, c89, c99	VL3_3r	162	163	164	VH3_3-23	165	166	167, 168, 85, 125, 79, 169, 170, 171, 172, 172, 173

[188] Table 4, below correlates the SEQ ID NOs: with the anti-IL-23p19consensus CDRs.

Table 4

CDR family	Clone#	Light Germlines	LCDR1	LCDR2	LCDR3	Heavy Germlines	HCDR1	HCDR2	HCDR3
A	c148, c151, c155, c162, c164, c166, c169, c171, c183, c186, c255, c26, c261, c360	VKIII_A27	174	175	176	VH3_3-23	177	178	179, 180, 181, 182, 183, 184, 185, 186, 187, 188
B	c145, c149, c163, c167, c172, c176, c263, c33, c351, c373	VKIII_L2	189	190	191	VH3_3-23	192	193	194, 195, 196, 197, 198, 199, 200, 201, 202
C	c124, c180, c260, c262, c264, c265, c266, c368	VKIII_L6	203	204	205	VH3_3-23	206	207	208, 209, 210, 211, 212, 213, 214, 215
D	c103, c190, c197, c206, c272, c287, c302, c303	VKII_A17	216	217	218	VH3_3-23	219	220	221, 222, 223
E	c115, c120, c138, c140, c141, c144, c150, c154, c158, c159, c161, c165, c170, c178, c179, c182, c185, c188, c189, c194, c198, c201, c205, c208, c211, c270, c271, c274, c275, c278, c280, c281, c282, c285, c289, c298, c352, c363, c367, c375, c376, c377, c41, c43, c467, c471, c472, c473, c474, c476, c478, c481, c483, c486, c489, c491, c492, c495, c497, c499, c501, c502, c503, c505, c506, c507, c511, c526, c528, c535, c591	VKII_A19	224	225	226	VH3_3-23	227	228	229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260
F	c139, c181	VKI_A20	261	262	263	VH3_3-23	264	265	266, 267
G	c364, c366	VKI_L1	268	269	270	VH3_3-23	271	272	273, 274
H	c130, c153, c160, c175, c299, c358, c439	VKI_L12	275	276	277	VH3_3-23	278	279	280, 281, 282, 283, 284

I	c102, c1220, c173, c174, c267, c273, c276, c277, c290, c347, c348, c354, c355, c36, c361, c362, c371, c504	VKI_L5	285	286	287	VH3_3-23	289	290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300
J	c131, c357	VKI_L8	301	302	303	VH3_3-23	304	305	306, 307
K	c114, c119, c122, c125, c129, c132, c135, c136, c142, c143, c152, c168, c184, c187, c27, c28, c301, c350, c365, c369, c370, c379, c490, c512	VKI_O12 VKI_O18	308	309	310	VH3_3-23	311	312	313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332
L	c110, c156, c157, c359, c496, c498, c538	VL1_1c	338	339	340	VH3_3-23	341	342	343, 344, 345, 346
M	c126, c127, c128, c494, c508, c540	VL1_1g	347	348	349	VH3_3-23	350	351	352, 353, 354, 355, 356, 357
N	c101, c137, c146, c279, c283, c284, c288, c29, c304, c305, c349, c374, c468, c469, c470, c475, c477, c479, c482, c484, c485, c487, c493, c500, c509, c510, c523, c534, c536, c537, c539	VL2_2a2, VL2_2b2	358	359	360	VH3_3-23	361	362	363, 364, 365, 366, 367
O	c123, c134, c251, c252, c253, c254, c256, c257, c259, c300, c356, c40	VL3_3r	368	369	370	VH3_3-23	371	372	373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383

Table 5 SEQ ID NOs: for anti-IL-17A Fabs and scFvs

Clone#	Light Germline	LCDR1	LCDR2	LCDR3	Heavy Germline	HCDR1	HCDR2	HCDR3
87	VK-IL1	RASQDITNYLA SEQ ID NO:20	SASNLQS SEQ ID NO:21	QQYESYPLT SEQ ID NO:22	VH3_3-23	AYVMD SEQ ID NO:23	SIGSSGGQTPYADSVKG SEQ ID NO:24	NEGLVGAFDI SEQ ID NO:17
200	VK-IL1	RASQDITNYLA SEQ ID NO:20	SASNLQS SEQ ID NO:21	QQYESYPIT SEQ ID NO:384	VH3_3-23	AYVMD SEQ ID NO:23	SIGSSGGQTPYADSVKG SEQ ID NO:24	NEGLVGAFDI SEQ ID NO:17
209	VK-IL1	RASQDITNYLA SEQ ID NO:20	SASNLQS SEQ ID NO:21	QQYESYPLT SEQ ID NO:22	VH3_3-23	AYVMD SEQ ID NO:23	SIGSSGGQTPYADSVKG SEQ ID NO:24	NEGLVGAFDI SEQ ID NO:17
632	VK-IL1	RASHDINTYLA SEQ ID NO:25	AASNLQS SEQ ID NO:26	LQYDTFPLT SEQ ID NO:27	VH3_3-23	PYSMM SEQ ID NO:28	YIYSSGGWTTYADSVKG SEQ ID NO:29	DTSLWFGDNDAFDI SEQ ID NO:19
389	VKI_O12	RASQTISIFLN SEQ ID NO:30	AASSLQS SEQ ID NO:26	QQSYSTPRT SEQ ID NO:31	VH3_3-23	IYTMQ SEQ ID NO:32	SISPSGGVTMYADSVKG SEQ ID NO:33	DFLEWLLLTSNYGMDV SEQ ID NO:18
631	VKI_O12	RASQTISIFLN SEQ ID NO:30	AASSLQS SEQ ID NO:26	QQSYSTPRT SEQ ID NO:31	VH3_3-23	EYNMN SEQ ID NO:34	WIGPSGGQTDYADSVKG SEQ ID NO:35	DFLEWLLLTSNYGMDV SEQ ID NO:18

Table 6 Sequences: for anti-IL-23p19 Fabs and scFvs

Clone#	Light Germline	LCDR1	LCDR2	LCDR3	Heavy Germline	HCDR1	HCDR2	HCDR3
29	VL2_2a2	TGTSSDVGGYNYVS (SEQ ID NO: 2086)	EVSNRPS (SEQ ID NO: 2087)	SSYTSSSTLFYV (SEQ ID NO: 2088)	VH3_3	AYGMD (SEQ ID NO:2089)	SISPSGGQTKYADSVKG (SEQ ID NO: 2090)	DLGGGYYYYYGMDV (SEQ ID NO:2091)
305	VL2_2a2	TGTSSDVGGYNYVS (SEQ ID NO: 2086)	EVSNRPS (SEQ ID NO: 2087)	SSYTSSSTLFYV (SEQ ID NO: 2088)	VH3_3	AYGMD (SEQ ID NO: 2089)	SISPSGGRTKYADSVKG (SEQ ID NO: 2092)	DLGGGYYYYYGMDV (SEQ ID NO: 2091)
361	VKI_L5	RASQGIGYWLA (SEQ ID NO: 2093)	VSSTLQR (SEQ ID NO: 2094)	QQAKAFPLT (SEQ ID NO: 2095)	VH3_3	SYAMQ (SEQ ID NO: 2096)	SIYPSGGKTFYADSVKG (SEQ ID NO: 2097)	HKGSSWYSAAFDI (SEQ ID NO: 2098)
490	VKI_O12	RASQSISGYLH (SEQ ID NO:2099)	ATSNLHS (SEQ ID NO: 2100)	QQSFSTPPT (SEQ ID NO: 2101)	VH3_3	AYSMT (SEQ ID NO: 2102)	GIYSFGGFTWYADSVKG (SEQ ID NO: 2103)	VGLWAFDI (SEQ ID NO: 2104)
472	VKII_A19	RSSQSLLHTNGYNYLD (SEQ ID NO: 2105)	LGSYRA S (SEQ ID NO: 2106)	MQGLQTPIT (SEQ ID NO: 2107)	VH3_3	HYWME (SEQ ID NO: 2108)	VIGPSGGTTRYADSVKG (SEQ ID NO: 2109)	GGPGWYFDL (SEQ ID NO: 2110)

[189] Further characterization of the bispecific molecules described herein is shown in the Examples that follow and in the Figures.

[190] Thus, the invention provides an antibody that binds a polypeptide comprising IL-17A (SEQ ID NO:2), wherein the antibody comprises: a light chain variable region comprising selected from the group consisting of the ligst chain variable regions listed in Table 1; and a heavy chain variable region seleceted from the group consisting of the heavy chain variable regions listed in Table 1. Within an embodiment, the antibody is an antibody fragment. Within another embodiment the antibody is selected from the groups consisting of: Fv, Fab, Fab', F(ab)2, F(ab') 2, scFv, and diabody. Within another embodiment the antibody is a bispecific molecule. Within another embodiment the antibody also binds IL-23p19 (SEQ ID NO: 4).

[191] The invention provides an anti-IL-17A antibody comprising: a) a light chain variable region comprising: i) a LCDR1 comprising SEQ ID NO: 12; ii) a LCDR2 comprising SEQ ID NO: 13; and iii) a LCDR3 comprising SEQ ID NO: 14; and b) a heavy chain variable region comprising: i) a HCDR1 comprising SEQ ID NO: 15; ii) a HCDr2 comprising SEQ ID NO: 16; and wherin the HCDR3 is the HCDR3 comprising SEQ ID NO: 17, 18, or 19.

[192] Within an aspect the invention provides an anti-IL-17A antibody wherein the antibody comprises the a LCDR1, a LCDR2, a LCDR3, a HCDR1, a HCDR2 and a HCDR3 of the consensus CDRs of the CDR families listed in Table 3.

[193] Within an aspet the invention provides aAn anti-IL17A antibody wherein the antibody comprises a variable light chain comprising at least 90% sequence identity to the variable light chain selected from the group consisting of the following SEQ ID NOs: 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 810, 812, 814, 816, 818, 820, 822, 824, 826, 828,830, 832, 834, 836, 838, 840, 842, 844, 846, 848, 850, 852, 854, 856, 858, 860, 862, 864, 866, 868, 870, 872, 874, 876, 878, 880, 882, 884, 886, 888, 890, 892, 894, 896, 898, 900, 902, 904, 906, 908, 910, 912, 914, 916, 1196, 921, 923, 926, 927, 930, 932, 934, 935, 937, 941, 942, 944, 946, 949, 952, 953, 955, 957, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1007, 1009, 1011, 1013, 1015, 1017, 1019, 1021, 1023, 1025, 1027, 1029, 1031, 1033, 1035, 1037, 1039, 1041, 1043, 1045, 1047, 1049, 1051, 1053, 1055, 1057, 1059, 1061, 1063, 1065, 1068, 1070, 1072, 1074, 1076, 1078, 1080, 1082, 1084, 1086, 1088, 1090, 1092, 1094, 1096, 1098, 1100, 1102, 1104, 1106, 1108, 1110, 1112, 1114, 1116, 1118, 1120, 1122, 1124, 1126, 1128, 1130, 1132, 1134, 1136, 1138, 1140, 1142, 1144, 1146, 1148, 1150, 1152, 1154, 1156, 1158, 1160, 1162, 1164, 1166, 1168, 1170, 1172, 1174, 1176, 1178, 1180, 1182, 1184, 1186, 1188, 1190, 1192, and 1194. Within an embodiment the antibody further comprises a variable heavy chain comprising at least 90% sequence identity to the variable heavy chain selected from the group consisting of the following SEQ ID NOs: 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863,

865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 918, 919, 920, 922, 924, 925, 928, 929, 931, 933, 936, 938, 939, 940, 943, 945, 947, 948, 950, 951, 954, 956, 958, 960, 962, 964, 966, 968, 970, 972, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, 1000, 1002, 1004, 1006, 1008, 1010, 1012, 1014, 1016, 1018, 1020, 1022, 1024, 1026, 1028, 1030, 1032, 1034, 1036, 1038, 1040, 1042, 1044, 1046, 1048, 1050, 1052, 1054, 1056, 1058, 1060, 1062, 1064, 1066, 1067, 1069, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1115, 1117, 1119, 1121, 1123, 1125, 1127, 1129, 1131, 1133, 1135, 1137, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, and 1195.

[194] Within an aspect, the invention provides an anti-IL-17A antibody wherein the antibody comprises a variable heavy chain comprising at least 90% sequence identity to the variable heavy chain selected from the group consisting of the following SEQ ID NOs: 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 918, 919, 920, 922, 924, 925, 928, 929, 931, 933, 936, 938, 939, 940, 943, 945, 947, 948, 950, 951, 954, 956, 958, 960, 962, 964, 966, 968, 970, 972, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, 1000, 1002, 1004, 1006, 1008, 1010, 1012, 1014, 1016, 1018, 1020, 1022, 1024, 1026, 1028, 1030, 1032, 1034, 1036, 1038, 1040, 1042, 1044, 1046, 1048, 1050, 1052, 1054, 1056, 1058, 1060, 1062, 1064, 1066, 1067, 1069, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1115, 1117, 1119, 1121, 1123, 1125, 1127, 1129, 1131, 1133, 1135, 1137, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, and 1195. Within an embodiment, the antibody further comprises a variable light chain comprising at least 90% sequence identity to the variable light chain selected from the group consisting of the following SEQ ID NOs: 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 918, 919, 920, 922, 924, 925, 928, 929, 931, 933, 936, 938, 939, 940, 943, 945, 947, 948, 950, 951, 954, 956, 958, 960, 962, 964, 966, 968, 970, 972, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, 1000, 1002, 1004, 1006, 1008, 1010, 1012, 1014, 1016, 1018, 1020, 1022, 1024, 1026, 1028, 1030, 1032, 1034, 1036, 1038, 1040, 1042, 1044, 1046, 1048, 1050, 1052, 1054, 1056, 1058, 1060, 1062, 1064, 1066, 1067, 1069, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1115, 1117, 1119, 1121, 1123, 1125, 1127, 1129,

1131, 1133, 1135, 1137, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, and 1195. Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer. Within an embodiment the antibody further comprises an anti-Il-23p19 antibody. Within an embodiment the the anti-IL-23p19 antibody comprises a light chain variable region comprising at least 90% sequence identity to the variable light chain selected from the group consisting of the following SEQ ID NOs: 1641, 1643, 1645, 1647, 1649, 1651, 1653, 1655, 1657, 1659, 1661, 1663 1665, 1667, 1669, 1671, 1673, 1675, 1677, 1679, 1681, 1683, 1685, 1687, 1689, 1691, 1693, 1695, 1697, 1699, 1701, 1703, 1705, 1707, 1709, 1711, 1713, 1715, 1717, 1719, 1721, 1723, 1725, 1727, 1729, 1731, 1733, 1735, 1737,1739, 1741, 1743, 1745, 1747, 1749, 1751, 1753, 1755, 1757, 1759, 1761, 1763, 1765, 1767, 1769, 1771, 1773, 1775, 1777, 1779, 1781, 1783, 1785, 1787, 1789, 1791, 1793, 1795, 1797, 1799, 1801, 1803, 1805, 1807, 1809, 1811, 1813, 1815, 1817, 1819, 1822, 1824, 1826, 1828, 1830, 1832, 1834, 1836, 1838, 1840, 1842, 1844, 1846, 1848, 1850, 1852, 1854, 1856, 1858, 1860, 1862, 1864, 1866, 1868, 1870, 1872, 1874, 1876, 1878, 1880, 1882, 1884, 1886, 1888, 1890, 1892, 1894, 1896, 1897, 2085, 1898, 1900, 1902, 1904, 1906, 1908, 1910, 1912, 1914, 1916, 1918, 1920, 1922, 1924, 1926, 1928, 1930, 1932, 1934, 1936, 1938, 1940, 1942, 1944, 1946, 1948, 1950, 1952, 1954, 1957, 1959 1961, 1963, 1965, 1967, 1969, 1972, 1974, 1976, 1978, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002, 2004, 2006, 2008, 2010, 2012, 2014, 2016, 2018, 2020, 2022, 2024, 2026, 2028, 2030, 2032, 2034, 2036, 2038, 2040, 2042, 2044, 2046, 2048, 2050, 2052, 2054, 2056, 2058, 2060, 2062, 2064, 2066, 2068, 2070, 2072, 2074, 2076, 2078, 2080, and 2082; and wherein the anti-IL-23p19 antibody further comprisies a heavy chain variable chain comprising at least 90% sequence identity to the variable heavy chain selected from the following SEQ ID NOs: 1642, 1644, 1646, 1648, 1650, 1652, 1654, 1656, 1658, 1660, 1662, 1664, 1666, 1668, 1670, 1672, 1674, 1676, 1678, 1680, 1682, 1684, 1686, 1688, 1690, 1692, 1694, 1696, 1698, 1700, 1702, 1704, 1706, 1708, 1710, 1712, 1714, 1716, 1718, 1720, 1722, 1724, 1726, 1728, 1730, 1732, 1734, 1736, 1738, 1740, 1742, 1744, 1746, 1748, 1750, 1752, 1754, 1756, 1758, 1760, 1762, 1764, 1766, 1768, 1770, 1772, 1774, 1776, 1778, 1780, 1782, 1784, 1786, 1788, 1790, 1792, 1794, 1796, 1798, 1800, 1802, 1804, 1806, 1808, 1810, 1812, 1814, 1816, 1818, 1820, 1821, 1823, 1825, 1827, 1829, 1831, 1833, 1835, 1837, 1839, 1841, 1843, 1845, 1847, 1849, 1851, 1853, 1855, 1857, 1859, 1861, 1863, 1865, 1867, 1869, 1871, 1873, 1875, 1877, 1879, 1881, 1883, 1885, 1887, 1889, 1891, 1893, 1895, 1899, 1901, 1903, 1905, 1907, 1909,1911, 1913, 1915, 1917, 1919, 1921, 1923, 1925, 1927, 1929, 1931, 1933, 1935, 1937, 1939, 1941, 1943, 1945, 1947, 1949, 1951, 1953, 1955, 1956, 1958, 1960, 1962, 1964, 1966, 1968, 1970, 1971, 1973, 1975, 1977, 1979, 1981, 1983, 1985, 1987, 1989, 1991, 1993, 1995, 1997, 1999, 2001, 2003, 2005, 2007, 2009, 2011, 2013, 2015, 2017, 2019, 2021, 2023, 2025, 2027, 2029, 2031, 2033, 2035, 2037, 2039, 2041,

2043, 2045, 2047, 2049, 2051, 2053, 2055, 2057, 2059, 2061, 2063, 2065, 2067, 2069, 2071, 2073, 2075, 2077, 2079, 2081, and 2083.

[195] The invention provides an anti-IL-23p19 antibody wherein the antibody comprises the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of the consensus CDRs of the CDR families listed in Table 4.

[196] Within an aspect the invention provides an anti-IL-23p19 antibody comprisin a light chain variable region comprising at least 90% sequence identity to the variable light chain selected from the group consisting of the following SEQ ID NOs: 1641, 1643, 1645, 1647, 1649, 1651, 1653, 1655, 1657, 1659, 1661, 1663 1665, 1667, 1669, 1671, 1673, 1675, 1677, 1679, 1681, 1683, 1685, 1687, 1689, 1691, 1693, 1695, 1697, 1699, 1701, 1703, 1705, 1707, 1709, 1711, 1713, 1715, 1717, 1719, 1721, 1723, 1725, 1727, 1729, 1731, 1733, 1735, 1737,1739, 1741, 1743, 1745, 1747, 1749, 1751, 1753, 1755, 1757, 1759, 1761, 1763, 1765, 1767, 1769, 1771, 1773, 1775, 1777, 1779, 1781, 1783, 1785, 1787, 1789, 1791, 1793, 1795, 1797, 1799, 1801, 1803, 1805, 1807, 1809, 1811, 1813, 1815, 1817, 1819, 1822, 1824, 1826, 1828, 1830, 1832, 1834, 1836, 1838, 1840, 1842, 1844, 1846, 1848, 1850, 1852, 1854, 1856, 1858, 1860, 1862, 1864, 1866, 1868, 1870, 1872, 1874, 1876, 1878, 1880, 1882, 1884, 1886, 1888, 1890, 1892, 1894, 1896, 1897, 2085, 1898, 1900, 1902, 1904, 1906, 1908, 1910, 1912, 1914, 1916, 1918, 1920, 1922, 1924, 1926, 1928, 1930, 1932, 1934, 1936, 1938, 1940, 1942, 1944, 1946, 1948, 1950, 1952, 1954, 1957, 1959 1961, 1963, 1965, 1967, 1969, 1972, 1974, 1976, 1978, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002, 2004, 2006, 2008, 2010, 2012, 2014, 2016, 2018, 2020, 2022, 2024, 2026, 2028, 2030, 2032, 2034, 2036, 2038, 2040, 2042, 2044, 2046, 2048, 2050, 2052, 2054, 2056, 2058, 2060, 2062, 2064, 2066, 2068, 2070, 2072, 2074, 2076, 2078, 2080, and 2082. Within an embodiment, the anti-IL-23p19 antibody further comprises a heavy chain variable region comprising at least 90% sequence identity to the variable heavy chain selected from the group consisting of the following SEQ ID NOs: 1642, 1644, 1646, 1648, 1650, 1652, 1654, 1656, 1658, 1660, 1662, 1664, 1666, 1668, 1670, 1672, 1674, 1676, 1678, 1680, 1682, 1684, 1686, 1688, 1690, 1692, 1694, 1696, 1698, 1700, 1702, 1704, 1706, 1708, 1710, 1712, 1714, 1716, 1718, 1720, 1722, 1724, 1726, 1728, 1730, 1732, 1734, 1736, 1738, 1740, 1742, 1744, 1746, 1748, 1750, 1752, 1754, 1756, 1758, 1760, 1762, 1764, 1766, 1768, 1770, 1772, 1774, 1776, 1778, 1780, 1782, 1784, 1786, 1788, 1790, 1792, 1794, 1796, 1798, 1800, 1802, 1804, 1806, 1808, 1810, 1812, 1814, 1816, 1818, 1820, 1821, 1823, 1825, 1827, 1829, 1831, 1833, 1835, 1837, 1839, 1841, 1843, 1845, 1847, 1849, 1851, 1853, 1855, 1857, 1859, 1861, 1863, 1865, 1867, 1869, 1871, 1873, 1875, 1877, 1879, 1881, 1883, 1885, 1887, 1889, 1891, 1893, 1895, 1899, 1901, 1903, 1905, 1907, 1909,1911, 1913, 1915, 1917, 1919, 1921, 1923, 1925, 1927, 1929, 1931, 1933, 1935, 1937, 1939, 1941, 1943, 1945, 1947, 1949, 1951, 1953, 1955, 1956, 1958, 1960, 1962, 1964, 1966, 1968, 1970, 1971, 1973, 1975, 1977, 1979, 1981, 1983, 1985, 1987, 1989, 1991, 1993, 1995, 1997, 1999, 2001, 2003, 2005, 2007, 2009, 2011, 2013, 2015, 2017, 2019, 2021, 2023, 2025, 2027,

2029, 2031, 2033, 2035, 2037, 2039, 2041, 2043, 2045, 2047, 2049, 2051, 2053, 2055, 2057, 2059, 2061, 2063, 2065, 2067, 2069, 2071, 2073, 2075, 2077, 2079, 2081, and 2083.

[197] Within an aspect the invention provides an anti-IL-23p19 antibody comprising a heavy chain variable region comprising at least 90% sequence identity to the variable heavy chain selected from the group consisting of the following SEQ ID NOs: 1642, 1644, 1646, 1648, 1650, 1652, 1654, 1656, 1658, 1660, 1662, 1664, 1666, 1668, 1670, 1672, 1674, 1676, 1678, 1680, 1682, 1684, 1686, 1688, 1690, 1692, 1694, 1696, 1698, 1700, 1702, 1704, 1706, 1708, 1710, 1712, 1714, 1716, 1718, 1720, 1722, 1724, 1726, 1728, 1730, 1732, 1734, 1736, 1738, 1740, 1742, 1744, 1746, 1748, 1750, 1752, 1754, 1756, 1758, 1760, 1762, 1764, 1766, 1768, 1770, 1772, 1774, 1776, 1778, 1780, 1782, 1784, 1786, 1788, 1790, 1792, 1794, 1796, 1798, 1800, 1802, 1804, 1806, 1808, 1810, 1812, 1814, 1816, 1818, 1820, 1821, 1823, 1825, 1827, 1829, 1831, 1833, 1835, 1837, 1839, 1841, 1843, 1845, 1847, 1849, 1851, 1853, 1855, 1857, 1859, 1861, 1863, 1865, 1867, 1869, 1871, 1873, 1875, 1877, 1879, 1881, 1883, 1885, 1887, 1889, 1891, 1893, 1895, 1899, 1901, 1903, 1905, 1907, 1909,1911, 1913, 1915, 1917, 1919, 1921, 1923, 1925, 1927, 1929, 1931, 1933, 1935, 1937, 1939, 1941, 1943, 1945, 1947, 1949, 1951, 1953, 1955, 1956, 1958, 1960, 1962, 1964, 1966, 1968, 1970, 1971, 1973, 1975, 1977, 1979, 1981, 1983, 1985, 1987, 1989, 1991, 1993, 1995, 1997, 1999, 2001, 2003, 2005, 2007, 2009, 2011, 2013, 2015, 2017, 2019, 2021, 2023, 2025, 2027, 2029, 2031, 2033, 2035, 2037, 2039, 2041, 2043, 2045, 2047, 2049, 2051, 2053, 2055, 2057, 2059, 2061, 2063, 2065, 2067, 2069, 2071, 2073, 2075, 2077, 2079, 2081, and 2083. Within an embodiment, the anti-IL-23p19 antibody further comprises a light chain variable region comprising at least 90% sequence identity to the variable light chain seledted from the group consisting of the following SEQ ID NO: 1641, 1643, 1645, 1647, 1649, 1651, 1653, 1655, 1657, 1659, 1661, 1663 1665, 1667, 1669, 1671, 1673, 1675, 1677, 1679, 1681, 1683, 1685, 1687, 1689, 1691, 1693, 1695, 1697, 1699, 1701, 1703, 1705, 1707, 1709, 1711, 1713, 1715, 1717, 1719, 1721, 1723, 1725, 1727, 1729, 1731, 1733, 1735, 1737,1739, 1741, 1743, 1745, 1747, 1749, 1751, 1753, 1755, 1757, 1759, 1761, 1763, 1765, 1767, 1769, 1771, 1773, 1775, 1777, 1779, 1781, 1783, 1785, 1787, 1789, 1791, 1793, 1795, 1797, 1799, 1801, 1803, 1805, 1807, 1809, 1811, 1813, 1815, 1817, 1819, 1822, 1824, 1826, 1828, 1830, 1832, 1834, 1836, 1838, 1840, 1842, 1844, 1846, 1848, 1850, 1852, 1854, 1856, 1858, 1860, 1862, 1864, 1866, 1868, 1870, 1872, 1874, 1876, 1878, 1880, 1882, 1884, 1886, 1888, 1890, 1892, 1894, 1896, 1897, 2085, 1898, 1900, 1902, 1904, 1906, 1908, 1910, 1912, 1914, 1916, 1918, 1920, 1922, 1924, 1926, 1928, 1930, 1932, 1934, 1936, 1938, 1940, 1942, 1944, 1946, 1948, 1950, 1952, 1954, 1957, 1959 1961, 1963, 1965, 1967, 1969, 1972, 1974, 1976, 1978, 1980, 1982, 1984, 1986, 1988, 1990, 1992, 1994, 1996, 1998, 2000, 2002, 2004, 2006, 2008, 2010, 2012, 2014, 2016, 2018, 2020, 2022, 2024, 2026, 2028, 2030, 2032, 2034, 2036, 2038, 2040, 2042, 2044, 2046, 2048, 2050, 2052, 2054, 2056, 2058, 2060, 2062, 2064, 2066, 2068, 2070, 2072, 2074, 2076, 2078, 2080, and 2082. Within an embodiment, the anti-IL-17A antbody comprising a variable light chain region comprising at least

90% sequence identity to the variable light chain selected from the group consisitingof the following SEQ ID NOs: 790, 792, 794, 796, 798, 800, 802, 804, 806, 808, 810, 812, 814, 816, 818, 820, 822, 824, 826, 828,830, 832, 834, 836, 838, 840, 842, 844, 846, 848, 850, 852, 854, 856, 858, 860, 862, 864, 866, 868, 870, 872, 874, 876, 878, 880, 882, 884, 886, 888, 890, 892, 894, 896, 898, 900, 902, 904, 906, 908, 910, 912, 914, 916, 1196, 921, 923, 926, 927, 930, 932, 934, 935, 937, 941, 942, 944, 946, 949, 952, 953, 955, 957, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1007, 1009, 1011, 1013, 1015, 1017, 1019, 1021, 1023, 1025, 1027, 1029, 1031, 1033, 1035, 1037, 1039, 1041, 1043, 1045, 1047, 1049, 1051, 1053, 1055, 1057, 1059, 1061, 1063, 1065, 1068, 1070, 1072, 1074, 1076, 1078, 1080, 1082, 1084, 1086, 1088, 1090, 1092, 1094, 1096, 1098, 1100, 1102, 1104, 1106, 1108, 1110, 1112, 1114, 1116, 1118, 1120, 1122, 1124, 1126, 1128, 1130, 1132, 1134, 1136, 1138, 1140, 1142, 1144, 1146, 1148, 1150, 1152, 1154, 1156, 1158, 1160, 1162, 1164, 1166, 1168, 1170, 1172, 1174, 1176, 1178, 1180, 1182, 1184, 1186, 1188, 1190, 1192, and 1194, and wherein the anti-IL-17A further comprises an a variable heavy chain comprising at least 90% sequence identity to the variable heavy chain selected from the group consisting of the following SEQ ID NOs: 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 918, 919, 920, 922, 924, 925, 928, 929, 931, 933, 936, 938, 939, 940, 943, 945, 947, 948, 950, 951, 954, 956, 958, 960, 962, 964, 966, 968, 970, 972, 974, 976, 978, 980, 982, 984, 986, 988, 990, 992, 994, 996, 998, 1000, 1002, 1004, 1006, 1008, 1010, 1012, 1014, 1016, 1018, 1020, 1022, 1024, 1026, 1028, 1030, 1032, 1034, 1036, 1038, 1040, 1042, 1044, 1046, 1048, 1050, 1052, 1054, 1056, 1058, 1060, 1062, 1064, 1066, 1067, 1069, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1115, 1117, 1119, 1121, 1123, 1125, 1127, 1129, 1131, 1133, 1135, 1137, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, and 1195. Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer.

[198] Within an aspect the invention provides anti-IL-17A antibody selected from the group consisting of: a) an anti-IL-17A antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 389 as shown in Table 5; b) a) an anti-IL-17A antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 632 as shown in Table 5; and c) an anti-IL-17A antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 631 as shown in Table 5. Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer. Within an embodiment, the antibody further comprises an anti-IL-23p19 antibody selected from the group consisting of: a) an anti-IL-23p19 antibody comprising the

LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 361 as shown in Table 6; b) a) an anti- IL-23p19 antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 490 as shown in Table 6; and c) an anti-IL-23p19antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 472 as shown in Table 6.

[199] Within an aspect the invention provides an anti-IL-23p19 antibody selected from the group consisting of: a) an anti-IL-23p19 antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 361 as shown in Table 6; b) a) an anti- IL-23p19 antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 490 as shown in Table 6; and c) an anti-IL-23p19antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 472 as shown in Table 6. Within an embodiment. the anti-Il-23p19 antibody according to claim 20 further comprising an anti-IL-17A antibody selected from the group consisting of: a) an anti-IL-17A antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 389 as shown in Table 5; b) a) an anti-IL-17A antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 632 as shown in Table 5; and c) an anti-IL-17A antibody comprising the LCDR1, LCDR2, LCDR3, HCDR1, HCDR2 and HCDR3 of clone 631 as shown in Table 5. Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer.

[200] The invention provides an anti-IL-17A antibody comprising the antibody deposited with ATCC as clone c631.1 HL scFv (MVE #228) given ATCC Patent Deposit Designation PTA-8819). Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer. Within an embodiment, the anti-IL-17A antibody urther comprising an anti-IL-23p19 antibody selected the group consisting of: a) clone c305 HL scFv (MVE #158) given ATCC Patent Deposit Designation PTA-8818); and b) clone c472.2 LH scFv (MVE # 263) given ATCC Patent Deposit Designation PTA-8820). Within an embodiment, the light and heavy chains of the scFv can be reversed.

[201] The invention provides an anti-IL-17A antibody comprising the antibody deposited with ATCC as clone c632.1 LH scFv (MVE #258) given ATCC Patent Deposit Designation PTA-8821). Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer. Within an embodiment, the anti-IL-17A antibody further comprising an anti-IL-23p19 antibody of clone c305 HL scFv (MVE #158) given ATCC Patent Deposit Designation PTA-8818). Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer. Within an embodiment, the anti-IL-17A antibody further comprising an anti-IL-23p19 antibody of clone c472.2 LH scFv (MVE # 263) given ATCC Patent Deposit Designation PTA-8820). Within an embodiment, the light and heavy chains of the scFv can be reversed.

[202] Within an aspect the invention provides an anti-IL-23p19 antibody comprising the antibody deposited with ATCC as clone c305 HL scFv (MVE #158) given ATCC Patent Deposit

Designation PTA-8818). Within an embodiment, the antibody further comprising an anti-IL-17A antibody selected from the group consisting of: a) clone c631.1 HL scFvn (MVE #228) given ATCC Patent Deposit Designation PTA-8819); and b) clone c632.1 LH scFv (MVE #258) given ATCC Patent Deposit Designation PTA-8821). Within an embodiment, the light and heavey chains of the scFv can be reversed. Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer.

[203] Within an aspect the invention provides an anti-IL-23p19 antibody comprising the antibody deposited with ATCC as clone c472.2 LH scFv (MVE # 263) given ATCC Patent Deposit Designation PTA-8820). Within an embodiment, the antibody further comprising an anti-IL-17A antibody selected from the group consisting of: a) clone c631.1 HL scFvn (MVE #228) given ATCC Patent Deposit Designation PTA-8819); and b) clone c632.1 LH scFv (MVE #258) given ATCC Patent Deposit Designation PTA-8821). Within an embodiment, the light and heavey chains of the scFv can be reversed. Within an embodiment, the anti-IL-17A antibodies anti-IL17A binding entities bind the IL17A/F heterodimer.

[204] Within an aspect the anti-IL-17A and anti-IL-23p19 antibodies comprise antibody fragments. Within an embodiment the antibody is selected from the group consisting of: a) Fv; b) Fab; c) Fab'; d) F(ab)2; e) F(ab')2; and f) scFv. Within an embodiment the antibody further comprises one or more Fc polypeptides.

[205] Within an aspect the anti-IL-17A and anti-IL-23p19 antibodies comprise a bispecific multivalent antibody. Within an embodiment the antibody is selected from the group consisting of: a) Fv; b) Fab; c) Fab'; d) F(ab)2; e) F(ab')2; and f) scFv. Within an embodiment the antibody further comprises one or more Fc polypeptides.

[206] Within an embodiment the invention provides an anti-IL-17A antibody capable of competing for binding to any of the anti-IL-17A antibodies described in Tables 1, 3, 5 or in the Examples.

[207] Within an embodiment the invention provides an anti-IL-23p19 antibody capable of competing for binding to any of the anti-23p19 antibodies described in Tables 2, 4, 6 or in the Examples.

[208] Within an aspect the invention provides a method for inhibiting IL-17A production by T cells comprising treating said T cells with an antagonist of IL-17 and IL-23. Within an embodiment the method comprises any one antibody described herein, or a combination of antibodies described herein.

[209] Within an aspect the invention provides a method of inhibiting IL-17F production by T cells comprinsing treating the T cells with an anti-IL-23p19 antibody. Within an embodiment the method comprises any one anti-IL-23p19 antibody described herein, or a combination of anti-IL-23p19 antibodies described herein.

[210] Within an aspect the invention provides a method for the treatment of a disease characterized by elevated expression of IL-17A, IL-17F,or IL-23 in a mammalian subject, comprising administering to said subject an antibody of any of the antibodies or combination of antibodies described herein.

[211] Within an aspect the invention provides a method for treating disease wherein said disease is selected from multiple sclerosis (MS), chronic inflammation, autoimmune diabetes, autoimmune ocular disease, rheumatoid arthritis (RA) and other arthritic conditions, asthma, systhemic lupus erythrematosus, psoriasis, Crohn's Disease, ulcerative colitis, irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) comprising administering any antibody or combination of antibodies described herein.

[212] Within an aspect the invention provides a method for preventing, inhibiting, or reducing relapse in multiple sclerosis comprising administering a combination of an anti-IL-17A antibody and anti-IL-23p19 antibody. Within an embodiment the anti-IL-17A antibody and the anti-IL-23p19 antibody are co-administered. Within an embodiment the anti-IL-17A antibody and the anti-IL-23p19 antibody are administered in one entity. Within another embodiment the entity is a bispecific molecule.

[213] Within an aspect the invention provides a method for preventing, inhibiting, or reducing IBD comprising administering a combination of an anti-IL-17A antibody and an anti-IL-23p19 anibody. Within an embodiment the anti-IL-17A antibody and the anti-IL-23p19 antibody are co-administered. Within an embodiment the anti-IL-17A antibody and the anti-IL-23p19 antibody are administered in one entity. Within another embodiment the entity is a bispecific molecule.

Nucleic Acids

[214] The invention also includes nucleic acids encoding the heavy chain and/or light chain of the antibodies of the invention. Nucleic acids of the invention include nucleic acids having at least 80%, more preferably at least about 90%, more preferably at least about 95%, and most preferably at least about 98% homology to nucleic acids of the invention. The terms "percent similarity", "percent identity" and "percent homology" when referring to a particular sequence are used as set forth in the University of Wisconsin GCG software program. Nucleic acids of the invention also include complementary nucleic acids. In some instances, the sequences will be fully complementary (no mismatches) when aligned. In other instances, there may be up to about a 20% mismatch in the sequences. In some embodiments of the invention are provided nucleic acids encoding both a heavy chain and a light chain of an antibody of the invention. One skilled in the art will know that the nucleic acid sequences provided herein can be exploited using codon optimization, degereate sequence, silent mutations, and other DNA techniques to optimize expression in a particular hosts. The invention encompasses such sequence modifications. The present invention provides

polynucleotide molecules, including DNA and RNA molecules, that encode the anti-IL-17A and anti-IL-23p19 antibodies disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules.

[215] Nucleic acids of the invention can be cloned into a vector, such as a plasmid, cosmid, bacmid, phage, artificial chromosome (BAC, YAC) or virus, into which another genetic sequence or element (either DNA or RNA) may be inserted so as to bring about the replication of the attached sequence or element. In some embodiments, the expression vector contains a constitutively active promoter segment (such as but not limited to CMV, SV40, Elongation Factor or LTR sequences) or an inducible promoter sequence such as the steroid inducible pIND vector (Invitrogen), where the expression of the nucleic acid can be regulated. Expression vectors of the invention may further comprise regulatory sequences, for example, an internal ribosomal entry site. The expression vector can be introduced into a cell by transfection, for example.

Methods of Producing Antibodies to IL-17 and IL-23

[216] The invention also provides methods of producing monoclonal antibodies that specifically bind to IL-17 and IL-23, either singly or together. Antibodies of the invention may be produced in vivo or in vitro. One strategy for generating antibodies against both IL-17 and IL-23 involves immunizing animals with both IL-17 and IL-23. In some embodiments, animals are immunized with the monomeric or multimeric form of oth IL-17 and IL-23. Animals so immunized will produce antibodies against both IL-17 and IL-23. Standard methods are known for creating monoclonal antibodies including, but are not limited to, the hybridoma technique (see Kohler & Milstein, (1975) *Nature 256*:495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor *et al.* (1983) *Immunol. Today* 4:72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.* in MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., 1985, pp. 77-96).

[217] Both IL-17 and IL-23 may be purified from cells or from recombinant systems using a variety of well-known techniques for isolating and purifying proteins. For example, but not by way of limitation, both IL-17 and IL-23 may be isolated based on the apparent molecular weight of the protein by running the protein on an SDS-PAGE gel and blotting the proteins onto a membrane. Thereafter, the appropriate size band corresponding to either protein may be cut from the membrane and used as an immunogen in animals directly, or by first extracting or eluting the protein from the membrane. As an alternative example, the protein may be isolated by size-exclusion chromatography alone or in combination with other means of isolation and purification.

[218] The invention also provides methods of producing monoclonal antibodies that specifically bind to homodimeric, heterodimeric, and/or multimeric forms of both IL-17 and IL-

23p19. These different forms may be purified from cells or from recombinant systems using a variety of well-known techniques for isolating and purifying proteins. For example, but not by way of limitation, both IL-17 and IL-23p19 may be isolated based on the apparent molecular weight of the protein by running the protein on an SDS-PAGE gel and blotting the proteins onto a membrane. Thereafter, the appropriate size band corresponding to each may be cut from the membrane and used as an immunogen in animals directly, or by first extracting or eluting the protein from the membrane. As an alternative example, the protein may be isolated by size-exclusion chromatography alone or in combination with other means of isolation and purification.

[219] Other means of purification are available in such standard reference texts as Zola, *Monoclonal Antibodies: Preparation And Use Of Monoclonal Antibodies And Engineered ntibody Derivatives (Basics: From Background To Bench)* Springer-Verlag Ltd., New York, 2000; *Basic Methods In Antibody Production And Characterization*, Chapter 11, "Antibody Purification Methods," Howard and Bethell, Eds., CRC Press, 2000; *Antibody Engineering (Springer Lab Manual.)*, Kontermann and Dubel, Eds., Springer-Verlag, 2001.

[220] For *in vivo* antibody production, animals are generally immunized with either IL-17 or IL-23 or an immunogenic portion of either. The antigen is generally combined with an adjuvant to promote immunogenicity. Adjuvants vary according to the species used for immunization. Examples of adjuvants include, but are not limited to: Complete Freund's Adjuvant ("CFA"), Incomplete Freund's Adjuvant ("IFA"), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions), peptides, oil emulsions, keyhole limpet hemocyanin ("KLH"), dinitrophenol ("DNP"), and potentially useful human adjuvants such as Bacille Calmette-Guerin ("BCG") and corynebacterium parvum. Such adjuvants are also well known in the art. Immunization may be accomplished using well-known procedures. The dose and immunization regimen will depend on the species of mammal immunized, its immune status, body weight, and/or calculated surface area, etc. Typically, blood serum is sampled from the immunized mammals and assayed for anti-IL-17 and IL-23p19 antibodies using appropriate screening assays as described below, for example.

[221] A common method for producing humanized antibodies is to graft CDR sequences from a MAb (produced by immunizing a rodent host) onto a human Ig backbone, and transfection of the chimeric genes into Chinese Hamster Ovary (CHO) cells which in turn produce a functional Ab that is secreted by the CHO cells (Shields, R. L., *et al.* (1995) *Int Arch. Allergy Immunol. 107*:412-413). The methods described within this application are also useful for generating genetic alterations within Ig genes or chimeric Igs transfected within host cells such as rodent cell lines, plants, yeast and prokaryotes (Frigerio L, *et al.* (2000) *Plant Physiol. 123*:1483-1494).

[222] Splenocytes from immunized animals may be immortalized by fusing the splenocytes (containing the antibody-producing B cells) with an immortal cell line such as a myeloma line.

Typically, myeloma cell line is from the same species as the splenocyte donor. In one embodiment, the immortal cell line is sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). In some embodiments, the myeloma cells are negative for Epstein-Barr virus (EBV) infection. In preferred embodiments, the myeloma cells are HAT-sensitive, EBV negative and Ig expression negative. Any suitable myeloma may be used. Murine hybridomas may be generated using mouse myeloma cell lines (e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines). These murine myeloma lines are available from the ATCC. These myeloma cells are fused to the donor splenocytes polyethylene glycol ("PEG"), preferably 1500 molecular weight polyethylene glycol ("PEG 1500"). Hybridoma cells resulting from the fusion are selected in HAT medium which kills unfused and unproductively fused myeloma cells. Unfused splenocytes die over a short period of time in culture. In some embodiments, the myeloma cells do not express immunoglobulin genes.

[223] Hybridomas producing a desired antibody which are detected by screening assays such as those described below may be used to produce antibodies in culture or in animals. For example, the hybridoma cells may be cultured in a nutrient medium under conditions and for a time sufficient to allow the hybridoma cells to secrete the monoclonal antibodies into the culture medium. These techniques and culture media are well known by those skilled in the art. Alternatively, the hybridoma cells may be injected into the peritoneum of an unimmunized animal. The cells proliferate in the peritoneal cavity and secrete the antibody, which accumulates as ascites fluid. The ascites fluid may be withdrawn from the peritoneal cavity with a syringe as a rich source of the monoclonal antibody.

[224] Another non-limiting method for producing human antibodies is described in U.S. Pat. No. 5,789,650 which describes transgenic mammals that produce antibodies of another species (*e.g.*, humans) with their own endogenous immunoglobulin genes being inactivated. The genes for the heterologous antibodies are encoded by human immunoglobulin genes. The transgenes containing the unrearranged immunoglobulin encoding regions are introduced into a non-human animal. The resulting transgenic animals are capable of functionally rearranging the transgenic immunoglobulin sequences and producing a repertoire of antibodies of various isotypes encoded by human immunoglobulin genes. The B-cells from the transgenic animals are subsequently immortalized by any of a variety of methods, including fusion with an immortalizing cell line (*e.g.*, a myeloma cell).

[225] The antibodies of the present invention may also be prepared in vitro using a variety of techniques known in the art. For example, but not by way of limitation, fully human monoclonal antibodies against IL-17 and IL-23p19 may be prepared by using *in vitro*-primed human splenocytes (Boerner *et al.* (1991) *J. Immunol. 147*:86-95).

[226] Alternatively, for example, the antibodies of the invention may be prepared by "repertoire cloning" (Persson *et al.* (1991) *Proc. Nat. Acad. Sci. USA 88*:2432-2436; and Huang and

Stollar (1991) *J. Immunol. Methods 141*:227-236). Further, U.S. Pat. No. 5,798,230 describes preparation of human monoclonal antibodies from human B antibody-producing B cells that are immortalized by infection with an Epstein-Barr virus that expresses Epstein-Barr virus nuclear antigen 2 (EBNA2). EBNA2, required for immortalization, is then inactivated resulting in increased antibody titers.

[227] In another embodiment, antibodies of the invention are formed by in vitro immunization of peripheral blood mononuclear cells ("PBMCs"). This may be accomplished by any means known in the art, such as, for example, using methods described in the literature (Zafiropoulos *et al.* (1997) *J. Immunological Methods 200*:181-190).

[228] In a specific embodiment, bispecific and single chain antibodies that bind both IL-17 and IL-23 are made. One method of the present invention is a method for producing a bispecific IL-17/IL-23 antibody. The method comprises fusing hybridoma cells that secrete a monoclonal antibody that binds IL-17, with hybridoma cells that secrete a monoclonal antibody that binds IL-23p19, thereby preparing a hybrid hybridoma that secretes a bispecific IL-17/IL-23 monclonal antibody. In one embodiment, the method comprises fusing hybridoma cells that secrete an antagonistic (or agonistic) IL-17 MAb, with hybridoma cells that secrete an antagonistic (or agonistic) IL-23p19 MAb. Conventional techniques for conducting such a fusion, and for isolating the desired hybrid hybridoma, include those described elsewhere herein, and those illustrated in the examples below.

[229] U.S. Pat. No. 6,060,285 discloses a process for the production of bispecific antibodies, in which at least the genes for the light chain and the variable portion of the heavy chain of an antibody having a first specificity are transfected into a hybridoma cell secreting an antibody having a second specificity. When the transfected hybridoma cells are cultured, bispecific antibodies are produced, and may be isolated by various means known in the art. Production of the bispecific molecules described herein in Chinese Hamster Ovary cells showed about 35 mg/ml from an unoptimized pool.

[230] Other investigators have used chemical coupling of antibody fragments to prepare antigen-binding molecules having specificity for two different antigens (Brennan *et al., Science 229*:81 1985; Glennie *et al., J. Immunol. 139*:2367, 1987). U.S. Pat. No. 6,010,902 also discusses techniques known in the art by which bispecific antibodies can be prepared, for example by the use of heterobifunctional cross-linking reagents such as GMBS (maleimidobutryloxy succinimide) or SPDP (N-succinimidyl 3-(2-pyridyldithio)propionate). (See, *e.g.*, Hardy, "Purification And Coupling Of Fluorescent Proteins For Use In Flow Cytometry", Handbook Of Experimental Immunology, 4.sup.th Ed., Volume 1, Immunochemistry, Weir et al. (eds.), pp. 31.4-31.12, 1986).

[231] The ability to produce antibodies via recombinant DNA technology has facilitated production of bispecific antibodies. Kostelny *et al.* utilized the leucine zipper moieties from the fos

and jun proteins (which preferentially form heterodimers) to produce bispecific antibodies able to bind both the cell surface molecule CD3 and the receptor for IL-2 (*J. Immunol. 148*:1547; 1992).

[232] Single chain antibodies may be formed by linking heavy and light chain variable region (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable region polypeptides (V.sub.L and V.sub.H). The resulting antibody fragments can form dimers or higher oligomers, depending on such factors as the length of a flexible linker between the two variable domains (Kortt *et al., Protein Engineering 10*:423, 1997). In particular embodiments, two or more scFvs are joined by use of a chemical cross-linking agent.

[233] Techniques developed for the production of single chain antibodies can be adapted to produce single chain antibodies of the present invention, that bind both IL-17 and IL-23. Such techniques include those described in U.S. Pat. No. 4,946,778; Bird (*Science 242:*423, 1988); Huston et al. (*Proc. Natl. Acad. Sci. USA 85*:5879, 1988); and Ward *et al. (Nature 334*:544, 1989). Once desired single chain antibodies are identified (for example, from a phage-display library), those of skill in the art can further manipulate the DNA encoding the single chain antibody(ies) to yield bispecific antibodies, including bispecific antibodies having Fc regions.

[234] Single chain antibodies against IL-17 and IL-23 may be concatamerized in either order (i.e., anti-IL-17-anti-IL-23 or anti-IL-23-anti-IL-17). In particular embodiments, starting materials for preparing a bispecific IL-17/IL-23 antibody include an antagonistic (or agonistic) single chain antibody directed against IL-17 and an antagonistic (or agonistic) single chain antibody directed against IL-23p19.

[235] U.S. Pat. No. 5,582,996 discloses the use of complementary interactive domains (such as leucine zipper moieties or other lock and key interactive domain structures) to facilitate heterodimer formation in the production of bispecific antibodies. The complementary interactive domain(s) may be inserted between an Fab fragment and another portion of a heavy chain (i.e., C_{H1} or C_{H2} regions of the heavy chain). The use of two different Fab fragments and complementary interactive domains that preferentially heterodimerize will result in bispecific antibody molecules. Cysteine residues may be introduced into the complementary interactive domains to allow disulphide bonding between the complementary interactive domains and stabilize the resulting bispecific antibodies.

[236] Tetravalent, bispecific molecules can be prepared by fusion of DNA encoding the heavy chain of an $F(ab')_2$ fragment of an antibody with either DNA encoding the heavy chain of a second $F(ab')_2$ molecule (in which the CH1 domain is replaced by a CH3 domain), or with DNA encoding a single chain Fv fragment of an antibody, as described in U.S. Pat. No. 5,959,083. Expression of the resultant fusion genes in mammalian cells, together with the genes for the

corresponding light chains, yields tetravalent bispecific molecules having specificity for selected antigens.

[237] Bispecific antibodies can also be produced as described in U.S. Pat. No. 5,807,706, which is incorporated by reference herein. Generally, the method involves introducing a protuberance in a first polypeptide and a corresponding cavity in a second polypeptide, polypeptides interface. The protuberance and cavity are positioned so as to promote heteromultimer formation and hinder homomultimer formation. The protuberance is created by replacing amino acids having small side chains with amino acids having larger side chains. The cavity is created by the opposite approach, i.e., replacing amino acids having relatively large side chains with amino acids having smaller side chains.

[238] The protuberance and cavity can be generated by conventional methods for making amino acid substitutions in polypeptides. For example, a nucleic acid encoding a polypeptide may be altered by conventional in vitro mutagenesis techniques. Alternatively, a polypeptide incorporating a desired amino acid substitution may be prepared by peptide synthesis. Amino acids chosen for substitution are located at the interface between the first and second polypeptides.

Screening for Antibody Specificity

[239] Screening for antibodies that specifically bind to IL-17 and/or IL-23p19 may be accomplished using the procedures and assays known in the art and those described herein. For example, an enzyme-linked immunosorbent assay (ELISA) can be used in which microtiter plates are coated with either or both IL-17A and/or IL-23 p19. In some embodiments, antibodies that bind both IL-17 and IL-23p19 from positively reacting clones can be further screened for reactivity in an ELISA-based assay using microtiter plates coated with the other forms IL-17 and IL-23p19, or other IL-17 family members. Clones that produce antibodies that are reactive to another form or family members are eliminated, and clones that produce antibodies that are reactive to both IL-17 and IL-23p19 may be selected for further expansion and development. Reactivity with both IL-17 and IL-23p19 and not another family member confirms specificity of reactivity for both IL-17 and IL-23p19.

Anti-IL-17 and IL-23p19 Antibody-Producing Cells

[240] Antibody-producing cells of the invention include any insect expression cell line known, such as for example, Spodoptera frugiperda cells. The expression cell lines may also be yeast cell lines, such as, for example, Saccharomyces cerevisiae and Schizosaccharomyces pombe cells. The expression cells may also be mammalian cells such as, for example, hybridoma cells (e.g., NS0 cells), Chinese hamster ovary cells, baby hamster kidney cells, human embryonic kidney line 293, normal dog kidney cell lines, normal cat kidney cell lines, monkey kidney cells, African green monkey kidney cells, COS cells, and non-tumorigenic mouse myoblast G8 cells, fibroblast cell lines, myeloma cell lines, mouse NIH/3T3 cells, LMTK31 cells, mouse sertoli cells, human cervical

carcinoma cells, buffalo rat liver cells, human lung cells, human liver cells, mouse mammary tumor cells, TRI cells, MRC 5 cells, and FS4 cells.

Antibody Purification

[241] Methods of antibody purification are known in the art. In some embodiments of the invention, methods for antibody purification include filtration, affinity column chromatography, cation exchange chromatography, anion exchange chromatography, and concentration. The filtration step preferably comprises ultrafiltration, and more preferably ultrafiltration and diafiltration. Filtration is preferably performed at least about 5-50 times, more preferably 10 to 30 times, and most preferably 14 to 27 times. Affinity column chromatography, may be performed using, for example, PROSEP Affinity Chromatography (Millipore, Billerica, Mass.). In a preferred embodiment, the affinity chromatography step comprises PROSEP-VA column chromatography. Eluate may be washed in a solvent detergent. Cation exchange chromatography may include, for example, SP-Sepharose Cation Exchange Chromatography. Anion exchange chromatography may include, for example but not limited to, Q-Sepharose Fast Flow Anion Exchange. The anion exchange step is preferably non-binding, thereby allowing removal of contaminants including DNA and BSA. The antibody product is preferably nanofiltered, for example, using a Pall DV 20 Nanofilter. The antibody product may be concentrated, for example, using ultrafiltration and diafiltration. The method may further comprise a step of size exclusion chromatography to remove aggregates.

Therapeutic Uses of the IL-17 and IL-23p19 Antibodies

[242] Antibodies that bind to both IL-17A and/or IL-23 can be used to modulate the immune system by binding IL-17A, IL-17A/F and/or IL-23p19 (either singly or together as with a bispecific antibody or scFV), and thus, preventing the binding of IL-17A or IL-17F with either IL-17RA and/or IL-17RC and IL-23 with its receptor (IL-12RB1/IL-23R) or any other receptor that they may bind, especially an IL-17 receptor family member. The antibodies of the invention can also be used to treat a subject which produces an excess of either IL-17 and/or IL-23. Suitable subjects include mammals, such as humans. For example, the antibodies of the invention are useful in binding, blocking, inhibiting, reducing, antagonizing or neutralizing IL-17 and IL-23 (either singly or together as with a bispecific antibody or scFV), in the treatment of inflammation and inflammatory diseases such as multiple sclerosis, demyelinating diseases, autoimmune ocular disease, uveitis, scleritis, cancer (characterized by IL-17 and IL-23 expression), psoriasis, IBS, inflammatory bowel disease (IBD), colitis, promotion of tumor growth, arthritis, or degenerative joint disease and other inflammatory conditions disclosed herein.

[243] Within embodiments, the antibodies of the invention bind to, block, inhibit, reduce, antagonize or neutralize IL-23p19 and IL-17 either singly or together (as with a bispecific antibody or scFV), *in vivo*.

[244] Moreover, the antibodies of the invention are useful to:

[245] (1) Block, inhibit, reduce, antagonize or neutralize signaling via IL-17 and IL-23 in the treatment of cancer, acute inflammation, and chronic inflammatory diseases such as inflammatory bowel disease (IBD), IBS, chronic colitis, splenomegaly, rheumatoid arthritis, and other diseases associated with the induction of acute-phase response, as well as in treatment of autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), demyelinating diseases, autoimmune ocular disease, uveitis, scleritis, systemic Lupus erythematosus (SLE), myasthenia gravis, rheumatoid arthritis, IBS and IBD to prevent or inhibit signaling in immune cells (e.g. lymphocytes, monocytes, leukocytes) via their receptors (e.g. IL-17RA and IL-17RC). Blocking, inhibiting, reducing, or antagonizing signaling via IL-17RA and IL-17RC, using the antibodies of the present invention, may also benefit diseases of the pancreas, kidney, pituitary and neuronal cells. IDDM, non-insulin dependent diabetes mellitus (NIDDM), pancreatitis, and pancreatic carcinoma may benefit.

[246] The antibodies described herein can be used to bind, block, inhibit, reduce, antagonize or neutralize IL-23 and IL-17 activity, either singly or together as with a bispecific antibody or scFV, tascFv, biscFc or BiAb (including Fc fusion proteins) in the treatment of multiple sclerosis, cancer, autoimmune disease, atopic disease, NIDDM, pancreatitis and kidney dysfunction as described above. The antibodies of the present invention are useful as antagonists of IL-17 or IL-23. Such antagonistic effects can be achieved by direct neutralization or binding of IL-17 and IL-23 p19.

[247] Antibodies herein can also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. For instance, antibodies or binding polypeptides which recognize IL-17 or IL-23 can be used to identify or treat tissues or organs that express a corresponding anti-complementary molecule. More specifically, antibodies to IL-17 or IL-23 or bioactive fragments or portions thereof, can be coupled to detectable or cytotoxic molecules and delivered to a mammal having cells, tissues or organs that express these cytokines IL-17 or IL-23 -expressing cancers.

[248] Suitable detectable molecules may be directly or indirectly attached to the antagonists of the present invention, such as "binding polypeptides," (including binding peptides disclosed above), antibodies, or bioactive fragments or portions thereof. Suitable detectable molecules include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. Suitable cytotoxic molecules may be directly or indirectly attached to the polypeptide or antibody, and include bacterial or plant toxins (for instance, diphtheria toxin, *Pseudomonas* exotoxin, ricin, abrin and the like), as well as therapeutic radionuclides, such as

iodine-131, rhenium-188 or yttrium-90 (either directly attached to the polypeptide or antibody, or indirectly attached through means of a chelating moiety, for instance). Binding polypeptides or antibodies may also be conjugated to cytotoxic drugs, such as adriamycin. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/ anticomplementary pair, where the other member is bound to the binding polypeptide or antibody portion. For these purposes, biotin/streptavidin is an exemplary complementary/ anticomplementary pair.

[249] In another embodiment, binding polypeptide-toxin fusion proteins or antibody-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for instance, to treat cancer cells or tissues). Alternatively, if the binding polypeptide has multiple functional domains (i.e., an activation domain or a ligand binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule or a complementary molecule to a cell or tissue type of interest. In instances where the fusion protein including only a single domain includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule fusion proteins thus represent a generic targeting vehicle for cell/tissue-specific delivery of generic anti-complementary-detectable/ cytotoxic molecule conjugates.

[250] Inflammation is a protective response by an organism to fend off an invading agent. Inflammation is a cascading event that involves many cellular and humoral mediators. On one hand, suppression of inflammatory responses can leave a host immunocompromised; however, if left unchecked, inflammation can lead to serious complications including chronic inflammatory diseases (e.g., asthma, psoriasis, arthritis, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and the like), septic shock and multiple organ failure. Importantly, these diverse disease states share common inflammatory mediators. The collective diseases that are characterized by inflammation have a large impact on human morbidity and mortality. Therefore it is clear that anti-inflammatory proteins, such as antagonists to IL-17A and IL-23p19, such as IL-17A and IL-23p19 antibodies, could have crucial therapeutic potential for a vast number of human and animal diseases, from asthma and allergy to autoimmunity, cancers, and septic shock.

1. Arthritis

[251] Arthritis, including osteoarthritis, rheumatoid arthritis, arthritic joints as a result of injury, and the like, are common inflammatory conditions which would benefit from the therapeutic use of anti-inflammatory proteins, such as the antagonists of the present invention. For example, rheumatoid arthritis (RA) is a systemic disease that affects the entire body and is one of the most common forms of arthritis. It is characterized by the inflammation of the membrane lining the joint, which causes pain, stiffness, warmth, redness and swelling. Inflammatory cells release enzymes that may digest bone and cartilage. As a result of rheumatoid arthritis, the inflamed joint lining, the

synovium, can invade and damage bone and cartilage leading to joint deterioration and severe pain amongst other physiologic effects. The involved joint can lose its shape and alignment, resulting in pain and loss of movement.

[252] Rheumatoid arthritis (RA) is an immune-mediated disease particularly characterized by inflammation and subsequent tissue damage leading to severe disability and increased mortality. A variety of cytokines are produced locally in the rheumatoid joints. Numerous studies have demonstrated that IL-1 and TNF-alpha, two prototypic pro-inflammatory cytokines, play an important role in the mechanisms involved in synovial inflammation and in progressive joint destruction. Indeed, the administration of TNF-alpha and IL-1 inhibitors in patients with RA has led to a dramatic improvement of clinical and biological signs of inflammation and a reduction of radiological signs of bone erosion and cartilage destruction. However, despite these encouraging results, a significant percentage of patients do not respond to these agents, suggesting that other mediators are also involved in the pathophysiology of arthritis (Gabay, *Expert. Opin. Biol. Ther. 2*(2):135-149 (2002). One of those mediators could be IL-17 or IL-23, as demonstrated in several reports to play a role in rheumatoid arthritis. For example, IL-17 and IL-23p19 are overexpressed in the synovium and synovial fibroblasts of patients with rheumatoid arthritis compared to individuals without rheumatoid arthritis. Furthermore, IL-17 and IL-23p19 have been demonstrated to promote matrix degradation and enhance the expression of inflammatory, matrix-destructive cytokines when added to synovium/synoviocyte cultures. (Murphy et al, J. Exp. Med 198:1951 (2003); reviewed in Lubberts *et al, Arthritis Res Ther. 7*:29 (2005) and Kim et al, Rheumatology,46:57 (2007)). Therefore, such a molecule that binds or inhibits IL-17 or IL-23 activity, such as the antagonists of the present invention, could serve as a valuable therapeutic to reduce inflammation in rheumatoid arthritis, and other arthritic diseases.

[253] There are several animal models for rheumatoid arthritis known in the art. For example, in the collagen-induced arthritis (CIA) model, mice develop chronic inflammatory arthritis that closely resembles human rheumatoid arthritis. Since CIA shares similar immunological and pathological features with RA, this makes it an ideal model for screening potential human anti-inflammatory compounds. The CIA model is a well-known model in mice that depends on both an immune response, and an inflammatory response, in order to occur. The immune response comprises the interaction of B-cells and CD4+ T-cells in response to collagen, which is given as antigen, and leads to the production of anti-collagen antibodies. The inflammatory phase is the result of tissue responses from mediators of inflammation, as a consequence of some of these antibodies cross-reacting to the mouse's native collagen and activating the complement cascade. An advantage in using the CIA model is that the basic mechanisms of pathogenesis are known. The relevant T-cell and B-cell epitopes on type II collagen have been identified, and various immunological (e.g., delayed-type hypersensitivity and anti-collagen antibody) and inflammatory (e.g., cytokines,

chemokines, and matrix-degrading enzymes) parameters relating to immune-mediated arthritis have been determined, and can thus be used to assess test compound efficacy in the CIA model (Wooley, *Curr. Opin. Rheum. 3*:407-20 (1999); Williams *et al., Immunol.* 89:9784-788 (1992); Myers *et al., Life Sci. 61*:1861-78 (1997); and Wang *et al., Immunol. 92*:8955-959 (1995)).

[254] One group has shown that an anti-mouse IL-17 antibody reduces symptoms in a mouse CIA-model relative to control mice, and another group has shown that deficiency of IL-23p19 is protective in CIA (Murphy et al, J. Exp. Med 198:1951 (2003)), thus showing conceptually that antagonists of the present invention may be beneficial in treating human disease. The administration of a single mouse-IL-17-specific rat antisera reduced the symptoms of arthritis in the animals when introduced prophylactically or after symptoms of arthritis were already present in the model (Lubberts *et al, Arthritis Rheum. 50*:650-9 (2004)).

[255] As described in the Examples below, both IL-17 and IL-23p19 are overexpressed in CIA. Therefore, antagonists of the present invention can be used to neutralize IL-17 and/or IL-23p19 in the treatment of specific human diseases such as arthritis, psoriasis, psoriatic arthritis, endotoxemia, inflammatory bowel disease (IBD), IBS, colitis, and other inflammatory conditions disclosed herein.

[256] The administration of antagonists of the present invention to these CIA model mice is used to evaluate the use of these antagonists to ameliorate symptoms and alter the course of disease. Moreover, results showing inhibition of IL-17 and/or IL-23 signalling by these antagonists would provide proof of concept that IL-17 and IL-23p19 antagonists, such as those disclosed herein, can also be used to ameliorate symptoms and alter the course of disease. By way of example and without limitation, the injection of 10 - 200 ug of an anti-IL-17 and anti-IL-23p19 per mouse (one to seven times a week for up to but not limited to 4 weeks via s.c., i.p., or i.m route of administration) can significantly reduce the disease score (paw score, incident of inflammation, or disease). Depending on the initiation of administration (e.g. prior to or at the time of collagen immunization, or at any time point following the second collagen immunization, including those time points at which the disease has already progressed), antagonists of the present invention can be efficacious in preventing rheumatoid arthritis, as well as preventing its progression.

2. Inflammatory Bowel Disease IBD

[257] In the United States approximately 1.35 million in the US (more than 1.9 million in the G7 nations) have Inflammatory Bowel Disease (IBD) which can affect either colon and rectum (Ulcerative colitis) or both, small and large intestine (Crohn's Disease). In both Crohn's disease and ulcerative colitis, the tissue damage results from an inappropriate or exaggerated immune response to antigens of the gut microflora. Despite having a common basis in overresponsiveness to luminal antigens, Crohn's disease and ulcerative colitis are immunologically distinct entities. Crohn's disease is more associated with a Th1 T cell-mediated response, characterized by enhanced production of interferon-[gamma] and tumor necrosis factor-[alpha]. Interleukin (IL)-12 and, possibly, IL-23 govern

the Th1 cell differentiation, but optimal induction and stabilization of polarized Th1 cells would require additional cytokines, such as IL-15, IL-18 and IL-21. In ulcerative colitis, the local immune response is less polarized, but it is characterized by CD1-reactive natural killer T cell production of IL-13. Beyond these differences, Crohn's disease and ulcerative colitis share important end-stage effector pathways of intestinal injury, which are mediated by an active cross-talk between immune and non-immune mucosal cells. As shown in the Examples and references below, IL-17 and IL-23 are both overexpressed in intestines and/or serum from humans with IBD and in mouse models of IBD. (Nielson et al, Scand J Gastroenterol. 38:180 (2003); Schmidt et al, Inflamm. Bowel Dis. 11:16 (2005); Fuss et al, Inflamm Bowel Dis. 12:9 (2006)). Moreover, neutralization of IL-17 and/or IL-23p19 reduces disease symptoms and pathology in animals models of IBD (Yen et al, J. Clin. Invest. 116:1310 (2006); Zhang et al, Inflamm Bowel Dis. 12:382 (2006)). Hue et al, J Exp Med. 203:2473 (2006); Kullberg et al, J Exp Med. 203:2485 (2006); Elson et al, Gastroenterology. 132:2359 (2007)

[258] As shown in the Examples below, both IL-17 and IL-23p19 expression are increased in the DSS colitis model and in the T cell transfer colitis model, and treatment wth a combination of an anti-IL-17A antibody and an anti-IL23p19 antibody is more efficacious in the oxazalone IBD model than treatment with either antibody alone. Thus, antagonists of the present invention could serve as a valuable therapeutic to reduce inflammation and pathological effects in IBD and related diseases.

[259] Crohn's disease is a chronic, relapsing-remitting inflammatory disease of the intestinal tract. It is a chronic disease that often occurs at an early age, thus requiring patients to be treated for decades. In contrast to the more limited tissue layer involvement of ulcerative colitis (i.e. mucosa and submucosa of the colon), Crohn's disease can extend through all layers of the intestinal wall of both the large and small intestine. Symptoms of Crohn's disease include diarrhea, weight loss, blood, and abdominal pain. Complications are very serious and include intestinal fistulas, abscesses, and obstructions.

[260] In healthy people, there is continuous, clinically undetected immune response to these antigens. In Crohn's disease, the response is prolonged and amplified. The precise cause nor the specific antigenic trigger has been identified, though it has been hypothesized to be a combination of a genetic predisposition plus an environmental trigger, such as exposure to endogenous or exogenous intestinal antigens. Therefore, in an effort to inhibit this characteristic aberrant immune response, immunosuppressive agents are used to treat CD patients. However, it is clear from the side effects of these drugs and the response/failure rate, that more selective and efficacious therapies are needed.

[261] Many immune cells, including neutrophils, macrophages, B and T lymphocytes, and mast cell, are present in the mucosal layer of healthy intestines. The intact epithelium lining the mucosa prevents these cells from being overstimulated by the large antigenic load to which the GI tract is exposed on a daily basis. It is thought that Crohn's disease patients have increased intestinal

permeability (perhaps because of the above mentioned genetic predisposition) that exposes the immune cells to numerous antigens. IL-23 and IL-17 appear to play a role in the overactive response and ensuing disease progression.

[262] Ulcerative colitis (UC) is an inflammatory disease of the large intestine, commonly called the colon, characterized by inflammation and ulceration of the mucosa or innermost lining of the colon. This inflammation causes the colon to empty frequently, resulting in diarrhea. Symptoms include loosening of the stool and associated abdominal cramping, fever and weight loss.

[263] Although the exact cause of UC is unknown, recent research suggests that the body's natural defenses are operating against proteins in the body which the body thinks are foreign (an "autoimmune reaction"). Perhaps because they resemble bacterial proteins in the gut, these proteins may either instigate or stimulate the inflammatory process that begins to destroy the lining of the colon. As the lining of the colon is destroyed, ulcers form releasing mucus, pus and blood. The disease usually begins in the rectal area and may eventually extend through the entire large bowel. Repeated episodes of inflammation lead to thickening of the wall of the intestine and rectum with scar tissue. Death of colon tissue or sepsis may occur with severe disease. The symptoms of ulcerative colitis vary in severity and their onset may be gradual or sudden. Attacks may be provoked by many factors, including respiratory infections or stress.

[264] Although there is currently no cure for UC available, treatments are focused on suppressing the abnormal inflammatory process in the colon lining. Treatments including corticosteroids, immunosuppressives (eg. azathioprine, mercaptopurine, and methotrexate) and aminosalicytates are available to treat the disease. However, the long-term use of immunosuppressives such as corticosteroids and azathioprine can result in serious side effects including thinning of bones, cataracts, infection, and liver and bone marrow effects. In the patients in whom current therapies are not successful, surgery is an option. The surgery involves the removal of the entire colon and the rectum.

[265] There are several animal models that can partially mimic chronic ulcerative colitis. Some of the most widely used models are the is the 2,4,6-trinitrobenesulfonic acid/ethanol (TNBS) induced colitis model or the oxazalone model, which induce chronic inflammation and ulceration in the colon. When TNBS or oxazalone is introduced into the colon of susceptible mice via intra-rectal instillation, it induces T-cell mediated immune response in the colonic mucosa, in this case leading to a massive mucosal inflammation characterized by the dense infiltration of T-cells and macrophages throughout the entire wall of the large bowel. Moreover, this histopathologic picture is accompanies by the clinical picture of progressive weight loss (wasting), bloody diarrhea, rectal prolapse, and large bowel wall thickening (Neurath *et al. Intern. Rev. Immunol. 19:*51-62, 2000).

[266] Another colitis model uses dextran sulfate sodium (DSS), which induces an acute colitis manifested by bloody diarrhea, weight loss, shortening of the colon and mucosal ulceration

with neutrophil infiltration. DSS-induced colitis is characterized histologically by infiltration of inflammatory cells into the lamina propria, with lymphoid hyperplasia, focal crypt damage, and epithelial ulceration. These changes are thought to develop due to a toxic effect of DSS on the epithelium and by phagocytosis of lamina propria cells and production of TNF-alpha and IFN-gamma. Despite its common use, several issues regarding the mechanisms of DSS about the relevance to the human disease remain unresolved. DSS is regarded as a T cell-independent model because it is observed in T cell-deficient animals such as SCID mice.

[267] The administration of antagonists of the present invention to these TNBS, DSS, oxazalone, or T cell transfer models can be used to evaluate the use of those antagonists to ameliorate symptoms and alter the course of gastrointestinal disease. Moreover, the results showing inhibition of IL-17 and IL-23 signalling provide proof of concept that other IL-17/IL-23 antagonists can also be used to ameliorate symptoms in the colitis/IBD models and alter the course of disease. See Example 28.

3. Psoriasis

[268] Psoriasis is a chronic skin condition that affects more than seven million Americans. Psoriasis occurs when new skin cells grow abnormally, resulting in inflamed, swollen, and scaly patches of skin where the old skin has not shed quickly enough. Plaque psoriasis, the most common form, is characterized by inflamed patches of skin ("lesions") topped with silvery white scales. Psoriasis may be limited to a few plaques or involve moderate to extensive areas of skin, appearing most commonly on the scalp, knees, elbows and trunk. Although it is highly visible, psoriasis is not a contagious disease. The pathogenesis of the diseases involves chronic inflammation of the affected tissues. IL-17 and IL-23 are both overexpressed in psoriatic skin compared to nonpsoriatic skin and both cytokines are thought to have direct effects on the development, progression, and pathology of psoriasis (Li et al, J Huazhong Univ Sci Technolog Med Sci. 24:294 (2004); Piskin et al, J. Immunol. 176:1908 (2006), J Am Acad Dermatol. 57:1059 (2007); Wilson et al, Nature Immunol 8:950 (2007); Li et al, J Huazhong Univ Sci Technolog Med Sci. 27:330 (2007); Chen et al, J Huazhong Univ Sci Technolog Med Sci. 26:750 (2006)).

[269] Therefore, antagonists of the present invention could serve as a valuable therapeutic to reduce inflammation and pathological effects in psoriasis, other inflammatory skin diseases, skin and mucosal allergies, and related diseases.

[270] Psoriasis is a T-cell mediated inflammatory disorder of the skin that can cause considerable discomfort. It is a disease for which there is no cure and affects people of all ages. Psoriasis affects approximately two percent of the populations of European and North America. Although individuals with mild psoriasis can often control their disease with topical agents, more than one million patients worldwide require ultraviolet or systemic immunosuppressive therapy. Unfortunately, the inconvenience and risks of ultraviolet radiation and the toxicities of many therapies

limit their long-term use. Moreover, patients usually have recurrence of psoriasis, and in some cases rebound, shortly after stopping immunosuppressive therapy.

[271] In addition to other disease models described herein, the activity of antagonists of the present invention on inflammatory tissue derived from human psoriatic lesions can be measured *in vivo* using a severe combined immune deficient (SCID) mouse model. Several mouse models have been developed in which human cells are implanted into immunodeficient mice (collectively referred to as xenograft models); see, for example, Cattan AR, Douglas E, *Leuk. Res. 18:*513-22 (1994) and Flavell, DJ, *Hematological Oncology 14*:67-82 (1996). As an *in vivo* xenograft model for psoriasis, human psoriatic skin tissue is implanted into the SCID mouse model, and challenged with an appropriate antagonist. Moreover, other psoriasis animal models in ther art may be used to evaluate the present antagonists, such as human psoriatic skin grafts implanted into AGR129 mouse model, and challenged with an appropriate antagonist (e.g., see, Boyman, O. *et al., J. Exp. Med.* 199:731 (2004), incorporated herein by reference). IL-17/IL-23 antibodies or binding peptides that bind, block, inhibit, reduce, antagonize or neutralize the activity of IL-17, IL-23 or both IL-17 and IL-23 are preferred antagonists. Similarly, tissues or cells derived from human colitis, IBD, arthritis, or other inflammatory lestions can be used in the SCID model to assess the anti-inflammatory properties of the IL-17 and IL-23 antagonists described herein.

[272] Efficacy of treatment is measured and statistically evaluated as increased anti-inflammatory effect within the treated population over time using methods well known in the art. Some exemplary methods include, but are not limited to measuring for example, in a psoriasis model, epidermal thickness, the number of inflammatory cells in the upper dermis, and the grades of parakeratosis. Such methods are known in the art and described herein. For example, see Zeigler, M. *et al. Lab Invest 81:*1253 (2001); Zollner, T. M. *et al. J. Clin. Invest. 109:*671 (2002); Yamanaka, N. et al. *Microbio.l Immunol. 45:*507 (2001); Raychaudhuri, S. P. *et al. Br. J. Dermatol. 144:*931 (2001); Boehncke, W. H *et al. Arch. Dermatol. Res. 291:*104, (1999); Boehncke, W. H et al.. *J. Invest. Dermatol. 116:*596 (2001); Nickoloff, B. J. *et al. Am. J. Pathol. 146:*580 (1995); Boehncke, W. H *et al. J. Cutan. Pathol. 24*:1, (1997); Sugai, J., M. *et al. J. Dermatol. Sci. 17:*85 (1998); and Villadsen L.S. *et al. J. Clin. Invest. 112*:1571 (2003). Inflammation may also be monitored over time using well-known methods such as flow cytometry (or PCR) to quantitate the number of inflammatory or lesional cells present in a sample, score (weight loss, diarrhea, rectal bleeding, colon length) for IBD. For example, therapeutic strategies appropriate for testing in such a model include direct treatment using IL-17 and IL-23 antagonists (singly or together), or related conjugates or antagonists based on the disrupting interaction of IL-17 and IL-23 with their receptors.

4. Atopic Dermatitis.

[273] AD is a common chronic inflammatory disease that is characterized by hyperactivated cytokines of the helper T cell subset 2 (Th2). Although the exact etiology of AD is

unknown, multiple factors have been implicated, including hyperactive Th2 immune responses, autoimmunity, infection, allergens, and genetic predisposition. Key features of the disease include xerosis (dryness of the skin), pruritus (itchiness of the skin), conjunctivitis, inflammatory skin lesions, *Staphylococcus aureus* infection, elevated blood eosinophilia, elevation of serum IgE and IgG1, and chronic dermatitis with T cell, mast cell, macrophage and eosinophil infiltration. Colonization or infection with *S. aureus* has been recognized to exacerbate AD and perpetuate chronicity of this skin disease.

[274] AD is often found in patients with asthma and allergic rhinitis, and is frequently the initial manifestation of allergic disease. About 20% of the population in Western countries suffer from these allergic diseases, and the incidence of AD in developed countries is rising for unknown reasons. AD typically begins in childhood and can often persist through adolescence into adulthood. Current treatments for AD include topical corticosteroids, oral cyclosporin A, non-corticosteroid immunosuppressants such as tacrolimus (FK506 in ointment form), and interferon-gamma. Despite the variety of treatments for AD, many patients' symptoms do not improve, or they have adverse reactions to medications, requiring the search for other, more effective therapeutic agents. The antagonists of the present invention can be used to neutralize IL-17 and IL-23p19 in the treatment of specific human diseases such as atoptic dermatitis, inflammatory skin conditions, and other inflammatory conditions disclosed herein.

5. Asthma

[275] IL-17 plays an important role in allergen-induced T cell activation and neutrophilic influx in the airways. The receptor for IL-17 is expressed in the airways (Yao, et al. Immunity 3:811 (1995)) and IL-17 mediated neutrophil recruitment in allergic asthma is largely induced by the chemoattractant IL-8, GRO-alpha and macrophage inflammatory protein-2 (MIP-2) produced by IL-17 stimulated human bronchial epithelial cells (HBECs) and human bronichial fibroblasts (Yao, et al. J Immunol 155:5483 (1995)); Molet, et al. J Allergy Clin Immunol 108:430 (2001)). IL-17 also stimulates HBECs to release IL-6, a neutrophil-activating factor (Fossiez, et al, J Exp Med 183:2593 (1996), and Linden, et al. Int Arch Allergy Immunol 126:179 (2001)) and has been shown to synergize with TNF-alpha to prolong the survival of human neutrophils in vitro (Laan, et al. Eur Respir J 21:387 (2003)). Moreover, IL-17 is capable of amplifying the inflammatory responses in asthma by its ability to enhance the secretion of cytokines implicated in airway remodeling such as the profibrotic cytokines, IL-6 and IL-11 and inflammatory mediators granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) (Molet, et al. J Allergy Clin Immunol 108:430 (2001)).

[276] Clinical evidence shows that acute, severe exacerbations of asthma are associated with recruitment and activation of neutrophils in the airways, thus IL-17 is likely to play a significant role in asthma. Furthermore, since IL-23 is important in the maintenance and differentiation of IL-17

producing cells (e.g. Th17 cells), IL-23 is also likely to play a role in asthma. Patients with mild asthma display a detectable increase in the local concentration of free, soluble IL-17 protein (Molet, et al. J Allergy Clin Immunol 108:430 (2001)) while healthy human volunteers with induced, severe airway inflammation due to the exposure to a swine confinement, display a pronounced increase in the concentration of free, soluble IL-17 protein in the bronchoalveolar space (Fossiez et al, J Exp Med 183:2593 (1996), and Linden, et al. Int Arch Allergy Immunol 126:179 (2001)). Furthermore, IL-17 levels in sputum have correlated with individuals who have increased airway hyper-reactivity Barczyk, et al. Respir Med 97:726 (2003).

[277] In animal models of airway hyper-responsiveness, chronic inhalation of ovalbumin by sensitized mice resulted in bronchial eosinophilic inflammation and early induction of IL-17 mRNA expression in inflamed lung tissue, together with a bronchial neutrophilia Hellings, et al. Am J Respir Cell Mol Biol 28:42 (2003). Anti-IL-17 monoclonal antibodies strongly reduced bronchial neutrophilic influx but significantly enhanced IL-5 levels in both bronchoalveolar lavage fluid and serum, and aggravated allergen-induced bronchial eosinophilic influx, suggesting that IL-17 may be involved in determining the balance between neutrophil and eosinophil accumulation following antigen insult Id..

[278] Apart from asthma, several chronic inflammatory airway diseases are characterized by neutrophil recruitment in the airways and both IL-17 and IL-23 have been reported to play an important role in the pathogenesis of respiratory conditions such as chronic obstructive pulmonary disease (COPD), bacterial pneumonia and cystic fibrosis (Linden, et al. Eur Respir J 15:973 (2000), Ye, et al. Am J Respir Cell Mol Biol 25:335 (2001), Rahman, et al. Clin Immunol 115:268 (2005); Dubin and Kolls, Am. J. Physiol. Lung Cell Mol. Physiol. 292: L519-28 (2007); McAllister et al. J. Immunol. 175:404-412 (2005)). An anti-IL-17 and/or anti-IL-23 therapeutic molecule could be demonstrated to be efficacious for chronic inflammatory airway disease in an in vitro model of inflammation. The ability of antagonists to IL-17 and/or IL-23 activity to inhibit IL-17 or and/or IL-23 signalling to induce cytokine and chemokine production from cultured HBECs or bronchial fibroblasts could be used as a measure of efficacy for such antagonists in the prevention of the production of inflammatory mediators directly resulting from IL-17 and/or IL-23 stimulation. If the addition of antagonists to IL-17 and/or IL-23 activity markedly reduces the production and expression of inflammatory mediators, it would be expected to be efficacious in inflammatory aspects associated with chronic airway inflammation.

6. Multiple Sclerosis

[279] Multiple sclerosis is a relatively commonly occurring autoimmune disease characterized by demyelination and chronic inflammation of the central nervous system (CNS). Although the mechanisms underlying disease initiation are not clearly understood, the disease processes that contribute to clinical progression of multiple sclerosis are inflammation, demyelination,

and axonal loss, or neurodegeneration. Macrophages and microglia are the main immune cells of the CNS. These cells, as well as T cells, neutrophils, astrocytes, and microglia, can contribute to the immune-related pathology of, e.g., multiple sclerosis. Furthermore, T cell reactivity/autoimmunity to several myelin proteins, including myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte protein (MOG), and perhaps other myelin proteins, have been implicated in the induction and perpetuation of disease state and pathology of multiple sclerosis. This interaction of autoreactive T cells and myelin proteins can result in the release of proinflammatory cytokines, including TNF-a, IFN-g, and IL-17, among others. Additional consequences are the proliferation of T cells, activation of B cells and macrophages, upregulation of chemokines and adhesion molecules, and the disruption of the blood-brain barrier. The ensuing pathology is a loss of oligodendrocytes and axons, and the formation of a demyelinated "plaque". The plaque consists of a lesion in which the myelin sheath is now absent and the demyelinated axons are embedded within glial scar tissue. Demyelination can also occur as the result of specific recognition and opsinization of myelin antigens by autoantibodies, followed by complement- and/or activated macrophage-mediated destruction. It is this axonal loss and neurodegeneration that is thought to be primarily responsible for the irreversible neurological impairment that is observed in progressive multiple sclerosis.

[280] There is a large amount of clinical and pathological heterogeneity in the course of human multiple sclerosis. Symptoms most often begin between the ages of 18 and 50 years old, but can begin at any age. The clinical symptoms of multiple sclerosis can vary from mild vision disturbances and headaches, to blindness, severe ataxia and paralysis. The majority of the patients (approximately 70 – 75%) have relapsing-remitting multiple sclerosis, in which disease symptoms can recur within a matter of hours to days, followed by a much slower recovery; the absence of symptoms during stages of remission is not uncommon. The incidence and frequency of relapses and remissions can vary greatly, but as time progresses, the recovery phases can be incomplete and slow to occur. This worsening of disease in these cases is classified as secondary-progressive multiple sclerosis, and occurs in approximately 10 - 15% of multiple sclerosis patients. Another 10 – 15% of patients are diagnosed with primary-progressive multiple sclerosis, in which disease symptoms and physical impairment progress at a steady rate throughout the disease process.

[281] Both IL-23 and IL-17 are overexpressed in the central nervous system of humans with multiple sclerosis and in mice undergoing an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). (See Example 37). The overexpression is observed in mice when the EAE is induced by either myelin oligodendrocyte glycoprotein (MOG) 35-55 peptide- or proteolipid peptide (PLP). Furthermore, neutralization of either IL-23p19 or IL-17 results in amelioration of EAE symptoms in mice (Park et al, *Nat Immunol. 6*:1133 (2005);); Chen *et al, J Clin Invest. 116*:1317 (2006)).

[282] IL-17R has recently been shown to be expressed on human brain-derived microvascular endothelial cells, and human Th17 cells readily migrate across the blood brain barrier (BBB) in vitro, kill neurons, and produce granzyme B (Kebir et al, Nature Med 13:1173; 2007). In addition, Kebir et al report that CD45RO+/IL-17+ cells are present in active human MS lesions by IHC and treatment of human BBB cells with IL17 in vitro results in increased permeability to CD4+ cells and albumin, thus suggesting a detrimental breakdown in this barrier.

[283] The ability of antagonists to IL-17 and/or IL-23 to inhibit IL-17 or and/or IL-23 signalling-induced cytokine and chemokine production could be used as a treatment of multiple sclerosis. The addition of antagonists to IL-17 and/or IL-23 activity markedly reduces the production and expression of inflammatory mediators (i.e. CNS-infiltrating immune cells; CNS expression of inflammatory cytokines/chemokines, etc.) and symptoms of multiple sclerosis (e.g. paralysis; ataxia; weight loss, etc). These results indicate that antagonists to IL-17 and/or IL-23 activity would be efficacious in the treatment of humans, such as in treating multiple slcerosis and in preventing, reducing, inhibiting, blocking, ameliorating relapse in some multiple sclerosis patients.

7. Cancer

[284] Chronic inflammation has long been associated with increased incidence of malignancy and similarities in the regulatory mechanisms have been suggested for more than a century. Infiltration of innate immune cells, elevated activities of matrix metalloproteases (MMP) and increased angiogenesis and vasculature density are a few examples of the similarities between chronic and tumour-associated inflammation. Conversely, the elimination of early malignant lesions by immune surveillance, which relies on the cytotoxic activity of tumour-infiltrating T cells or intra-epithelial lymphocytes, is thought to be rate-limiting for the risk to develop cancer.

[285] There are numerous publications describing important roles for IL-23 and IL-17 in tumor biology and/or angiogenesis. Both IL-23 and IL-17 have been published to be upregulated in several human tumors and cancers, including but not limited to those of the colon, breast, ovarian, cervical, prostate, lung, and stomach, as well as melanoma and T cell lymphoma (Tartour et al, *Cancer Res. 59*:3698 (1999); Kato *et al, Biochem. Biophys. Re.s Commun. 282*:735 (2001); Steiner *et al, Prostate. 56*:171 (2003); Langowski *et al*, *Nature*, 442:461-5, (2006)). Thus, neutralization of both IL-17 and a key upstream regulator of IL-17, IL-23p19, is a potent and effective means of treating cancer and other neoplastic diseases. Therefore, neutralizing both IL-17 and IL-23 with antagonists of the present invention (i.e. a single neutralizing entity or antibody to IL-17 and IL-23 or an antagonistic molecule that will neutralize both together, such as a bispecific antibody or bispecific scFv) will have better efficacy in these diseases than antagonists directed toward either of IL-17 or IL-23 alone.

[286] Angiogenesis refers to the formation of new capillaries from preexisting vessels. There are several reports that angiogenesis plays important roles in hematological malignancies and

solid tumors. The initiation of angiogenesis and the switch to the angiogenic phenotype requires a change between proangiogenic factors and angiogenic inhibitors (Folkman, *Nat. Med.*1:27 (1995)). IL-17 acts as a stimulatory hematopoietic cytokine by initiating proliferation of mature neutrophils and by expanding myeloid progenitors. It has been well documented that IL-17 has pro-angiogenic activities and stimulates the migration of vascular endothelial cells, which are associated with tumor promotion (Numasaki *et al, Blood*, 101:2620 (2003); Yang *et al, J. Biol. Chem.*, 278:33232 (2003); Fujino *et al, Gut*, 52:65 (2003)). In vitro angiogenic activity can be suppressed by neutralizing IL-17 with a neutralizing anti-IL-17 monoclonal antibody, further supporting the role of IL-17 in this action. It is also able to selectively enhance mitogenic activity of basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF), and IL-17 may also promote bFGF-, HGF- and VEGF-mediated angiogenesis via bFGF-, HGF- and VEGF-induced growth of vascular endothelial cells (Takahashi *et al, Immunol Lett.* 98:189 (2005)). IL-17 has been reported to augment the secretion of several angiogenic CXC chemokines (e.g. CXCL1, CXCL5, CXCL6, and CXCL8) in non-small cell lung cancer (NSCLC) lines. Endothelial cell chemotactic activity (a measure of net angiogenic potential) is increased in response to conditioned medium from NSCLC stimulated with recombinant IL-17. NSCLC lines transfected with IL-17 grew more rapidly versus controls when transplanted in SCID mice (Numasaki *et al, J Immunol.* 175:6177 (2005)). Furthermore, IL-17 has been reported to be associated with increased IL-6 at the site of tumors and is well reported to increase MMP-9 expression. MMP-9 is an important modulator in diseases of inflammation, autoimmunity, and cancer. These reports, therefore, clearly implicate a pro-angiogenic and tumor promoting action for IL-17. Therefore, neutralizing both IL-17 and IL-23 with antagonists of the present invention (i.e. a single neutralizing entity or antibody to IL-17 and IL-23 or an antagonistic molecule that will neutralize both together, such as a bispecific antibody or bispecific scFv) will have better efficacy than antagonists directed toward either of IL-17 or IL-23 alone.

[287] Similar to IL-17, IL-23 promotes inflammatory responses including upregulation of MMP-9, and is also reported to increase angiogenesis and reduce CD8+ T-cell infiltration. Taken together, these actions can lead to enhanced initiation, progression, and/or maintenance of tumors, cancers, and other transformed growths. That IL-23 plays an important role in cancerous diseases is supported by the observation that neutralization of IL-23 with a monoclonal antibody or with genetic deletion in mice reduces tumor growth in several murine tumor models (Langowksi *et al. Nature*, 442: 461-5 (2006)). Efficacy is associated with reduced IL-17 expression and reductions in IL-17-related tumorogenic biomarkers, such as granulocyte infiltration, G-CSF and MMP-9. Therefore, neutralizing both IL-17 and IL-23 with antagonists of the present invention (i.e. a single neutralizing entity or antibody to IL-17 and IL-23 or an antagonistic molecule that will neutralize both together, such as a bispecific antibody or bispecific scFv) will have better efficacy in these diseases than antagonists directed toward either of IL-17 or IL-23 alone.

8. Irritable Bowel Syndrome (IBS)

[288] Irritable bowel syndrome (IBS) represents a disease characterized by abdominal pain or discomfort and an erratic bowel habit. IBS patients can be characterized into three main groups based on bowel habits: those with predominantly loose or frequent stools, those with predominantly hard or infrequent stools, and those with variable or normal stools (Talley *et al., Expert Opin. Emerg. Drugs* 7:91-8 (2002)). Altered intestinal motility, abnormalities in epithelial function, abnormal transit of stool and gas, and stress, may contribute to symptoms, while visceral hypersensitivity is a key feature in most patients. Genetic factors affecting pain-signaling and disturbances in central processing of afferent signals are postulated to predispose individuals to IBS following specific environmental exposures. Studies have also demonstrated that inflammatory responses in the colon may contribute to increased sensitivity of smooth muscle and enteric nerves and therefore perturb sensory-motor functions in the intestine (Collins *et al., Can. J. Gastroenterol. 15 Suppl. B*:14B-16B (2001)). There is clinical overlap between IBS and IBD, with IBS-like symptoms frequently reported in patients before the diagnosis of IBD, and a higher than expected IBS symptoms in patients in remission from established IBD. Thus, these conditions may coexist with a higher than expected frequency, or may exist on a continuum, with IBS and IBD at different ends of the same spectrum. However, it should be noted that in most IBS patients, colonic biopsy specimens appear normal. Nevertheless, IBS significantly affects a very large number of individuals (U.S. prevalence in 2000, approximately 16 million individuals), resulting in a total cost burden of 1.7 billion dollars (year 2000). Thus, among the most prevalent and costly gastrointestinal diseases and disorders, IBS is second only to gastroesophageal reflux disease (GERD). Yet unlike GERD, treatment for IBS remains unsatisfactory ((Talley *et al., Expert Opin. Emerg. Drugs* 7:91-8 (2002)); Farhadi *et al., Expert Opin. Investig. Drugs 10*:1211-22 (2001); Collins *et al., Can. J. Gastroenterol. 15 Suppl. B*:14B-16B (2001)), demonstrating that IBS clearly represents an unmet medical need.

[289] Converging disease models have been proposed that postulate an enhanced responsiveness of neural, immune or neuroimmune circuits in the central nervous system (CNS) or in the gut to central (psychosocial) or peripheral (tissue irritation, inflammation, infection) perturbations of normal homeostasis (Talley *et al., Expert Opin. Emerg. Drugs* 7:91-8 (2002)). This enhanced responsiveness results in dysregulation of gut motility, epithelial function (immune, permeability), and visceral hypersensitivity, which in turn results in IBS symptoms.

[290] There may be a role for a number of different molecules in the pathogenesis of IBS including a role for molecules that stimulate neurons and those that are involved in initiation of inflammatory process, including IL-17A and IL-23p19.

[291] Efficacy of inhibitors of these molecules could be tested *in vivo* in animal models of disease. Several animal models have been proposed that mimic key features of IBS and involve centrally targeted stimuli (stress) or peripherally targeted stimuli (infection, inflammation). Two

examples of in vivo animal models that can be used to determine the effectiveness of inhibitors in the treatment of IBS are (i) models focusing on primary CNS-directed pathogeneisis of IBS (stress models), and (ii) models focusing on gut-directed inducers of stress (i.e. gut inflammation, infection or physical stress). It should be noted however, that events within the CNS or in the gastrointestinal (GI) tract do not occur in isolation and that symptoms of IBS most likely result from a complex interaction between signals from the CNS on the GI and vice versa.

[292] Thus, in summary, there are several molecules and pathogenic pathways that are shared by IL-17 and IL-23 which play important roles in the devopment, progression, and maintenance of both autoimmune diseases and cancerous diseases. These include the pro-angiogenic roles of IL-17 and IL-23; enhanced MMP-9 levels and activity by IL-17 and IL-23; IL-23, TGF-b and IL-6-mediated production and/or maintenance of Th17 cells; roles of TGF-b and IL-6 in the generation of Foxp3+ regulatory T cells; and additional pathways and molecules. Therefore, the IL-17/IL-23 axis represents an important link to the inappropriate and pathogenic T cell responses associated with autoimmune diseases, tumour-promoting pro-inflammatory processes, and the failure of the adaptive immune surveillance to infiltrate tumours. Therefore, neutralizing both IL-17 and IL-23 with antagonists of the present invention (i.e. a single neutralizing entity or antibody to IL-17 and IL-23 or an antagonistic molecule that will neutralize both together, such as a bispecific antibody or bispecific scFv) will have better efficacy in these diseases than antagonists directed toward either of IL-17 or IL-23 alone.

Autoimmune and Idiopathic Neuropathies

[293] Idiopathic inflammatory-demyelinating diseases (IIDDs) include a broad spectrum of central nervous system disorders that can usually be differentiated on the basis of clinical, imaging, laboratory and pathological findings. However, there can be a considerable overlap between at least some of these disorders, leading to misdiagnoses or diagnostic uncertainty. The relapsing-remitting and secondary progressive forms of multiple sclerosis (MS) are the most common IIDDs. Other MS phenotypes include those with a progressive course from onset (primary progressive and progressive relapsing) or with a benign course continuing for years after onset (benign MS). Uncommon forms of IIDDs can be classified clinically into: (1) fulminant or acute IIDDs, such as the Marburg variant of MS, Baló's concentric sclerosis, Schilder's disease, and acute disseminated encephalomyelitis; (2) monosymptomatic IIDDs, such as those involving the spinal cord (transverse myelitis), optic nerve (optic neuritis) or brainstem and cerebellum; and (3) IIDDs with a restricted topographical distribution, including Devic's neuromyelitis optica, recurrent optic neuritis and relapsing transverse myelitis. Other forms of IIDD, which are classified clinically and radiologically as pseudotumoral, can have different forms of presentation and clinical courses. Although some of these uncommon IIDDs are variants of MS, others probably correspond to different entities. MR imaging of the brain

and spine is the imaging technique of choice for diagnosing these disorders, and together with the clinical and laboratory findings can accurately classify them.

[294] Idiopathic neuropathy is a disorder that affects the peripheral nerves and has no identifiable primary cause. Peripheral neuropathy can be caused by disease; nerve compression, entrapment, or laceration; exposure to toxins; or inflammation, but these causes are oftentimes not apparent, and thus, the resulting diagnosis may be idiopathic neuropathy. Peripheral neuropathy also can be classified by where it occurs in the body. Nerve damage that occurs in one area of the body is called mononeuropathy, in many areas, polyneuropathy. When the disorder occurs in the same places on both sides of the body, the condition is called symmetric neuropathy. It also can be categorized by cause (when known), such as diabetic neuropathy and nutritional neuropathy. When a cause cannot be identified, the condition is called idiopathic neuropathy.

[295] According to this definition, a third of all neuropathies can be classified as idiopathic neuropathies. The nervous system is divided into the central nervous system (CNS) and the peripheral nervous system (PNS). Peripheral nerve cells have three main parts: cell body, axons, and dendrites (nerve/muscle junctions). Any part of the nerve can be affected, but damage to axons is most common. The axon transmits signals from nerve cell to nerve cell or muscle. Most axons are surrounded by myelin, which facilitates signal transmission. The brain and spinal cord compose the CNS, and the nerves that lead to or branch off the CNS compose the PNS. Peripheral neuropathies encompass a wide range of disorders in which peripheral nerves are damaged. It may also be referred to as peripheral neuritis (inflammation of peripheral nerves), or if many nerves are involved, the terms polyneuropathy or polyneuritis may be used.

[296] There are no known causes for idiopathic neuropathies, and therefore they are considered primary diseases. If a cause is detected, then the neuropathy is secondary to that, and not idiopathic. Nonetheless, there are many different peripheral neuropathies, among them the idiopathic type, which demonstrates the functional diversity of PNS activities. Symptoms may involve sensory, motor, or autonomic functions. Symptoms are classified based on the affected nerve type and the duration of disease development. Acute development refers to symptoms that have appeared within days, and subacute refers to those that have evolved over a number of weeks. Early chronic symptoms are those that take months to a few years to develop, and late chronic are the ones that have been present for several years.

[297] Prognosis and complications depend on the type and severity of the neuropathy. Idiopathic neuropathies range from a reversible problem to a potentially fatal complication. In the best-case scenario, a damaged nerve regenerates. Nerve cells cannot be replaced if they are killed, but they are capable of recovering from damage. The extent of recovery is tied to the extent of the damage, to the patient's age, and to the general health status. Recovery can take weeks to years due to the slow neuronal regrowth rate. Full recovery may not be achieved in some cases.

[298] Treatment for idiopathic neuropathies is mostly symptomatic. Treatment options for reducing pain include medication, injection therapy, and physical therapy. Surgery may be needed to treat some causes of neuropathy. Because analgesics (aspirin, ibuprofen) are usually ineffective against pain caused by neuropathy, treatment often involves medications that target nerve cells. However, the current treatments are not satisfactorily effective, do not adequately improve the actual pathology, and have significant side effects. In summary, neuropathies affect a significant portion of the population, are debilitating, and there are very few effective therapies. It is clear that more effective, safer and targeted therapies are needed such as IL-23/IL-17A antagonists described herein. Given the roles of IL-23 and IL-17A in inflammation, multiple sclerosis, and the processes surrounding these diseases, antagonizing these molecules is expected to be beneficial in the various neuropathies described here.

Methods for measuring the effects of the anti-IL-17A and anti-IL-23A antibodies and bispecific molecules described herein include the following assays.

1) Effects of IL-17A and IL-23 on Lamina Propria T cells and Monocytes/Macrophages from Normal and Human IBD Samples:

[299] Dysregulated or sustained immune-mediated inflammation may contribute to the symptoms and pathology associated with IBD by way of tissue damage or permanent skewing to inappropriate or prolonged immune responses. This model can determine the potential down-stream consequences of exposure of disease-associated T cells and monocytes to IL-17A and IL-23 which may be present in the immediate environmental cytokine mileu of the intestinal tissue.

[300] Therapeutics that would be efficacious in human IBD in vivo, would work in ex vivo models by inhibiting and/or neutralizing the production and/or presence of inflammatory mediators (including but not limited to IL-1b, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, MIP family members, MCP-1, G- and GM-CSF, etc.).

[301] In this model, T cells and monocytes/macrophages are isolated from intestinal biopsy samples by carefully mincing biopsies with scissors in HBSS, treating with collagense and Dispase II and incubating for 1 hr at 37°C in a shaker. The cell suspension is filtered through nylon mesh to remove debris and cell clumps and washed multiple times in HBSS. T cells and macrophage/monocytes can be isolated using direct cell sorting or bead-depletion/enrichment protocols. Isolated cells are incubated in the presence of IL-17A and IL-23. This induces the production of inflammatory mediators by T cells and monocytes/macrophages or results in skewing subsequent T cell responses to highly pro-inflammatory responses. Comparisons between the types of inflammatory mediators produced by cells from IBD patients and those from cells of normal individuals can be made and might suggest that T cells and monocyte/macrophages from IBD patients produce a more pro-inflammatory profile in the presence of IL-17A and IL-23. The addition of

antibodies to IL-17A and IL-23p19 to neutralize the production of downstream inflammatory mediators may be efficacious in the therapeutic treatment of patients with IBD.

2) Efficacy of Antibodies that Antagonize Both IL-17A and IL-23 in Irritable Bowl Syndrome ("IBS"): CNS-Directed Pathogenesis:

[302] A model focusing on primary CNS-directed pathogenesis of IBS which employs stress stimuli to induce symptoms characteristic of IBS. The neonatal psychosocial stress model mimics some clinical features associated with IBS patients including visceral hyperalgesia, diarrhea and stress-sensitivity. Daily separation of the litter from their mothers for 180 minutes each day during postnatal days 4-18 will result in an alteration of maternal behaviour and significantly reduce times of the licking/grooming behaviour. The stress on the neonates results in permanent changes in the CNS resulting in altered stress-induced visceral and somatic pain sensitivity. Colonic motor function in response to stress is enhanced in these animals and preliminary data shows evidence of increased intestinal permeability (Mayer et al., Eur. J. surg. Suppl. (587): 3-9 (2002)). Treatment with the antibodies of the present invention and subsequent analysis of colonic motor function, epithelial permeability and response to stress stimuli could determine efficacy in this animal model of IBS. Decreases in the incidence of symptoms following treatment with these inhibitors may suggest potential efficacy in the treatment of IBS.

3) Efficacy of Antibodies that Antagonize IL-17A and IL-23p19 in Irritable Bowl Syndrome ("IBS"): Primary Gut-Directed Inducers of Stress:

[303] This is a model focusing on primary gut-directed inducers of stress (ie. gut inflammation, infection or physical stress). Animal studies have indicated that low-grade inflammation or immune activation may be a basis for altered motility, and/or afferent and epithelial function of the gut (Mayer et al., Eur. J. Surg. Suppl. (587): 3-9 (2002)). In this model, daily colon irritation is produced in neonatal animals (days 8-21) in the form of daily intracolonic injection of mustard oil. Mustard oil is a neural stimulant and has been shown to induce visceral hyperalgesia following intracolonic administration. This model mimics key features of the IBS including visceral hypersensitivity and alteration in bowel habits. Animals also present with diarrhea or constipation, a key feature of IBS patients (Mayer et al., Eur. J. surg. Suppl. (587): 3-9 (2002)); (Kimball et al., Am. J. Physiol. Gastrointest. Liver Pathol. 288: G1266 (2005)). An antibody of the present invention could be delivered to determine changes in the development of symptoms associated with this model. The antagonists described herein may be efficacious in decreasing the incidence or magnitude of visceral hypersensitivity and altered gut motility following therapeutic treatment for IBS.

4) Efficacy of IL-23A/IL-17A Antagonists in a Non-Human Primate Model of Ulcerative colitis :

[304] Several examples of spontaneous colitis have been described in the New-World monkeys (Mast, et al. 1993. In: Clapp NK (ed) A Primate Model for the Study of Colitis and Colonic

Carcinoma. Boca Raton: CRC Press). Amongst these, the most realistic spontaneous model occurs in the cotton top tamarin, which develops a disease that is unique both in its clinical and histological resemblance to human disease, in its associated complications and in its response to treatment (Warren BF and Watkins PE. J. Pathol. 172:313-316 (1994)). Affected animals develop a diffuse mucosal disease, with diffuse chronic inflammation, extensive crypt distortion and neutrophil infiltration within the lamina propria. In more severe cases the disease progresses to cryptitis and crypt abscesses and in some cases may progress to colon cancer. Disease progression can be measured by repeated endoscopy and biopsy. Treatments which have been shown to be efficacious in treating human disease, including 5-amino salicylic acid (5-ASA), predinisolone and TNF-α antagonists have also been shown to have efficacy in this model (Warren BF. Aliment. Pharmacol. Ther. 10 (Suppl.2): 45-47 (1996)), with efficacy read out by endoscopy, biopsy, changes in body weight and stool consistency.

[305] For these studies, unrelated naive cotton top tamarin (Saguinus oedipus) are used (randomly divided between vehicle and antagonist). The daily diet consists of commercial food pellets for nonhuman primates supplemented with rice and fresh fruit. Drinking water is provided ad libitum. Animals are recruited to the study after a positive diagnosis of ulcerative colitis by endoscopy, biopsy and histological evaluation. Animals are assigned to a treatment group and are treated therapeutically with subcutaneous or intravenous administration of IL-23/IL-17A antagonists or vehicle to determine whether these treatments are efficacious in this model of ulcerative colitis.

[306] To evaluate efficacy, animals are weighed daily and stool consistency measured on a daily basis over a 30 day period. Animals are evaluated by endoscopy and biopsy midway through the study and on study completion. Disease scores are calculated based on change in body weight, stool consistency and histological evaluation.

[307] Animals treated therapeutically with the IL-23A/IL-17A antagonists are expected to have reduced severity of disease as evidenced by lower average clinical scores compared to animals treated with vehicle alone. The rate of lesion load and/or volume in the colon is measured by biopsy after endoscopy.

5) Efficacy of IL-23A/IL-17A Antagonists in a Human (hu)-SCID Mouse Model of Psoriasis:

[308] A preclinical human severe combined immunodeficiency (huSCID) model for may be used as a model to evaluate efficacy of the IL-23A/IL-17A antagonists described herein for use in human psoriasis. SCID mice have been developed and are available from several commercial sources. These mice contain a genetic defect in a recombinase gene which prevents the expression of mature T cells and B cells. The resulting immunodeficiency allows for the survival of human cells in these mice. Human full-thickness xenografts of chronic plaque-stage psoriasis and nonlesional skin are excised from the inner aspect of the upper arm of patients under local anesthesia. The xenografts are transplanted onto the back of 6–8-wk-old anesthetized C.B17 SCID mice (Charles River). Spindle-

shaped pieces of full-thickness skin measuring 1 cm in diameter are grafted onto corresponding excisional full-thickness defects of the shaved central dorsum of the mice and fixed by 6-0 atraumatic monofilament sutures. The grafts are protected from injury by suturing a skin pouch over the transplanted area using the adjacent lateral skin. The sutures and "pouches" are left in place until they resolve after approximately 2–3 weeks. In parallel, 2 106 of the donors' peripheral blood mononuclear cells (PBMC) are injected intraperitoneally in 100 uL PBS. These cells are prepared from peripheral blood taken at the time of skin excision by Ficoll density gradient sedimentation, frozen in medium containing 90% fetal bovine serum (FBS) and 10% dimethylsulfoxide, and stored at -80°C. Forty-eight hours prior to injection the PBMC are thawed and cultured at 37°C in an atmosphere containing 5% CO2. The medium used is supplemented RPMI-1640. Following a 4 week graft acceptance and healing period, graded doses of IL-23A/IL-17A antagonists or vehicle are injected intravenously, intraperitoneally or subcutaneously into engrafted animals. Each treatment is administered to mice that contain replicate grafts from the same psoriasis patient. At timepoints following engraftment, mice are sacrificed and the skin grafts collected into 10% formalin for histopathologic analysis. Subsequently, routine hematoxylin and eosin stainings are performed and the grafts analyzed for changes in pathology (e.g. epidermal differentiation, inflammatory infiltrate, epidermal thickness) by a blinded investigator, as described (Boehncke W-H, et al. 1994, Arch Dermatol Res 286: 325). Treatment of mice with IL23A/IL17A antagonists may reduce psoriaform changes in the mice (e.g. akanthosis, papillomatosis, and parakeratosis) compared to vehicle-treated engrafted huSCID mice. This would indicate that the neutralizing entities described herein are beneficial in treating humans with psoriatic conditions.

6) Efficacy of IL-23A/IL-17A Antagonists in Human Rheumatoid Arthritis Explant Cultures:

[309] Human synovial tissue from rheumatoid arthritis patients is obtained at joint replacement surgery . The tissue is cut into small pieces, weighed and placed into culture wells and cultured with or without activating compounds (e.g. PBS control; activated human T cells; human PBMC's; LPS; TNF-alpha; rhIL-23; rhIL-17A; etc), and in the presence of IL-23/IL-17A antagonists described herein or PBS vehicle. Each condition is cultured in replicates of 6 to 8 from 4 donors. Following an incubation period of 24 – 72 hours at 37 degrees C in an 95%O2/5%CO2 incubator, supernatants are collected and frozen at -80 degrees C for subsequent analysis of concentrations of a large number of inflammatory cytokines, chemokines, growth factors, metalloproteinases, and other inflammatory mediators. Synovial explants cultured in the presence of activating compounds will produce increased concentrations of inflammatory mediators compared to explants cultured in PBS alone. Addition of IL-23/IL-17A antagonists can result in a dose-dependent reduction of the inflammatory mediators produced by the explant cultures. Therefore, IL-23/IL-17A neutralizing entities described herein would be expected to be functionally efficacious in the treatment of human

arthritis by their ability to reduce the levels of inflammatory mediators associated with the pathobiology of the disease.

7) Efficacy of IL-23A/IL-17A Antagonists in a Non-Human Primate Model of Multiple Sclerosis:

[310] The common marmoset (Callithrix jacchus) is a non-human primate that shares several similarities to the human immune system (Brok, et al, 2001. Immunol. Rev. 183:173-185). The marmoset is highly susceptible to experimental autoimmune encephalomyelitis (EAE), thus offering a preclinical model for evaluating the efficacy of potential therapeutics for use in humans with multiple sclerosis (MS) ('t Hart, et al. 2004. Drug Discov. Today 9:517-524). Furthermore, the development of lesions within the white matter of the brain resembles those observed in the brains of humans with multiple sclerosis and can be visualized with magnetic resonance imaging (MRI) techniques similar to those used for the diagnosis of human MS ('t Hart, et al. 2004. Trends Mol. Med. 10:85-91).

[311] For these studies, unrelated naive common marmosets (C. jacchus) are used (randomly divided between vehicle and antagonist). The daily diet consists of commercial food pellets for nonhuman primates supplemented with rice and fresh fruit. Drinking water is provided ad libitum. EAE is induced by immunization with recombinant human (rh) MOG as an emulsion with CFA, because with this protocol, the animals develop the large and relatively homogeneous lesions needed for MRI analysis, as well as the clinical and pathological signs of myelin-induced EAE ('t Hart, et al. 2004. Lancet Neurol. 3:588-597; 't Hart, et al. 2004. Trends Mol. Med. 10:85-91). Animals are treated with prophylactically or therapeutically with subcutaneous or intravenous administration of IL-23/IL-17A antagonists to determine whether these treatments are efficacious in this model of multiple sclerosis.

[312] To evaluate efficacy, clinical signs of EAE are recorded twice daily, using a scoring system described previously ('t Hart, et al. 1998. Am. J. Pathol. 153:649-663). In addition, in vivo brain imaging is performed approximately every 2 weeks using clinically relevant MRI techniques to detect lesions. The lesions are evaluated for the following: (1) T2-weighted (T2W) MRI to assess the spatial distribution and size of lesions; and (2) quantitative T2 relaxation time imaging to determine the extent of CNS inflammation. Brain MRI is continued until the end of the observation period to detect the effect of prophylactic or therapeutic delivery of IL-23A/IL-17A antagonists on the development and/or progression of lesions.

[313] Animals treated prophlyactically with the IL-23A/IL-17A antagonists may have a significant delay in onset of disease as measured by clinical scores and MRI lesion analysis compared to animals treated with vehicle. In addition, animals treated prophylactically or therapeutically with the IL-23A/IL-17A antagonists may have reduced severity of disease as evidenced by lower average clinical scores, fewer MRI lesions, less MRI lesion volume, and smaller lesion load compared to

animals treated with vehicle alone. The rate of lesion load and/or volume is significantly less in animals treated with the IL-23A/IL-17A antagonists, indicating less progressive disease. The MRI T2 relaxation times of the lesions in vehicle-treated monkeys increases significantly with disease progression, whereas there is significantly less increase in T2 relaxation times in the animals treated with the IL-23A/IL-17A antagonists, thus indicating suppression of inflammatory activity in the lesions. The relative change in GE-T1W values is used to estimate blood-brain barrier (BBB) leakage, and in this study, there is significantly less BBB permeability in the IL-23A/IL-17A antagonist-treated animals compared to vehicle-treated animals. Moreover, the peak of BBB permeability is expected to occur significantly later in the IL-23A/IL-17A-antagonist treated animals than vehicle-treated animals, which would further indicate a delay in disease. In summary, the reduced disease onset, disease severity and disease progression in animals treated prophylactically or therapeutically with IL-23A/IL-17A antagonists in this non-human primate model of multiple sclerosis would indicate that these antagonists are expected to be beneficial in humans with multiple sclerosis.

Pharmaceutical Compositions

[314] For pharmaceutical use, the antibodies of the present invention are formulated for parenteral, including intravenous or subcutaneous, delivery according to conventional methods. Intravenous administration will be by bolus injection, controlled release, e.g, using mini-pumps or other appropriate technology, or by infusion over a typical period of one to several hours. In general, pharmaceutical formulations will include a hematopoietic protein in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc. When utilizing such a combination therapy, the cytokines may be combined in a single formulation or may be administered in separate formulations. Methods of formulation are well known in the art and are disclosed, for example, in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., Easton PA, 1990, which is incorporated herein by reference. Therapeutic doses will generally be in the range of 0.1 to 100 mg/kg of patient weight per day, preferably 0.5-20 mg/kg per day, with the exact dose determined by the clinician according to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, etc. The antibodies of the present invention can also be administered in combination with other cytokines such as IL-3, -6 and -11; stem cell factor; erythropoietin; G-CSF and GM-CSF. Within regimens of combination therapy, daily doses of other cytokines are commonly known by one skilled in the art, or can be determined without undue experimentation. Combination therapy with EPO, for example, is indicated in anemic patients with low EPO levels.

[315] Administration of antibodies of the invention to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion

through a regional catheter, or by direct intralesional injection, topically or by nasal inhalation. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses.

[316] Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, "Oral Delivery of Microencapsulated Proteins," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe and Illum, *Adv. Drug Deliv. Rev. 35*:199 (1999)). Dry or liquid particles comprising antibodies of the invention can be prepared and inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (*e.g.,* Pettit and Gombotz, *TIBTECH 16*:343 (1998); Patton *et al., Adv. Drug Deliv. Rev. 35*:235 (1999)). Studies have shown that proteins as large as 48,000 kDa have been delivered across skin at therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of trascutaneous administration (Mitragotri *et al., Science 269*:850 (1995)). Transdermal delivery using electroporation provides another means to administer a molecule having IL-17 and IL-23p19 binding activity (Potts *et al., Pharm. Biotechnol. 10*:213 (1997)).

[317] A pharmaceutical composition comprising one or more antibodies of the invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a pharmaceutically acceptable carrier if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995).

[318] For purposes of therapy, antibodies of the invention and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of a therapeutic molecule of the present invention and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response. Effective treatment may be assessed in a variety of ways. In one embodiment, effective treatment is determined by reduced inflammation. In other embodiments, effective treatment is marked by inhibition of inflammation. In still other embodiments, effective therapy is measured by increased well-being of the patient including such

signs as weight gain, regained strength, decreased pain, thriving, and subjective indications from the patient of better health.

[319] The antibody fragments described herein, including scFvs, Fabs, diabodies, etc. can be fused to an entity to extend their half-life. See, for example, Kubetzko S. *et al. J Biol Chem 281*:35186 (2006) [Modified p185Her2 (Her2)-specific scFv 4D5 and measured binding affinity, functional affinity, tumor distribution, and PK. In tumor-bearing mice, 20kD PEGylation of the scFv monomer and dimer extended serum half-life and tumor distribution.]; Yang K *et al. Protein Engineering 16*:761 (2003) [A 20kD or 40kD-PEGylated anti-TNF-a scFv (D2E7/Humira) retained affinity for target and pharmacological activity. Following iv administration in mice, 40kD PEGylated scFv had a 200-fold increased circulating half-life and an 800-fold increased AUC as compared to the unmodified scFv.]; Chapman AP et al. Nature Biotech 17:780 (1999). [Fab utilized in the experiments exploring affinities and half-life of random versus targeted PEG attachment and different sizes of the PEG molecule. Demonstrates site-specific attachment of PEG moieties to the hinge region cysteine residue results in higher binding affinities for target as compared to randomly attached PEGs. Binding affinity as measured by Biacore analysis was identical for parent Ig and the Fab molecules modified by attachment of 5kD, 25kD and branched 40kD PEGs. 25kD and 40kD-PEGylated Fabs had a comparable or increased half-life and an AUC of 50-70% of that of the parent Ig molecule in mice and monkeys]; Muller D et al. J Biol Chem 282:12650 (2007). [Fused human serum albumin (HSA) to several bispecific antibodies to carcinoembryonic antigen (CEA) and CD3 and compared activity and pharmacokinetics. scFv2, scDb, and taFv (constructs shown in paper) fused to HSA retained full binding capacity and activity. Half-life in serum of the HSA constructs increased 5-11-fold and the AUC increased 6-7-fold compared to their respective unmodified parent molecule]; and Kipriyanov SM et al. J Mol Biol 293:41 (1999). [Anti-CD3/CD-19 tandem diabody molecule demonstrated to have higher affinity, slower dissociation, higher stability in serum, higher in vivo stability, and longer in vivo half-life as compared to a single chain Fv fragments and diabodies.]

[320] Methods of measuring the activity of the anti-IL-17A antibodies, anti-IL-23p19 antibodies and anti-IL-17A/anti-IL-23p19 antibodies described herein are known in the art. For example, Baby Hamster Kidney (BHK) cells that have been transfected with expression vectors encoding human IL-17R can be used and their ability to bind biotinylated human IL-17 in the presence of anti-IL-17A antibodies can be measured. Briefly, cells are harvested with versene, counted and diluted to 10^7 cells per ml in staining media (SM), which is HBSS plus 1 mg/ml bovine serum albumin (BSA), 10 mM Hepes, and 0.1% sodium azide (w/v). Biotinylated human IL-17 (SEQ ID NO:2) is incubated with the cells on ice for 30 minutes at various concentrations. After 30 minutes, excess cytokine is washed away with SM and the cells were incubated with a 1:100 dilution of streptavidin conjugated to phycoerythrin (SA-PE) for 30 minutes on ice. Excess SA-PE is washed away and cells were analyzed by flow cytometry. The amount of cytokine binding is quantitated from

the mean fluorescence intensity of the cytokine staining. A range of concentrations of an antagonist of the present invention (i.e. an antibody) to human IL-17 is included in the binding reactions.

[321] The present invention also contemplates chemically modified polypeptides having binding IL-17 and IL-23 activity such as anti-IL-17A and IL-23p19 antibodies, which a polypeptide is linked with a polymer, as discussed above.

[322] Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

[323] As an illustration, pharmaceutical compositions may be supplied as a kit comprising a container that comprises an antibody of the invention. Antibodies of the invention can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic polypeptide. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition. Moreover, such information may include a statement that the antibody composition is contraindicated in patients with known hypersensitivity to IL-17 and IL-23.

[324] A pharmaceutical composition comprising antibodies of the invention can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions, aerosols, droplets, topological solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al., Pharm. Biotechnol. 10*:239 (1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)). Other solid forms include creams, pastes, other topological applications, and the like.

[325] Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al., Eur. J. Clin. Microbiol. Infect. Dis. 12 (Suppl. 1)*:S61 (1993), Kim, *Drugs 46*:618 (1993), and Ranade, "Site-Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are

biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm. A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy *et al., Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al., American J. Hosp. Pharm. 46*:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

[326] Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al., Ann. N.Y. Acad. Sci. 446*:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 μm) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 μm are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

[327] The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al., Biochim. Biophys. Acta 802*:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al., Biochim. Biophys. Acta 1068*:133 (1991); Allen *et al., Biochim. Biophys. Acta 1150*:9 (1993)).

[328] Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa *et al.*, Japanese Patent 04-244,018; Kato *et al., Biol. Pharm. Bull. 16*:960 (1993)). These formulations were prepared by mixing soybean phospatidylcholine, α-tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu *et al., Biol. Pharm. Bull. 20*:881 (1997)).

[329] Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies of the present invention, antibody fragments, carbohydrates, vitamins, and transport proteins. For example, liposomes can be modified with branched type galactosyllipid

derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, *Crit. Rev. Ther. Drug Carrier Syst. 14:*287 (1997); Murahashi *et al., Biol. Pharm. Bull. 20:*259 (1997)). Similarly, Wu *et al., Hepatology 27:*772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be inhibited by preinjection of asialofetuin (Murahashi *et al., Biol. Pharm. Bull. 20:*259 (1997)). Polyaconitylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al., Proc. Nat'l Acad. Sci. USA 94:*11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

[330] In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al., Adv. Drug Deliv. Rev. 32:*99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym *et al., Adv. Drug Deliv. Rev. 32*:99 (1998)).

[331] The antibodies of the invention can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al., Infect. Immun. 31*:1099 (1981), Anderson *et al., Cancer Res. 50:*1853 (1990), and Cohen *et al., Biochim. Biophys. Acta 1063*:95 (1991), Alving *et al.* "Preparation and Use of Liposomes in Immunological Studies," in *Liposome Technology,* 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al., Meth. Enzymol. 149*:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al., Biochim. Biophys. Acta 1150:*9 (1993)).

[332] Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz and Pettit, *Bioconjugate Chem. 6:*332 (1995); Ranade, "Role of Polymers in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al., Science 281:*1161 (1998); Putney and Burke, *Nature Biotechnology 16:*153 (1998); Putney, Curr. Opin. Chem. Biol. 2:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for intravenous administration of therapeutic proteins (see, for example, Gref *et al., Pharm. Biotechnol. 10:*167 (1997)).

[333] Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

[334] The present invention contemplates antagonists of IL-17 and IL-23 and methods and therapeutic uses comprising an such antagonists as described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

[335] The invention is further illustrated by the following non-limiting examples.

EXAMPLE 1

Panning for Antibodies that bind IL-17A and IL-23p19

[336] Human antibody phage and phagemid libraries were screened for Fab molecules that bind to either IL-17A or IL-23p19. These human antibody libraries combine human gene fragments encoding the light-chain variable region and a portion of the heavy-chain variable region and are combined with synthetic DNA encoding human antibody sequences with diversity introduced at strategic sites. These diverse gene products are displayed on phage and phagemid libraries as Fab antibody fragments. The libraries were provided by Dyax (Dyax Corp., Cambridge).

[337] General methods of panning against target antigen using the Dyax Fab libraries have previously been described (See de Haard, et al. J Biol Chem 1999, 274:18218–18230; and Joystock, et al. J Immunol Methods. 2004, 289(1-2):65-80). IL-23 and IL-17A cytokines were used as single targets for screening the human Fab libraries Two selection formats of panning were employed for antibody rescue. 1.) Immunotube panning: IL-17A or IL-23 was coated on a polystyrene surface for panning against the human libraries. Once adsorbed, incubation of the libraries was followed by washing and elution steps for identification of antigen-specific antibodies. 2.) Bead-based panning: IL-17A and IL-23 were both biotinylated at ZymoGenetics. Streptavidin beads were employed for panning of biotinylated targets. Antibody selection was performed on bead-bound targets as well as soluble target with bead rescue.

[338] Upon elution, bacterial cells were infected with phage. Plasmid was retrieved from infected cells and DNA was then modified for expression of soluble Fab fragments. Antibodies were identified for target-specific binding and neutralization in the plate ELISAs described in Examples 2-5 herein.

EXAMPLE 2

Identification of Anti-IL-17A antibodies in a Plate-Based Binding Assay

Materials and Methods:

[339] Costar 96-well plates (Corning Inc. Life Sciences, Lowell, MA, Catalog #9018) were coated with 50 ul IL-17A (ZymoGenetics Inc., Seattle, WA) or IL-17F (ZymoGenetics Inc., Seattle, WA) at 2 ug/ml in 0.1M $NaHCO_3$, pH 9.6 overnight at 4°C. The next day, plates were washed three times with 0.1% Tween-20/PBS (PBST) (ZymoGenetics Inc., Seattle, WA). Each well was filled with 350 ul of 2% milk (#170-6404, Bio-Rad Hercules, CA)/PBST for one hour at RT for blocking. Assay plates were then washed three times with PBST. Each well was filled with 50 ul of 2% milk/PBST, followed by the addition of 25 ul of Fab or scFv supernatant. Wells were then mixed and then incubated for one hour at RT. Plates were washed three times with PBST. For Fab detection, 50 ul of (1:4000) anti-Human Fab specific pAb-HRP (, Pierce, Rockford, IL, Catalog #31482) in 2%

milk/PBST was added to each well for one hour at RT. For scFv detection, 50 ul of (1:4000) anti-His tag mAb-HRP (Sigma, St. Louis, MO, Catalog #A7058) in 2% milk/PBST was added to each well for one hour at RT. Plates were then washed three times with PBST. 50 ul of TMB (TMBW-1000-01, BioFX Laboratories, Owings Mills, MD) was added to each well to develop for 20 – 30 min, followed by the addition of 50 ul of stop buffer (STPR-1000-01, BioFX Laboratories) to quench the reaction. Plates were then read at 450nm on a plate reader.

[340] Antibody clones in wells displaying higher signal on the IL-17A ELISA over the IL-17F ELISA were chosen for further analysis and manipulation.

EXAMPLE 3

Identification of Neutralizing Anti-IL-17 Antibodies in a Plate-based Neutralization Assay

Materials and Methods:

[341] Costar 96-well plates (Corning Inc. Life Sciences, Lowell, MA, Catalog #9018) were coated with 50 ul of anti-human IgG Fcγ-specific antibody (Jackson Immunology, Catalog #109-005-098) at 1 ug/ml in 0.1M NaHCO3, pH 9.6 overnight at 4°C. The next day, plates were washed three times with 0.1% Tween-20/PBS (PBST). 50 ul of IL-17RA (ZymoGenetics Inc., Seattle, WA) at 0.25 ug/ml in PBS was added to each well, followed by a one-hour incubation at room temperature (RT). Plates were then washed three times with PBST. Each well was filled with 350 ul of 2% milk (Bio-Rad, Hercules, CA, Catalog #170-6404,)/PBST for one hour at RT for blocking. During plate blocking, adjacent plates were set up for pre-incubation of biotinylated IL-17A with Fab or scFv supernatant. Pre-incubation plates were filled with 75 ul of Fab supernatant, followed by the addition of 25 ul of biotinylated IL-17A (ZymoGenetics Inc., Seattle, WA) at 0.10 ug/ml in 4% milk/PBST. Each well was mixed and then incubated for 30min at RT. Assay plates were then washed three times with PBST, and the volume of the supernatant/IL-17A complex transferred from the pre-incubation plate to the assay plate, followed by a one-hour incubation at RT. Plates were then washed three times with PBST. 50 ul of (1:3000) Streptavidin-HRP (Pierce, Rockford, IL, Catalog #21124C) in PBST added to each well to incubate for one hour. Plates then washed three times with PBST. 50 ul of TMB (TMBW-1000-01, BioFX Laboratories, Owings Mills, MD) added to each well to develop for 20 – 30 min, followed by the addition of 50 ul of stop buffer (STPR-1000-01, BioFX Laboratories, Owings Mills, MD) to quench the reaction. Plates were then read at 450nm on a plate reader.

[342] Anti-IL-17A antagonists that bound and neutralized their targets in Examples 2 and 3 herein are listed in Tables 7, below. In the Table, some of the DNA sequences contain stop codons in the normal reading frame for the protein. However, these sequences are expressed as full length antibody poly-peptide sequences with the help of suppressor tRNA genes that read through the stops and replace that codon with an amino acid. This is evidenced by gels which show an expressed protein at the normal length for an antibody for these clones. In the table below, if a cluster is

represented only by a DNA sequence with a stop in it, that DNA sequence is presented with the stops converted to the appropriate amino acid (via in-silico suppression).

Table 7 lists the information for the anti-IL-17A antagonists

clone	molecule	orientation	Stop codon suppressed	germline
c83	Fab	LH	no	VL3_3r
c84	Fab	LH	no	VL1_1c
c85	Fab	LH	no	VKI_O12
c86	Fab	LH	no	VKI_O18
c87	Fab	LH	no	VKI_L1
c88	Fab	LH	no	VKI_O12
c89	Fab	LH	no	VL3_3r
c90	scFv mixed	LH	no	VKI_O12
c91	Fab	LH	no	VKI_O18
c92	Fab	LH	no	VKI_L5
c93	Fab	LH	no	VKII_A19
c94	Fab	LH	no	VKI_O12
c95	Fab	LH	no	VKI_O12
c96	Fab	LH	no	VKI_L4
c97	Fab, scFv	VH/VL, LH	no	VKI_O18
c98	Fab, scFv	LH	no	VKI_O18
c99	Fab	LH	no	VL3_3r
c100	Fab	LH	no	VKI_L12
c157	Fab	LH	no	VL1_1c
c168	Fab	LH	no	VKI_O12
c191	scFv VL/VH	LH	no	VKI_O18
c192	scFv VH/VL, scFv mixed	HL	no	VL3_3r
c193	scFv VH/VL,scFv mixed	HL	no	VL3_3r
c195	scFv VL/VH	LH	no	VL3_3r
c196	scFv VH/VL,scFv mixed	HL	no	VL3_3r
c199	scFv VL/VH	LH	no	VKI_O18
c200	scFv VH/VL	HL	no	VKI_L1
c202	scFv VL/VH	LH	no	VKI_O18
c203	scFv VH/VL	HL	no	VL3_3r
c204	scFv VH/VL	HL	no	VKI_L1
c207	scFv VL/VH	LH	no	VKI_O18
c209	scFv VH/VL,scFv mixed	HL	no	VKI_L1
c210	scFv VH/VL	HL	no	VL3_3r
c212	scFv VL/VH	LH	no	VKI_O18
c213	scFv VL/VH	LH	no	VKI_O18
c214	scFv VH/VL	HL	no	VKI_O18
c215	scFv VH/VL	HL	no	VKI_O18
c218	scFv VL/VH	LH	no	VL3_3r
c219	scFv VH/VL	HL	no	VL3_3r
c220	scFv mixed	HL	no	VL3_3r
c221	scFv mixed	HL	no	VL3_3r
c223	scFv mixed	HL	no	VKI_L1
c224	scFv mixed	HL	no	VKI_O18
c225	scFv mixed	HL	no	VL3_3r

c226	scFv mixed	HL	no	VKI_L1
c227	scFv mixed	HL	no	VL3_3r
c229	scFv mixed	HL	no	VKI_O12
c230	scFv mixed	LH	no	VKI_O18
c231	scFv mixed	HL	no	VL3_3r
c232	scFv mixed	LH	no	VKI_O18
c233	scFv mixed	HL	no	VKI_O18
c234	scFv mixed	HL	no	VKI_L1
c235	scFv mixed	HL	no	VL3_3r
c236	scFv mixed	HL	no	VKI_O18
c237	scFv mixed	HL	no	VL3_3r
c238	scFv mixed	LH	no	VL3_3r
c239	scFv mixed	HL	no	VL3_3r
c240	scFv mixed	HL	no	VL3_3r
c245	scFv mixed	HL	no	VL3_3r
c246	scFv mixed	HL	no	VKI_O18
c248	scFv mixed	HL	no	VL3_3r
c249	scFv mixed	HL	no	VL3_3r
c250	scFv mixed	HL	no	VKI_L12
c296	Fab	LH	no	VH3_3-23
c380	Fab	LH	no	VH3_3-23
c381	Fab	LH	no	VH3_3-23
c382	Fab	LH	no	VKI_O12
c383	Fab	LH	no	VL1_1c
c384	Fab	LH	no	VH3_3-23
c385	Fab	LH	no	VKIII_A27
c386	Fab	LH	no	VKI_O12
c387	Fab	LH	no	VH3_3-23
c388	Fab	LH	no	VKI_O18
c389	Fab	LH	no	VKI_O12
c390	Fab	LH	no	VKI_O18
c391	Fab	LH	no	VKIII_A27
c392	Fab	LH	no	VL1_1g
c393	Fab	LH	no	VH3_3-23
c394	Fab	LH	no	VH3_3-23
c396	Fab	LH	no	VKIII_A27
c397	Fab	LH	no	VKI_L8
c398	Fab	LH	no	VKI_L8
c399	Fab	LH	no	VKIII_L25
c400	Fab	LH	no	VH3_3-23
c401	Fab	LH	no	VKI_A30
c402	Fab	LH	no	VH3_3-23
c403	Fab	LH	no	VKIII_L2
c404	Fab	LH	no	VKI_L12
c405	Fab	LH	no	VKIII_L6
c406	Fab	LH	no	VKI_L12
c407	Fab	LH	no	VH3_3-23
c408	Fab	LH	no	VKI_L12
c409	Fab	LH	no	VKIII_L6
c410	Fab	LH	no	VKI_O12
c411	scFv mixed	HL	no	VKI_O12
c412	scFv mixed	LH	yes	VKII_A19
c416	scFv mixed	HL	no	VKI_O12
c417	scFv mixed	LH	no	VKI_O12
c418	scFv mixed	HL	no	VKI_O12
c419	scFv mixed	LH	no	VKI_O12
c420	scFv mixed	HL	no	VL3_3r
c421	scFv mixed	HL	no	VKI_O12
c422	scFv mixed	HL	no	VL3_3r
c424	scFv mixed	LH	no	VKI_O18

c425	scFv mixed	HL	no	VKI_O12
c426	Fab	LH	no	VL1_1g
c427	Fab	LH	no	VL1_1g
c428	Fab	LH	no	VKI_O12
c429	Fab	LH	no	VL1_1c
c430	Fab	LH	no	VL6_6a
c431	Fab	LH	no	VKI_O12
c432	Fab	LH	no	VL1_1g
c433	Fab	LH	no	VL1_1g
c434	Fab	LH	no	VL3_3l
c435	Fab	LH	no	VKI_O12
c436	Fab	LH	no	VKII_A19
c437	Fab	LH	no	VL1_1g
c438	Fab	LH	no	VKI_L5
c440	Fab	LH	no	VKI_L12
c441	Fab	LH	no	VL3_3r
c442	Fab	LH	no	VKII_A19
c443	Fab	LH	no	VL3_3r
c444	Fab	LH	no	VL1_1g
c445	Fab	LH	no	VKI_O12
c446	Fab	LH	no	VL1_1c
c447	Fab	LH	no	VKI_O12
c448	Fab	LH	no	VL1_1g
c449	Fab	LH	no	VL1_1g
c450	Fab	LH	no	VKI_O12
c451	Fab	LH	no	VL1_1c
c452	Fab	LH	no	VL1_1g
c453	Fab	LH	no	VKI_O12
c454	Fab	LH	no	VL1_1g
c455	Fab	LH	no	VL1_1g
c456	Fab	LH	no	VKI_L11
c457	Fab	LH	no	VKI_L1
c458	Fab	LH	no	VL2_2a2
c459	Fab	LH	no	VL1_1g
c460	Fab	LH	no	VKI_L1
c461	Fab	LH	no	VL1_1g
c462	Fab	LH	no	VKI_O12
c463	Fab	LH	no	VKI_O12
c464	Fab	LH	no	VL3_3h
c465	Fab	LH	no	VL1_1c
c466	Fab	LH	no	VH3_3-23
c513	Fab	LH	no	VKI_O12
c514	Fab	LH	no	VKII_A17
c515	Fab	LH	no	VKI_O12
c516	Fab	LH	no	VKI_L12
c517	Fab	LH	no	VKI_L12
c518	Fab	LH	no	VKI_O12
c519	Fab	LH	no	VKI_O12
c520	Fab	LH	no	VKI_O12
c521	Fab	LH	no	VKI_L1
c522	Fab	LH	no	VKIII_L2
c529	scFv mixed	LH	yes	VKI_O12
c541	scFv mixed	LH	yes	VKI_L1
c542	scFv mixed	HL	no	VL3_3r
c543	scFv mixed	HL	no	VKI_O18
c545	scFv VL/VH	LH	yes	VKI_L12
c546	scFv mixed	HL	no	VKI_L1
c547	scFv mixed	HL	no	VKI_L1
c548	scFv mixed	HL	no	VKI_L1
c549	scFv mixed	HL	no	VKI_L1

c550	scFv mixed	HL	no	VKI_O12
c551	scFv mixed	HL	no	VKI_L1
c552	scFv mixed	HL	no	VKI_L1
c553	scFv mixed	HL	no	VKI_L1
c554	scFv mixed	HL	no	VKI_L1
c555	scFv mixed	HL	no	VKI_L1
c556	scFv mixed	HL	no	VKI_L1
c557	scFv mixed	HL	no	VL1_1g
c559	scFv mixed	HL	no	VKI_L1
c560	scFv mixed	HL	no	VKI_L1
c561	scFv mixed	HL	no	VKI_L1
c562	scFv mixed	LH	yes	VKI_L1
c563	scFv mixed	LH	yes	VKI_L1
c564	scFv mixed	LH	yes	VKI_L1
c565	scFv mixed	LH	yes	VKI_L1
c566	scFv mixed	LH	yes	VKI_L1
c567	scFv mixed	LH	yes	VKIII_A27
c570	scFv mixed	HL	no	VKI_L1
c571	scFv mixed	HL	no	VKI_L1
c572	scFv mixed	HL	no	VKI_L1
c573	scFv mixed	HL	no	VKI_L1
c574	scFv mixed	HL	yes	VKI_L1
c575	scFv mixed	HL	no	VL1_1g
c576	scFv mixed	HL	no	VKI_L1
c577	scFv mixed	HL	no	VKI_L1
c578	scFv mixed	LH	yes	VKI_L1
c579	scFv mixed	HL	no	VL3_3r
c580	scFv mixed	HL	no	VKI_L1
c581	scFv mixed	LH	no	VL3_3r
c582	scFv mixed	HL	no	VKI_O12
c583	scFv mixed	LH	no	VKI_O12
c584	scFv mixed	LH	yes	VKI_L1
c585	scFv mixed	HL	yes	VKI_L1
c586	scFv mixed	LH	yes	VKI_L1
c587	scFv mixed	HL	yes	VKI_L1
c588	scFv mixed	HL	yes	VKI_L1
c589	scFv mixed	HL	yes	VKI_L1
c590	scFv mixed	LH	yes	VKI_L1
c592	scFv mixed	HL	yes	VKI_L1
c593	scFv mixed	HL	no	VKI_L1
c594	scFv mixed	HL	yes	VKI_L1
c595	scFv mixed	HL	yes	VKI_L1
c596	scFv mixed	HL	no	VKI_L1
c631	Fab	HL	no	VKI_O12
c632	scFv mixed	LH	no	VKI_L1

EXAMPLE 4

Identification of Anti-IL-23A antibodies in a Plate-Based Binding Assay

Materials and Methods:

[343] Costar 96-well plates (Corning Inc. Life Sciences, Lowell, MA, Catalog #9018) were coated with 50 ul IL-23 (# 1290-IL-010/CF, R&D Systems, Minneapolis, MN, or produced at ZymoGenetics, Inc., Seattle, WA) or IL-12 (#219-IL/CF, R&D Systems, Minneapolis, MN) at 4

ug/ml in 0.1M NaHCO3, pH 9.6, overnight at 4°C. The next day, plates were washed three times with 0.1% Tween-20/PBS (ZymoGenetics, Inc., Seattle, WA). Each well was filled with 350 ul of 2% milk (Bio-Rad, Hercules, CA, Catalog #170-6404,)/PBST for one hour at RT for blocking. Assay plates were then washed three times with PBST. Each well was filled with 50 ul of 2% milk/PBST, followed by the addition of 25 ul of Fab or scFv supernatant. Well were mixed and then incubated for one hour at RT. Plates were washed three times with PBST. For Fab detection, 50 ul of (1:4000) anti-Human Fab specific pAb-HRP (Pierce, Rockford, IL, Catalog #31482,) in 2% milk/PBST was added to each well for one hour at RT. For scFv detection with R&D IL-23, 50 ul of (1:4000) anti-His tag mAb-HRP (Sigma, St. Louis, MO, Catalog #A7058) in 2% milk/PBST was added to each well for one hour at RT. Plates were washed three times with PBST. 50 ul of TMB (TMBW-1000-01, BioFX Laboratories, Owings Mills, MD) added to each well to develop for 20 – 30 min, followed by the addition of 50 ul of stop buffer (STPR-1000-01, BioFX Laboratories, Owings Mills, MD) to quench the reaction. Plates were then read at 450nm on a plate reader.

[344] Wells that showed greater binding on the plate coated with the IL-23 heterodimer than on the plate coated with the IL-12 heterodimer were chosen for further analysis and manipulation.

EXAMPLE 5

Identification of Neutralizing Anti-IL-23A Antibodies in a Plate-based Neutralization Assay

Materials and Methods:

[345] Costar 96-well plates (Corning Inc. Life Sciences, Lowell, MA, Catalog #9018) were coated with 50 ul of anti-human IgG Fcγ-specific antibody (Jackson Immunology, West Grove, PA, Catalog #109-005-098) at 1 ug/ml in 0.1M NaHCO3, pH 9.6 overnight at 4°C. The next day, plates were washed three times with 0.1% Tween-20/PBS (PBST). 50 ul of IL-23R (, R&D Systems, Minneapolis, MN, Catalog #1400-IR-050, or produced at ZymoGenetics Inc., Seattle, WA) at 0.25 ug/ml in PBS was added to each well, followed by a one-hour incubation at room temperature (RT). Plates were then washed three times with PBST. Each well was filled with 350 ul of 2% milk (Bio-Rad, Hercules, CA, Catalog #170-6404,)/PBST for one hour at RT for blocking. During plate blocking, adjacent plates were set up for pre-incubation of biotinylated IL-23 with Fab or scFv supernatant. Pre-incubation plates were filled with 75 ul of Fab or scFv supernatant, followed by the addition of 25 ul of biotinylated IL-23 heterodimer or p19 (ZymoGenetics Inc., Seattle, WA) at 0.10 ug/ml in 4% milk/PBST. Each well was mixed and then incubated for 30min at RT. Assay plates were then washed three times with PBST, and the volume of the supernatant/IL-23 complex transferred from the pre-incubation plate to the assay plate, followed by a one-hour incubation at RT. Plates were then washed three times with PBST. 50 ul of (1:3000) Streptavidin-HRP (Pierce, Catalog #21124,) in PBST added to each well to incubate for one hour. Plates were then washed three times with PBST.

50 ul of TMB (TMBW-1000-01, BioFX Laboratories) added to each well to develop for 20 – 30 min, followed by the addition of 50 ul of stop buffer (STPR-1000-01, BioFX Laboratories) to quench the reaction. Plates were then read at 450nm on a plate reader.

[346] Anti-IL-23p19 antagonists that bound and neutralized their targets in Examples 4 and 5 are herein are listed in Table 8, below. In the Table, some of the DNA sequences contain stop codons in the normal reading frame for the protein. However, these sequences are expressed as full length antibody poly-peptide sequences with the help of suppressor tRNA genes that read through the stops and replace that codon with an amino acid. This is evidenced by gels which show an expressed protein at the normal length for an antibody for these clones. In the table below, if a cluster is represented only by a DNA sequence with a stop in it, that DNA sequence is presented with the stops converted to the appropriate amino acid (via in-silico suppression).

Table 8 lists the information for the anti-IL-23p19 antagonists

clone	molecule	orientation	Stop codon suppressed	germline
c26	phagemid	LH	no	VKIII_A27
c27	Fab	LH	no	VKI_O12
c28	phagemid	LH	no	VKI_O12
c29	Fab,phagemid,scFv VH/VL,scFv mixed	LH	no	VL2_2a2
c33	phagemid	LH	no	VKIII_L2
c36	Fab	LH	no	VKI_L5
c40	phagemid, Fab	LH	no	VL3_3r
c41	phagemid, Fab, scFv mixed	LH	no	VKII_A19
c43	phagemid	LH	no	VKII_A19
c101	Fab	LH	no	VL2_2a2
c102	Fab, phagemid	LH	no	VKI_L5
c103	Fab, phagemid	LH	no	VKII_A17
c110	Fab	LH	no	VL1_1c
c114	Fab	LH	no	VKI_O12
c115	Fab	LH	no	VKII_A19
c119	Fab	LH	no	VKI_O12
c120	Fab	LH	no	VKII_A19
c121	Fab	LH	no	VKI_L11
c122	Fab	LH	no	VKI_O12
c123	Fab	LH,	no	VL3_3r
c124	Fab	LH	no	VKIII_L6
c125	Fab	LH	no	VKI_O12
c126	Fab	LH	no	VL1_1g
c127	Fab	LH	no	VL1_1g
c128	Fab	LH	no	VL1_1g
c129	Fab	LH	no	VKI_O12
c130	Fab	LH	no	VKI_L12
c131	Fab	LH	no	VKI_L8
c132	Fab	LH	no	VKI_O12
c134	Fab	LH	no	VL3_3r
c135	Fab	LH	no	VKI_O12
c136	Fab	LH	no	VKI_O12
c137	Fab	LH	no	VL2_2a2

c138	Fab	LH	no	VKII_A19
c139	Fab	LH	no	VKI_A20
c140	Fab, phagemid	LH	no	VKII_A19
c141	Fab, phagemid	LH	no	VKII_A19
c142	Fab	LH	no	VKI_O12
c143	Fab	LH	no	VKI_O12
c144	Fab	LH	no	VKII_A19
c145	Fab	LH	no	VKIII_L2
c146	Fab	LH	no	VL2_2a2
c148	Fab	LH	no	VKIII_A27
c149	Fab	LH	no	VKIII_L2
c150	Fab	LH	no	VKII_A19
c151	Fab	LH	no	VKIII_A27
c152	Fab	LH	no	VKI_O12
c153	Fab	LH	no	VKI_L12
c154	Fab	LH	no	VKII_A19
c155	Fab	LH	no	VKIII_A27
c156	Fab	LH	no	VL1_1c
c157	Fab	LH	no	VL1_1c
c158	Fab	LH	no	VKII_A19
c159	Fab	LH	no	VKII_A19
c160	Fab	LH	no	VKI_L12
c161	Fab	LH	no	VKII_A19
c162	Fab	LH	no	VKIII_A27
c163	Fab	LH	no	VKIII_L2
c164	Fab	LH	no	VKIII_A27
c165	Fab	LH	no	VKII_A19
c166	Fab	LH	no	VKIII_A27
c167	Fab	LH	no	VKIII_L2
c168	Fab, phagemid	LH	no	VKI_O12
c169	Fab	LH	no	VKIII_A27
c170	Fab	LH	no	VKII_A19
c171	Fab	LH	no	VKIII_A27
c172	Fab	LH	no	VKIII_L2
c173	Fab	LH	no	VKI_L5
c174	Fab	LH	no	VKI_L5
c175	Fab	LH	no	VKI_L12
c176	Fab	LH	no	VKIII_L2
c178	Fab, phagemid	LH	no	VKII_A19
c179	Fab	LH	no	VKII_A19
c180	Fab	LH	no	VKIII_L6
c181	Fab	LH	no	VKI_A20
c182	Fab	LH	no	VKII_A19
c183	Fab	LH	no	VKIII_A27
c184	Fab	LH	no	VKI_O12
c185	Fab	LH	no	VKII_A19
c186	Fab	LH	no	VKIII_A27
c187	Fab	LH	no	VKI_O12
c188	Fab	LH	no	VKII_A19
c189	Fab	LH	no	VKII_A19
c190	scFv VH/VL	HL	no	VKII_A17
c194	scFv VH/VL	HL	no	VKII_A19
c197	scFv VH/VL, scFv mixed	HL	no	VKII_A17
c198	scFv VH/VL	HL	no	VKII_A19
c201	scFv VH/VL	HL	no	VKII_A19
c205	scFv VH/VL	HL	no	VKII_A19
c206	scFv VH/VL	HL	no	VKII_A17
c208	scFv VH/VL	HL	no	VH3_3-23
c211	scFv VH/VL	HL	no	VKII_A19

c251	Fab	LH	no	VL3_3r
c252	Fab	LH	no	VL3_3r
c253	Fab	LH	no	VL3_3r
c254	Fab	LH	no	VL3_3r
c255	Fab	LH	no	VKIII_A27
c256	Fab	LH	no	VL3_3r
c257	Fab	LH	no	VL3_3r
c259	Fab	LH	no	VL3_3r
c260	Fab	LH	no	VKIII_L6
c261	Fab	LH	no	VKIII_A27
c262	Fab	LH	no	VKIII_L6
c263	Fab	LH	no	VKIII_L2
c264	Fab	LH	no	VKIII_L6
c265	Fab	LH	no	VKIII_L6
c266	Fab	LH	no	VKIII_L6
c267	Fab	LH	no	VKI_L5
c270	scFv mixed	HL	no	VKII_A19
c271	scFv mixed	HL	no	VKII_A19
c272	scFv mixed	HL	no	VKII_A17
c273	scFv mixed	HL	no	VKI_L5
c274	scFv mixed	HL	no	VKII_A19
c275	scFv mixed	LH	no	VKII_A19
c276	scFv mixed	HL	no	VKI_L5
c277	scFv mixed	LH	no	VKI_L5
c278	scFv mixed	HL	no	VKII_A19
c279	scFv mixed	HL	no	VL2_2a2
c280	scFv mixed	HL	no	VKII_A19
c281	scFv mixed	HL	no	VKII_A19
c282	scFv mixed	HL	no	VKII_A19
c283	scFv mixed	LH	no	VL2_2a2
c284	scFv mixed	HL	no	VL2_2a2
c285	scFv mixed	HL	no	VKII_A19
c287	scFv mixed	HL	no	VKII_A17
c288	scFv mixed	HL	no	VL2_2a2
c289	scFv mixed	LH	no	VKII_A19
c290	Fab	LH	no	VKI_L5
c298	Fab	UK	no	VH3_3-23
c300	Fab	UK	yes	VL3_3r
c301	Fab	LH	no	VKI_O12
c302	scFv VL/VH	LH	no	VKII_A17
c303	scFv VL/VH	LH	no	VKII_A17
c304	scFv VH/VL	HL	no	VL2_2a2
c305	scFv VH/VL,scFv mixed	HL	no	VL2_2a2
c347	Fab	LH	no	VKI_L5
c348	Fab	LH	no	VKI_L5
c349	Fab	LH	no	VL2_2b2
c350	Fab	LH	no	VKI_O18
c351	Fab	LH	no	VKIII_L2
c352	Fab	LH	no	VKII_A19
c353	Fab	LH	no	VKI_O12
c354	Fab	LH	no	VKI_L5
c355	Fab	LH	no	VKI_L5
c356	Fab	LH	no	VL3_3r
c357	Fab	LH	no	VKI_L8
c358	Fab	LH	no	VKI_L12
c359	Fab	LH	yes	VL1_1c
c360	Fab	LH	no	VKIII_A27
c361	Fab	LH	no	VKI_L5
c362	Fab	LH	no	VKI_L5

c363	Fab	LH	no	VKII_A19
c364	Fab	LH	no	VKI_L1
c365	Fab	LH	no	VKI_O12
c366	Fab	LH	no	VKI_L1
c367	phagemid	LH	no	VKII_A19
c368	Fab	LH	no	VKIII_L6
c369	Fab	LH	no	VKI_O12
c370	phagemid	LH	no	VKI_O12
c371	phagemid	LH	no	VKI_L5
c372	phagemid	LH	no	VH3_3-23
c373	phagemid	LH	no	VKIII_L2
c374	phagemid	LH	no	VL2_2a2
c375	phagemid	LH	no	VKII_A19
c376	phagemid	LH	no	VKII_A19
c377	Fab	LH	no	VKII_A19
c378	phagemid	LH	no	VL4_4b
c379	phagemid	LH	no	VKI_O12
c439	Fab	LH	no	VH3_3-23
c467	scFv mixed	HL	no	VKII_A19
c468	scFv mixed	HL	no	VL2_2a2
c469	scFv mixed	HL	no	VL2_2a2
c470	scFv mixed	HL	no	VL2_2a2
c471	scFv mixed	HL	no	VKII_A19
c472	scFv mixed	LH	no	VKII_A19
c473	scFv mixed	HL	no	VKII_A19
c474	scFv mixed	HL	no	VKII_A19
c475	scFv mixed	HL	no	VL2_2a2
c476	scFv mixed	HL	no	VKII_A19
c477	scFv mixed	HL	no	VL2_2a2
c478	scFv mixed	HL	no	VKII_A19
c479	scFv mixed	LH	no	VL2_2a2
c481	scFv mixed	HL	no	VKII_A19
c482	scFv mixed	LH	no	VL2_2a2
c483	scFv mixed	HL	no	VKII_A19
c484	scFv mixed	LH	no	VL2_2a2
c485	scFv mixed	HL	no	VL2_2a2
c486	scFv mixed	HL	no	VKII_A19
c487	scFv mixed	HL	no	VL2_2a2
c489	scFv mixed	LH	no	VKII_A19
c490	scFv mixed	HL	no	VKI_O12
c491	scFv mixed	HL	no	VKII_A19
c492	scFv mixed	LH	no	VKII_A19
c493	scFv mixed	HL	no	VL2_2a2
c494	scFv mixed	HL	no	VL1_1g
c495	scFv mixed	HL	no	VKII_A19
c496	scFv mixed	HL	no	VL1_1c
c497	scFv mixed	HL	no	VKII_A19
c498	scFv mixed	HL	no	VL1_1c
c499	scFv mixed	HL	no	VKII_A19
c500	scFv mixed	HL	no	VL2_2a2
c501	scFv mixed	HL	no	VKII_A19
c502	scFv mixed	HL	no	VKII_A19
c503	scFv mixed	HL	no	VKII_A19
c504	scFv mixed	HL	no	VKI_L5
c505	scFv mixed	HL	no	VKII_A19
c506	scFv mixed	HL	no	VKII_A19
c507	scFv mixed	HL	no	VKII_A19
c508	scFv mixed	HL	no	VL1_1g
c509	scFv mixed	HL	no	VL2_2a2

c510	scFv mixed	HL	no	VL2_2a2
c511	scFv mixed	HL	no	VKII_A19
c512	scFv mixed	HL	no	VKI_O12
c523	scFv mixed	HL	yes	VL2_2a2
c526	scFv mixed	HL	yes	VKII_A19
c528	scFv mixed	LH	no	VKII_A19
c534	scFv mixed	HL	yes	VL2_2a2
c535	scFv mixed	LH	yes	VKII_A19
c536	scFv mixed	HL	yes	VL2_2a2
c537	scFv mixed	LH	yes	VL2_2a2
c538	scFv mixed	HL	no	VL1_1c
c539	scFv mixed	LH	yes	VL2_2a2
c540	scFv mixed	LH	yes	VL1_1g
c591	scFv mixed	HL	yes	VKII_A19
c1220	scFv VL/VH	LH	no	VKI_L5

Example 6

Construction of Anti-IL17A/IL23A Bispecific Molecule Panel

[347] Exemplary expression construct formats for an anti-IL17A/IL23A bispecific molecule include tandem single chain FvFcs (tascFvFcs), bispecific single chain FvFcs (biscFvFcs) and bispecific antibodies (biAb). The definitions of these molecular formats are as follows: A tascFvFc has two scFvs side by side connected by a tether and fused directly to an Fc. A biscFvFcs has both an amino-terminal and carboxyl-terminal scFv fused to an Fc with the carboxyl-terminal connected via a linker. Both tascFvFcs and biscFvFcs, for the purposes of this example, have an effector minus Fc (Fc_5). The Fc_5 used in constructing the tascFvFcs molecules used the amino acid sequence of SEQ ID NO: 11 (the polynucleotide sequence of SEQ ID NO: 12). The Fc_5 used in the constructing the biscFvFcs molecules used the amino acid sequence of SEQ ID NO: 333 (same as SEQ ID NO: 11, but excluding the carboxyl terminal lysine). Either Fc_5 (i.e., SEQ ID NO: 11 or SEQ ID NO: 333) could be used for construction of tascFvFcs and biscFvFcs molecules. A biAb is a whole immunoglobulin with a carboxyl-terminal scFv connected via a linker. In the case of biAbs described in this example the heavy chain used was human gamma1 (IgG1.1) and the light chains were either kappa or lambda as appropriate to the variable region of the antibody. The molecules are shown in Figure 2.

[348] For the purposes of describing tandem single chain Fv molecules, a tether is defined as a polypeptide that connects two single chain Fv's and the linker refers to the polypeptide that connects the two variable domains comprising a single chain Fv and for any polypeptide linking an scFv to an Fc or immunoglobulin heavy chain. The linker sequence used in the scFv molecules described in this Example is $(G_4S)_5$ (SEQ ID NO: 2120). There are seven tether sequences used in the tascFvFc molecules described below: Lambda stump long (SEQ ID NO: 2121), Lambda stump short (SEQ ID NO: 2122), Kappa stump long (SEQ ID NO: SEQ ID NO: 2123), Kappa stump short (SEQ ID NO: 2124), $(G_4S)_2$(SEQ ID NO: 2125), CH1 stump long (SEQ ID NO: 2126), and CH1 stump

short (SEQ ID NO: 2127). The scFv sequences were fused to Fc_5 (SEQ ID NO: 11 or SEQ ID NO: 333), with out without a carboxyl terminal lysine, an effector minus mutation of human gamma1 Fc.

[349] Two anti-IL23A scFv molecules, c305.1 scFv and c472.2 scFv and two anti-IL17A scFv molecules, c631.1 and c632.1, were obtained from the Dyax phage library as described in Examples 1-5 above. In the construction of the bispecific molecules, c305.1 scFv was always in the variable heavy / variable light orientation; c631.1 was always in the variable heavy / variable light orientation; and c632.1 was always in the variable light / variable heavy orientation.

[350] In addition to the molecules described above there was also one biAb constructed with an additional anti-IL17A Fab (c389.2 heavy chain Fd and light chain) also derived from the Dyax library as described but not converted to an scFv. c389 was converted to human gamma1 and kappa chains and the c305.1 scFv fused to the carboxyl terminus of the heavy chain.

[351] Table 9 shows the SEQ ID NOs: that correspond to the scFv clones used herein, the tether or stump components, and the Multi-Valent Clones ("MVC") generated.

Table 9: SEQ ID NOs corresponding to scFv clones used herein, the tether or stump components, and the Multi-Valent Clones ("MVC") generated

Protein name	SEQ ID NO: of polypeptide	SEQ ID NO: of polynucleotide
(c305.1 single chain Fv (scFv)	2111	2165
c631.1 scFv VL	2112	2166
c631.1 scFv VH	2113	2167
c632.1 scFv VL	2114	2168
c632.1 scFv VH	2115	2169
c472.2 scFv VL	2116	2170
c472.2 scF VH	2117	2171
C389.2 VL	2118	2172
C389.2 VH	2119	2173
$(G_4S)_5$ stump	2120	2174
Lambda stump long	2121	2175
Lambda stump short	2122	2176
Kappa stump long	2123	2177
Kappa stump short	2124	2178
$(G_4S)2$	2125	2179
CH1 stump long	2126	2180
CH1 stump short	2127	2181
MVC # 241	2128	2182
MVC #242	2129	2183
MVC #243	2130	2184
MVC #244	2131	2185
MVC #245	2132	2186
MVC #246	2133	2187
MVC #256	2134	2188
MVC #257	2135	2189
MVC #258	2136	2190
MVC #259	2137	2191
MVC #260	2138	2192
MVC #261	2139	2193
MVC #360	2140	2194
MVC #361	2141	2195
MVC #362	2142	2196

MVC #247	2143	2197
MVC #248	2144	2198
MVC #249	2145	2199
MVC #250	2146	2200
MVC #262	2147	2201
MVC #263	2148	2202
MVC #264	2149	2203
MVC #265	2150	2204
MVC #226	2151	2205
MVC #227	2152	2206
MVC #253	2153	2207
MVC #268	2154	2208
MVC #281	2155	2209
MVC #251	2156	2210
MVC #252	2157	2211
MVC #254	2158	2212
MVC #255	2159	2213
MVC #266	2160	2214
MVC #267	2161	2215
MVC #269	2162	2216
MVC #270	2163	2217
MVC #164	2164	2218

Tandem Single Chain Fv Fc Construction:

[352] Two PCR fragments were generated, one for each scFv and assembled into tandem scFv by the following method: The scFv at the 5' end had oligo sequence overlapping mouse 26-10 VH signal sequence and with tether region between the two single chain Fv's. The 3' scFv had oligo sequence overlapping the tether and the Fc5 fusion partner. These scFv fragments were assembled by PCR. The tandem scFv fragment was inserted at the NotI site of pZMP31-ms 26-10VH-Fc5 by yeast recombination as described below.

[353] c305.1 was already an intact single chain Fv so only one PCR was necessary to generate the PCR fragment. scFv fragments of c631.1 and c632.1 were each constructed by overlapping three PCR fragments, one each for the variable heavy and variable light regions and one for the 25mer G4S linker via PCR.

[354] The PCR amplification reaction conditions were as follows: 1 cycle, 95 °C, 2 minutes; 30 cycles, 95 °C, 15 seconds, followed by 55 °C, 30 seconds, followed by 68°C, 1 minute per kb. The PCR reaction mixture was run on a 1% agarose gel and the DNA fragment corresponding to the expected size was extracted from the gel using the GE Healthcare Illustra GFX™ PCR DNA and Gel Band Purification Kit (Cat. No. 27-9602-01).

[355] The cDNA's were cloned into the vector pZMP31-ms 26-10VH-Fc5 by yeast recombination, This vector was derived from the pZMP31 by the addition of mouse 26-10 VH signal sequence and Fc5 with a NotI restriction site between for insertion of cDNA. pZMP31 is a mammalian expression vector containing an expression cassette having the chimeric CMV enhancer/MPSV promoter, a NotI site for linearization for insertion of cDNA, a poliovirus internal ribosome entry site (IRES), a DHFR cDNA, and the SV40 terminator; an E. coli origin of replication,

and S. cerevisiae URA3 and CEN-ARS genes. This vector was derived from pZMP21 (US Patent 7,262,025).

[356] The pZMP31-ms 26-10VH-Fc5 plasmid was digested with NotI prior to recombination in yeast with the gel-extracted anti-IL17A and anti-IL23A scFv PCR fragments. One hundred µl of electrocompetent yeast (S. cerevisiae SF838-9D, URA-) were combined with approximately 12 µl of each gel-extracted anti-IL17A and anti-IL23A scFv DNA inserts and approximately 100 ng of NotI digested pZMP31-ms 26-10VH-Fc5 vector. The mix was transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed using power supply (BioRad Laboratories, Hercules, CA) settings of 0.75 kV (5 kV/cm), ∞ ohms, and 25 µF. Six hundred µl of 1.2 M sorbitol was added to the cuvette, and the yeast was plated in 300 µl aliquots onto two URA-D plates and incubated at 30°C. After about 72 hours, the Ura+ yeast transformants from a single plate were resuspended in 1 ml H2O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 0.5 ml of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The 500 µl of the lysis mixture was added to an Eppendorf tube containing 250 µl acid-washed glass beads and 300 µl phenol-chloroform, was vortexed for 3 minutes, and spun for 5 minutes in an Eppendorf centrifuge at maximum speed. Three hundred µl of the aqueous phase was transferred to a fresh tube, and the DNA was precipitated with 600 µl ethanol, followed by centrifugation for 30 minutes at maximum speed. The tube was decanted and the pellet was washed with 1 mL of 70% ethanol. The tube was decanted and the DNA pellet was resuspended in 10 µl water.

[357] Transformation of electrocompetent E. coli host cells (DH10B, Invitrogen, Carlsbad, CA) was done using 1 µl of the yeast DNA preparation and 20 µl of E. coli cells. The cells were electropulsed at 2.0 kV, 25 µF, and 400 ohms. Following electroporation, 1 ml SOC (2% Bacto™ Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose) was added and the cells were plated in 50 µl and 200 µl aliquots on two LB AMP plates (LB broth (Lennox), 1.8% Bacto™ Agar (Difco), 100 mg/L Ampicillin).

[358] The plasmid was extracted from six E. coli clones for each construct, subjected to sequence analysis and one clone containing the correct sequence was selected for further use. Large-scale plasmid DNA was isolated using a commercially available kit (QIAGEN Plasmid Mega Kit, Qiagen, Valencia, CA) according to manufacturer's instructions.

Table 10: Tandem Single Chain FvFc (tascFvFc) Construction (polynucleotide sequences)

Final construct (MVC) #/ SEQ ID NO:	**scFv1 (farthest from Fc) Template/ SEQ ID NO:**	**Tether Tether / SEQ ID NO:**	**scFv2 (closest to Fc) Template / SEQ ID NO:**
241 / SEQ ID NO: 2182	c305.1 scFv/ SEQ ID NO: 2165	Lambda stump long/ SEQ ID NO: 2175	c631.1V_H - V_L scFv of SEQ ID NOs: 2167 and 2166
242 / SEQ ID NO: 2183	c305.1 scFv/ SEQ ID NO: 2165	Lambda stump short/ SEQ ID NO: 2176	c631.1V_H- V_L scFv of SEQ ID NOs: 2167 and 2166
243 / SEQ ID NO: 2184	c631.1V_H - V_L scFv of SEQ ID NOs: 2167 and 2166	Kappa stump Long/ SEQ ID NO: 2177	c305.1 scFv/ SEQ ID NO: 2165
244 / SEQ ID NO: 2185	c631.1V_H - V_L scFv of SEQ ID NOs: 2167 and 2166	Kappa stump Short/ SEQ ID NO: 2178	c305.1 scFv/ SEQ ID NO: 2165
245 / SEQ ID NO: 2186	c305.1 scFv/ SEQ ID NO: 2165	$(G_4S)_2$/ SEQ ID NO: 2179	c631.1V_H and V_L scFv of SEQ ID NOs: 2167 and 2166
246 / SEQ ID NO: 2187	c631.1V_H - V_L scFv of SEQ ID NOs: 2167 and 2166	$(G_4S)_2$/ SEQ ID NO: 2179	c305.1 scFv/ SEQ ID NO: 2165
256 / SEQ ID NO: 2188	c305.1 scFv/ SEQ ID NO: 2165	Lambda stump long/ SEQ ID NO: 2175	c632.1 V_L - V_H scFv of SEQ ID NOs: 2168 and 2169
257 / SEQ ID NO: 2189	c305.1 scFv/ SEQ ID NO: 2165	Lambda stump short/ SEQ ID NO: 2716	c632.1 V_L - V_H scFv of SEQ ID NOs: 2168 and 2169
258 / SEQ ID NO: 2190	c632.1V_H - V_L scFv of SEQ ID NOs: 2168 and 2169	CH1 stump long/ SEQ ID NO: 2180	c305.1 scFv/ SEQ ID NO: 2165
259 / SEQ ID NO: 2191	c632.1 V_L - V_H scFv of SEQ ID NOs: 2168 and 2169	CH1 stump short/ SEQ ID NO: 2181	c305.1 scFv/ SEQ ID NO: 2165
260 / SEQ ID NO: 2192	c305.1 scFv/ SEQ ID NO: 2165	$(G_4S)_2$/ SEQ ID NO: 2179	c632.1V_H - V_L scFv of SEQ ID NOs: 2168 and 2169
261 / SEQ ID NO: 2193	c632.1V_H- V_L scFv of SEQ ID NOs: 2168 and 2169	$(G_4S)_2$/ SEQ ID NO: 2179	c305.1 scFv/ SEQ ID NO: 2165
360 / SEQ ID NO: 2194	c472.2 V_L - V_H / SEQ ID NOs: 2170 and 2171	$(G_4S)_2$/ SEQ ID NO: 2179	c632.1V_H - V_L scFv of SEQ ID NOs: 2168 and 2169
361 / SEQ ID NO: 2195	c631.1V_H - V_L scFv of SEQ ID NOs: 2167 and 2166	$(G_4S)_2$/ SEQ ID NO: 2179	c472.2 V_L - V_H scFv of SEQ ID NOs: 2170 and 2171
362 / SEQ ID NO: 2196	c632.1V_H - V_L scFv of SEQ ID NOs: 2168 and 2169	$(G_4S)_2$/ SEQ ID NO: 2179	c472.2 V_L - V_H scFv of SEQ ID NOs: 2170 and 2171

Bispecific Single Chain FvFc (biscFvFc) Construction:

[359] Assembly of the scFv's c305.1, c631.1 and c632.1 for the biscFvFc constructs were described in the previous section. PCR fragments encoding each scFv and Fc5 fragment were cloned into the vector pZMP31, similar to the previous vector but without the ms 26-10VH signal sequence and Fc5. The ms 26-10VH signal sequence was added to each 5' scFv via oligo addition and PCR. The bispecifc cDNA's were inserted at a unique EcoRI restriction site. Three PCR fragments were generated using PCR conditions described in the tascFvFc section above, one for each scFv and one for the Fc5 region. The 5' scFv had oligo sequence overlapping the 5' non-translated region and Fc5. The Fc5 region had oligo sequence overlapping the 5' scFv and the carboxyl-terminal linker sequence. The 3' scFv had oligo sequence overlapping the carboxyl-terminal linker and the poliovirus internal ribosome entry site of pZMP31.

[360] The pZMP31 plasmid was digested with EcoRI prior to recombination in yeast with the gel-extracted anti-IL17A scFv, anti-IL23A scFv, and Fc5 PCR fragments. The recombination and transfer to E. coli of approximately10 µl of each gel-extracted DNA insert and approximately 100 ng vector was performed as described for tascFvFc in the paragraphs above. DNA sequence analysis on plasmid DNA from six E. coli colonies for each construct was performed as previously described.

Table 11: Bispecific Single Chain FvFc (biscFvFc) Construction (polynucleotide sequences)

MVC#/ SEQ ID NO:	**scFv1 (N-terminal to Fc) Template/ SEQ ID NO:**	**scFv2 (C-terminal to Fc) Template/ SEQ ID NO:**
247/ SEQ ID NO: 2197	c305.1 scFv/ SEQ ID NO: 2165	c631.1V_H - V_L scFv of SEQ ID NOs: 2167 and 2166
248/ SEQ ID NO: 2198	c631.1V_H- V_L scFv of SEQ ID NOs: 2167 and 2166	c305.1 scFv/ SEQ ID NO: 2165
249/ SEQ ID NO: 2199	c305.1 scFv/ SEQ ID NO: 2165	c631.1V_H - V_L scFv of SEQ ID NOs: 2167 and 2166
250/ SEQ ID NO: 2200	c631.1V_H - V_L scFv of SEQ ID NOs: 2167 and 2166	c305.1 scFv/ SEQ ID NO: 2165
262/ SEQ ID NO: 2201	c305.1 scFv/ SEQ ID NO: 2165	c632.1V_H - V_L scFv of SEQ ID NOs: 2168 and 2169
263/ SEQ ID NO: 2202	c632.1V_H - V_L scFv of SEQ ID NOs: 2168 and 2169	c305.1 scFv/ SEQ ID NO: 2165
264/ SEQ ID NO: 2203	c305.1 scFv/ SEQ ID NO: 2165	c632.1V_H - V_L scFv of SEQ ID NOs: 2168 and 2169
265/ SEQ ID NO: 2204	c632.1V_H - V_L scFv of SEQ ID NOs: 2168 and 2169	c305.1 scFv/ SEQ ID NO: 2165

* All biscFvFcs have the same Fc sequence (Fc_5, SEQ ID NO:333)

Bispecific Antibody (biAb) Construction:

[361] The biAb molecules were made by two constructs, heavy chains in the pZMP31 vector described in the biscFvFc section and light chains in a modified vector, pZMP31zeo. This vector was modified by the substitution of the zeoR gene for dhfr. Assembly of the scFv's c305.1, c631.1 and c632.1 for the biAb heavy chain constructs were described in the tascFvFc section. For construction of the biAb heavy chain, three PCR fragments were generated, heavy chain variable region, human wild type IgG1 constant region, and carboxyl-terminal scFv. The heavy variable region at the 5' end had oligo sequence overlapping the 5' non-translated region of the vector and the IgG1 fragment downstream. The IgG1 fragment had oligo sequence overlapping the variable heavy sequence and the carboxyl-terminal linker sequence. The 3' scFv had oligo sequence overlapping the carboxyl-terminal linker and the poliovirus internal ribosome entry site of pZMP31.

[362] For the light chains, there were two types of variable region, lambda (c305.1) or kappa (c632.1, and c631.1). All of the light chain variable regions were cloned into the vector, pZMP31zeo-kappa constant region, at a unique EcoRI. PCR fragments for each light chain variable region were generated with oligo sequence overlapping 5' non-translated region and the kappa constant region. The ms 26-10VH was added via oligo addition and PCR. For c305.1 an additional construct was made with the lambda variable region fused to lambda constant region inserted into pZMP31zeo signal sequence. Two PCR fragments were generated with the c305.1 lambda variable region fragment overlapping the 5' non-translated region and the lambda constant region and the lambda constant region fragment overlapping the c305.1 variable region and the poiliovirus internal ribosome entry site of pZMP31zeo. PCR reactions were carried out as described in the section on tascFvFc.

[363] pZMP31 or pZMP31-Zeo plasmid as appropriate was digested with EcoRI prior to recombination in yeast with gel-extracted PCR fragments of the biAb heavy chain or the biAb light chain fragments. Recombination and transfer to E. coli of approximately 10 µl of each gel-extracted DNA insert and approximately 100 ng vector was performed as described for tascFvFc in the paragraphs above. DNA sequence analysis on plasmid DNA from six E. coli colonies for each construct was performed as previously described.

Table 12: Bispecific Antibody (biAb) Construction Light Chain (polynucleotide sequences)

MVC#/ SEQ ID NO:	Template/ SEQ ID NO:	Constant Region
226/ SEQ ID NO: 2205	c305.1 V_L/ SEQ ID NO: 1904	Lambda
227/ SEQ ID NO: 2206	c305.1 V_L/ SEQ ID NO: 1904	Kappa
253/ SEQ ID NO: 2207	c631.1 V_L/ SEQ ID NO: 2166	Kappa
268/ SEQ ID NO: 2208	c632.1 V_L / SEQ ID NO: 2168	Kappa
281/ SEQ ID NO: 2209	c389.2 V_L/ 2246	Kappa

Table 13: Bispecific Antibody (biAb) Construction Heavy Chain with C-Terminus scFv (polynucleotide sequences)

MVC#/ SEQ ID NO:	V_H	scFv
MVC#/ SEQ ID NO:	**Template/ SEQ ID NO:**	**Template/ SEQ ID NO:**
251/ SEQ ID NO:2210	c305.1 V_H / SEQ ID NO: 1905	c631.1 V_H - V_L/ scFv of SEQ ID NOs: 2167 and 2166
252/ SEQ ID NO: 2211	c305.1 V_H / SEQ ID NO: 1905	c631.1V_H - V_L/ scFv of SEQ ID NOs: 2167 and 2166
254/ SEQ ID NO: 2212	c631.1V_H / SEQ ID NO: 2167	c305.1 scFv/ SEQ ID NO: 2165
255/ SEQ ID NO: 2213	c631.1V_H / SEQ ID NO: 2167	c305.1 scFv/ SEQ ID NO: 2165
266/ SEQ ID NO: 2214	c305.1 V_H / SEQ ID NO: 1905	c632.1 V_L - V_H/ scFv of SEQ ID NOs: 2168 and 2169
267/ SEQ ID NO: 2215	c305.1 V_H / SEQ ID NO: 1905	c632.1 V_L - V_H/ scFv of SEQ ID NOs: 2168 and 2169
269/ SEQ ID NO: 2216	c632.1 V_H/ SEQ ID NO: 2169	c305.1 scFv/ SEQ ID NO: 2165
270/ SEQ ID NO: 2217	c632.1 V_H/ SEQ ID NO: 2169	c305.1 scFv/ SEQ ID NO: 2165
164/ SEQ ID NO: 2218	c389.2 V_H / SEQ ID NO: 2173	c305.1 scFv/ SEQ ID NO: 2165

* All biAbs have the same IgG1 sequence, SEQ ID NO:334 for polynucleotide and SEQ ID NO: 335 for polypeptide

EXAMPLE 7

Expression of tascFv Fc and biscFv Fc molecules in CHO cells

[364] Four replicates of a 100 µg aliquot of a tasc or bisc construct are digested with 100 units of Pvu I at 37°C for three hours, precipitated with IPA, and spun down in a 1.5 mL microfuge tube. The supernatant is decanted off each pellet, 1 mL of 70% ethanol is added and then allowed to incubate for 5 minutes at room temperature. The tubes are spun in a microfuge for 5 minutes at

14,000 RPM and the supernatant decanted off the pellets. Each pellet is resuspended in 500 µl of ZF1 media in a sterile environment and allowed to incubate at room temperature for 30 minutes. 5E6 to 1E7 5xSA cells per replicate are spun down in each of four tubes and resuspended using the DNA-media solution. Each DNA/cell mixture is placed in a 0.4 cm gap cuvette and electroporated using the following parameters: 950 µF, high capacitance, and 300 V. The contents of the cuvettes are removed, pooled, diluted into a 125 mL shake flask containing 25 mLs of ZF1 media. The flask is placed in an incubator on a shaker at 37°C, 6% CO2, and shaking at 120 RPM. The cell line is then subjected to methotrexate (MTX) selection and expanded to higher volumes.

[365] Production of each molecule is accomplished by seeding a selected cell line into a 3L spinner flask at 4E5 cells/mL in 1500 mL of ZF1 media. The spinner is spun at 85 RPM for 120 hours at 37°C and 6% CO2, is harvested, filtered through a 1.2 µm and a 0.2 µm filter, and delivered for purification.

EXAMPLE 8

Expression of biAb molecules in CHO cells

[366] Four replicates of a 100 µg aliquot of biab DNA, comprised of 50 µg of heavy-chain plasmid and 50 µg of light-chain plasmid of a biab construct pair, are digested with 100 units of Pvu I at 37°C for three hours, precipitated with IPA, and spun down in a 1.5 mL microfuge tube. The supernatant is decanted off each pellet, 1 mL of 70% ethanol is added and then allowed to incubate for 5 minutes at room temperature. The tubes are spun in a microfuge for 5 minutes at 14,000 RPM and the supernatant decanted off the pellets. Each pellet is resuspended in 500 µl of ZF1 media in a sterile environment and allowed to incubate at room temperature for 30 minutes. 5E6 to 1E7 5xSA cells per replicate are spun down in each of four tubes and be resuspended using the DNA-media solution. Each DNA/cell mixture is placed in a 0.4 cm gap cuvette and electroporated using the following parameters: 950 µF, high capacitance, and 300 V. The contents of the cuvettes are removed, pooled, diluted into a 125 mL shake flask containing 25 mLs of ZF1 media. The flask is placed in an incubator on a shaker at 37°C, 6% CO2, and shaking at 120 RPM. The cell line is then subjected to methotrexate (MTX) selection and expanded to higher volumes.

[367] Production of a Biab is accomplished by seeding a selected cell line into a 3L spinner flask at 4E5 cells/mL in 1500 mL of ZF1 media. The spinner is spun for 120 hours at 85 RPM, 37°C, and 6% CO2, is harvested, filtered through a 1.2 µm and a 0.2 µm filter, and delivered for purification.

EXAMPLE 9

Purification of Bispecific Anti-IL17A/Anti-IL23 Molecules Produced in CHO Cells

[368] Conditioned media are harvested from cultures described in the previous example, sterile filtered using 0.2μm filters and adjusted to pH 7.4. The protein is purified from the filtered media using a combination of POROS® A50 Protein A Affinity Chromatography (Applied Biosciences, Foster City, CA) and Superdex 200 Size Exclusion Chromatography (GE Healthcare, Piscataway, NJ.) The 4ml POROS® A50 column (10mm x 50mm) is pre-eluted with three column volumes (CV) of 25 mM citrate-phosphate (1.61 mM sodium citrate – 23.4 mM sodium phosphate,) 250 mM ammonium sulfate pH 3 buffer and equilibrated with 20 CV 25 mM citrate-phosphate, 250 mM ammonium Sulfate pH 7.4. Direct loading to the column at 1500 cm/hr at 4°C captured the fusion proteins in the conditioned media. After loading is complete, the column is washed with 10 CV of 25 mM citrate-phosphate, 250 mM ammonium sulfate pH 7.4 buffer following which the bound protein is eluted at 1500 cm/hr with a 10 CV gradient from pH 7.4 to pH 3 formed using the citrate-phosphate-ammonium sulfate buffers. Fractions of 2.0 ml each are collected into tubes containing 200μl of 2.0 M Tris, pH 8.0 and mixed immediately in order to neutralize the eluted proteins. The fractions are pooled based on A280 and non-reducing SDS-PAGE.

[369] The target-containing pool is concentrated by ultrafiltration using Amicon Ultra-15 10K NWML centrifugal devices (Millipore), to < 3% of the volume of an appropriate size Superdex 200 column. The concentrate is injected to the size exclusion column equilibrated in 35 mM Sodium Phosphate, 120 mM NaCl pH 7.3 and eluted isocratically at 30 cm/hr. The fractions containing purified target are pooled based on A280 and SDS PAGE, filtered through a 0.2 μm filter and frozen as aliquots at –80oC. The concentration of the final purified protein is determined by UV absorption at 280 nm. The overall process recovery is approximately 50%.

Analysis of Purified Bispecific Anti-IL17A/Anti-IL23 Molecules

[370] The recombinant proteins are analyzed by SDS-PAGE (4-12% BisTris, Invitrogen, Carlsbad, CA) with 0.1% Coomassie R250 staining for protein and immunoblotting with Anti-IgG-HRP. The purified protein is electrophoresed and transferred to nitrocellulose (0.2 μm; Invitrogen, Carlsbad, CA) at ambient temperature at 600 mA for 45 minutes in a buffer containing 25 mM Tris base, 200 mM glycine, and 20% methanol. The filters are then blocked with 10% non-fat dry milk in 50 mM Tris, 150 mM NaCl, 5 mM EDTA, 0.05% Igepal (TBS) for 15 minutes at room temperature. The nitrocellulose is quickly rinsed, and the IgG-HRP antibody (1:10,000) is added. The blots are incubated overnight at 4°C, with gentle shaking. Following the incubation, the blots are washed three times for 10 minutes each in TBS, and then quickly rinsed in H2O. The blots are developed using commercially available chemiluminescent substrate reagents (Pierce SuperSignal), and the signal is

captured using ImageQuant instrument and software (GE Healthcare, Piscataway, NJ.) The purified proteins appeared as bands at the expected Molecular Weights on both the non-reducing Coomassie stained gel and on the immunoblot. The proteins had the correct NH2 termini and the correct amino acid compositions.

EXAMPLE 10

Identification of the Binding Epitopes of Anti-IL17 Molecules for Human IL-17A and Anti-IL23 Molecules for Human IL23A

A) Identification of the Binding Epitope of Anti-IL17 Molecules for Human IL-17A by JPT Peptide Microarray

[371] Background: IL-17A has the highest homology with IL-17F, as they are 50% identical at the protein level. The crystal structure of IL-17F has been solved and reveals that IL-17F is a structural homolog of the cysteine knot family of proteins and dimerizes in a parallel fashion (See 1. Hymowitz, D. G., et. al EMBO 20: 5332-5341 (2001), and Weaver, C. T., et. al Annu. Rev. Immunol 25: 821-852 (2007). In the binding epitope determination of anti-IL-17A molecules, the IL-17F structural information was utilized as a platform for the peptide peptide microarray epitope mapping data interpretation.

[372] The binding epitope of anti-IL-17A molecules were determined using a peptide microarray immunoassay. In this Example, the JPT Peptide Microarray Technology (JPT Peptide Technologies GmbH, Berlin, Germany) was used to evaluate three anti-IL-17A molecules: IL-17A scFv (A2027F, which is a c631scFv), IL-17A scFv (A2031F, which is a c632 scFv) and IL-17A bisc (A2103F, which is a c87/c305 biscFv-Fc 25mer, shown herein as MVC #177, having the amino acid sequence of SEQ ID NO: 2239).

[373] Epitope mapping was determined by detecting anti-IL-17A molecule binding specific peptides from human IL-17A on RepliTope ™ slides (JPT Peptide Technologies GmbH). Prior to running slides, each anti-IL-17A molecule was tested on a dot blot to determine approximate working concentrations of primary and secondary antibodies. Each experiment was run in duplicate.

[374] The RepliTope™ peptide microarray was prepared as a series of overlapping 15 amino acid peptide fragments of human IL-17A (11 aa overlaps and 4 aa shifts). Both control spots and peptides were printed in three identical replicate arrays per slide for intra-plate reproducibility. The control spots consisted of: human IgG, mouse IgG, anti – IL-17A scFv (A2027F), anti-IL-17A scFv (A2028F), anti-IL-23A/17A bisc and anti-IL-23A/IL-17A bisc (A2124F).

[375] The RepliTope ™ slides were incubated for one hour with anti-IL-17A molecules diluted with an Elisa Plate Block solution with 1% BSA. The scFv molecules contained a poly-

histidine affinity tag and anti-IL17A molecules (A2027F and A2031F) were stained with an anti-His6 secondary antibody as described in the method outline below. The anti-IL-17A molecule (A2103F) contained a human Fc domain that was used. A goat anti-human HRP fab fragment specific secondary antibody was used for detection. Following th tyramide amplification and TNT wash steps the slides were developed with DAKO permanent red by incubating at RT until spots became visible but before background staining developed (1-30 min). Color development was stopped by rinsing with dH2O. Slides were dried with nitrogen and peptide spots were visualized with both a bright field and a fluorescent microscope. Plate images were scanned under 4X magnification using fluorescence imaging and MetaMorph software version 7.1. Reactive peptide spots were determined by visual analysis.

[376] Epitope assignments were based on testing at least two concentrations (10 and 50 μg/mL) of binding molecules, with both spot intensity and appropriate control staining used as criteria for positive spot identification. Excessive test molecule concentrations caused non-specific staining of irrelevant IgG's in the control panel, so the working concentration of each molecule studied was carefully titrated.

[377] All of the positive control spots stained as expected. However, as stated, occasional non-specific interaction was observed when a high concentration of the test molecule was used.

Results:

[378] 1) Anti-IL-17A scFv (A2027F) bound to peptides #9 (TNTNPKRSSDYYNRS, SEQ ID NO: 2219) and #10 (PKRSSDYYNRSTSPW, SEQ ID NO: 2220) encompassing the putative intracellular loop between β-sheet (0) and α-helix and extending to α -helix region. These regions are predicted to be on the surface of the IL-17A. A part of the peptide #10 (SEQ ID NO:2220) (i.e., STSPW or from amino acid 11 to amino acid number 15 of SEQ ID NO: 2220) is known to be the surface of the bioactive of IL-17F. Hence, the binding of A2027F to the peptides #9 (TNTNPKRSSDYYNRS, SEQ ID NO: 2219) and #10 (PKRSSDYYNRSTSPW, SEQ ID NO: 2220) are considered to be specific.

[379] 2) Anti-IL-17A scFv (A2031F): In addition to peptides #9 (SEQ ID NO: 2219) and #10 (SEQ ID NO: 2220) , this molecule also binds to the peptide #7 (NLNIHNRNTNTNPKR , SEQ ID NO:2221), which is also predicted to be the surface of the IL-17A dimer. It is considered as a specific binding event.

[380] 3) Anti-IL-23A/IL-17A bisc (A2103F): The binding pattern is same as the anti-IL-17A scFv (A2027F).

Conclusion:

[381] The JPT Peptide Microarray system was used to identify the epitope for anti-IL-17A molecules binding to human IL-17A. Results obtained with purified anti- IL-17A molecules demonstrate that the combined peptide sequence of peptides # 9 and #10

(TNTNPKRSSDYYNRSTSPW, SEQ ID NO: 2222) is a common binding sequence recognized by all three anti-IL-17A molecules described herein. This sequence begins with a putative loop between β-sheet (0) and α-helix and it extends to α -helix. In addition to the peptide of SEQ ID NO: 2222, anti-IL-17A, scFv (A2031F) extends its binding to a predicted β sheet (0) and a front end of α -helix, which is the amino acid sequence of NLNIHNRNTNTNPKR, SEQ ID NO: 2221). Thus, these results show that for clones IL-17A scFv (A2027F), IL-17A scFv (A2031F) and IL-17A bisc (A2103F), the anti-IL-17A molecules recognizes at least one amino acid of SEQ ID NO: 2222. In addition, the IL-17A scFv (A2031F) recognizes at least one amino acid of SEQ ID NO:2221. As such, the invention provides anti-IL-17A antibodies that are capable of binding an immobilized peptide consisting of the amino acid sequence of TNTNPKRSSDYYNRSTSPW, SEQ ID NO: 2222. In an embodiment, said anti-IL-17A antibody is selected from the group consisting of: clone c631.1 VHVL scFv-pARB013 in zGold5 strain (MVE #228) was given ATCC Patent Deposit Designation PTA-8819; and clone c632.1 VLVH scFv-pARB013 in DH10B strain (MVE #258) was given ATCC Patent Deposit Designation PTA-8821); or clone c87 as described herein. The invention further provides anti-IL-17A antibodies that are additionally capable of binding an immobilized peptide consisting of the amino acid sequence of NLNIHNRNTNTNPKR, SEQ ID NO: 2221. Within an embodiment, the anti-IL-17A antibody is clone c632.1 VLVH scFv-pARB013 in DH10B strain (MVE #258) was given ATCC Patent Deposit Designation PTA-8821).

B) Identification of the Binding Epitope of Anti-IL23 Molecules for Human IL-23A by JPT Peptide Microarray

[382] Background: IL-23 is a binary complex consisting of a four-helix bundle cytokine (p19) and a soluble class I cytokine receptor (p40). The IL-23 structure closely resembles that of IL-12. They share the common p40 subunit, and IL-23 p19 overlaps well with IL-12 p35 (See Lupardus, P. J., et. al JMB 382: 931-941(2008). The p19 subunit of the IL-23 contains 178-aa residues in a four-helix bundle with α-helices named A, B, C, and D, whereas the p40 subunit is an elongated molecule consisting of three IgG-like domains, D1, D2, and D3, each forming β-strands. The A and D helices of the p19 subunit are known to interact with the p40 subunit. Crystal structures of IL-23 and its complex with a neutralizing antibody reveal that the binding site of the antibody is located exclusively on the p19 subunit (See Beyer, B. M., et. al JMB 382: 942-955 (2008). Therefore, only the p19 subunit was used for the binding epitope determination of anti-IL23 molecules.

[383] The binding sites of anti-IL-23 molecules were determined using a peptide microarray immunoassay. In this Example, the JPT Peptide Microarray Technology (JPT Peptide Technologies GmbH, Berlin, Germany) was used to evaluate two anti-IL-23 molecules: IL-23 scFv (A1996F, which is a c305 scFv), IL-23 scFv (A2088F, which is a c472 scFv).

[384] Epitope mapping was determined by detecting anti-IL-23 molecule binding to specific peptides from human IL-23(p19 subunit) on RepliTope ™ slides (JPT Peptide Technologies GmbH). Prior to running slides, each anti-IL-23 molecule was tested on a dot blot to determine approximate working concentrations of primary and secondary antibodies. Each experiment was run in duplicate. The optimal working concentration for A2088F was determined to be 0.05ug/ml. Each experiment was run in duplicate.

[385] The RepliTope™ peptide microarray was prepared as a series of overlapping 15 amino acid peptide fragments of human IL-23 containing11 aa overlaps and 4 aa shifts. Both control spots and peptides were printed as three identical replicate arrays per slide for intra-plate reproducibility. All peptides and the control antibodies including the binding molecules were covalently attached by selective immobilization chemistry using the amino-function of the lysine-side chains. The six control spots consisted of: human IgG, mouse IgG, anti- IL-23 scFv (A1996F), anti-IL-23 scFv (A2114F), anti-IL-23/17A bisc (A2107F) and anti-IL-23/IL-17A bisc (A2124F).

[386] The RepliTope ™ slides were incubated for one hour with anti-IL-23 molecules diluted with an Elisa Plate Block solution with 1% BSA. An anti-His6 secondary antibody was used for detection as described in the method outline below. Following the tyramide amplification and TNT wash steps, the slides were developed with DAKO permanent red by incubating at RT until spots became visible but before background staining developed (1-30 min). Color development was stopped by rinsing with dH2O. Slides were dried with nitrogen and peptide spots were visualized with both a bright field and a fluorescent microscope. Plate images were scanned under 4X magnification using fluorescence imaging and MetaMorph software version 7.1. Images were visualized to facilitate analysis of the anti-IL23 binding peptides using Adobe Photoshop. Binding peptide spots were determined by visual analysis.

[387] Epitope assignments were based on testing the binding molecules at a range of concentrations. Both spot intensity and appropriate control staining was used as criteria for positive spot identification. High test molecule concentrations caused non-specific staining of irrelevant IgG's in the control panel, so the working concentration of each molecule studied was carefully titrated.

[388] All of the positive control spots, anti- IL-23 scFv (A1996F), anti-IL-23 scFv (A2114F), anti-IL-23/17A bisc and (A2107F) and anti-IL-23/IL-17A bisc (A2124F) and the negative control spots, (human IgG and mouse IgG) stained as expected. However, as stated, occasional non-specific interaction was observed when a high concentration of the test molecule was used.

[389] The anti-IL-23 molecules used in this study were of the scFv forms.

Results:

[390] 1) Anti-IL-23 scFv (A1996F): The crystal structure of IL-23 (composed of a p19 and a p40 subunit) reveals that A and D helices of the p19 subunit interact with the p40 subunit and helices B and C are involved in receptor interaction. In addition, the crystal structure of IL-23

and its complex with a Fab (antigen-binding fragment) shows that the Fab binding site at the end of helix D and along the intra loop between B and C (2). Anti-IL-23 scFv binds to the peptides encompassing the putative α-helix B (peptide #19: LQRIHQGLIFYEKLL, SEQ ID NO:2223; peptide #20: HQGLIFYEKLLGSDI, SEQ ID NO: 2224; and peptide #21: IFYEKLLGSDIFTGE, SEQ ID NO: 2225), intra loop between α-helix B and C (peptide #22: KLLGSDIFTGEPSLL, SEQ ID NO: 2226), α-helix C (peptide # 25: SLLPDSPVGQLHASL, SEQ ID NO: 2227; peptide #26: DSPVGQLHASLLGLS, SEQ ID NO: 2228; and peptide #27: GQLHASLLGLSQLLQ, SEQ ID NO: 2229), intracellular loop between α -helix C and D (peptide #32: WETQQIPSLSPSQPW, SEQ ID NO: 2230) and extending to the part of α -helix D (peptide #33: QIPSLSPSQPWQRLL, SEQ ID NO: 2231) of the p19 subunit. These results indicate that binding site is centered on the intra loop between α –helices B and C and along the helix B and a portion of the helix D. This binding pattern is similar to the published results.

[391] 2) Anti-IL-23 scFv (A2088F): The binding pattern was similar to the anti-IL-23 scFv (A1996F). But this molecule extends its binding to the post α -helix C region (peptide # 28: ASLLGLSQLLQPEGH, SEQ ID NO: 2235).

Conclusion:

[392] The JPT Peptide Microarray system was used to determine the epitope binding characteristics of anti-IL-23 molecules to human IL-23 Results obtained with purified anti- IL-23 molecules demonstrate that the peptide sequence of LQRIHQGLIFYEKLLGSDIFTGE (SEQ ID NO: 2236) (α-helix B and a part of intra loop between α-helix Band C), the peptide sequence of SLLPDSPVGQLHASLLGLSQLLQPEG (SEQ ID NO: 2237) (entire α-helix C and post α-helix C), and the peptide sequence of WETQQIPSLSPSQPWQRLL (SEQ ID NO: 2238) (intra loop b/w C&D helixes and a part of a-helix D) are common binding sequences recognized by anti-IL-23 molecules.

[393] Thus these anti-IL23 antibodies bind at least one amino acid from the peptide sequence of LQRIHQGLIFYEKLLGSDIFTGE (SEQ ID NO: 2236) (α-helix B and a part of intra loop between α-helix Band C), and at least one amino acid from the peptide sequence of SLLPDSPVGQLHASLLGLSQLLQPEG (SEQ ID NO: 2237) (entire α-helix C and post α-helix C), and at least one amino acid from the peptide sequence of WETQQIPSLSPSQPWQRLL (SEQ ID NO: 2238). As such, the invention provides anti-IL-23A antibodies that are capable of binding an immobilized peptide consisting of the amino acid sequence of LQRIHQGLIFYEKLLGSDIFTGE (SEQ ID NO: 2236) (α-helix B and a part of intra loop between α-helix Band C), and an immobilized peptide consisting of the amino acid sequence of SLLPDSPVGQLHASLLGLSQLLQPEG (SEQ ID NO: 2237) (entire α-helix C and post α-helix C), and an immobilized peptide consisting of the amino acid sequence of WETQQIPSLSPSQPWQRLL (SEQ ID NO: 2238). In an embodiment, the antti-IL-23A antibody is selected from the group consisting of: clone c305 VHVL scFv-pARB013 in DH10B

strain (MVE #158) having ATCC Patent Deposit Designation PTA-8818; and clone c472.2 VLVH scFv-pARB013 in DH10B strain (MVE # 263) having ATCC Patent Deposit Designation PTA-8820.

EXAMPLE 11

Inhibition of Activation by Human IL-17A and Human IL-17AF in Murine Nih3t3 Cells Using an Antagonist to Human IL-17

A) Creation of a Stable Nih3t3 Assay Clone Expressing the nfkb Transcription Factor

[394] The murine nih3t3 cell line described above was stably transfected with the kz170 nfkb reporter construct, containing a neomycin-selectable marker. The Neo resistant transfection pool was plated at clonal density. Clones were isolated using cloning rings and screened by luciferase assay using the human IL-17 ligand as an inducer. Clones with the highest mean fluorescence intensity (MFI) (via NfkB luciferase) and the lowest background were selected. A stable transfectant cell line was selected and called nih3t3/kz170.

[395] Antibodies to human IL-17A were used as antagonists of human IL-17A or human IL-17AF activation of nfkb elements in a luciferase assay. In this assay, EC50 levels of human IL-17A- or IL-17AF-mediated nfkb activation in the murine nih3t3/kz170 assay cell line is measured. For highly effective antibodies, when used at approx. 10μg/mL concentration, the antibody completely neutralized activity induced by human IL-17A or IL-17AF, with the inhibition of activity decreasing in a dose dependent fashion at the lower concentrations. An isotype-matched negative control mAb, tested at the concentrations described above, provided no inhibition of activity. These results demonstrate that antibodies IL-17A are able to antagonize the activity of the pro-inflammatory cytokines, IL-17A and IL-17AF. Inhibition in this assay was not observed when anti-human antibodies to other human-specific ligand members of the IL-17 family, besides IL-17A, were added instead of the anti-human IL-17A antibody, including IL-17FF homodimer. There was also no neutralization to murine IL-17A or murine IL-17F for the anti-human IL-17A-containing antibodies described herein (data not shown), Tables 14 - 16 below shows representative example data for the ability of neutralizing IL-17A antagonist positive controls, and anti-IL-17A antibodies described herein. The data demonstrate that these neutralizing antibodies are able to reduce the activity induced by human IL-17A and human IL-17AF.

TABLE 14: Neutralization of human IL-17A-induced luciferase activity with IL-17A scFv molecules

scFv #	average IC50 (nM)
IL-17RA-Fc5	1.26
c416.1_1	1.8
c421.1_1	0.58
c421.1_2	1.1
c419.1_2	7.9
c559.1_4	3.7
c596.1_4	0.63
c631.1_2	0.57
c632.2_1	1.8
c632.1 ZGOLD	0.72
c632.1 ZGOLD	0.062

TABLE 15: Neutralization of human IL-17A-induced luciferase activity with anti-IL-17A/anti-IL-23p19 antibodies

MVC #	average IC50 (nM)	Construct
IL-17RA-Fc5 CONTROL	1.23	IL-17RA-Fc5 CONTROL
232	1.4	c305_6_c389 E.coli (A2016F)
173		c305_c87-Fc5 CHO (A2038F)
174		c87_c305-Fc5 CHO (A2039F)
174		c87_c305-Fc5 CHO (A2040F)
241	0.15	c305.1_c631.1 tascFv-Fc5 (long stump)
242	0.15	c305.1_c631.1 tascFv-Fc5 (short stump)
243	0.36	c631.1_c305.1 tascFv-Fc5 (long stump)
245	0.17	c305.1_c631.1 tascFv-Fc5 (10mer)
247	0.35	c305.1_c631.1 biscFv-Fc5 (5mer)
256	0.41	c305.1_c632.1 tascFv-Fc5 (long stump)
258	0.24	c632.1_c305.1 tascFv-Fc5 (long stump)
246	0.38	c631.1_c305.1 tascFv-Fc5 (10mer)
248	0.22	c631.1_c305.1 biscFv-Fc5 (5mer)
249	0.57	c305.1_c631.1 biscFv-Fc5 (10mer)
250	0.24	c631.1_c305.1 biscFv-Fc5 (10mer)
257	0.42	c305.1_c632.1 tascFv-Fc5 (short stump)
260	0.4	c305.1_c632.1 tascFv-Fc5 (10mer)
259	0.28	c632.1_c305.1 tascFv-Fc5 (short stump)
261	0.34	c632.1_c305.1 tascFv-Fc5 (10mer)
263	0.32	c632.1_c305.1 biscFv-Fc5 (5mer)
251+226	0.43	c305.1_c631.1 bi Ab

TABLE 16: Neutralization of human IL-17AF-induced luciferase activity with anti-IL-17A/anti-IL-23p19 antibodies

MVC #	average IC50 (nM)	Construct
IL-17RA-Fc5 CONTROL	does not neutralize (can not calculate IC50)	IL-17RA-Fc5 CONTROL
174	29	c87_c305-Fc5 CHO
241	8.2	c305.1_c631.1 tascFv-Fc5 (long stump)
242	8.0	c305.1_c631.1 tascFv-Fc5 (short stump)
243	14	c631.1_c305.1 tascFv-Fc5 (long stump)
245	6.3	c305.1_c631.1 tascFv-Fc5 (10mer)
247	15	c305.1_c631.1 biscFv-Fc5 (5mer)
256	1.2	c305.1_c632.1 tascFv-Fc5 (long stump)
258	0.44	c632.1_c305.1 tascFv-Fc5 (long stump)
246	11	c631.1_c305.1 tascFv-Fc5 (10mer)
248	6.1	c631.1_c305.1 biscFv-Fc5 (5mer)
249	35	c305.1_c631.1 biscFv-Fc5 (10mer)
250	6.8	c631.1_c305.1 biscFv-Fc5 (10mer)
257	0.99	c305.1_c632.1 tascFv-Fc5 (short stump)
260	0.58	c305.1_c632.1 tascFv-Fc5 (10mer)
259	0.33	c632.1_c305.1 tascFv-Fc5 (short stump)
261	0.28	c632.1_c305.1 tascFv-Fc5 (10mer)
263	0.39	c632.1_c305.1 biscFv-Fc5 (5mer)
251+226	11	c305.1_c631.1 bi Ab

[396] See Figure 11. Note: for Figure 11: "biscFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was ~0.14-0.2 nM in this assay; "biAb" was the MVC construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb) and the IC_{50} range for this molecule in this assay was ~0.09-0.15 nM; "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was 0.10-0.08 nM.

[397] See Figure 12. Note: for Figure 12: "biscFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was ~0.4-0.6 nM in this assay; "biAb" was the MVC construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb) and the IC_{50} range for this molecule in this assay was ~0.13-0.2 nM; "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was 0.2-0.28 nM.

[398] See Figure 13. Note: for Figure 13: "biscFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was ~0.16-0.21 nM in this assay; "biAb" was the MVC construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb) and the IC_{50} range for this molecule in this assay was ~0.35-0.65 nM; "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was 0.13-0.2 nM.

[399] In a similar assay run for 4 hours with nih3T3 cells transfected with kz170 (NfkB luciferase) reporter neutralizing 0.375 nM of human IL-17AA, the IC_{50} of the IL-17RA was 0.8nM; the IC_{50} of c631.1 alone was 0.6nM; and the IC_{50} of c632.1 was 0.6nM. Likewise, when the assay was run for 4 hours with NIH-3T3 cells transfected with kz170 (NfkB luciferase) reporter neutralizing 0.75 nM of human IL-17AF, the IC_{50} of c631.1 alone was 7.5 nM; and the IC_{50} of c632.1 was 0.8nM.

EXAMPLE 12

Neutralization of human IL-17A-induced pIkB activity with IL-17A scFv's

[400] The NIH-3T3/KZ142.8/huIL-17RCx4 transfected cell line was generated as described in WO 2005/123778, filed June 10, 2005. On day one NIH-3T3/KZ142.8/huIL-17RCx4 cells were plated out at 7,500 cells/well in growth media (DMEM with L-Glutamine plus 5% fetal bovine serum, 1% Sodium Pyruvate, 1μM MTX) in 96-well, flat-bottom tissue culture plates. On day two cells were switched to assay media (DMEM with L-Glutamine plus 0.1% BSA and 10mM HEPES). On day three serial dilutions of human IL-17A, IL-17F, or IL17AF (E.coli material and 293F B material, ZymoGenetics Inc., Seattle, WA) were made up in assay media and added to the plates containing the cells and incubated together at 37°C for 10 minutes. Additionally the assay was also used to measure neutralization of IL-17 activities. A sub-maximal concentration (either EC_{50} or EC_{90}, effective concentration at 50 and 90 percent, respectively) of IL-17A, IL-17F, or IL-17AF was combined with serial dilutions of the human IL-17RA-Fc soluble receptor (ZGI), anti-human IL-17A monoclonal antibody (R&D, MAB317), anti-human IL-17A polyclonal antibody (R&D, AF317NA), or the anti-IL-17A, anti-IL-17F, anti-IL-23p19, and anti-IL-17A/anti-IL-23p19 antagonists described herein, and incubated together at 37°C for 30 minutes in assay media prior to addition to cells. Following pre-incubation, treatments were added to the plates containing the cells and incubated together at 37°C for 10 minutes.

[401] Following incubation, cells were washed with ice-cold wash buffer and put on ice to stop the reaction according to manufacturer's instructions (BIO-PLEX Cell Lysis Kit, BIO-RAD Laboratories, Hercules, CA). 50 μL/well lysis buffer was added to each well; lysates were pipetted up and down five times while on ice, then agitated on a microplate platform shaker for 20 minutes at 300 rpm and 4°C. Plates were centrifuged at 4500 rpm at 4°C for 20 minutes. Supernatants were collected and transferred to a new micro titer plate for storage at -20°C.

[402] Capture beads (BIO-PLEX Phospho-Iκβ-α Assay, BIO-RAD Laboratories) were combined with 50 µL of 1:1 diluted lysates and added to a 96-well filter plate according to manufacture's instructions (BIO-PLEX Phosphoprotein Detection Kit, BIO-RAD Laboratories). The aluminum foil-covered plate was incubated overnight at room temperature, with shaking at 300 rpm. The plate was transferred to a microtiter vacuum apparatus and washed three times with wash buffer. After addition of 25 µL/well detection antibody, the foil-covered plate was incubated at room temperature for 30 minutes with shaking at 300 rpm. The plate was filtered and washed three times with wash buffer. Streptavidin-PE (50 µL/well) was added, and the foil-covered plate was incubated at room temperature for 15 minutes with shaking at 300 rpm. The plate was filtered and washed two times with bead resuspension buffer. After the final wash, beads were resuspended in 125 µL/well of bead suspension buffer, shaken for 30 seconds, and read on an array reader (BIO-PLEX, BIO-RAD Laboratories) according to the manufacture's instructions. Data were analyzed using analytical software (BIO-PLEX MANAGER 3.0, BIO-RAD Laboratories). Increases in the level of the phosphorylated Iκβ-α transcription factor present in the lysates were indicative of an IL-17A, IL-17F, or IL-17AF receptor-ligand interaction. For the neutralization assay, decreases in the level of the phosphorylated Iκβ-α transcription factor present in the lysates were indicative of neutralization of the IL-17 receptor-ligand interactions. IC_{50} (inhibitory concentration at 50 percent) values were calculated using GraphPad Prism®4 software (GraphPad Software, Inc., San Diego CA) and expressed as molar ratios for each reagent in the neutralization assay.

[403] IL-17A, IL-17F, and IL-17AF induced Iκβ-α phosphorylation in a dose dependent manner with an EC_{90} concentration determined to be approximately 0.05 – 0.5 nM. IL-17A was neutralized by the anti-human IL-17A polyclonal antibody (R&D Systems; Minneapolis, MN) at a 1:1 molar ratio, by the human IL-17RA-Fc soluble receptor ZymoGenetics) at a 7:1 molar ratio, and by the anti-human IL-17A monoclonal antibody at a 50:1 molar ratio. TABLEs 17 -19 below shows IC50 values of representative IL-17A neutralizing entities described herein, as compared to the IL-17RA-Fc and IL-17A polyclonal antibody controls. Results demonstrate that the anti-IL-17A clones were effective at reducing the signals induced by human IL-17A and IL-17AF. There was no neutralization activity against human IL-17F or murine IL-17A (data not shown).

TABLE 17: Neutralization of human IL-17A-induced pIkB activity with IL-17A scFv's

scFv #	average IC50 (nM)
IL-17 polyAb (R&D)	1.2
IL-17RA-Fc5	2.9
c555.1	30
c97.2.7	32
c202.1.6	101
c548.1	42
c550.1	35
c411.1_1	15
c416.1_1	13
c416.1_2	16
c421.1_1	15
c421.1_2	13
c425.2_1	19
c419.1_1	36
c419.1_2	18
c559.1_3	13
c559.1_4	16
c571.1_3	14
c424.1_2	Non neutral
c596.1_3	Non neutral
c596.1_4	23
c631.1_2	0.85
c632.2_1	1.16
c631.1 E.coli (A2027F)	0.36
c632.1 ZGOLD (A2028F)	0.74
c632.1 ZGOLD (A2029F)	0.84
c632.1 BL21 (A2031F)	0.53

TABLE 18: Neutralization of human IL-17A-induced pIkB activity with anti-IL-17A/anti-IL-23p19 antibodies

MVC #	average IC50 (nM)	Construct
IL-17RA-Fc5 CONTROL	3.1	IL-17RA-Fc5 CONTROL (ZymoGenetics)
IL-17 polyAb	1.2	IL-17 polyAb CONTROL (R&D)
232	1.3	c305_6_c389 E.coli ta scFv
173	4.1	c305_c87-Fc5 CHO
174	0.53	c87_c305-Fc5 CHO
174	0.76	c87_c305-Fc5 CHO
241	0.16	c305.1_c631.1 tascFv-Fc5 (long stump)
242	0.24	c305.1_c631.1 tascFv-Fc5 (short stump)
243	0.41	c631.1_c305.1 tascFv-Fc5 (long stump)
245	0.20	c305.1_c631.1 tascFv-Fc5 (10mer)
247	0.33	c305.1_c631.1 biscFv-Fc5 (5mer)
256	0.51	c305.1_c632.1 tascFv-Fc5 (long stump)
258	0.39	c632.1_c305.1 tascFv-Fc5 (long stump)
246	0.71	c631.1_c305.1 tascFv-Fc5 (10mer)
248	0.39	c631.1_c305.1 biscFv-Fc5 (5mer)
249	0.91	c305.1_c631.1 biscFv-Fc5 (10mer)
250	0.38	c631.1_c305.1 biscFv-Fc5 (10mer)
257	0.79	c305.1_c632.1 tascFv-Fc5 (short stump)
260	0.63	c305.1_c632.1 tascFv-Fc5 (10mer)
259	0.32	c632.1_c305.1 tascFv-Fc5 (short stump)
261	0.43	c632.1_c305.1 tascFv-Fc5 (10mer)
263	0.37	c632.1_c305.1 biscFv-Fc5 (5mer)
251+226	0.83	c305.1_c631.1 bi Ab

TABLE 19: Neutralization of human IL-17A-induced pIkB activity with IL-17A scFv's or IL-23A/IL-17A antagonists

scFv #	average IC50 (nM)
IL-17RA-Fc5	Non neutralizing (could not calculate)
c631.1 E.coli (A2027F)	16
c632.1 BL21 (A2031F)	1.1
c632.1_c305.1 tascFv-Fc5 (long stump) MVC #258	0.78

EXAMPLE 13

Neutralization of huIL-17-Induced Cytokine Production in Human Small Airway Epithelial Cells (SAEC)

[404] Treatment of human small airway epithelial cells (SAEC) with rhIL-17 induces the production of cytokines G-CSF, IL-6, and IL-8, which in turn, play a role in the pathology associated with the diseases for which an IL17/IL23p19 neutralizing entity would be efficacious. The ability of any of the neutralizing entities described herein to inhibit rhIL-17-mediated production of these cytokines was measured in this bioassay, thus being predictive of in vivo efficacy against these cytokines as well.

[405] Method: SAEC (cells and growth media purchased from Lonza Walkersville, Inc.) were plated at 8,000 cells/well in 96-well flat bottom tissue culture multi-well plates, and placed in a 37 degrees C, 5% CO2 incubator. The following day, cells were treated with a dose range of the neutralizing entity in combination with 0.25 – 0.5 nM rhIL-17. The ligand and neutralizing entity were incubated together for 30 minutes at 37 degrees C before adding to the cells. Duplicate or triplicate wells were set up for each dose. After 24 hours, supernatants were collected, and stored at -80 degrees C if not used directly. Before taking supernatants, wells were scanned by inverted microscope to make note of which wells had considerable cell death. Those wells were not included in the final calculations. Supernatants were then assayed for cytokines huG-CSF, huIL-6, and huIL-8 in a multiplex bead-based assay system (Bio-Rad Laboratories), and IC50 determined.

[406] Results: In the presence of rhIL-17, the neutralizing entitities described herein were efficacious at reducing huG-CSF cytokine production with IC50 values ranging from 0.01 – < 10 nM, as seen in the tables below. These data demonstrate that the IL-17A neutralizing entities were effective at neutralizing the biological effects of IL-17A on human primary cells.

Table 20: Neutralization of 0.25 nM IL-17A induced huG-CSF production in 24 hour SAEC supernatants with IL-17A scFv's

Neutralizing entity	IC50 (nM)
IL17A scFv # c631.1	0.30
IL17A scFv # c632.2	0.53
IL17A scFv # c421.1	5.24
IL17A scFv # c209.1	9.47
IL23/IL17A ta scFV #c305_6 and #c389	2.56

TABLES 21a and 21b: Neutralization of human IL-17A-induced huG-CSF production in 24 hour SAEC supernatants with anti-IL-17A/anti-IL-23p19 antibodies

MVC #	average IC50 (nM)	Construct
IL-17RA-Fc5 CONTROL	0.29	
174	1.56	c87_c305-Fc5 CHO (A2039F)
241	0.0077	c305.1_c631.1 tascFv-Fc5 (long stump)
242	0.019	c305.1_c631.1 tascFv-Fc5 (short stump)
243	0.073	c631.1_c305.1 tascFv-Fc5 (long stump)
245	0.013	c305.1_c631.1 tascFv-Fc5 (10mer)
247	0.062	c305.1_c631.1 biscFv-Fc5 (5mer)
256	0.108	c305.1_c632.1 tascFv-Fc5 (long stump)
258	0.015	c632.1_c305.1 tascFv-Fc5 (long stump)
246	0.013	c631.1_c305.1 tascFv-Fc5 (10mer)
248	0.022	c631.1_c305.1 biscFv-Fc5 (5mer)
249	0.095	c305.1_c631.1 biscFv-Fc5 (10mer)
250	0.025	c631.1_c305.1 biscFv-Fc5 (10mer)
257	0.081	c305.1_c632.1 tascFv-Fc5 (short stump)
260	0.191	c305.1_c632.1 tascFv-Fc5 (10mer)
259	0.014	c632.1_c305.1 tascFv-Fc5 (short stump)
261	0.016	c632.1_c305.1 tascFv-Fc5 (10mer)
263	0.042	c632.1_c305.1 biscFv-Fc5 (5mer)
251+226	0.053	c305.1_c631.1 bi Ab

[407] In this assay, the tasc, bisc, and BiAb molecules described herein showed the following values. See Table 21b, below

Table 21b:

Target	Construct	IL-17A SAEC Primary Cells (24hr) (nM)
IL-17A	IL-17RA	1.4
IL-17A	c632.1	2.7
IL-17A/IL-23	c632.1/c305 TascFv-Fc	0.01
IL-17A/IL-23	c305/c632.1 BiscFv-Fc	0.04
IL-17A/IL-23	c305/c632.1 BiAb	0.04

[408] These results show a 60-fold increase in IL-17A neutralization of the bispecific molecules over the monovalent scFv.

[409] See also Figure 14: Note: for Figure 14: "biscvFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer); "biAb" was the MVc construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb); "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer).

EXAMPLE 14

Determination of Ability of Anti-Human IL-17A Neutralizing Entities to Cross-React and Neutralize Cynomolgus Monkey IL-17A-Induced Activity in IL-17A Bioassays

[410] Species cross-reactivity studies (especially for non-human primate cross-reactivity) are an important activity to demonstrate for therapeutic antagonist development strategies. In order to determine whether the anti-human IL-17A neutralizing entities described herein may cross-react and neutralize the activity induced by cynomolgus IL-17A (and therefore, justify the cynomolgus monkey as a viable test species), it was first necessary to demonstrate comparable activities induced by both recombinant human and cynomolgus monkey IL-17A (ZymoGenetics, Inc., Seattle, WA) in the bioassays employed here for testing neutralization. The methods for IL-17A activity assays described in EXAMPLES 11, 12 and 13were used with a full range (0 – 112 nM) of either recombinant human IL-17A (ZymoGenetics, Inc.) or recombinant cynomolgus IL-17A. Results indicated that the activity induced by the human and cynomolgus IL-17A proteins used here were nearly identical in all three assays, yielding indistinguishable curves and EC50 values (identical between species for each experiments, and ranged from 0.22 – 0.41 nM amongst independent replicate experiments performed on different days).

[411] Human IL-17RA-Fc was able to neutralize the effects of either human IL-17A or cynomolgus IL-17A with IC50 values nearly identical for either species (i.e. 2.9 nM for neutralization against human IL-17A and 2.8 nM for cynomolgus IL-17A). The IL-17A neutralizing entities

described herein were also tested for species cross-reactivity of neutralizing ability and though there was a range of neutralizing capabilities, the antagonists that worked best to neutralize human IL-17A were also able to effectively neutralize activity induced by cynomologous IL-17A. Results are shown in Table 22. Thus, there was clearly primate species cross-reactivity in the ability of the anti-IL-17A neutralizing entities to inhibit IL-17A-mediated biological activity.

TABLE 22: Neutralization of cynomolgus IL-17A-induced luciferase activity

scFv #	average IC50 (nM)
IL-17RA-Fc5	0.72
c416.1_2	51
c421.1_2	6.5
c425.2_1	32
c559.1_3	218
c571.1_3	21
c631.1_2	0.8
c632.2_1	0.90

EXAMPLE 15

Neutralization of huIL17A-Induced G-CSF and IL-6 Cytokine Production in U373MG and U87MG Human Glioblastoma Cells

[412] rhIL-17A treatment induces the production of cytokine IL-6 in human glioblastoma cells U373MG, and of cytokines G-CSF and IL-6 in human glioblastoma cells U87MG. The cell lines are available from commercial vendors such as ATCC (Manassas, VA). These cytokines, in turn, play a role in the pathology associated with the diseases for which an IL-17A/IL23p19 antagonist would be efficacious. The ability of any of the antagonists described herein to inhibit rhIL-17A-mediated production of G-CSF and IL-6 was measured in this bioassay, thus being predictive of in vivo efficacy against this cytokine as well.

[413] Method: Cells were plated in media (MEM w/Earle's salts, 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 uM NEAA) at 3,000-7,000 cells/well in 96-well flat bottom tissue culture multi-well plates and placed in a 370C, 5% CO2 incubator from 1hr-O/N. Doses of the neutralizing entities were prepared in culture media with FCS concentration reduced to 2%. The cells were treated with a dose range of the neutralizing entity in combination with 0.20–0.5 nM rhIL-17A. The ligand and neutralizing entity were incubated together for 30 minutes at 370C before adding to the cells. Supernatants were collected after 24 hours and assayed for huG-CSF and huIL-6 using a bead-based assay system (Bio-Rad Laboratories), and IC50 determined.

[414] Results: In the presence of rhIL-17A, the IL-17A antagonists described herein were efficacious at reducing huIL-6 cytokine production by U373MG cells, with IC50 values ranging from

2.3-45 nM, as seen in the table below. For U87MG cells, neutralization of huG-CSF cytokine production with IC50 values ranging from 0.17-0.52 nM were obtained. These data demonstrate that the IL-17A neutralizing entities were effective at neutralizing the biological effects of IL17A on human glioblastoma cell lines.

Table 23

Neutralization of 0.2 nM hIL-17A induced huG-CSF production in 24hr U87MG supernatants		Neutralization of 0.5 nM hIL-17A induced huIL-6 production in 24hr U373MG supernatants	
Neutralizing entity	IC50 (nM)	Neutralizing entity	IC50(nM)
IL17A scFv 632.1	.17	IL17A scFv c631.1	2.6
IL-23/IL-17A c87_c305.1-Fc5 (MVC 174)	.52	IL17A scFv c632.1	2.3
		IL17AscFv c421.1	44.6
		IL-17A scFv c209.1	41.7

[415] See Figure 15. Note: for Figure 15: "biscvFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was ~0.085-0.15 nM in this assay; "biAb" was the MVc construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb) and the IC_{50} range for this molecule in this assay was ~0.08-0.3 nM; "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was 0.09-0.15 nM.

EXAMPLE 16

Measurement of Binding Affinities of Anti-IL-17A Molecules to IL-17A Via Surface Plasmon Resonance (Biacore)

[416] Anti-IL-17A entities described herein were evaluated for their binding affinities to IL-17A and IL-17AF using surface plasmon resonance and Biacore T-100 instrument (GE Healthcare). Recombinant human IL-17A or IL-17AF was immobilized to the sensor chip, followed by passing the antagonists over the immobilized ligand to attain affinity measurements. These methods allow for binding affinity measurements of the IL-17A neutralizing entities for their respective ligand (IL-17A) and also demonstrate specificity for IL-17A by displaying no binding to similar but different ligands, such as other members of the IL-17 family (including IL-17F).

[417] To determine the best conditions for immobilization, a series of pH scouting experiments were performed. For these experiments, recombinant human IL-17A (ZGI lot A1781F) was diluted to 100 nM in five different immobilization buffers; Acetate-4.0, Acetate-4.5, Acetate-5.0, Acetate-5.5, and Borate-8.5. Using Immobilization Scouting Wizard, these conditions were tested and at the end Acetate-4.0 was the best condition for immobilization.

[418] For the immobilization procedure, IL-17A or IL-17AF protein was diluted to 10 ug/ml in Acetate-4.5, and then immobilized onto a Series S Sensor Chip (CM5, GE Healthcare / Biacore #BR-1006-68) using the amine coupling kit and Biacore Immobilization Wizard. Briefly, the level of immobilization was targeted to 300 Biacore Resonance Units (RU), and IL-17A was only injected over an active flow cell. After the immobilization procedure, active sites on the flow cell were blocked with ethanolamine. Non-specifically bound protein was removed by washing with 50mM NaOH. The final immobilization level was 466 RU. The reference cell was activated and then blocked with ethanolamine.

[419] For the kinetic run, serial dilutions of the control proteins, IL-17RA-Fc (ZGI lot A1763F) or IL-17A monoclonal antibody (R&D MAB317), or anti-IL-17A neutralizing entities described herein, were prepared in 1X HBS-EP+ buffer. Duplicate injections of each concentration were performed. The analyte injections were at 30 ul/min with a dissociation time of 600 seconds. Buffer injections were also performed to allow for subtraction of instrument noise and drift.

[420] Regeneration buffers supplied in the Regeneration Scouting Kit (GE Healthcare / Biacore # BR-1005-56) were used to determine regeneration conditions. Standard Biacore methods were performed to define the best regeneration conditions for an IL-17A surface using IL-17RA-Fc at 100 nM. Mildest conditions were tested first and moved up in strength. The final regeneration condition chosen was a 1-60 sec pulse of 10mM H3PO4 followed by a 1-60 sec pulse of 1X HSB-EP buffer at 50 ul/min.

[421] As a check for IL-17A specific interactions, similar procedures were followed using immobilized recombinant human IL-17F (ZGI lot A1275F).

[422] Data were analyzed using Biacore Evaluation software (v.1.1.1) to define the kinetic values of the interaction of IL-17A with the control proteins (IL-17A monoclonal antibody and IL-17RA-Fc) and IL-17A neutralizing entities described herein. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Binding curves were normalized by double-referencing, and duplicate injection curves were checked for reproducibility. The resulting binding curves were globally fit to the bivalent interaction model.

[423] The data demonstrated high affinity binding of human IL-17A to the control antagonist proteins (IL-17RA-Fc and anti-IL-17A monoclonal antibody) and to the IL-17A neutralizing entities described herein. There were some IL-17A scFv's tested that also bound with high affinity to the IL-17AF heterodimer (c631 and c 632). There was, however, no binding of the

control IL-17A antagonist proteins or the anti-IL-17A neutralizing entities to IL-17F homodimer, thus providing evidence for specificity of targeting binding sites on IL-17A.

[424] Specifically, human IL-17A demonstrated dissociation equilibrium constants (KD) for IL-17R-Fc to be approximately 5 nM and approximately 2 nM for the IL-17A monoclonal antibody. Neutralizing entities described herein displayed a large range of affinities, but were well within this range (0.1 – <15 nM for IL-17A homodimer; 0.1 – 100 nM for IL-17AF heterodimer), thus demonstrating comparable binding affinities, albeit more diversity in IL-17AF heterodimer binding.

EXAMPLE 17

Off-Rate Analysis of Anti-IL-17A Molecules (from supernatants) to IL-17A Via Surface Plasmon Resonance (Biacore)

[425] Anti-IL-17A entities described herein were evaluated for binding off-rates to IL-17A and IL-17AF using surface plasmon resonance and Biacore T-100 instrument (GE Healthcare). Off-rate analysis is thought to help estimate the interaction that occurs in vivo, since a slow off-rate would predict a greater degree of interaction over long period of time. For these experiments, recombinant human IL-17A or IL-17AF was immobilized to the sensor chip, followed by passing the antagonists over the immobilized ligand to attain off-rate analyses.

[426] To determine the best conditions for immobilization, a series of pH scouting experiments were performed. For these experiments, recombinant human IL-17A (ZGI lot A1781F) was diluted to 100 nM in five different immobilization buffers; Acetate-4.0, Acetate-4.5, Acetate-5.0, Acetate-5.5, and Borate-8.5. Using Immobilization Scouting Wizard, these conditions were tested and at the end, Acetate-4.0 was the best condition for immobilization.

[427] For the immobilization procedure, native human IL-17A was diluted to 10ug/ml in Acetate-4.0 and was immobilized onto a Series S Sensor Chip (CM5, GE Healthcare / Biacore #BR-1006-68) using the amine coupling kit and Biacore Immobilization Wizard. Briefly, one flow cell was used as the reference, and therefore was only activated and then blocked with ethanolamine. The native form of IL-17A was immobilized to another flow cell with a target immobilization level of 300 Biacore Resonance Units (RU). Analytes were injected only over active flow cell. After the immobilization, the active sites on the flow cell were blocked with ethanolamine. Non-specifically bound protein was removed by washing with 50mM NaOH. The final immobilization levels fell between 331 and 497 RU.

[428] Samples of the anti-IL-17A neutralizing entity supernatants or proteins were diluted 1:3 in 1X HBS-EP+ buffer (GE Healthcare / Biacore, #BR-1006-69) supplemented with 0.1% BSA. IL-23R-Fc was also prepared at 100nM in 1X HBS-EP+, 0.1%BSA buffer as a positive control. The

analyte injections were at 30uL/min with association time of 300 seconds and dissociation time of 300 seconds. Media control injections were also performed to allow for subtraction of instrument noise and drift.

[429] Regeneration buffers supplied in a regeneration scouting kit were used to determine regeneration conditions. Using this procedure, the optimal regeneration condition was found to be 60 seconds of 10mM H3PO4 followed with 60 seconds of 1X HBS-EP+, 0.1% BSA buffer injections at a flow rate of 50ul/min.

[430] Data were evaluated using Biacore Evaluation software to define the off-rate of the interactions of anti-IL-17A neutralizing entities to immobilized IL-17A. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Binding curves were normalized by double-referencing and the resulting binding curves were globally-fit to the 1:1 binding interaction model using the Biacore Evaluation Software v1.1.1. The IL-17A neutralizing entities were ranked first according to their off-rates (slowest to fastest) and then to their binding levels (highest to lowest binding RUs) during the association phase. Off-rate values were also obtained. All samples were compared to the positive control, IL-17RA-Fc (off-rate of approximately 2.5 x 10-5 sec-1). Neutralizing entities described herein displayed a large range of off-rates to human IL-17A and human IL-17AF (0.01 to 10 x 10^{-5} sec-1).

EXAMPLE 18

Cell-Based Bioassay to Evaluate Activity of a Anti-Mouse IL-23p19 to Mouse IL-23

[431] A bioassay was previously developed for use in testing anti-IL-23 neutralizing antibodies by cloning the full-length human DCRS2 receptor (IL-23R, SEQ ID NO:9) along with endogenously expressed mouse IL-12RB1 into a BaF3 cell line to determine if mouse IL-23 can bind human DCRS2 and induce proliferation. Then a second proliferation assay was run with mouse IL-23 and a monoclonal antibody to mouse IL-23p19 to determine its ability to specifically neutralize mouse IL-23p19. An appropriate negative control cell line was used. See U.S. Patent Application Serial Number 11/762,738, filed June 13, 2007 for a detailed description of the construction of the Baf3/KZ134 cell line.

A) Alamar Blue Proliferation Assay to Determine Species Specificity of IL-23 on BaF3 cells Expressing Human DCRS2

[432] To determine whether human DCRS2 and mouse IL-12RB1 endogenously expressed in murine BaF3 cells can bind mouse IL-23, and perhaps human IL-23, an Alamar Blue proliferation assay was run. Recombinant human IL-23 (R&D Systems, Cat.#1290-IL) and mouse IL-23 (R&D

Systems, Cat.# 1887-ML) were run at concentrations of 200, 100, 50, 25, 12.5 , 6.3, and 3.1 ng/mL. A positive control of mouse IL-3 was run at concentrations of 20, 10, 5, 2.5, 1.25, 0.6, 0.3, and 0.15 pg/mL. Negative controls were run in parallel using mouse IL-3-free media only. Samples were plated into 96-well flat-bottomed plates (Bectin-Dickinson, Franklin Lakes, NJ) in a volume of 100 µL. The cells were washed 3 times in IL-3 free media and counted using a hemocytometer. Cells were resuspended in IL-3-free media and plated at a concentration of 5000 cells per well in 100 µL into the plate containing the samples for total well volume of 200 µL. The assay plates are incubated at 37°C, 5% CO_2 for 3 days at which time Alamar Blue (Accumed, Chicago, IL) is added at 20 µL/well. Alamar Blue gives a fluorometric readout based on number of live cells, and thus is a direct measurement of cell proliferation in comparison to a negative control. Plates are again incubated at 37°C, 5% CO_2 for 24 hours. Plates are read on the Wallac 1420 microplate reader (PerkinElmer Life Sciences, Boston, MA) at wavelengths 544 (Excitation), and 590 (Emission).

[433] Results show that both human and mouse IL-23 cause similar proliferative responses on the BaF3 WSX1/DCRS2 cell line. Human and mouse IL-23 were negative on the BaF3/KZ134 cell line, which shows that the response is limited to the presence of DCRS2 expression. A second BaF3 cell line expressing human DCRS2 was also tested and was found to proliferate in response to human and mouse IL-23, similar to the BaF3 WSX1/DCRS2 cell line.

B) Alamar Blue Proliferation Assay to Determine Effects of a Neutralizing Antibody to Mouse IL-23/p19 on BaF3 Cells Expressing Human DCRS2

[434] Another proliferation assay was run to test the ability of an antibody to neutralize mouse IL-23. The antibody tested was rat anti-mouse IL-23/p19 clone G23-8 (Cat.#16-723285, eBioscience, San Diego, CA). Mouse IL-23 concentrations of 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15, and 0.08 ng/mL were run. In order to determine whether the anti-IL-23/p19 antibody was specific for the p19 sub-unit and did not cross-react with with the IL-12/23p40 subunit, a range of recombinant mouse IL-23p40 concentrations (up to 200 ng/mL) was also tested. Concentrations of the anti-mouse IL-23/p19 antibody were run at 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15, and 0.08 ug/mL, and a negative control antibody, Rat IgG1 anti-mouse CD115 (Cat. #MCA1848, Serotec, Raleigh, NC) was also run at the same dilutions as the anti-mouse IL-23/p19 antibody. Mouse IL-23 was added to each well containing either the anti-mouse IL-23/p19 antibody or mouse CD115 antibody for a final concentration of 1.5 ng/mL mouse IL-23. Wells without antibody were also set up with mouse IL-23 at 1.5 ng/mL, which is approximately 80% of the maximum IL-23 response. A positive control of mouse IL-3 was run at concentrations of 20, 10, 5, 2.5, 1.25, 0.6, 0.3, and 0.15 pg/mL. Negative controls were run in parallel using mouse IL-3-free media only. Samples were plated into 96-well flat-bottomed plates (Bectin-Dickinson, Franklin Lakes, NJ) in a volume of 100 µL.

[435] The cells were washed 3 times in IL-3-free media and counted using a hemocytometer. Cells were resuspended in IL-3-free media and plated at a concentration of 5000 cells

per well in 100 µL into the plate containing the samples for a final well volume of 200 µL. The assay plates were incubated at 37°C, 5% CO_2 for 3 days at which time Alamar Blue (Accumed, Chicago, IL) was added at 20 µL /well. Alamar Blue gives a fluorometric readout based on number of live cells, and thus is a direct measurement of cell proliferation in comparison to a negative control. Plates were again incubated at 37°C, 5% CO_2 for 24 hours. Plates were read on the Wallac 1420 microplate reader (PerkinElmer Life Sciences, Boston, MA) at wavelengths 544 (Excitation), and 590 (Emission).

[436] The results show that the anti-mouse IL-23/p19 antibody neutralized the mouse IL-23 proliferative response in a dose-dependent manner. There was no neutralization of the recombinant mouse IL-12/IL-23p40, thus indicates that the anti-mouse IL-23p19 antibody is specific for the p19 subunit of IL-23. The CD115 antibody slightly inhibits the proliferative response at the highest concentration only. These assays show that the human DCRS2 receptor can cause signaling with mouse IL-23 in a BaF3 proliferation assay system and can be used to screen neutralizing antibodies to mouse IL-23. Furthermore, these results demonstrate that the anti-mouse IL-23p19 antibody is specific to the p19 subunit of mouse IL-23.

EXAMPLE 19

Cell-Based Bioassay to Evaluate Neutralizing Activity of an Anti-Human IL-23p19 to Human IL-23

[437] A bioassay for testing human IL-23p19 neutralizing antibodies was developed. An IL-3-dependent mouse cell line expressing human DCRS2 (IL-23R, 10) was tested with recombinant human IL-23 in a proliferation assay to determine whether the human DCRS2 receptor along with endogenously expressed mouse IL-12RB1 can bind human IL-23 and cause cell signaling and proliferation. As shown in Example 8 above, the cell line and resulting bioassay was found to be appropriate for the testing of both mouse and human IL-23 activity and IL-23 neutralizing activity. Therefore, once the ability of human IL-23 to bind human DCRS2 was established, as shown in that example, a second proliferation assay was run with human IL-23 and a monoclonal antibody to human IL-23/p19 to determine its ability and specificity to neutralize human IL-23p19.

A) Construction of BaF3 Cells Expressing Full - Length DCRS2 and Construction of Baf3/KZ134 Cell Line

[438] The BaF3 assay cell line was a previously utilized cell line and was chosen for use in these assays because it expresses human DCRS2. A previously utilized BaF3 cell line that does not express human DCRS2 was selected for use as a negative control. The assay cell line was constructed by sequentially placing an expression vector (pZP7Z) containing human WSX1 and an expression vector (pZP7NX) containing human DCRS2 into BaF3 cells. These expression vectors and

subsequent cell lines were built using the steps outlined in Example 18. The negative control cell line, BaF3/KZ134, was constructed using the steps outlined in Example 18.

B) Alamar Blue Proliferation Assay to Determine Effects of a Neutralizing Antibody to Human IL-23p19 on BaF3 cells Expressing Human DCRS2

[439] Another proliferation assay was run to test the ability of an antibody to neutralize human IL-23, via IL-23/p19. Recombinant human IL-23 concentrations of 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15, and 0.08 ng/mL were run. In order to determine whether the anti-IL-23p19 antibody was specific for the p19 subunit and did not cross-react with the IL-12/IL-23p40 subunit, a range of recombinant human IL-12 concentrations (up to 200 ng/mL) was also tested. Concentrations of the anti-human IL-23p19 antibody were run at 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15, and 0.08 ug/mL, and a negative control antibody was also run at the same dilutions as the anti-human IL-23/p19 antibody. Human IL-23 was added to each well containing either the anti-human IL-23/p19 antibody or negative control antibody for a final concentration of 1.5 ng/mL human IL-23. Wells without antibody were also set up with human IL-23 at 1.5 ng/mL, which is approximately 80% of the maximum IL-23 response. A positive control of human IL-3 was run at concentrations of 20, 10, 5, 2.5, 1.25, 0.6, 0.3, and 0.15 pg/mL. Negative controls were run in parallel using human IL-3-free media only. Samples were plated into 96-well flat-bottomed plates (Bectin-Dickinson, Franklin Lakes, NJ) in a volume of 100 µL.

[440] The cells were washed 3 times in IL-3-free media and counted using a hemocytometer. Cells were resuspended in IL-3-free media and plated at a concentration of 5000 cells per well in 100 µL into the plate containing the samples for a final well volume of 200 µL. The assay plates were incubated at 37°C, 5% CO_2 for 3 days at which time Alamar Blue (Accumed, Chicago, IL) was added at 20 µL /well. Alamar Blue gives a fluorometric readout based on number of live cells, and thus is a direct measurement of cell proliferation in comparison to a negative control. Plates were again incubated at 37°C, 5% CO_2 for 24 hours. Plates were read on the Wallac 1420 microplate reader (PerkinElmer Life Sciences, Boston, MA) at wavelengths 544 (Excitation), and 590 (Emission).

[441] The results show that the anti-human IL-23p19 antibody neutralizes the human IL-23 proliferative response in a dose-dependent manner. There was no neutralization of the exogenously added recombinant human IL-12, thus indicating that the anti-human IL-23p19 antibody is specific for the p19 subunit of IL-23. Therefore, these results demonstrate that the human DCRS2 receptor can cause signaling with human IL-23 in a BaF3 proliferation assay system and can be used to screen neutralizing antibodies to mouse IL-23. Furthermore, these results demonstrate that the anti-human IL-23/p19 antibody is specific to the p19 subunit of IL-23.

EXAMPLE 20

Neutralization of human IL-23-induced pSTAT3 activity with anti-IL-23A scFvs and with anti-IL-17A/anti-IL-23p19 bispecific antibodies

[442] The Baf3/KZ134/huIL-23R/huIL-12Rβ1 Clone 6 transfected cell line was generated as described herein. Baf3/KZ134/huIL-23R/huIL-12RB1 Clone 6 cells were washed two times with assay media (RPMI 1640 with L-Glutamine plus 10% fetal bovine serum, 1% Sodium Pyruvate, and 2uM β-Mercaptoethanol) before being plated out at 30,000 cells/well in 96-well, round-bottom tissue culture plates. Serial dilutions of recombinant human IL-23 (CHO material, ZymoGenetics Inc., Seattle, WA,) or eBioscience's Insect heterodimer material, San Diego, CA) were made up in assay media and added to the plates containing the cells and incubated together at 37°C for 15 minutes. Additionally the assay was also used to measure neutralization of IL-23 activity. A half maximal concentration (EC_{50}, effective concentration at 50 percent) of IL-23 was combined with serial dilutions of anti-human IL-12 p40 monoclonal antibody (Pharmingen, Franklin Lakes, NJ), anti-human IL-23 p19 polyclonal antibody (R&D, AF1716), and human IL-23R-Fc Soluble Receptor (ZymoGenetics Inc., Seattle, WA,) and incubated together at 37°C for 30 minutes in assay media prior to addition to cells. Following pre-incubation, treatments were added to the plates containing the cells and incubated together at 37°C for 15 minutes.

[443] Following incubation, cells were washed with ice-cold wash buffer and put on ice to stop the reaction according to manufacturer's instructions (BIO-PLEX Cell Lysis Kit, BIO-RAD Laboratories, Hercules, CA). Cells were then spun down at 2000 rpm at 4°C for 5 minutes prior to dumping the media. 50 µL/well lysis buffer was added to each well; lysates were pipetted up and down five times while on ice, then agitated on a microplate platform shaker for 20 minutes at 300 rpm and 4°C. Plates were centrifuged at 4500 rpm at 4°C for 20 minutes. Supernatants were collected and transferred to a new micro titer plate for storage at -20°C.

[444] Capture beads (BIO-PLEX Phospho-STAT3 Assay, BIO-RAD Laboratories) were combined with 50 µL of 1:1 diluted lysates and added to a 96-well filter plate according to manufacture's instructions (BIO-PLEX Phosphoprotein Detection Kit, BIO-RAD Laboratories). The aluminum foil-covered plate was incubated overnight at room temperature, with shaking at 300 rpm. The plate was transferred to a microtiter vacuum apparatus and washed three times with wash buffer. After addition of 25 µL/well detection antibody, the foil-covered plate was incubated at room temperature for 30 minutes with shaking at 300 rpm. The plate was filtered and washed three times with wash buffer. Streptavidin-PE (50 µL/well) was added, and the foil-covered plate was incubated at room temperature for 15 minutes with shaking at 300 rpm. The plate was filtered and washed two times with bead resuspension buffer. After the final wash, beads were resuspended in 125 µL/well of bead suspension buffer, shaken for 30 seconds, and read on an array reader (BIO-PLEX, BIO-RAD Laboratories) according to the manufacture's instructions. Data were analyzed using analytical

software (BIO-PLEX MANAGER 3.0, BIO-RAD Laboratories). Increases in the level of the phosphorylated STAT3 transcription factor present in the lysates were indicative of an IL-23 receptor-ligand interaction. For the neutralization assay, decreases in the level of the phosphorylated STAT3 transcription factor present in the lysates were indicative of neutralization of the IL-23 receptor-ligand interaction. IC_{50} (inhibitory concentration at 50 percent) values were calculated using GraphPad Prism®4 software (GraphPad Software, Inc., San Diego CA) and expressed as molar ratios for each reagent in the neutralization assay.

[445] IL-23 induced STAT3 phosphorylation in a dose dependent manner with an EC_{50} concentration determined to be 20 pM for the ZymoGenetics Inc, material and 30 pM for eBioscience heterodimer. Tables 24 and 25 present example IC50 data for the IL-23 positive controls (IL-23 p19 polyAb and IL-23R-Fc) and IL-23 neutralizing entities described herein. These data indicate that the IL-23 neutralizing entities were efficacious and were equally or better at reducing the effects of IL-23 as the controls.

TABLE 24: Neutralization of human IL-23-induced pSTAT3 activity with IL-23A scFv's

scFv #	average IC50 (nM)
IL-23 polyAb (R&D Systems)	55
IL-23R-Fc CONTROL (A1913F)	1.71
c305.1_2 (SQ22)	0.14
c305.1	0.13
c305.1	0.19
c305.1	0.15
c305-Fc5 293	0.04
c305 monomer 293	0.12
c304 (SQ22)	31
c272.1	25
c29.4_1	.31
c305.2_1	0.14
c472.2_7	.066

TABLE 25: Neutralization of human IL-23-induced pSTAT3 activity with anti-IL-17A/anti-IL-23p19 antibodies

MVC #	average IC50 (nM)	Construct
IL-23p19 polyAb	55	IL-23p19 polyAb CONTROL (R&D)
IL-23R-Fc5	1.9	IL-23R-Fc5 CONTROL (ZGI)
232	0.32	c305_6_c389 E.coli (A2016F)
173	0.065	c305_c87-Fc5 CHO (A2038F)
174 (first lot)	0.084	c87_c305-Fc5 CHO (A2039F)
174 (second lot)	0.11	c87_c305-Fc5 CHO (A2040F)
241	0.029	c305.1_c631.1 tascFv-Fc5 (long stump)
242	0.058	c305.1_c631.1 tascFv-Fc5 (short stump)
243	0.082	c631.1_c305.1 tascFv-Fc5 (long stump)
245	0.052	c305.1_c631.1 tascFv-Fc5 (10mer)
247	0.052	c305.1_c631.1 biscFv-Fc5 (5mer)
256	0.050	c305.1_c632.1 tascFv-Fc5 (long stump)
258	0.040	c632.1_c305.1 tascFv-Fc5 (long stump)

EXAMPLE 21

Bioassays to Measure Neutralization of IL-23 activity

[446] **Leukopheresis PBMC:** To obtain a consistent pool of PBMC's, normal human donors were voluntarily apheresed. The leukopheresis PBMC were poured into a sterile 500 ml plastic bottle, diluted to 400 ml with room temperature PBS + 1 mM EDTA and transferred to 250 ml conical tubes. The 250 ml tubes were centrifuged at 1500 rpm for 10 minutes to pellet the cells. The cell supernatant was then removed and discarded. The cell pellets were then combined and suspended in 400 ml PBS + 1 mM EDTA. The cell suspension (25 ml/tube) was overlaid onto Ficoll (20 ml/tube) in 50 ml conical tubes (total of 16 tubes). The tubes were centrifuged at 2000 rpm for 20 minutes at room temperature. The interface layer ("buffy coat") containing the white blood cells and residual platelets was collected, pooled and washed repeatedly with PBS + 1 mM EDTA until the majority of the platelets had been removed. The white blood cells were then suspended in 100 ml of ice-cold Cryopreservation medium (70% RPMI + 20% FCS + 10% DMSO) and distributed into sterile cryovials (1 ml cells/vial). The cryovials were placed in a -80° C freezer for 24 hours before transfer to a liquid-nitrogen freezer. The white blood-cell yield from a typical apheresis is 0.5 – 1.0 x

10^{10} cells. Apheresis cells processed in this manner contain T cells, B cells, NK cells, monocytes and dendritic cells.

[447] **Preparation of PHA blasts:** T cells must be activated in order to express the IL-12 receptor and be able to respond to IL-12 and IL-23. In this assay, they are atctivated with phytohemagglutinin (a polyclonal antibody T cell activator). Cryopreserved leukopheresis PBMC were thawed, transferred to a sterile 50 ml conical tube, washed once with 50 ml of warm RPMI + 10% heat-inactivated FBS + 1 ug/ml DNAse I (Calbiochem, San Diego, CA), resuspended in 50 ml of fresh RPMI/FBS/DNAse medium and incubated in a 37° F water bath for at least 1 hour to allow the cells to recover from being thawed. The cells were then centrifuged and the cell-supernatant discarded. The cell pellet was resuspended in RPMI + 10% FBS and distributed into sterile 75 cm^2 tissue culture flasks (1 x 10^7 cells/flask in 40 ml/flask). PHA-L (5 mg/ml stock in PBS) was added to the cells at a final concentration of 5 ug/ml. The cells were then cultured at 37°C in a humidified incubator for a total of 5 days. The cells were "rested" for some experiments by harvesting the cells on the afternoon of day 4, replacing the culture medium with fresh RPMI + 10% FBS without PHA-L (40 ml/flask) and returning the cells to their flasks and incubating at 37°C the cells in a humidified incubator for the remainder of the 5 day culture period.

[448] **IL-12 and IL-23 bioassays:** Four in vitro assays for detection of human IL-12 and IL-23 bioactivity on normal human T cells have been established: 1) IFN-gamma and MIP-1alpha production, 2) proliferation ([3H]-incorporation) 3) IL-17A and IL-17F production (IL-23 only) and 4) STAT3 activation. Human PHA blasts (activated T cells) were harvested on day 5 of culture, suspended in fresh RPMI + 10% FBS and plated at the desired cell number per well in 96 well plates.

[449] **IFN-gamma Production:** For the IFN-gamma production assay, the cells were plated at 1 x 10^6/well in flat-bottom 96-well plates. The cells were cultured at 37° C in a final volume of 200 ul/well with either medium alone, human IL-2 alone (10 ng/ml; R & D Systems, Minneapolis. MN), human IL-12 alone (graded doses; Invitrogen, Carlsbad, CA), human IL-23 alone (graded doses; CHO-derived, ZymoGenetics, Inc., Seattle, WA), anti-human CD28 mAb alone (graded doses; clone 28.2, e-Biosciences, San Diego, CA), or each cytokine in combination with anti-human CD28 mAb. Triplicate wells were set up for each culture condition. For the IFN-gamma production assay, cell supernatants (120 ul/well) were harvested after 24 – 48 hours of culturing the cells at 37°C in a humidified incubator. Human IFN-gamma and MIP-1alpha concentrations in these supernatants (pooled for each triplicate) were measured using a commercial Luminex bead-based ELISA kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions.

[450] Effects of IL-23 on IFN-gamma and MIP-1alpha production were enhanced by culturing the cells with plate-immobilized anti-human CD3 mAb (5ug/ml) and soluble anti-human CD28mAb (1ug/ml) as well as harvesting the supernatants (120ul/well) after 48 hrs of culture at 37°C the cells in a humidified incubator. Human IFN-gamma concentrations in these supernatants (pooled

for each triplicate) were measured using a commercial Luminex bead-based ELISA kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions.

[451] [3H]-incorportaion Assay: For the [3H]-incorporation assay the cells were plated at 2 x 10^5 cells/well in U-bottom 96-well plates. The cells were cultured at 37 degrees C for 72 hours. The cells were pulsed with 1 uCi/well of [^{3}H]-Thymidine (Amersham, Piscataway, NJ) for the last 8 hours of this culture period. The cells were then harvested onto glass-fiber filters and the CPMs of [^{3}H] incorporated were quantitated using a beta counter (Topcount NXT, Packard, Waltham, MA).

[452] For each of these above endpoint parameters, there was neutralization of activity mediated by IL-23 in the presence of anti-IL23p19 neutralizing entities described herein at IC50 values that ranged from 0.5 to ~100 nM. If IC50 values could not be obtained from the curves, percent inhibition was determined. In this case, IL-23p19 antagonists were able to neutralize with percent inhibitions ranging from 20 – 80% and were comparable with positive control neutralizers (IL-23R-Fc5 (ZymoGenetics); IL-23p19 polyclonal Ab (R&D)). Molecules that contained cluster i.d. c305 or c472 were the best neutralizers and had IC50 values or percent inhibition values in the lower range. There was no effect of the anti-IL-23p19 antagonists on neutralizing the effects mediated by IL-12, indicating specificity of the antagonists to IL-23p19.

[453] IL-17A, IL-17F, and IL-17AF Production: For the IL-17A and IL-17F production assay, the CD4+ cells isolated (Miltenyi Biotec MACS Cell Separation Kit for negative CD4+ cell isolation) from whole PBMCs and were plated at 1 x 105/well in round-bottom 96-well plates. Anti-human CD3 mAb (5.0ug/ml from BD Bioscience) in 50ul/well 2-4hr to bind the anti-CD3 to the plate and washed with PBS. Then cells were cultured for 48 hours at 37o C in a final volume of 100 ul/well with a Th17 condition media of anti-human CD28 mAb (1.0ug/ml from BD Bioscience), anti-human IL-4 mAb (2.0ug/ml from BD Bioscience), anti-human IFN-gamma mAb (2.0ug/ml from BD Bioscience), recombinant human IL-1alpha (1.0ng/ml; R&D Systems), recombinant human IL-1beta (5.0ng/ml, R&D Systems). After 48hours, cells were centrifuged, existing media removed, and cells washed once with RPMI complete. Human IL-23 (graded doses; ZGI; CHO-derived) was added to the wells in a final volume of 200ul/well. Duplicate or triplicate wells were set up for each culture condition. For the IL-17A, IL-17F, and IL-17AF production assay, cell supernatants (175 ul/well) were harvested after 48 hours of culturing the cells at 37°C in a humidified incubator. Human IL-17A, IL-17F and IL-17AF concentrations in these supernatants were measured using an in-house bead-based Luminex assay.

[454] Effects of IL-23 on IL-17A, IL-17F and IL-17AF production were enhanced and background levels were decreased by skewing the cells for 48 in media optimized for Th17 development prior to washing cells and adding IL-23 +/- IL-23 antagonists. The media contained anti-CD3, anti-CD28, anti-IL-4, anti-IFN-gamma, rhIL-1alpha, and rhIL-1beta. Antagonists were pre-incubated with IL-23 for 30 min at 37oC and then added to the cells together after the 48hour skewing

period. There was neutralization of activity mediated by IL-23 in the presence of anti-IL-23A or IL-23A/IL-17A neutralizing entities described herein at IC50 values that ranged from 0.5 to ~100 nM. If IC50 values could not be obtained from the curves, percent inhibition was determined. In this case, IL-23p19 antagonists were able to neutralize with percent inhibitions ranging from 20 – 100% and were comparable with positive control neutralizers (IL-23R-Fc5 (ZymoGenetics); IL-23p19 polyclonal Ab (R&D)). Molecules that contained cluster i.d. c305 or c472 were the best neutralizers and had IC50 values or percent inhibition values in the lower range. There was no effect of the anti-IL-23A or IL-23A/IL-17A antagonists on neutralizing the effects mediated by IL-12, indicating specificity of the antagonists to the p19 subunit of IL-23 (IL-23A).

[455] **Phosphorylated STAT3 Bioassay:** For the IL-12- or IL-23 mediated phosphorylated STAT3 bioassay, cells were plated at 2 x 10^5 cells/well in U-bottom 96-well plates. Serial dilutions of human IL-12 (R&D, Minneapolis, MN) or recombinant human IL-23 (CHO-derived material, ZymoGenetics Inc, Seattle, WA, or eBioscience's Insect heterodimer material, San Diego, CA) were prepared in assay media (RPMI 1640 with L-Glutamine plus 10% fetal bovine serum), added to the plates containing the cells and incubated together at 37°C for 15 minutes. Additionally, the assay was also used to measure neutralization of IL-12 and IL-23 activity using either commercially-available neutralizing reagents (as "controls") or the anti-IL-23p19-containing neutralizing entities described herein. A half-maximal concentration (EC_{50}, effective concentration at 50 percent) of IL-12 or IL-23 were combined with serial dilutions of anti-human IL-12 p40 monoclonal antibody (Pharmingen, Franklin Lakes, NJ), anti-human IL-23 p19 polyclonal antibody (R&D, AF1716), human IL-23R-Fc Soluble Receptor (ZymoGenetics Inc.), or any of the neutralizing entities described herein, and incubated together at 37°C for 30 minutes in assay media prior to addition to cells. Following pre-incubation, treatments were added to the plates containing the cells and incubated together at 37°C for 15 minutes.

[456] Following incubation, cells were washed with ice-cold wash buffer and put on ice to stop the reaction, according to manufacturer's instructions (BIO-PLEX Cell Lysis Kit, BIO-RAD Laboratories, Hercules, CA). Cells were then spun down at 2000 rpm at 4°C for 5 minutes prior to removing the media. 50 ul/well lysis buffer was added to each well; lysates were pipetted up and down five times while on ice, then agitated on a microplate platform shaker for 20 minutes at 300 rpm and 4°C. Plates were centrifuged at 4500 rpm at 4°C for 20 minutes. Supernatants were collected and transferred to a new micro titer plate for storage at -20°C until analyzed.

[457] Capture beads (BIO-PLEX Phospho-STAT3 Assay, BIO-RAD Laboratories) were combined with 50 ul of 1:1 diluted lysates and added to a 96-well filter plate according to manufacture's instructions (BIO-PLEX Phosphoprotein Detection Kit, BIO-RAD Laboratories). The aluminum foil-covered plate was incubated overnight at room temperature, with shaking at 300 rpm. The plate was transferred to a microtiter vacuum apparatus and washed three times with wash buffer.

After addition of 25 µL/well detection antibody, the foil-covered plate was incubated at room temperature for 30 minutes with shaking at 300 rpm. The plate was filtered and washed three times with wash buffer. Streptavidin-PE (50 ul/well) was added, and the foil-covered plate was incubated at room temperature for 15 minutes with shaking at 300 rpm. The plate was filtered and washed two times with bead resuspension buffer. After the final wash, beads were resuspended in 125 ul/well of bead suspension buffer, shaken for 30 seconds, and read on an array reader (BIO-PLEX, BIO-RAD Laboratories) according to the manufacture's instructions. Data were analyzed using analytical software (BIO-PLEX MANAGER 3.0, BIO-RAD Laboratories).

[458] Increases in the level of the phosphorylated STAT3 transcription factor present in the lysates were indicative of an IL-12 or IL-23 receptor-ligand interaction. For the neutralization assay, decreases in the level of the phosphorylated STAT3 transcription factor present in the lysates were indicative of neutralization of the IL-12 or IL-23 receptor-ligand interaction. IC_{50} (inhibitory concentration at 50 percent) values were calculated using GraphPad Prism®4 software (GraphPad Software, Inc., San Diego CA) and expressed as molar ratios for each reagent and/or neutralizing entity in the neutralization assay.

[459] IL-12 and IL-23 both induced STAT3 phosphorylation in a dose dependent manner with variation from donor to donor in PHA-activated human T cells. EC50 values for IL-23 were in the range of 12 – 53 pM. IL-12 and IL-23 were both neutralized by the anti-human IL-12 p40 monoclonal antibody, whereas only IL-23 was neutralized by the anti-human IL-23 p19 polyclonal antibody and human IL-23R-Fc controls, and by the anti-IL23p19 neutralizing entities described herein.

[460] For just one example, for one donor an EC_{50} concentration was determined to be 200pM for IL-12 and 20pM for IL-23 (using ZGI CHO-derived protein, ZymoGenetics Inc., Seattle, WA) and 30pM for IL-23 (using the eBioscience heterodimer). IL-12 and IL-23 were both neutralized by the commercially available anti-human IL-12 p40 monoclonal antibody. Only IL-23 was neutralized by the commercially available anti-human IL-23 p19 polyclonal antibody and human IL-23R-Fc soluble receptor (as "controls"), indicating that this assay is specific for its intended cytokine activity/neutralization use. Results indicated that the efficacious neutralizing entities described herein were equally or better than the commercially available reagents at neutralizing the effects of rhIL-23. (see tables below for representative example neutralizing values). Also see Figure 6. Furthermore, results indicated that the neutralizing entities specifically inhibited rhIL-23 and not IL-12 (data not shown; all data graphs indicated absolutely no neutralizing ability of anti-IL-23p19 clones against IL-12 induced activity).

[461] Also see Figure 6. Note: for Figure 6: "biscvFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was ~0.065-0.09 nM in this assay; "biAb" was the MVc construct #266 and #226 described herein

(c305Mab/lambda-c632scFv biAb) and the IC_{50} range for this molecule in this assay was ~.01-0.19 nM; "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was 0.055-0.08 nM.

TABLE 26: Neutralization of human IL-23-induced pSTAT3 activity in human PHA blasts with IL-23A scFv's

scFv #	average IC50 (nM)
IL-23R-Fc CONTROL (A1913F)	1.87
c305.1 (A1996F)	0.12
c305-Fc5 293 (A2020F)	0.090
p40 mAb	0.041
c472.2_7	0.091

TABLE 27: Neutralization of human IL-23-induced pSTAT3 activity in human PHA blasts with IL-23A/IL-17A antagonists

MVC #	A lot #	average IC50 (nM)	Construct
IL-23R-Fc5		1.87	IL-23R-Fc5 CONTROL
174	A2039F	0.122465	c87_c305-Fc5 CHO (A2039F)
258	A2069F	0.051 nM	c632.1_c305.1 tascFv-Fc5 (long stump)

[462] A similar assay was perfomed in which bsAbs were pre-incubated with high concentraions of hIL-17A (30 nM) for 30 minuntes at 37°C prior to 15 min pSTAT3 assay. Results are shown in Figure 7. Note: for Figure 7: "biscvFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was ~0.055-0.082 nM in this assay; "biAb" was the MVc construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb) and the IC_{50} range for this molecule in this assay was ~.01-0.17 nM; "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was 0.095-0.13 nM.

Human NK cell IL-23 Bioassay:

[463] Human PBMCs from Donor #E012 were used from a Leukopheresis stock. CD56+ cells were positively selected from PBMCs using a Miltenyi isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). NK cells were activated with human IL-2 for 24hrs and then cultured with human IL-18. IL-23 A2091F (Human) and A1922F (Cyno) was added to the cells at 10pM or 20pM. 100nM of IL-23 antagonists and control proteins were added to 10pM of IL-23 and incubated for

30min. at 37°C then added to cells. Antagonists tested included MVC262 (c305.1/c632.1 5mer Fc5 bisc), MVC266/226 (c305 biAb 5 mer c632), MVC264 (c305.1-c632.1 10mer Fc5 bisc), MVC261 (c632.1-c305.1 10mer Fc5 tasc), and MVC270/268 (biAb c632.1 VLK VH IgG1 10mer c305.1). Controls included anti-IL-12p40, IL-23R Fc5, IL-23scFv c305.1, and IL-17AscFv c632. Supernatants were collected at 72hrs and analyzed for IL-17A, IL-17F, and IL-17AF.

[464] See Figure 10: Note: for Figure 10 and Table XX below: "biscvFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer); "biAb" was the MVc construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb); "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer). Also see Table 28:

[465] Table 28: IL-23p19/IL-17A bsAbs inhibit hIL-23-mediated production of IL-17A, IL-17F, and IL-17AF in human NK cells. hIL-23 induces IL-17A, IL-17F and IL-17A/F production in primary human NK cells which is neutralized with bsAbs and to a lesser extent, IL23p19 scFv and IL-17A scFv.

Table 28

	Average % Inhibition of Cytokine Production in Presence of hIL-23		
	IL-17A	IL-17F	IL-17A/F
+hIL-23R-Fc	37.7%	58.7%	33.1%
+IL-23p19 scFv	69.1%	88.1%	48.6%
+IL-17A scFv	60.8%	63.8%	24.0%
+tascFv-Fc	97.0%	90.1%	69.7%
+biscFv-Fc	95.7%	92.9%	71.5%
+biAb	97.4%	95.7%	77.5%

EXAMPLE 22

Determination of Ability of Anti-Human IL-23 Neutralizing Entities to Cross-React and Neutralize Cynomolgus Monkey IL-23-Induced Activity in IL-23 Bioassays

[466] Similar experiments as described above in EXAMPLE 14 (Cynomolgus IL-17A experiments) were performed with human versus cynomolgus IL-23, using IL-23 activity assays described in EXAMPLES 20 and 21 using a full range (0 – 4050 pM) of either recombinant human IL-23 (ZymoGenetics, Inc., Seattle, WA)) or recombinant cynomolgus IL-23 ((ZymoGenetics, Inc., Seattle, WA)). Results indicated that the activities induced by these two species of IL-23 were

identical for the luciferase-based assay (EC50, in one experiment for example, of 85 pM for both species of IL-23) and nearly identical for the pSTAT3-based assay (46 and 39 pM for human and cynomolgus IL-23 EC50 values, respectively).

[467] In experiments designed to evaluate the ability of the IL-23 neutralizing entities described herein to neutralize both human and cynomolgus IL-23, results were similar to those described above for cynomolgus IL-17A: i.e. although there was a range of neutralizing capacities, those that neutralized human IL-23 best also showed the best neutralization against cynomolgus IL-23-induced activity. Results are presented in Table 29. These results demonstrated cynomolgus monkey cross-reactivity for the ability to inhibit IL-23-mediated biological activity.

TABLE 29: Neutralization of human IL-23-induced pSTAT3 activity with IL-23A scFv's

scFv #	average IC50 (nM)
IL-23A polyAb (R&D Systems)	102
IL-23R-Fc CONTROL (A1913F)	0.8
c305.1_2 (SQ22)	0.15
c305.1 (A1996F)	0.14
c472.2_7	0.10

EXAMPLE 23

Neutralization of Human IL-23 mediated IL-17A, IL-17F, and IL-17AF Production in Murine Splenocytes

[468] Recombinant human IL-23 (rhIL-23) induces the production of IL-17A, IL-17F, IL17A/F heterodimer (IL-17AF or IL17A/F) and GM-CSF in murine splenocytes. To evaluate antagonists to rhIL-23, we examined the neutralization of murine IL-17A, IL-17F, IL17A/F and GM-CSF production in rhIL-23 treated murine splenocytes. Antagonists to rhIL-23 are compared to the soluble receptor rhIL23RA-Fc (ZGI).

Experimental protocol:

[469] A single cell suspension of splenocytes is prepared from whole spleens harvested from either C57BL/6 or BALB/c mice. After red blood cell lysis with ACK buffer (0.010 M KHCO3, 0.0001 M EDTA, 0.150 M NH4Cl), splenocytes are washed and resuspended in RPMI buffer (containing 1% non-essential amino acids, 1% Sodium Pyruvate, 2.5 mM HEPES, 1% L-glutamine, 0.00035% 2-mercaptoethanol, 1% Pen/Strep, 10% FCS and 50 ng/ml human IL-2 (R&D Systems, Minneapolis, MN)). Cells are seeded at 500,000 cells per well in a 96-well round bottom plate. In a separate plate, rhIL-23 at a concentration of 10 pM is pre-incubated for 30 – 90 minutes at

37degreesC with 3-fold serial dilutions of examples of antagonists listed in Table 30. Concentrations of the antagonists range from 0 – 343 nM. The IL-23 ligand plus antagonists are then added to the splenocytes and incubated at 37 degrees C, 5% CO2 for 24-72 hours. The supernatants are collected and frozen at -80 degrees C until ready to process. The levels of murine IL-17A, IL-17F, IL17A/F, and GM-CSF protein in the supernatants are measured using bead-based sandwich ELISAs. A commercial kit (Upstate) is used to measure IL-17A and GM-CSF protein. A bead-based ELISA developed in-house using an antibody to IL-17F (R&D Systems, Minneapolis, MN) conjugated to a bead is used to measure IL-17F. A combination of the commercial beads used to measure IL17A with a secondary antibody to IL17F (R&D Systems, Minneapolis, MN) is used to measure IL17A/F heterodimer. IC50 values for each antagonist are calculated as the amount of antagonist needed to neutralize 50% of the activity of rhIL-23.

Results:

[470] In the presence of rhIL-23, the example antagonists described in Table 30 are efficacious at reducing murine IL-17A, IL-17F, IL-17A/F and GM-CSF production with IC50 values of 0.08 – 30.8 nM. The multivalent molecules described herein are able to be evaluated in this murine-IL17-production assay system, since the anti- human IL-17A component of the multivalent molecules does not bind or neutralize murine IL-17A, IL-17F or IL-17AF. (see Example 11) The term "MVC" in the table is a clone number for the tascFv, biscFv, or BiAb construct. (Example 6).

[471] In a similar assay measuring the inhibition of IL-23-mediated IL-17A/A production in murine splenocytes the IC_{50} of IL-23RA was 26nM; the IC_{50} of c305 was 3.4nM; and the IC_{50} of c472.1 was 0.08nM.

Table 30: IC50 values measured in the murine splenocyte assay.

Antagonist	MVC	SEQ ID	IC50 for neutralizing IL-17A	IC50 for neutralizing IL-17F	IC50 for neutralizing IL17A/F Het	IC50 for neutralizing GM-CSF
Anti-IL12p40 (Pharmingen, Franklin Lakes, NJ)			0.08nM	0.11nM		
Anti-IL23p19 polyAb (R&D)			21.2nM	29.0nM		
IL23RA-Fc (ZGI)			29nM	61nM	37.1nM	3.76nM
c305.1 (scFv)			5.5nM	5.6nM	2.7nM	3.64nM
c305.2 (scFv)			5.6nM	25.4nM	10.6nM	
c29.4 (scFv)			25.9nM	30.8nM	22.6nM	
c305.1-Fc			2.07nM			2.03nM
c305_6_c389 (tascFv)			6.48nM			
c472.1 (scFv)			0.25nM			
c305_c87-Fc (tascFv)	173		1.7nM			
c87_c305-Fc (tascFv)	174		2.4nM			
c87_c305-Fc (tascFv)	175		4.4nM			
c305.1_c631.1 tascFv-Fc5 (long stump)	241		0.43nM			
c305.1_c631.1 tascFv-Fc5 (short stump)	242		1.44nM			
c631.1_c305.1 tascFv-Fc5 (long stump)	243		1.07nM			
c305.1_c631.1 tascFv-Fc5 (10mer)	245		0.4nM			
c305.1_c631.1 biscFv-Fc5 (5mer)	247		0.085nM			
c305.1_c632.1 tascFv-Fc5 (long stump)	256		0.088nM			
c632.1_c305.1 tascFv-Fc5 (long stump)	258		0.11nM			
c631.1_c305.1 tascFv-Fc5 (10mer)	246		0.53nM			
c631.1_c305.1 biscFv-Fc5 (5mer)	248		0.19nM			
c305.1_c631.1 biscFv-Fc5 (10mer)	249		0.06nM			
c631.1_c305.1 biscFv-Fc5 (10mer)	250		0.92nM			

c305.1_c632.1 tascFv-Fc5 (short stump)	257	0.21nM
c305.1_c632.1 tascFv-Fc5 (10mer)	260	0.11nM
c632.1_c305.1 tascFv-Fc5 (short stump)	259	0.042nM
c632.1_c305.1 tascFv-Fc5 (10mer)	261	0.047nM
c632.1_c305.1 biscFv-Fc5 (5mer)	263	0.41nM
c305.1_c631.1 bi Ab	251+226	0.7nM

[472] In this assay, the tasc, bisc, and BiAb molecules described herein showed the following values. See Table 31, below.

Table 31:

Target	Construct	IL-23 Neutralization (24hr) (nM)
IL-23	IL-23R	26
IL-23	c305	3.4
IL-17A/IL-23	c632.1/c305 TascFv-Fc	0.04
IL-17A/IL-23	c305/c632.1 BiscFv-Fc	0.07
IL-17A/IL-23	c305/c632.1 BiAb	0.09

[473] These results show a 30-fold increase in IL-23 neutralization of the bispecific tetravalent molecules over the monovalent scFv.

[474] Also see Figure 8. Note: for Figure 8: "biscvFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was ~0.04-0.09 nM in this assay; "biAb" was the MVc construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb) and the IC_{50} range for this molecule in this assay was ~0.04-0.2 nM; "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was 0.02-0.1 nM. As this assay is based on primary cells, these ranges show a lot of variability.

[475] See Table 32, below. Human IL-23p19/IL-17A bsAbs inhibit hIL-23-mediated production of mIL-17A, IL-17F, and IL-17A/F in murine splenocytes. hIL-23 induces IL-17A, IL-17F and IL-17A/F production in 1o murine splenocytes (24 h) which is neutralized with bsAbs

Table 32

	IC50 (nM) Cytokine Production in Presence of hIL-23		
	IL-17A	IL-17F	IL-17A/F
+hIL-23R-Fc	7.2	82.0	26.0
+IL-23p19 scFv	0.80	11.2	3.45
+tascFv-Fc	0.025 (range is .020-0.1)	1.18 (range is 1-2)	0.39 (range is .3 - .75)
+biscFv-Fc	0.050 (range is 0.040-0.09)	1.76 (range is 1.5 – 2.7)	0.45 (range is0.35 - 0.85)
+biAb	0.058 (range is 0.04 - 0.24)	1.75 (range is 1.5-2.7)	0.78 (range is .65 -1.1)

EXAMPLE 24

Neutralization of Human IL-23 mediated IL-17A and IL-17F Production in Cynomolgus Splenocytes.

[476] Recombinant human IL-23 (rhIL-23) induces the production of IL-17A and IL-17F in cynomolgus splenocytes. To evaluate antagonists to IL-23, we examined the neutralization of IL-17A and IL-17F production in rhIL-23 treated cynomolgus splenocytes. Antagonists to rhIL-23 are compared to the soluble receptor IL23RA-Fc (ZGI).

Experimental protocol:

[477] The bioassay for the neutralization of rhIL-23 mediated IL-17A and IL-17F production in cynomolgus splenocytes is performed similarly to the bioassay in murine splenocytes outlined in Example 23 with the following changes. rhIL-23 at a concentration of 20pM is preincubated for 30-90 minutes at 37 degrees C with 3-fold serial dilutions of antagonists listed in Table 33, below. Cynomolgus IL-17A and IL-17F levels in the supernatants are measured using bead-based sandwich ELISAs developed at ZymoGenetics. An antibody to human IL-17A (R&D Systems, Minneapolis, MN) is conjugated to beads and the beads are used to measure IL-17A. Similarly, an antibody to human IL-17F (R&D Systems, Minneapolis, MN) is conjugated to beads and the beads are used to measure IL-17F. Both assays developed with antibodies to human IL-17A and IL-17F proteins cross-reacted with cynomolgus IL-17A and IL-17F proteins respectively. IC50 values for each antagonist are calculated as the amount of antagonist needed to neutralize 50% of the activity of rhIL-23.

Results:

[478] In the presence of rhIL-23, the example antagonists described in Table 33 are efficacious at reducing cynomolgus IL-17A and IL-17F production with IC50 values of 0.31 – 100.0 nM.

Table 33: IC50 values measured in the cynomologous splenocyte assay.

Antagonist	IC50 for neutralizing IL-23 mediated IL-17A production	IC50 for neutralizing IL-23 mediated IL-17F production
Anti-IL23p40 (eBioscience)	0.31nM	1.31nM
IL23RA-Fc (ZGI)	34.8nM	100nM
c305.1 (scFv)	15.4nM	41.6nM
c305_6_c389	2.2nM	

[479] See Figure 9. Note: for Figure 9: "biscvFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was ~0.07-0.25 nM in this assay; "biAb" was the MVc construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb) and the IC_{50} range for this molecule in this assay was ~0.04-0.24 nM; "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer) and the IC_{50} range for this molecule in this assay was 0.05-0.25 nM. As this assay is based on primary cells, these ranges show a lot of variability.

EXAMPLE 25

Measurement of Binding Affinities of Anti-IL-23p19 Molecules to IL-23 Via Surface Plasmon Resonance (Biacore)

[480] Anti-IL-23p19 entities described herein were evaluated for their binding affinities to IL-23 using surface plasmon resonance and Biacore T-100 instrument (GE Healthcare). Recombinant human IL-23 was immobilized to the sensor chip, followed by passing the antagonists over the immobilized ligand to attain affinity measurements. These methods allow for binding affinity measurements of the IL-23p19 neutralizing entities for their respective ligand (IL-23) and also demonstrate specificity for IL-23 by displaying no binding to similar but different ligands, such as IL-12.

[481] To determine the best conditions for immobilization, a series of pH scouting experiments were performed. For these experiments, recombinant human IL-23 (ZGI lot A1806F) was diluted to 100nM in five different immobilization buffers; Acetate-4.0, Acetate-4.5, Acetate-5.0, Acetate-5.5, and Borate-8.5. Using Immobilization Scouting Wizard, these conditions were tested and at the end Acetate-4.5 was the best condition for immobilization.

[482] For the immobilization procedure, IL-23 protein was diluted to 10ug/ml in Acetate-4.5, and then immobilized onto a Series S Sensor Chip (CM5, GE Healthcare / Biacore #BR-1006-68) using the amine coupling kit and Biacore Immobilization Wizard. Briefly, the level of immobilization was targeted to 300 Biacore Resonance Units (RU), and IL-23 was only injected over an active flow cell. After the immobilization procedure, active sites on the flow cell were blocked with ethanolamine. Non-specifically bound protein was removed by washing with 50mM NaOH. The final immobilization level was 466RU. The reference cell was activated and then blocked with ethanolamine.

[483] To determine the optimal contact time, RL (resonance signal of the ligand) testing was performed. Control proteins, soluble IL-23R-Fc (ZGI lot A1913F) and an anti-IL-23p19 polyclonal antibody (R&D Systems), were diluted to 100nM in 1X HSB-EP+ running buffer (GE Healthcare / Biacore, #BR-1006-69). The IL-23R-Fc or IL-23p19 polyclonal antibody were injected over both the active and reference cells for three different contact times. From this RL testing, the contact time for the IL-23p19 polyclonal antibody was determined to be 60 seconds and for IL-23R-Fc, it was 300 seconds.

[484] For the kinetic run, serial dilutions of the control proteins, IL-23R-Fc or IL-23p19 polyclonal antibody, or anti-IL-23p19 neutralizing entities described herein, were prepared in 1X HBS-EP+ buffer. Duplicate injections of each concentration were performed. The analyte injections were at 30ul/min with a dissociation time of 600 seconds. Buffer injections were also performed to allow for subtraction of instrument noise and drift.

[485] Regeneration buffers supplied in the Regeneration Scouting Kit (GE Healthcare / Biacore # BR-1005-56) were used to determine regeneration conditions. This run was performed manually, starting from the least mild condition. At the end of this procedure, the optimal regeneration condition was 60 seconds of 2M Magnesium Chloride followed with 60 seconds of 1X HBS-EP+ buffer injections at a flow rate of 50ul/min.

[486] As a check for IL-23 specific interactions, similar procedures were followed using immobilized recombinant human IL-12 (R&D Systems).

[487] Data were analyzed using Biacore Evaluation software (v.1.1.1) to define the kinetic values of the interaction of IL-23 with the control proteins (IL-23p19 polyclonal antibody and IL-23R-Fc) and IL-23p19 neutralizing entities described herein. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Binding curves were normalized by double-referencing, and duplicate injection curves were checked for reproducibility. The resulting binding curves were globally fit to the bivalent interaction model.

[488] The data demonstrated high affinity binding of human IL-23 to the control antagonist proteins, IL-23R-Fc and anti-IL-23p19 polyclonal antibody, with affinity constants (KD) of 0.8nM and 5.1nM, respectively. The IL-23p19 neutralizing entities described herein were within this range

(0.1 - <10 nM). There was no binding of the control IL-23 antagonist proteins or the anti-IL-23p19 neutralizing entities to IL-12, thus providing evidence for specificity of targeting IL-23.

EXAMPLE 26

Off-Rate Analysis of Anti-IL-23p19 Molecules (from supernatants) to IL-23 Via Surface Plasmon Resonance (Biacore)

[489] Anti-IL-23p19 entities described herein were evaluated for binding off-rates to IL-23 using surface plasmon resonance and Biacore T-100 instrument (GE Healthcare). Off-rate analysis is thought to help estimate the interaction that occurs in vivo, since a slow off-rate would predict a greater degree of interaction over long period of time. For these experiments, recombinant human IL-23 was immobilized to the sensor chip, followed by passing the antagonists over the immobilized ligand to attain off-rate analyses.

[490] To determine the best conditions for immobilization, a series of pH scouting experiments were performed. For these experiments, recombinant human IL-23 (ZGI lot A1806F) was diluted to 100nM in five different immobilization buffers; Acetate-4.0, Acetate-4.5, Acetate-5.0, Acetate-5.5, and Borate-8.5. Using immobilization scouting wizard, these conditions were tested and at the end Acetate-4.5 was the best condition for immobilization.

[491] For the immobilization procedure, native human IL-23 was diluted to 10ug/ml in Acetate-4.5 and was immobilized onto a Series S Sensor Chip (CM5, GE Healthcare / Biacore #BR-1006-68) using the amine coupling kit and Biacore Immobilization Wizard. Briefly,one flow cell was used as the reference, and therefore was only activated and then blocked with ethanolamine. The native form of IL-23 was immobilized to another flow cell with a target immobilization level of 300 Biacore Resonance Units (RU). Analytes were injected only over active flow cell. After the immobilization, the active sites on the flow cell were blocked with ethanolamine. Non-specifically bound protein was removed by washing with 50mM NaOH. The final immobilization levels fell between 415 and 496 RU.

[492] Samples of the anti-IL-23p19 neutralizing entity supernatants or proteins were diluted 1:3 in 1X HBS-EP+ buffer (GE Healthcare / Biacore, #BR-1006-69) supplemented with 0.1% BSA. IL-23R-Fc was also prepared at 100nM in 1X HBS-EP+, 0.1%BSA buffer as a positive control. The analyte injections were at 30uL/min with association time of 300 seconds and dissociation time of 300 seconds. Media control injections were also performed to allow for subtraction of instrument noise and drift.

[493] Regeneration buffers supplied in a regeneration scouting kit were used to determine regeneration conditions (RR-2007001). Using this procedure, the optimal regeneration condition was

found to be 60 seconds of 2M Magnesium Chloride followed with 60 seconds of 1X HBS-EP+, 0.1% BSA buffer injections at a flow rate of 50ul/min.

[494] Data were evaluated using Biacore Evaluation software to define the off-rate of the interactions of anti-IL-23p19 neutralizing entities to immobilized IL-23. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Binding curves were normalized by double-referencing and the resulting binding curves were globally-fit to the 1:1 binding interaction model using the Biacore Evaluation Software v1.1.1. The IL-23p19 neutralizing entities were ranked first according to their off-rates (slowest to fastest) and then to their binding levels (highest to lowest binding RUs) during the association phase. Off-rate values were also obtained. All samples were compared to the positive control, IL23R-Fc (which has an off-rate of approximately 4 x 10-4 sec-1). Neutralizing entities described herein displayed a large range of off-rates to human IL-23 (0.01 to 9 x 10-5 sec-1).

EXAMPLE 27

Measurement of Binding Affinities of Human IL23 Antagonists to the IL23 Via Surface Plasmon Resonance (Biacore)

[495] Human IL23 antagonists which have single IL23 binding domains were evaluated for their binding affinities to human recombinant IL-23 using surface plasmon resonance.

Affinity Determination

[496] Kinetic rate constants and equilibrium dissociation constants were measured for the interaction of IL23 antagonists with IL23 via surface plasmon resonance. The association rate constant (ka (M-1s-1)) is a value that reflects the rate of the antigen-antagonist complex formation. The dissociation rate constant (kd (s-1)) is a value that reflects the stability of this complex. By dividing the association rate constant by the dissociation rate constant (ka/kd) the equilibrium association constant (KA (M-1)) is obtained. By dividing the dissociation rate constant by the association rate constant (kd/ka) the equilibrium dissociation constant (KD (M)) is obtained. This value describes the binding affinity of the interaction. Interactions with the same KD can have widely variable association and dissociation rate constants. Consequently, measuring both the ka and kd helps to more uniquely describe the affinity of the interaction.

Materials and Methods

[497] A series of experiments were completed to measure the binding affinities of purified IL23 antagonists (c305 and c472) generated against human IL23 (ZymoGenetics). Binding kinetics

and affinity studies were performed on a Biacore T100™ system (GE Healthcare, Piscataway, NJ). Methods were programmed using Biacore T100TM Control Software, v 1.1.1.

[498] Two different experimental designs were used. The first method was performed by covalently immobilizing IL23 antagonists onto separate flow cells of the CM5 sensor chip using amine coupling chemistry (EDC:NHS) to a density of approximately 300 RU. Serial 1:2 dilutions of IL23 (ZymoGenetics) from 50 nM – 0.6 nM were injected over the surface and allowed to specifically bind to each IL23 antagonist that is on the sensor chip. Duplicate injections of each IL23 concentration were performed with an association time of 5 minutes and dissociation time of 10 minutes. Kinetic binding studies were performed with a flow rate of 30 µL/min. All binding experiments were performed at 25 °C in a buffer of 10 mM HEPES, 150 mM NaCl, 3 mM EDTA 0.05% Surfactant P20, pH 7.4. In order to regenerate the surface, flow cells were washed with 2M MgCl2 or 10 mM Glycine, pH 2.0 (c305 or c472, respectively) in between each cycle.

[499] The second method was performed by capturing IL23 antagonists on an anti-His/Myc immobilized CM5 chip since the IL23 antagonists have C-terminal His/Myc epitope tags. The IL23 lot (ZymoGenetics) used in the previous method also has a C-terminal His6 tag and cannot be used on His/Myc capture format. Therefore IL23 (R&D Systems, Inc.) with no epitope tags was used for the second method. Anti-His and anti-Myc antibodies were mixed in 1:1 molar ratio and covalently immobilized to a CM5 sensor chip using amine coupling chemistry to a density of approximately 7500RU. 300 nM of IL23 antagonists were injected on separate flow cells at 10ul/min for 1 minute, followed with a 1 minute stabilization period. Serial 1:3 dilutions of IL23 from 300 nM – 3.7 nM were injected over this surface and allowed to specifically bind to human IL23 antagonist captured on the sensor chip. Duplicate injections of each IL23 concentration were performed with an association time of 5 minutes and dissociation time of 30 minutes. Kinetic binding studies were performed with a flow rate of 30 µL/min. All binding experiments were performed at 25 °C in a buffer of 10 mM HEPES, 500 mM NaCl, 3 mM EDTA 0.05% Surfactant P20, 0.1mg/ml bovine serum albumin, pH 7.4. Between cycles, the flow cell was washed with 50 mM H3PO4 to regenerate the surface. This wash step removed the captured IL23 antagonist from the immobilized antibody surface, and allowed for the subsequent binding of the next sample.

[500] Data was compiled using Biacore T100TM Evaluation software (version 1.1.1). Data was processed by subtracting reference flow cell and blank injections. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Duplicate injection curves were checked for reproducibility. Since both IL23 antigen and the IL23 antagonists are monovalent molecules, the resulting binding curves were globally fitted to the 1:1 binding interaction model.

Results:

[501] Two IL23 antagonists were characterized for their binding affinity for IL23. Association rate constants (ka (M-1s-1)) and dissociation rate constants (kd (s-1)) were measured using two different experimental approaches. KD and KA were calculated from the ka and kd values. Two different experimental approaches yielded some variation in the dissociation rate constants of the IL23 antagonists, therefore the values for kd, KD and KA are reported as ranges. The data produced from both experimental approaches fit well to the 1:1 binding interaction model. Both IL23 antagonists have similar association rates (3.E+5 M-1s-1 for c305 and 4.E+5 M-1s-1 for c472). Equilibrium dissociation constants of IL23 to c305-IL23 antagonist is between 7.E-10 – 2.E-9 M and that of c472-IL23 antagonist is between 2.E-10 – 8.E-10 M.

Table 34:

ID #	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	K_A (M^{-1})
c305	3.E+5	2.E-4 – 5E-4	7.E-10 – 2.E-9	6.E+8 - 2.E+9
c472	4.E+5	6.E-5 – 3E-4	2.E-10 – 8.E-10	1.E+9 - 7.E+9

EXAMPLE 28

Measurement of Binding Affinities of Human IL17A Antagonists to the IL17A Via Surface Plasmon Resonance (Biacore)

[502] Human IL17A antagonists which have single IL17A binding domains were evaluated for their binding affinities to human recombinant IL-17A using surface plasmon resonance.

Affinity Determination

[503] Kinetic rate constants and equilibrium dissociation constants were measured for the interaction of IL17A antagonists with the IL17A via surface plasmon resonance. The association rate constant (ka (M-1s-1)) is a value that reflects the rate of the antigen-antagonist complex formation. The dissociation rate constant (kd (s-1)) is a value that reflects the stability of this complex. By dividing the association rate constant by the dissociation rate constant (ka/kd) the equilibrium association constant (KA (M-1)) is obtained. By dividing the dissociation rate constant by the association rate constant (kd/ka) the equilibrium dissociation constant (KD (M)) is obtained. This value describes the binding affinity of the interaction. Interactions with the same KD can have widely variable association and dissociation rate constants. Consequently, measuring both the ka and kd helps to more uniquely describe the affinity of the interaction.

Materials and Methods

[504] A series of experiments were completed to measure the binding affinities of purified IL17A antagonists (c631 and c632) generated against IL17A. Binding kinetics and affinity studies were performed on a Biacore T100™ system (GE Healthcare, Piscataway, NJ). Methods were programmed using Biacore T100TM Control Software, v 1.1.1. Human IL17A, IL17F and IL17A/F antigens (ZymoGenetics) were covalently immobilized onto separate flow cells of the CM5 sensor chip using amine coupling chemistry (EDC:NHS) to a density of approximately 300 RU. Serial 1:3 dilutions of human IL17A antagonists from 33.3 nM – 0.05 nM were injected over the surface and allowed to specifically bind to each ligand that is on the sensor chip. Duplicate injections of human IL17A antagonist concentration were performed with an association time of 5 minutes and dissociation time of 30 minutes. Kinetic binding studies were performed with a flow rate of 30 μL/min. All binding experiments were performed at 25 °C in a buffer of 10 mM HEPES, 500 mM NaCl, 3 mM EDTA 0.05% Surfactant P20, 0.1mg/ml bovine serum albumin, pH 7.4. In order to regenerate the surface, flow cells were washed with 10 mM H3PO4 in between each cycle.

[505] Data was compiled using Biacore T100TM Evaluation software (version 1.1.1). Data was processed by subtracting reference flow cell and blank injections. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Duplicate injection curves were checked for reproducibility. The target ligands, IL17A and IL17A/F, are dimeric molecules and were immobilized on the chip surface. The human IL17A antagonists tested are monovalent molecules and they were injected over the immobilized IL17A or IL17A/F. Therefore, the resulting binding curves were globally fitted to the 1:1 binding interaction model.

Results:

[506] Two IL17A antagonists were characterized for their binding affinity for IL17A, IL17F and IL17A/F. Association rate constants (ka (M-1s-1)) and dissociation rate constants (kd (s-1)) were measured for these human IL17A antagonists. KD and KA were calculated from the ka and kd values. The data fit well to the 1:1 binding interaction model. Under these assay conditions, no binding to IL17F antigen was detected. Both c631 and c632 showed similar affinity to IL17A antigen (KD=2.E-10M and 3.E-10M), whereas they had different affinities to IL17A/F antigen (KD=1.E-8M and 2.E-10M). This difference in affinity is primarily due to the difference in dissociation rates (kd) values.

Table 35

		IL17A Affinity Results				IL17A/F Affinity Results			
ID #	Lot #	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	K_A (M^{-1})	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	K_A (M^{-1})
c631	A2027F	5.E+5	8.E-5	2.E-10	6.E+9	3.E+5	4.E-3	1.E-8	8.E+7
c632	A2029F	2.E+5	6.E-5	3.E-10	3.E+9	2.E+5	3.E-5	2.E-10	7.E+9

EXAMPLE 29

Measurement of Binding Affinity of Anti-IL-17A/IL23p19 Molecule to both IL-17A and IL23 Via Surface Plasmon Resonance (Biacore)

[507] Anti-IL-17A/IL23p19 entity described herein was evaluated for its binding affinity to its targets IL-17A and IL23 using surface plasmon resonance and Biacore T-100 instrument (GE Healthcare). Recombinant human IL-17A was immobilized to the sensor chip, followed by passing the antagonists over the immobilized ligand to attain affinity measurements. This method allows for binding affinity measurement of the IL-17A/IL23p19 neutralizing entity for its respective ligand, IL-17A. Following capture of anti-IL17A/IL23p19 molecule on an IL17A immobilized surface, recombinant human IL23 was passed over at different concentrations. This method allows for demonstrating co-binding ability of the anti-IL17A/IL23p19 molecule to both of its targets (IL17A and IL23) and also for binding affinity measurement of the IL17A/IL23p19 molecule for IL23.

[508] To determine the best conditions for immobilization, a series of pH scouting experiments were performed. For these experiments, recombinant human IL-17A (ZGI lot A1781F) was diluted to 100 nM in five different immobilization buffers; Acetate-4.0, Acetate-4.5, Acetate-5.0, Acetate-5.5, and Borate-8.5. Using Immobilization Scouting Wizard, these conditions were tested and at the end Acetate-4.0 was the best condition for immobilization.

[509] For the immobilization procedure, IL-17A protein was diluted to 10 ug/ml in Acetate-4.5, and then immobilized onto a Series S Sensor Chip (CM5, GE Healthcare / Biacore #BR-1006-68) using the amine coupling kit and Biacore Immobilization Wizard. Briefly, the level of immobilization was targeted to 300 Biacore Resonance Units (RU), and IL-17A was only injected over an active flow cell. After the immobilization procedure, active sites on the flow cell were blocked with ethanolamine. Non-specifically bound protein was removed by washing with 50mM NaOH. The final immobilization level was 497 RU. The reference cell was activated and then blocked with ethanolamine.

[510] For the kinetic run, serial dilutions of the control protein (that was identified in-house), or IL-17RA-Fc (ZGI lot A1763F), or the anti-IL-17A/IL23p19 neutralizing molecule described herein, were prepared in 1X HBS-EP+ buffer containing final salt concentration of 500mM NaCl. The analyte injections were at 30 ul/min with association time of 300 seconds and dissociation time of 600 seconds. Buffer injections were also performed to allow for subtraction of instrument noise and drift.

[511] Regeneration buffers supplied in the Regeneration Scouting Kit (GE Healthcare / Biacore # BR-1005-56) were used to determine regeneration conditions. Standard Biacore methods were performed to define the best regeneration conditions for an IL-17A surface using IL-17RA-Fc at 100nM. Mildest conditions were tested first and moved up in strength. The final regeneration condition chosen was a 1-60 sec pulse of 10mM H3PO4 followed by a 1-60 sec pulse of 1X HSB-EP+, 500mM NaCl buffer at a flow rate of 50ul/min.

[512] After the binding affinity of the anti-IL17A/IL23p19 molecule to recombinant human IL17A was established, next the binding affinity of recombinant human IL23 to the anti-IL17A/IL23p19 entity was determined. Recombinant human IL17A immobilized surfaced used to capture anti-IL17A/IL23p19 molecule (that is prepared in 1XHBS-EP+, 500mM NaCl buffer) to achieve more than 50% binding to IL17A. Then recombinant human IL23 (A1806F) serially diluted in 1X HBS-EP+, 500mM NaCl and injected over the anti IL17A/IL23p19 captured surface. The analyte injections were at 30 ul/min with association time of 300 seconds and dissociation time of 600 seconds. Buffer injections were also performed to allow for subtraction of instrument noise and drift.

[513] Data were analyzed using Biacore Evaluation software (v.1.1.1) to define the kinetic values of the interaction of anti-IL-17A/IL23p19 neutralizing molecule with IL17A and IL23 described herein. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Binding curves were normalized by double-referencing. Duplicate injection curves were checked for reproducibility. The resulting binding curves were globally fit to the 1:1 binding interaction model (for anti-IL17A/IL23p19 - IL17A) or to the 1:1 binding with drifting baseline (for anti-IL17A/IL23p19 - IL23).

[514] The data demonstrate high affinity binding of the anti-IL17A/IL23p19 neutralizing molecule to both IL17A and IL23 at the same time. Specifically, the anti-IL-17A/IL23p19 molecule demonstrates dissociation equilibrium constants (KD) for the recombinant human IL-17A to be approximately 2nM and approximately 7nM for the recombinant human IL-23.

[515] In this assay, the tasc, bisc, and BiAb molecules described herein showed the following values. See Table 36, below

Table 36: Kinetic Analysis of tasc, bisc, and BiAb molecules

Target	Construct	K_D IL-17A(nM)	K_D IL-23 (nM)
IL-17A	IL-17RA	5.0	---
IL-23	IL-23R	---	0.8
IL-17A	c632.1	0.3	---
IL-23	c305	---	0.7
IL-17A/IL-23	c632.1/c305 TascFv-Fc	0.09	4.3
IL-17A/IL-23	c305/c632.1 BiscFv-Fc	0.28	6.4
IL-17A/IL-23	c305/c632.1 BiAb	0.06	6.5

[516] In studies of kinetic analysis of co-binding, it was observed that co-binding does not induce any significant changes in k_a. That is, the bispecific nature of the molecules described herein does not affect the binding of the molecules to either target. See Table 37 below.

Table 37 : Kinetic Analysis of Co-Binding

Target	Construct	IL-17A binding (RU) Initial binding	IL-17A binding (RU) Binding after IL-23	IL-23A binding (RU) Initial binding	IL-23 binding (RU) Binding after IL-17A
IL-17A/IL-23	c632.1/c305 TascFv-Fc	25	25	86	86
IL-17A/IL-23	c305/c632.1 BiscFv-Fc	13	13	54	64
IL-17A/IL-23	c305/c632.1 BiAb	29	27	66	64

[517] See Figure 16. Note: for Figure 16: "biscvFv-Fc" was the MVC construct # 264 described herein (c305/c632/10mer); "biAb" was the MVc construct #266 and #226 described herein (c305Mab/lambda-c632scFv biAb); "tascFv-Fc" was the MVC construct #261 as described herein (c305/c632/10mer).

EXAMPLE 30

Measurement of Binding Affinities of Human IL23/IL17A Antagonist to the IL23 and IL17A Via Surface Plasmon Resonance (Biacore)

[518] A human IL23/IL17A antagonist was evaluated for its binding affinities to human recombinant IL-23 and IL17A using surface plasmon resonance. This IL23/IL17A antagonist consists of two domains: an IL23 binding unit (c305) and an IL17A binding unit (c389) that are tethered by a linker. Besides binding affinities to IL23 and IL17A, simultaneous binding of this IL23/IL17A antagonist to both IL23 and IL17A was demonstrated.

Affinity Determination

[519] Kinetic rate constants, equilibrium association and dissociation constants were measured for the interaction of the IL23/IL17A antagonist (MVC# 232) with the IL23 and IL17A via surface plasmon resonance. The association rate constant (ka (M-1s-1)) is a value that reflects the rate of the antigen-antagonist complex formation. The dissociation rate constant (kd (s-1)) is a value that reflects the stability of this complex. By dividing the association rate constant by the dissociation rate constant (ka/kd) the equilibrium association constant (KA (M-1)) is obtained. By dividing the dissociation rate constant by the association rate constant (kd/ka) the equilibrium dissociation constant (KD (M)) is obtained. This value describes the binding affinity of the interaction. Interactions with the same KD can have widely variable association and dissociation rate constants. Consequently, measuring both the ka and kd helps to more uniquely describe the affinity of the interaction.

Materials and Methods

[520] A series of experiments were completed to measure the binding affinity of a purified IL23/IL17A antagonist (ZymoGenetics, MVC #232) generated against IL23 (ZymoGenetics) and IL17A (ZymoGenetics). Binding kinetics and affinity studies were performed on Biacore T100™ system (GE Healthcare, Piscataway, NJ). Methods were programmed using Biacore T100TM Control Software, v 1.1.1. Human IL17A was covalently immobilized onto a flow cell of the CM5 sensor chip using amine coupling chemistry (EDC:NHS) to a density of approximately 500 RU. Serial 1:3 dilutions of human the IL23/IL17A antagonist from 66.7 nM – 0.27 nM were injected over the surface and allowed to specifically bind to the IL17A immobilized on the sensor chip. Duplicate injections of IL23/IL17A antagonist were performed with an association time of 5 minutes and dissociation time of 10 minutes. Kinetic binding studies were performed with a flow rate of 30 μL/min. All binding experiments were performed at 25 °C in a buffer of 10 mM HEPES, 500 mM NaCl, 3 mM EDTA 0.05% Surfactant P20, pH 7.4. In order to regenerate the surface, flow cells were washed with 10 mM H3PO4 in between each cycle.

[521] For affinity measurement of IL23 binding to the IL23/IL17A antagonist, the same immobilized IL17A surface was used. The IL23/IL17A antagonist was captured at 600 nM for 300 seconds at 30 ul/min which was followed with a 60 second stabilization period. Serial 1:3 dilutions of the IL23 (ZymoGenetics) from 600 nM – 1.23 nM were injected over the surface and allowed to specifically bind to the IL23/IL17A antagonist with an association time of 5 minutes and dissociation time of 10 minutes. Kinetic binding studies were performed with a flow rate of 30 μL/min. All binding experiments were performed at 25 °C in a buffer of 10 mM HEPES, 500 mM NaCl, 3 mM EDTA 0.05% Surfactant P20, pH 7.4. In order to regenerate the surface, flow cells were washed with 10 mM H3PO4 in between each cycle.

[522] Data was compiled using Biacore T100TM Evaluation software (version 1.1.1). Data was processed by subtracting reference flow cell and blank injections. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Duplicate injection curves were checked for reproducibility. IL17A is a bivalent molecule and was immobilized on the chip surface while the IL23/IL17A antagonist has only one binding site for IL17A. Therefore, the resulting binding curves for the IL23/IL17A antagonist binding to IL17A were globally fitted to the 1:1 binding interaction model. In the case of the IL23-IL23/17A antagonist interaction, IL23 is monovalent and the IL23/IL17A antagonist has one binding site for IL23. Also, in the kinetics analysis performed using IL17A capture, there was a slight decay of signal due to dissociation of the IL23/IL17A antagonist from the IL17A surface while the IL23 binding was taking place. As a result, binding curves of IL23-IL23/IL17A antagonist interaction were fitted to a 1:1 drifting baseline model.

Results:

[523] A purified IL23/IL17A antagonist was characterized for its binding affinity for the IL17A and IL23. Association rate constants (ka (M-1s-1)) and dissociation rate constants (kd (s-1)) were measured for binding unit. KD and KA for each interaction were calculated from the ka and kd values. In these set of experiments, simultaneous binding of the IL23/IL17A antagonist to both IL17A and IL23 was demonstrated. Binding affinity of the IL23/17A antagonist to IL17A was determined by immobilizing IL17A and injecting the IL23/IL17A antagonist over this surface. The resulting data set fit well to the 1:1 binding model. Binding affinity of the IL23/17A antagonist to IL23 was determined through capturing the IL23/IL17A antagonist on IL17A immobilized surface. During the IL23 interaction with IL23/IL17 antagonist molecule there was a slow dissociation of the antagonist from the IL17A surface. This resulted in an apparently faster dissociation rate (1.E-3 s-1) of IL23–IL23/IL17 antagonist interaction. 1:1 drifting baseline model was chosen to address this issue. Under these assay conditions, the binding affinity of IL23/IL17A antagonist molecule to IL17A was 1.5E-9M and to IL23 was 6.9E-9M.

Table 38

IL23/IL17A Antagonist (MVC #232) tandem_c305_c389				
Antigen	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	K_A (M^{-1})
IL17A	7.5E+4	1.1E-4	1.5E-9	6.8E+8
IL23	1.6E+5	1.1E-3	6.9E-9	1.5E+8

EXAMPLE 31

Measurement of Binding Affinities of IL-17/IL-23p19 Antagonists to the IL-17A Antigen and the IL-23 Antigen Via Surface Plasmon Resonance (Biacore)

[524] This study was performed to evaluate the binding affinities of IL-17/IL-23p19 antagonists composed of IL-17 and IL-23p19 binding domains fused to the Fc domain in various configurations.

Affinity Determination

[525] Kinetic rate constants, equilibrium association constants, and equilibrium dissociation constants were measured for the interaction of IL-17/IL-23p19 antagonists with the IL-17A antigen and the IL-23 antigen via surface plasmon resonance. The association rate constant (ka (M-1s-1)) is a value that reflects the rate of the antigen-antagonist complex formation. The dissociation rate constant (kd (s-1)) is a value that reflects the stability of this complex. Equilibrium binding affinity is typically expressed as either a dissociation equilibrium constant (KD (M)) or an association equilibrium constant (KA (M-1)). KD is obtained by dividing the dissociation rate constant by the association rate constant (kd/ka), while KA is obtained by dividing the association rate constant by the dissociation rate constant (ka/kd). Antagonists with similar KD (or a similar KA) can have widely variable association and dissociation rate constants. Consequently, measuring the ka and kd as well as the KA or KD helps to more uniquely describe the affinity of the antagonist-antigen interaction.

Materials and Methods

[526] A series of experiments were completed to measure the binding affinities of purified IL-17/IL-23p19 antagonists to human recombinant IL-17A and human recombinant IL-23. Binding kinetics and affinity studies were performed on a Biacore T100™ system (GE Healthcare, Piscataway, NJ). Methods for the Biacore T100TM were programmed using Biacore T100TM Control Software, v 1.1.1. Since each of the IL-17/IL-23p19 antagonists contained a human Fc domain, goat anti-human IgG Fc-gamma (Jackson ImmunoResearch, West Grove, PA) could be used as a capture antibody for these studies. The capture antibody was covalently immobilized to a CM4

sensor chip using a mixture of 0.4 M EDC [N-ethyl-N'-(3-diethylamino-propyl) carbodiimide] and 0.1 M NHS (N-hydroxysuccinimide) to a density of approximately 4000 RU. After immobilization of the antibody, the active sites on the flow cell were blocked with 1 M ethanolamine. Each IL-17/IL-23p19 antagonist was captured onto a separate flow cell of the CM4 chip at an approximate density of 80-100 RU. Capture of the antagonist to the immobilized surface was performed at a flow rate of 10 μL/min. The Biacore instrument measures the mass of protein bound to the sensor chip surface, and thus, capture of the antagonist was verified for each cycle.

[527] For binding studies with IL-17A, serial 1:3 dilutions of the IL-17A antigen (ZymoGenetics) from 20 nM – 0.03 nM were injected over the surface and allowed to specifically bind to the IL-17/IL-23p19 antagonist captured on the sensor chip. Duplicate injections of each IL-17A antigen concentration were performed with an association time of 7 minutes and dissociation time of either 10 or 60 minutes. Kinetic binding studies were performed with a flow rate of 30 μL/min. All binding experiments were performed at 25 °C in a buffer of 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20 (GE Healthcare, Piscataway, NJ), 1 mg/mL bovine serum albumin, pH 7.4.

[528] For binding studies with IL-23, serial 1:3 dilutions of the IL-23 antigen (ZymoGenetics) from 50 nM – 0.07 nM were injected over the surface and allowed to specifically bind to the IL-17/IL-23p19 antagonist captured on the sensor chip. Duplicate injections of each IL-23 antigen concentration were performed with an association time of 7 minutes and dissociation time of 10 minutes. Kinetic binding studies were performed with a flow rate of 30 μL/min. All binding experiments were performed at 25 °C in a buffer of 10 mM HEPES, 500 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, 1 mg/mL bovine serum albumin, pH 7.4.

[529] Between cycles, the flow cell was washed with 20 mM hydrochloric acid to regenerate the surface. This wash step removed the captured IL-17/IL-23p19 antagonist from the immobilized antibody surface, and allowed for the subsequent binding of the next test sample. Data was compiled using the Biacore T100TM Evaluation software (version 1.1.1). Data was processed by subtracting reference flow cell and blank injections. Baseline stability was assessed to ensure that the regeneration step provided a consistent binding surface throughout the sequence of injections. Duplicate injection curves were checked for reproducibility. Since the IL-17A antigen is a dimer, the bivalent analyte model was determined to be appropriate for binding of the IL-17A antigen to the IL-17/IL-23p19 antagonists. Conversely, the IL-23 antigen is a monomer, and the 1:1 binding model was determined to be appropriate for binding of the IL-23 antigen to the IL-17/IL-23p19 antagonists. Binding curves were globally fit to the appropriate model.

Results:

[530] A total of eight IL-17/IL-23p19 antagonists were characterized for their binding affinity for the human IL-17A antigen. Association rate constants (ka (M-1s-1)) and dissociation rate constants (kd (s-1)) were measured for these antagonists. Since the IL-17A antigen is a dimer, there is the potential for multivalent interaction with the IL-17/IL-23p19 antagonist. The bivalent analyte binding model was chosen to address this issue. This model measures two values for both ka (ka1 and ka2) and for kd (kd1 and kd2). The first set of values (ka1 and kd1) describes the monovalent kinetics of the interaction. The affinity reported for these samples was derived from these values, and is designated KD1 and KA1. The second set of values (ka2 and kd2) refers to the avidity of the interaction and was not reported. The data fit well to the bivalent analyte model. The kinetics of the antagonists containing the c631.1 scFv ranged from 3E+06 to 6E+06 (M-1s-1) for ka1 and 4E-05 to 3E-06 (s-1) for kd1, resulting in a calculated KD1 from 1E-11 to 8E-13 (M) [KA1 from 1E+11 to 1E+12 (M-1)]. The kinetics of the antagonists containing the c632.1 scFv ranged from 4E+05 to 1E+06 (M-1s-1) for ka1 and 4E-05 to 2E-05 (s-1) for kd1, resulting in a calculated KD1 from 1E-10 to 2E-11 (M) [KA1 from 1E+10 to 5E+10 (M-1)]. The measured affinity of the IL-17/IL-23p19 antagonists is higher than the affinity measured for the single domain antagonists.

Table 39: Binding Affinity for IL-17A

Description	Multivalent Construct #	k_{a1} ($M^{-1}s^{-1}$)	k_{d1} (s^{-1})	K_{D1} (M)	K_{A1} (M^{-1})
c305.1_c631.1 tascFv-Fc5	241	3.E+06	5.E-06	2.E-12	6.E+11
c631.1_c305.1 tascFv-Fc5	243	6.E+06	2.E-05	3.E-12	3.E+11
c305.1_c632.1 tascFv-Fc5	256	1.E+06	2.E-05	2.E-11	5.E+10
c632.1_c305.1 tascFv-Fc5	258	4.E+05	4.E-05	1.E-10	1.E+10
c305.1_c631.1 biscFv-Fc5	249	4.E+06	3.E-05	8.E-12	1.E+11
c631.1_c305.1 biscFv-Fc5	250	4.E+06	3.E-06	8.E-13	1.E+12
c632.1_c305.1 biscFv-Fc5	263	4.E+05	3.E-05	8.E-11	1.E+10
c305.1_c631.1 bi Ab	226	4.E+06	4.E-05	1.E-11	1.E+11

[531] A total of eight IL-17/IL-23p19 antagonists were characterized for their binding affinity for the human IL-23 antigen. Association rate constants (ka (M-1s-1)) and dissociation rate constants (kd (s-1)) were measured for these antagonist. The KD and the KA were calculated from the ka and kd values. The data fit well to the 1:1 binding model. All eight IL-17/IL-23p19 antagonists had an equivalent binding affinity for IL-23: ka of 1E+05 to 2E+05 (M-1s-1), kd of 5E-04 to 7E-04 (s-1), KD of 3E-09 to 7E-09 (M), and KA of 1E+08 to 4E+08 (M-1). The assembly configuration does not significantly affect the affinity of the IL-17/IL-23p19 antagonists. The affinity measured for these constructs are slightly lower than the affinity measured for the single domain antagonist.

Table 40: Binding Affinity for IL-23

Description	Multivalent Construct #	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	K_A (M^{-1})
c305.1_c631.1 tascFv-Fc5	241	2.E+05	6.E-04	3.E-09	3.E+08
c631.1_c305.1 tascFv-Fc5	243	1.E+05	5.E-04	5.E-09	2.E+08
c305.1_c632.1 tascFv-Fc5	256	2.E+05	6.E-04	3.E-09	3.E+08
c632.1_c305.1 tascFv-Fc5	258	1.E+05	5.E-04	5.E-09	2.E+08
c305.1_c631.1 biscFv-Fc5	249	2.E+05	6.E-04	3.E-09	3.E+08
c631.1_c305.1 biscFv-Fc5	250	2.E+05	5.E-04	3.E-09	4.E+08
c632.1_c305.1 biscFv-Fc5	263	1.E+05	5.E-04	5.E-09	2.E+08
c305.1_c631.1 bi Ab	226	1.E+05	7.E-04	7.E-09	1.E+08

EXAMPLE 32

IL17A CH6 – IL17F CEE Heterodimer Expression in 293F

[532] Two expression plasmids, encoding IL17A C(H6) and IL17F C(EE), were each constructed in vector pZMP45 via homologous recombination in yeast. The IL17A C(H6) fragment was created with PCR, using a DNA fragment containing the IL17A sequence as template, a forward primer to create a 5' overlap in pZMP45, and reverse primers to create a Serine-Glycine linker, the 6xHistidine tag, and a 3' overlap in pZMP45.

[533] The IL17F C(EE) fragment was created with PCR, using a previously generated plasmid containing IL17F as the template, a forward primer o create a 5' overlap in pZMP45, and reverse primers to create a Serine-Glycine linker, a EE tag, and a 3' overlap in pZMP45.

[534] The PCR conditions, using Platinum® PCR SuperMix High Fidelity (Invitrogen, Cat. #12532-016), were as follows: 1 cycle 94°C for 2 min; 30 cycles 94°C for 30 sec., 55°C for 30 sec, 68°C for 45 sec; then hold at 4°C. The PCR reaction mixtures were then run on a 1% agarose gel with 1x TAE. The correct bands were excised and purified using Qiagen's gel purification kit (Qiagen, catalog #28704).

[535] Plasmid pZMP45 is a mammalian expression vector containing an expression cassette having the CMV promoter, Intron A, multiple restriction sites for insertion of coding sequences, and an otPA signal peptide sequence, the SV40 terminator, an E. coli origin of replication, and URA3 and CEN-ARS sequences required for selection and replication in S. cerevisiae.

[536] One hundred µL of electrocompetent yeast cells (S. cerevisiae) were combined with 10µl of purified DNA from above, mixed with 100ng of BglII-cut pZMP45 plasmid, and transferred to a 0.2cm electroporation cuvette. The yeast-DNA mixture was electropulsed at 0.75kV (5kV/cm), ∞ ohms, 25µF. To each cuvette was added 1 ml of 1.2M sorbitol, and the yeast were plated onto a URA-DS plate and incubated at 30°C. After about 72 hours, approximately 50µL packed yeast cells taken from the Ura+ yeast transformants of a single plate was resuspended in 100µL of lysis buffer

(2% Triton X-100, 1% SDS, 100mM NaCl, 10mM Tris, pH 8.0, 1mM EDTA), 100µL of Qiagen P1 buffer from a Qiagen miniprep kit (Qiagen, Valencia, CA, catalog #27104), and 20U of Zymolyase (Zymo Research, Orange, CA, catalog #1001). This mixture was incubated for 30 minutes at 37°C, and the remainder of the Qiagen miniprep protocol was performed, starting with the addition of reagent P2. The DNA was eluted with 40 µL EB reagent.

[537] Fifteen µL electrocompetent E. coli cells (DH12S, Invitrogen, Carlsbad, CA) were transformed with 2 µL yeast DNA in a 0.2 cm electroporation cuvette. The cells were electropulsed at 1.75kV, 25µF, and 400 ohms. Following electroporation, 1 ml SOC (2% Bacto‘ Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10mM NaCl, 2.5mM KCl, 10mM MgCl2, 10mM MgSO4, 20mM glucose) was added to the cuvette. This solution was plated on two LB AMP plates (LB broth (Lennox), 1.8% Bacto Agar (Difco), 100mg/L Ampicillin), one with 200 µL of transformants, the second with 100 µL.

[538] Individual clones were picked from the transformation plates, and were sequenced to identify one clone containing the correct expression construct for IL17A CH6 and one for IL17F CEE. Larger scale plasmid DNA was isolated using the Invitrogen mega prep kit (Invitrogen, Carlsbad, CA, catalog #457009) according to manufacturer’s instructions.

Transfection into 293F Cells:

[539] To test for expression of the IL17A CH6 - IL17F CEE heterodimer, 293F cells were transiently transfected using Lipofectamine2000 (Invitrogen, Carlsbad, CA, catalog #11668-019) and OptiMEM (Invitrogen, Carlsbad, CA, catalog #31985-070) and grown in a 12-well plate. IL17A was previously determined to be the less expressed protein of the two, so a DNA ratio of 3 parts IL17A CH6 to 1 part IL17F CEE was used for the transfection. 2µg plasmid DNA and one million cells were used for the transfection. After 96 hours, medium was harvested and prepared for a Western blot assay.

[540] Invitrogen materials and protocols were used for the Western blot with anti-6X histidine (R&D Systems, Minneapolis, MN, catalog #MAB050H) as the detection antibody for IL17A CH6, and with an in-house mouse mAb as the detection antibody for IL17F CEE, and Jackson HRP-goat anti mouse IgG (H+L) (catalog# 115-035-003) + BD Parm. HRP-anti mouse IgG2a (R19-15) (catalog #553391) as the secondary antibodies. Significant expression was observed, so a large scale transfection was done for protein acquisition.

EXAMPLE 33

Transient Expression of IL17A/IL17F heterodimer in 293F Cells

[541] IL17A/IL17F heterodimer (aka zcyto40f2 CH6 CEE) was produced transiently in 293F cells (Invitrogen, Carlsbad, CA Cat# R790-07). DNA of ZG Constructs 1788 (IL17A) and 1811 (IL17F) was prepared using a Qiafilter Plasmid Mega Kit (Qiagen, Valencia, CA Cat#12281). 293F suspension cells were cultured in 293 Freestyle medium (Invitrogen, Carlsbad, CA Cat# 12338-018) at 37° C, 6% CO2 in four 3 L spinner flask at 95 RPM. Fresh medium was added immediately prior to transfection to obtain a 1.5 liter working volume at a final density of 1 x10E6 cells/mL. For each spinner, 2 mL of Lipofectamine 2000 (Invitrogen, Carlsbad, CA Cat# 11668-019) was added to 20 mL Opti-MEM medium (Invitrogen, Carlsbad, CA Cat# 31985-070) and 3 mg total DNA (2.25 mg IL17A DNA + 0.75 mg IL17F DNA) was diluted in a separate tube of 20 mL Opti-MEM. Each tube was incubated separately at room temperature for 5 minutes, then combined and incubated together for an additional 30 minutes at room temperature with occasional gentle mixing. The lipid-DNA mixture was added to each spinner of 293F cells which were then returned to 37° C, 6% CO2 at 75 RPM. After approximately 96 hours, the conditioned medium was harvested, 0.2 μM filtered and submitted for protein purification.

EXAMPLE 34

Purification of IL17A_IL17F hetrodimer from co expression

[542] Purification from Constructs # 1788; ZYMO IL17A CH6 pZMP45 and # 1811; ZYMO IL17F CEE pZMP45.

Cation Exchange chromatography Capture -

[543] Conditioned media was adjusted to pH 5.0 by slow addition of concentrated Acetic acid to the well stirred media. The final concentration of Acetic acid was 0.015 M. A column, 2.0 cm. Dia. (Millipore) with 16 mL SP FF resin (GE Healthcare) was equilibrated with 0.02 M Acetic acid; 100 mM NaCl pH 5.0 buffer prior to loading at 10 ml/min. with the adjusted conditioned media, overnight at 4 C. Following complete sample load, the column was washed with 20 column volumes of equilibration buffer until the absorbance at 280nm was baseline stable. Bound target was eluted at a flow of 20 ml/min. by forming a 20 column volume gradient between equilibration buffer, and elution buffer of the following composition; 20 mM Acetic acid; 1M NaCl, pH 5.0. Fractions of 5 ml were collected throughout and were subsequently analyzed by Reverse phase HPLC, SDS-PAGE (Coomassie staining), and Western Blot, probing for either the His-tag or EE–tags present on each chain. A pool was made based on interpretation of all the analytical data. The elution chromatogram

was biphasic with an early and late peak. The analytical data pointed clearly to the later eluting peak being putative heterodimeric product.

mAb-affinity chromatography (anti-EE-tag):

[544] The cation exchange pool, comprising the late eluate peak profile, was adjusted to pH 7.5 with dropwise addition of an equimolar mixture of mono and di-basic Sodium Phosphate forms. The adjusted material was loaded at a flow rate of 1.0 ml/min to a 16 ml bed; 2cm dia. column of anti-EE-tag mAb derivitized Sepharose that had been fully equilibrated in 10 mM Sodium Phosphate; 10 mM Citric acid, 500 mM NaCl, pH 7.5 buffer. Following the sample load, the column was washed with 10 column volumes of equilibration buffer until the absorbance at 280nm was baseline stable. For column washing, the flow rate was increased to 10 ml/minute until at least 10 column volumes of equilibration buffer were passed. The bound protein fraction was eluted at 10 ml/min with a 5 column volume gradient formed between equilibration buffer and elution buffer of the same composition as equilibration buffer, but at pH 3.0. The fraction contents were mixed immediately with neutralization buffer: 2M pH 8.0 Tris buffer (0.4 ml to each fraction tube prior to elution). Each tube was immediately mixed to ensure complete pH equilibration to neutrality.

Size exclusion chromatography:

[545] The EE-affinity column eluate pool was concentrated down to 3.0 ml and injected on to a Superdex 200 SEC column (16/60 format, GE Healthcare) flowing at 1.5 ml/minute in mobile phase of the following composition: 35 mM Sodium Phosphate Buffer, 109 mM NaCl, pH 7.3. A single, symmetric (Gaussian) peak elutes at ~ 0.65 CV. The entire peak is pooled, and demonstrated to be 100 % complex forming with either anti-His mAb, or anti-EE mAb in an analytical size exclusion peak shift assay.

EXAMPLE 35
Inhibition of Inflammation and Inflammatory Mediators in an *Ex Vivo* Multiple Sclerosis Model

[546] Multiple sclerosis is a complex disease that is thought to be mediated by a number of factors, including the presence of lymphocytic and mononuclear cell inflammatory infiltrates and demyelination throughout the CNS. Microglia are macrophage-like cells that populate the central nervous system (CNS) and become activated upon injury or infection. Microglia have been implicated as playing critical roles in various CNS diseases including multiple sclerosis, and may be used to study mechanism(s) of initiation, progression, and therapy of the disease (Nagai *et al. Neurobiol. Dis.* 8:1057 (2001); Olson *et al. J Neurosci Methods* 128:33 (2003)). Immortalized human microglial cell

lines and/or established human astroglia cell lines can, therefore, be used to study some of the effects of inflammatory mediators on these cell types and their potential for neutralization. Inflammatory mediators (including but not limited to IL-1b, IL-6, IL-8, IL-12, IL-13, IL-15, IL-17 A and F, IL-18, IL-23, TNF-a, IFN-g, MIP family members, RANTES, IP-10, MCP-1, G- and GM-CSF, etc.) can contribute to the symptoms and pathology associated with MS by way of their effect(s) on activating inflammatory pathways and downstream effector cells.

[547] In order to evaluate the pro-inflammatory actions of IL-17 and IL-23, and the ability of an antagonist to these activities, such as molecules that bind IL-17 and IL-23, either singly or together, or antibodies thereto including the anti-IL-17A antibodies, anti-IL-23p19 antibodies, and anti-IL-17A/anti-IL-23p19 antibodies of the present invention to neutralize or decrease these effects, cultured glial cells may be treated with one of the following: vehicle; rhIL-17; rhIL-23; or any of the compounds known to induce an inflammatory response by microglial and/or astroglia cells (e.g. LPS, other TLR agonists, inflammatory cytokines, etc.). Cells may be obtained from various commercial sources such as ATCC (Manassas, VA) and/or ScienCell Research Laboratories (Carlsbad, CA). In addition, these are treated with or without an antagonist of either IL-17 or IL-23, alone or in combination. After varying times in culture (from 1 h to several days), supernatants and cells are collected and analyzed for levels and/or expression of inflammatory mediators, including those listed above. Levels of inflammatory cytokines and chemokines are elevated in the presence of rhIL-17 and/or IL-23 and/or other inflammatory stimulants compared to cultures treated with vehicle alone. The addition of antagonists to IL-17 and/or IL-23 activity, such as the antibodies of the present invention can reduce the production and expression of inflammatory mediators, and thus, may be efficacious in inflammatory aspects associated with human multiple sclerosis.

EXAMPLE 36

Decreased Disease Incidence and Progression, and Prevention of Relapse in Mouse Experimental Allergic Encephalomyelitis (EAE) as a Model of Multiple Sclerosis

A) Mouse Allergic Encephalomyelitis (EAE) Model

[548] To evaluate the effects of potential therapies for multiple sclerosis, the animal model of experimental autoimmune encephalomyelitis (EAE) is commonly used. For the relapsing-remitting EAE model, 9 to 10 week old female SJL mice (Jackson or Charles River Labs) were immunized subcutaneously with proteolipid peptide (PLP) emulsified in complete Freund's adjuvant, and with intravenous pertussis toxin. Within approximately 6 to 23 days, animals begin to show symptoms of weight loss and paralysis that are characteristic of this model. The extent of disease is evaluated daily in the mice by taking their body weights and assigning a clinical score (0-8) to each mouse, as detailed below. The typical pattern of disease symptoms in immunized, but otherwise untreated mice,

is one of weight loss and paralysis, followed by a period of disease symptom remission, and a subsequent relapse of disease symptoms. A pattern of relapses and remissions of disease symptoms ensues, which is also found in humans with this type of multiple sclerosis, known as relapsing-remitting disease. Chronic progressive and secondary progressive multiple sclerosis are also targeted indications for this therapeutic combination of IL-17 and IL-23/p19 neutralizing antibodies or a single neutralizing entity such as a bispecific antibody or scFV as described in this invention. These latter types of multiple sclerosis are tested in a similar manner using MOG35-55 peptide in C57BL/6 mice, instead of PLP in SJL mice.

[549] Commercially available neutralizing monoclonal antibodies to mouse IL-17 (goat anti-mouse IL-17A mAb; R&D Systems, Minneapolis, MN) and IL-23p19 (goat anti-mouse IL-23p19; eBiosciences, San Diego, CA) were administered separately or as a therapeutic combination (i.e., not as a single bispecific molecule), during remission from the first peak of EAE disease symptoms. The antibodies were delivered as intraperitoneal injections every other day, or as a similar dosing regimen. Groups receive either 25, 50 or 100 μg of each antibody, alone or as a therapeutic combination, per animal per dose, and control groups receive the vehicle control, PBS (Life Technologies, Rockville, MD) or antibody isotype control (rat IgG2A isotype control, R&D Systems, or rat IgG1, k isotype control, eBiosciences).

B) Monitoring Disease

[550] Animals can begin to show signs of paralysis and weight loss between approximately 6 and 23 days following PLP or MOG35-55 immunizations. Most animals develop symptoms within 11 – 17 days of the immunizations, but some may show symptoms sooner or later than this.

[551] All animals are observed, weighed, and assigned a clinical score daily to assess the status of disease.

C) Clinical Score

0 = Normal; healthy; 1 = slight tail weakness (tip of tail does not curl and); 2 = tail paralysis (unable to hold tail upright); 3 = tail paralysis and mild waddle; 4 = tail paralysis and severe waddle; 5 = tail paralysis and paralysis of one limb; 6 = tail paralysis and paralysis of any 2 limbs; 7 = tetraparesis (all 4 limbs paralysed); and 8 = moribund or dead.

[552] Blood was collected throughout the experiment to monitor serum levels of cytokine and levels of other mediators of disease. At the time of euthanasia, blood was collected for serum, and brain and spinal cord collected in 10% NBF for histology. In separate animals, tissues (including lymph nodes, brain, spinal cord, spleen, and others) were harvested for the quantification of mRNA by TaqMan quantitative real-time PCR.

D) Results

[553] Groups of mice (n=13-15 each) receiving the therapeutic combination of neutralizing monoclonal antibodies to IL-17 and IL-23p19 were characterized by a significant ($p<0.05$) reduction

in disease severity as evidenced by significant ($p<0.05$) reductions in clinical score and body weight loss compared to mice treated with PBS, either of the antibodies alone at similar doses as those used in the combination, or isotype control antibodies. Furthermore, the mice treated with the therapeutic antibody combination had a complete absence of disease relapse, whereas all other treatment groups had 35 – 85% of mice experiencing significant disease relapse. Therefore, the delivery of the therapeutic combination was significantly more efficacious in reducing active disease and importantly, in preventing disease relapse, than the delivery of either monoclonal antibody alone at similar doses. This is a very important finding since disease relapse is a hallmark of this disease model and of relapsing-remitting MS in humans. There was extensive infiltration of inflammatory cells into the CNS parenchyma for mice treated with only the PBS or isotype control antibody. The greatest overall reduction of inflammatory cell infiltrates in the CNS of mice were those treated with the therapeutic combination of neutralizing monoclonal antibodies to IL-17 and IL23/p19. Treatment with these therapeutic antibodies also resulted in significant reductions in serum IL-6, IL-13, IL-17A, IL-23, G-CSF, and TNF-a concentrations compared to PBS-treated mice. Samples were collected at the same time point following peak of first disease onset (day 27) and after the same number of antibody doses (11 doses). Draining lymph nodes were harvested from the mice at this same time point and cultured for 24 h with PLP139-151. Mice treated with the therapeutic antibody combination had a lower percentage of TNF-a containing draining lymph node cells compared to other groups of mice. Thus, the significant reductions in disease severity and disease relapse in the mice treated with the antibody combination were associated with reductions in CNS inflammatory infiltrates and inflammatory cytokine production, suggesting a mechanism of action for the observed efficacy.

[554] Taken together, these results indicate that the therapeutic combination of IL-17 and IL23/p19 neutralizing antibodies is more efficacious in the treatment of EAE as a model of human multiple sclerosis. See Figures 4a and 4b. The therapeutic combination can reduce clinical disease symptoms and works at the molecular level to reduce inflammation, inflammatory infiltrates, inflammatory cytokines/chemokines, and other mechanisms known to be affected in this manner.

[555] Thus, the anti-IL-17A antibodies, anti-IL-17F antibodies, anti-IL-23p19 antibodies, and anti-IL-17A/anti-IL-23p19 antibodies described herein may be used in combination therapy (as in combined administration) or as a single entity, such as a bispecific (tascFv, biscFv, or BiAb, including as an Fc fusion) to treat multiple sclerosis and to prevent relapse in this disease.

EXAMPLE 37

Murine IL23p19 and IL-17A mRNAs are Regulated in Select Tissues in a Murine Model of Experimental Allergic Encephalomyelitis (EAE) Compared to Non-Diseased Controls

[556] Tissues were obtained from mice at the peak of disease onset in the PLP EAE model. The model was performed following standard procedures of immunizing female SJL mice with PLP139-151, as described in Example 8 above, in the presence or absence of pertussis toxin, and included appropriate unimmunized, non-diseased controls. Tissues that were collected included brain, spinal cord and cervical lymph nodes. RNA was isolated from all tissues using standard procedures. In brief, tissues were collected and immediately frozen in liquid N2 and then transferred to -80^0C until processing. For processing, tissues were placed in Qiazol reagent (Qiagen, Valencia, CA) and RNA was isolated using the Qiagen Rneasy kit according to manufacturer's recommendations. Expression of murine IL23p19 and IL-17A mRNAs were measured with multiplex real-time quantitative RT-PCR method (TaqMan) and the ABI PRISM 7900 sequence detection system (PE Applied Biosystems). IL23p19 and IL-17A mRNA levels were normalized to the expression of the murine hypoxanthine guanine physphoribosyl transferase mRNA and determined by the comparative threshold cycle method (User Bulletin 2; PE Applied Biosystems). Primers specific to IL23p19 (SEQ ID NO: 4) and to IL-17A (SEQ ID NO: 2) were used. The results were as follows:

1) Murine IL23p19 mRNA expression was detected in all tissues tested including brain, spinal cord and cervical lymph nodes;

2) Murine IL23p19 mRNA levels were increased approximately 2.2-fold in the spinal cord of mice immunized with PLP compared to unimmunized controls;

3) Murine IL-17 mRNA expression was detected at very low levels in the lymph nodes and below the level of detection in the brain and spinal cord of unimmunized, control mice;

4) Murine IL-17 mRNA levels were increased approximately 104-fold in the brain tissue of mice immunized with PLP compared to unimmunized controls;

5) Murine IL-17 mRNA levels were increased approximately 695-fold in the spinal cord of mice immunized with PLP compared to unimmunized controls; and

6) Murine IL-17 mRNA levels were increased approximately 1.9-fold in the cervical lymph nodes of mice immunized with PLP compared to unimmunized controls.

[557] Because there are significantly higher levels of both IL-17 and IL-23p19 in the CNS of mice (i.e. as measured in the same mice) with PLP-induced relapsing-remitting EAE, this further supports the need to antagonize both of these cytokines in multiple sclerosis. Thus, antagonists of the present invention have a therapeutic advantage over IL-17 or IL-23 treatment alone.

EXAMPLE 38

IL-17 and IL-23p19 Are Overexpressed in Tissues From Mice With Collagen Induced Arthritis (CIA) Compared to Tissue From Non-diseased Mice

A) Experimental Protocol

[558] Tissues were obtained from mice with varying degrees of disease in the collagen-induced arthritis (CIA) model. The model was performed following standard procedures of immunizing male DBA/1J mice with collagen in complete Freund's adjuvant (CFA) in the tail, followed 3 weeks later by similar immunizations, but with collagen in incomplete Freund's adjuvant (IFA). Non-diseased age- and gender-matched DBA/1J mice were also included for comparison. Tissues isolated included affected paws. RNA was isolated from the tissues using standard procedures. In brief, tissues were collected and immediately frozen in liquid N2 and then transferred to –80°C until processing. For processing, tissues were placed in Qiazol reagent (Qiagen, Valencia, CA) and RNA was isolated using the Qiagen Rneasy kit according to manufacturer's recommendations. Expression of murine IL-17 and IL-23p19 mRNA was measured with multiplex real-time quantitative RT-PCR methods (TaqMan) and the ABI PRISM 7900 sequence detection system (PE Applied Biosystems). Murine IL-17 and IL-23/p19 mRNA levels were normalized to the expression of murine hypoxanthine guanine physphoribosyl transferase mRNA and determined by the comparative threshold cycle method (User Bulletin 2: PE Applied Biosystems). The primers and probe for murine IL-17 and IL-23/p19 were the same as described in Example 37.

B) Results

[559] Murine IL-17 and IL-23/p19 mRNA expression was detected in the tissues tested. Both IL-17 and IL-23/p19 mRNA were increased in the paws from mice in the CIA model of arthritis compared to tissues obtained from non-diseased controls. Murine IL-17 mRNA was increased in the paws approximately 8-fold in mice with more mild disease and approximately 9.3-fold in mice with more severe disease compared to non-diseased controls. Murine IL-23/p19 mRNA was increased in the paws approximately 2.4 -fold in mice with more mild disease and approximately 2.1-fold in mice with more severe disease compared to non-diseased controls.

[560] Because there are significantly higher levels of both IL-17 and IL-23/p19 in the affected paws of mice (i.e. as measured in the same mice) with CIA, this further supports the need to antagonize both of these cytokines in rheumatoid arthritis.

EXAMPLE 39

IL-17 and IL-23/p19 Are Overexpressed in Tissues From Mice With DSS-Induced Colitis Compared to Tissue From Non-diseased Mice

A) Experimental Protocol

[561] Tissues were obtained from mice in the dextran sodium sulfate (DSS) model of colitis. The model was performed following standard procedures of administering 1.5 – 2.5 % DSS in the drinking water of female C57BL/6 mice for 5 – 7 days (acute protocol), followed by cycles of regular water and DSS water (chronic protocol). Non-diseased age- and gender-matched C57BL/6 mice were also included for comparison. Tissues isolated included the descending colon and proximal colon. RNA was isolated from the tissues using standard procedures. In brief, tissues were collected and immediately frozen in liquid N2 and then transferred to –80°C until processing. For processing, tissues were placed in Qiazol reagent (Qiagen, Valencia, CA) and RNA was isolated using the Qiagen Rneasy kit according to manufacturer's recommendations. Expression of murine IL-17 and IL-23/p19 mRNA was measured with multiplex real-time quantitative RT-PCR methods (TaqMan) and the ABI PRISM 7900 sequence detection system (PE Applied Biosystems). Murine IL-17 and IL-23/p19 mRNA levels were normalized to the expression of murine hypoxanthine guanine physphoribosyl transferase mRNA and determined by the comparative threshold cycle method (User Bulletin 2: PE Applied Biosystems). The primers and probe for murine IL-17 and IL-23/p19 were the same as described in Example 37.

B) Results

[562] Murine IL-17 and IL-23/p19 mRNA expression was detected in the tissues tested. Both IL-17 and IL-23/p19 mRNA were increased in the descending and proximal colon from mice in the acute and chronic DSS colitis model, compared to tissues obtained from non-diseased controls. Murine IL-17 mRNA was increased approximately 50-fold in the descending colon and approximately 180-fold in the proximal colon of mice treated acutely with DSS compared to non-diseased controls. With chronic DSS treatment, IL-17 mRNA levels were approximately 21- and 22-fold higher in the descending and proximal colon, respectively, compared to non-diseased controls. Murine IL-23/p19 mRNA was increased approximately 2-fold in the descending colon and approximately 4.4-fold in the proximal colon of mice treated acutely with DSS compared to non-diseased controls. With chronic DSS treatment, IL-23/p19 mRNA levels were approximately 1.5- and 2-fold higher in the descending and proximal colon, respectively, compared to non-diseased controls. Because there are significantly higher levels of both IL-17 and IL-23/p19 in the colons of mice (i.e. as measured in the same mice) with DSS colitis, this further supports the need to antagonize both of these cytokines in inflammatory bowel disease.

EXAMPLE 40

IL-17 and IL-23/p19 Are Overexpressed in Tissues From Mouse Model of T-cell Adoptive Transfer Colitis Compared to Tissue From Non-diseased Mice

T-cell Adoptive Transfer Colitis Model

[563] Adoptive transfer of naive T cells into minor histocompatibility mismatched or syngeneic immunocompromised mice leads to development of colitis (Leach MW et al 1996, Powrie F et al, 1997) as well as skin lesions resembling psoriasis (Schon MP et al 1997, Davenport CM et al 2002). Transplantation of as low as 0.2 million CD4+CD25- T cells from Balb/C mice into immunocompromised C.B-17 SCID mice results in weight loss, hemoccult positive stool and development of skin lesions (the symptoms in these mice vary from colony to colony). These symptoms normally arise in mice between 7-10 weeks after transplantation.

[564] This model of colitis has some similarities to human Crohn's disease and has been used extensively to test efficacy of therapeutics for this disease in humans. For this experiment, mice (10 Balb/C females, 20 CB17 SCID female) were obtained from CRL. Mice were on tap water starting on day –6. Spleens from 10 Balb/C mice will be collected. CD4+ CD25- T-cell will be collected from pooled spleen (see below for methods). CB17 SCID mice will receive either 5x10^5 or 7.5x10^5 CD4+ CD25- T-cells from spleen via i.v. injection. All mice are weighed 3x week and carefully observed for weight loss. When weight loss is observed, the Disease Activity Index (DAI) score [stool consistency, body weight, and blood in stool] are measured. Any animal with DAI score of 4 or body weight loss of greater than 20% are euthanized. There are no whole splenocytes control.

[565] For LPS/IL-12 accelerated psoriasis, CB17 SCID mice will receive $5x10^5$ CD4+ CD25- T-cells from spleen via i.v. injection. On day 0, 7, and 14, mice are treated with 20ug of LPS (from salmonella) and 10ng of rm IL-12 in 100ul i.p. injection. All mice are weighed 3x week and carefully observed for weight loss. When ear thickening is observed, ear thickness are measured 3x week. Mice are carefully monitored for signs of psoriasis (hair loss, scratching, alopecia, etc).

[566] Treatment groups were as follows: Colitis: (Group I) received 0.5 mil CD4+ CD25 cells, with a N of 6 and (Group II) received 0.75 mil CD4+ CD25 cells, with a N of 6. Psoriasis (Group III) received 0.5 mil CD4+ CD25 cells, with a N of 4.

[567] For histology, the distal part of small intestine and entire colon are collected in 10% NBF then transfer to 70% ETOH after 24 hours. Samples are submitted for histological evidence of colitis including presence of granuloma. For GEMS, MLN, distal part of small intestine, proximal colon and distal colon are collected and snap frozen in liquid nitrogen.

[568] CD4+ CD25- T-cell isolation: Balb/C mice are sacrificed by CO2 asphyxiation and spleens are removed and single cell suspensions made. CD4 T cells are enriched by using a sterile

magnetic enrichment protocol. CD4+CD25-T cells are further enriched using a sterile magnetic enrichment protocol or by sorting using a cell sorter. Purity of T-cells are evaluated by flow.

[569] Adoptive Transfer of CD4+CD25-T cells into SCID mice: Immunocompromised C.B-17 SCID mice were injected i.v. with either 0.5 or 0.75 million enriched CD4+CD25- T cells on d0. Colitis animals were sacrificed. Mice started to show sign of BW loss around day 15. On day 21, mice were observed with soft stool and diarrhea and were sacrificed. Mice were bled for serum via retro-orbital bleed then euthanized by cervical dislocation. An entire intestine was removed and entire colon was excised for length measurement. The Colon was cut in half (proximal and distal) and snap frozen. The ileum proximal to cecum was removed and snap frozen. Fecal matter was carefully removed from all the intestinal tissues collected. Mesenteric LNs were collected and snap frozen. For Histology (2 out of 6 animals from each group) an entire intestine was removed and flushed with PBS followed by 10% NBF. The entire intestine, intact, was fixed overnight in 10% NBF and transferred to 70% ETOH and submitted for histology.

[570] Tissues were obtained from mice after onset of disease in a murine model of T-cell adoptive transfer colitis. The model was performed following standard procedures of adoptive transfer of naive T cells into minor histocompatibility mismatched or syngeneic immunocompromised mice and included appropriate immunocompromised, non-diseased controls. Tissues that were collected included the distal part of small intestine, proximal colon, distal colon and mesenteric lymph node. RNA was isolated from all tissues using standard procedures. In brief, tissues were collected and immediately frozen in liquid N2 and then transferred to –800C until processing. For processing, tissues were placed in Qiazol reagent (Qiagen, Valencia, CA) and RNA was isolated using the Qiagen Rneasy kit according to manufacturer's recommendations. Expression was measured with multiplex real-time quantitative RT-PCR method (TaqMan) and the ABI PRISM 7900 sequence detection system (PE Applied Biosystems). IL-17mRNA levels were normalized to the expression of the murine hypoxanthine guanine physphoribosyl transferase mRNA and determined by the comparative threshold cycle method (User Bulletin 2; PE Applied Biosystems). The primers and probe for murine IL-17A and IL-23p19 were the same as described in Example 37.

Results:

IL-17A mRNA:

[571] -In the distal colon, mice with colitis had levels of IL-17A mRNA that were approximately 9000-fold greater than levels from control mice (no colitis).

[572] -In the proximal colon, mice with colitis had levels of IL-17A mRNA that were approximately 18,900-fold greater than levels from control mice (no colitis).

[573] -In the small intestine, mice with colitis had levels of IL-17A mRNA that were approximately 990-fold greater than levels from control mice (no colitis).

[574] -In the mesenteric lymph nodes, mice with colitis had levels of IL-17A mRNA that were approximately 175-fold greater than levels from control mice (no colitis).

[575] -In all these samples, IL-17A mRNA was basically undetectable in control mice.

IL-17F mRNA

[576] -In the distal colon, mice with colitis had levels of IL-17F mRNA that were approximately 3.8-fold greater than levels from control mice (no colitis).

[577] -In the proximal colon, mice with colitis had levels of IL-17F mRNA that were approximately 7.4-fold greater than levels from control mice (no colitis).

[578] -In the small intestine, mice with colitis had levels of IL-17F mRNA that were approximately 4.8-fold greater than levels from control mice (no colitis).

[579] -In the mesenteric lymph nodes, mice with colitis had levels of IL-17F mRNA that were approximately 213-fold greater than levels from control mice (no colitis).

IL-23p19 mRNA

[580] -In the small intestine, mice with colitis had levels of IL-23p19 mRNA that were approximately 2-fold greater than levels from control mice (no colitis).

[581] -In the mesenteric lymph nodes, mice with colitis had levels of IL-17A mRNA that were approximately 1.5 -fold greater than levels from control mice (no colitis).

EXAMPLE 41

Combination of Anti-IL-17 and Anti-IL-23p19 mAb in Murine Colitis is More Efficacious Than Either mAb Alone

[582] IL-23 and IL-17 are important players in murine colitis and human IBD, via the actions of Th17 cells. IL-23 and IL-17 are upregulated in colitis and IBD, and neutralization of either cytokine alone is efficacious in several animal models of colitis (Fujino et al, *Gut*, 2003, 52:65-70; Schmidt et al, *Inflamm Bowel Dis*. 2005, 11:16-23; Yen et al, *J Clin Invest.* 2006, 116:1310-1316 ; Zhang et al, *Inflamm Bowel Dis.* 2006, 12:382-388; Kullberg et al, *J Exp Med.* 2006, 203:2485-94.). Since IL-23 is important for the maintenance, differentiation, and/or induction of Th17 cells, neutralization of both cytokines would be more efficacious at reducing disease than either cytokine alone. The current example provides data to support this in oxazalone-induced colitis model, which resembles human ulcerative colitis (UC).

[583] Methods: For this experiment, 40 C57BL/10 female mice (obtained from Harlan) were used. On day -5, mice were treated topically with 200ul of 3.0% (w/v) oxazalone in 100%

ethanol ("sensitization") on the abdomen. On day 0, all mice receive intrarectal injections (120 uL each) of 2.0% (w/v) oxazalone in 50% ethanol while under light isoflurane gas anesthesia ("challenge"). Mice were monitored for disease using a Disease Activity Index (DAI) score, which includes stool consistency, body weight, and blood in stool. For mAb treatments, mice were administered one of the following, via i.p. injection on days -5, -3, and -1: PBS, 50 ug neutralizing anti-mouse IL-17 (goat anti-mouse IL-17A mAb; R&D Systems, Minneapolis, MN), 50 ug neutralizing anti-mouse IL-23p19 mAb (goat anti-mouse IL-23p19; eBiosciences, San Diego, CA), or a combination of the anti- IL-17 + IL-23p19 mAb's.

[584] Mice were euthanized on day 2. Serum was collected and stored for later analysis; colons were removed and observed for any gross signs of colitis (lesion, colon shortening, and colon wall thickening). Colons were then cut longitudinally and processed for histology and for 24 h colon cultures.

[585] Results: There was a significant reduction ($p = 0.0216$; 54% reduction compared to PBS group) in DAI score and significant ($p < 0.05$) improvement in histological morphology (e.g. reduced colonic damage and reduced inflammation) in mice treated with the combination of anti-IL-17 + anti-IL-23p19 antibodies, compared to PBS and either mAb alone. Consistent with these benefits, mice treated with the combination of antibodies also had significant improvements ($p = 0.0112$) in colon shortening compared to PBS-treated mice and mice treated with either of the antibodies alone. In the production of inflammatory cyokines from colon cultures, oxazalone mice treated with the anti-IL-17 + IL-23p19 combination antibodies had less increases in IL-1b, IL-12, IL-13, IL-17, IL-23, and TNF-a compared to PBS-treated oxazalone mice. Treatment with the combination of anti-IL-17 + IL-23p19 antibodies also resulted in significant reductions in serum IL-12, IL-17, IL-23, and TNF-a concentrations compared to PBS-treated mice. See Figure 5.

[586] Therefore, the delivery of the therapeutic combination of anti-IL-17 and anti-IL-23p19 antibodies was significantly more efficacious in reducing colitis from several aspects: disease symptoms, pathology, and inflammatory cytokine production. Taken together, these results indicate that the therapeutic combination of IL-17 and IL-23/p19 neutralizing antibodies was more efficacious in the treatment of oxazalone colitis as a model of human IBD. The therapeutic combination reduced clinical disease symptoms and worked at the molecular level to reduce inflammation, tissue damage, inflammatory cytokines, and other mechanisms known to be affected in this manner.

EXAMPLE 42

Stability of scFv Molecules

[587] Various stability assays can be performed to measure the stability of the anti-IL-17 and anti-IL-23 molecules described herein. These include, for example, microcalorimetry, thermal

reversibility, analytical size exclusion, selection of buffer conditions for storage and analysis, and thermal challenge.

[588] A) Analytical size exclusion: Analysis of the protein expressed by the anti-IL-23 construct c305 was performed as follows. The column was a QC-PAK TSK300, 150 x 7.8mmID, 5µm particle column, (Tosoh Bioscience, Montgomeryville, PA). The column was at room temperature, with the sample compartment at 5° C. For the mobile phase, the buffer was 25mM Histidine, 250mM NaCl, pH 6.8, and the flow was 1ml/min. The resulting SEC profile showed a peak of about 65 mAu at 280 nM at about 4.9 minutes. See also Figure 2 of U.S. Provisional Application number 61/039,022, filed March 4, 2008. Said Figure 2 is incorporated herein by reference. Also see Figure 3 herein.

[589] B) Microcalorimetry (Differential Scanning Calorimetry (DSC)): Analysis of the protein expressed by the anti-IL-23 and anti-IL-17 scFv constructs was performed as follows. The samples were prepared in two different exchange buffers: 1) 25mM Histidine/125mM NaCl (pH 6.8); and 2) P 1.47mM Potassium Phosphate/ 7.04mM Sodium Phosphate/ 7.68mM Potassium Chloride/ 137mM Sodium Chloride, pH adjusted to 7.2. 5K MWCO membrane centrifuge tubes were used with a Beckman Ultracentrifuge 30k rpm, rotor chilled to 5° C. The filtrate was retained for reference cell and protein concentration adjustment. The protein concentration was adjusted and confirmed by A280 to 0.34mg/mL. The instrument used to measure the microcalorimetry was a VP-DSC single cell system from MicroCal (MicroCal, LLC, Northampton, MA). The data collection parameters were: Scan rate 90° C/min; Temp Scan range 10 to 85 ° C; Pre/Post hold of 10min; and samples were diluted to 0.34mg/mL. Results are shown in the Table 41 below:

Table 41 : scFv Stability measured by Differential Scanning Calorimetry (DSC) (analysis ~ .02-.04 mg/ml)

Target	Construct	Tm	Formulation
IL-23	c305	68°	Phosphate pH 7.2
IL-23	c305	73°	Histidine pH 6.8
IL-23	c472	71°	Phosphate pH 7.2
IL-23	c472	76°	Histidine pH 6.8
IL-17A	c632	58°	Histidine pH 6.8
IL-17A	c631	54°	Histidine pH 6.8

[590] C) Thermal Challenges: Proteins from anti-IL-17 constructs c632 and c631 and from anti-IL-23 constructs c305 and c472 were diuted to 4 ug/ml in Dulbecco's Phosphate Buffer Saline (PBS) (Lonza, MD, #17-512F). 50 ul of each protein was then transferred into a PCR tube (VWR, Batavia, IL, #20170-004). Each sample was incubated for 1hr at temperatures ranging from 25-99oC

using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, #4343176). Following incubation, samples were then chilled on ice for 20 minutes. Each sample was then analyzed via binding ELISA at corresponding EC50.

[591] i) Binding ELISA for IL-17A: Costar (Corning Life Sciences, Lowell, MA, #9018) 96-well plates were coated with 50 ul IL-17A (made in-house) or IL-17F (made in-house) at 2 ug/ml in 0.1M NaHCO3, pH 9.6 overnight at 4oC. The next day, plates were washed three times with 0.1% Tween-20/PBS (PBST). Each well was filled with 350 ul of 2% milk (#170-6404, Bio-Rad Hercules, CA)/PBST for one hour at RT for blocking. Assay plates were then washed three times with PBST. Each well was filled with 50 ul of 2% milk/PBST, followed by the addition of 25 ul of Fab or scFv supernatant. Wells were then mixed and then incubated for one hour at RT. Plates were washed three times with PBST. For Fab detection, 50 ul of (1:4000) anti-Human Fab specific pAb-HRP (#31482, Pierce, Rockville, IL) in 2% milk/PBST was added to each well for one hour at RT. For scFv detection, 50 ul of (1:4000) anti-His tag mAb-HRP (Sigma, St. Louis, MO, #A7058) in 2% milk/PBST was added to each well for one hour at RT. Plates were then washed three times with PBST. 50 ul of TMB (TMBW-1000-01, BioFX Laboratories, Owing Mills, MD) was added to each well to develop for 20 – 30 min, followed by the addition of 50 ul of stop buffer (STPR-1000-01, BioFX Laboratories, Owing Mills, MD) to quench the reaction. Plates were then read at 450nm on a plate reader.

[592] ii) Binding ELISA for IL-23: Costar (Corning Life Sciences, Lowell, MA, #9018) 96-well plates were coated with 50 ul IL-23 (# 1290-IL-010/CF, R&D Systems, Minneapolis, MN, or produced in-house) or IL-12 (#219-IL/CF, R&D Systems Minneapolis, MN) at 4 ug/ml in 0.1M NaHCO3, pH 9.6, overnight at 4oC. The next day, plates were washed three times with 0.1% Tween-20/PBS (PBST). Each well was filled with 350 ul of 2% milk (#170-6404, Bio-Rad, Hercules, CA)/PBST for one hour at RT for blocking. Assay plates were then washed three times with PBST. Each well was filled with 50 ul of 2% milk/PBST, followed by the addition of 25 ul of Fab or scFv supernatant. Well were mixed and then incubated for one hour at RT. Plates were washed three times with PBST. For Fab detection, 50 ul of (1:4000) anti-Human Fab specific pAb-HRP (#31482, Pierce, Rockville, IL)) in 2% milk/PBST was added to each well for one hour at RT. For scFv detection with R&D IL-23, 50 ul of (1:4000) anti-His tag mAb-HRP (Sigma, St. Louis, MO, #A7058) in 2% milk/PBST was added to each well for one hour at RT. Plates were washed three times with PBST. 50 ul of TMB (TMBW-1000-01, BioFX Laboratories, Owing Mills, MD) added to each well to develop for 20 – 30 min, followed by the addition of 50 ul of stop buffer (STPR-1000-01, BioFX Laboratories, Owing Mills, MD) to quench the reaction. Plates were then read at 450nm on a plate reader. Results are shown in Table 42 below.

Table 42: scFv Stability Measured by Thermal Challenge (PCR ThermoCycler; Challenge at 4 ug/ml for 1hr, cool to 4°C; Binding ELISA; Assay at EC_{50} of each construct)

Target	Construct	T_{50}	Formulation
IL-23	c305	66°	Phosphate pH 7.2
IL-23	c472	56°	Phosphate pH 7.2
IL-17A	c632	81°	Phosphate pH 7.2
IL-17A	c631	47°	Phosphate pH 7.2

[593] Thus, within an aspect, the invention provides an isolated anti-IL-17 antibody fragment comprising an anti-IL-17 antibody fragment having Tm of between about 50 to about 60 °C measured by DSC. Within an embodiment the antibody fragment comprisies a bispecific molecule. Within another aspect, the invention provides, an isolated anti-IL-23 antibody fragment comprising an anti-IL-23 antibody fragment having Tm of between about 60 to about 80 °C measured by DSC. Within another embodiment, the antibody fragment binds the IL-17A/F heterodimer. Within other embodiments the antibody fragment binds the IL-17A/F heterodimer. Within an embodiment, the antibody fragment comprisies a bispecific molecule.

[594] Within another aspect, the invention provides an isolated anti-IL-17 antibody fragment comprising an anti-IL-17 antibody fragment having T50 of between about 47 to about 85 °C measured by thermal challenge in phosphate buffer at pH 7.2. Within an embodiment, the antibody fragment comprisies a bispecific molecule.

[595] Within another aspect the invention provides an isolated anti-IL-23 antibody fragment comprising an anti-IL-23 antibody fragment having T50 of between about 50 to about 70 °C measured by thermal challenge in phosphate buffer at pH 7.2 Within an embodiment, the antibody fragment binds the IL-17A/F heterodimer. Within another embodiment the antibody fragment comprisies a bispecific molecule.

[596] Within another aspect, the invention provides an isolated anti-IL-17/anti-IL-23 bispecific molecule that is capable of neutralizing IL-17A (IC50) in the SAEC assay at between 0.001 to 2.5 nM. Within an embodiment the range is between 0.01 and 0.04 nM.

[597] Within another aspect, the invention provides an isolated anti-IL-17/anti-IL-23 bispecific molecule that is capable of neutralizing IL-23 (IC50) at between 0.01 to7.0 nM. Within an embodiment the range is between 0.041 and 0.09 nM. Within another embodiment, the antibody fragment binds the IL-17A/F heterodimer.

EXAMPLE 43

FCRN binding assay for measuring binding of IL-17IL-23 bispecific antagonists to FCRN at pH 6.0 and pH 7.4

[598] FcRn is the neonatal Fc receptor. It protects IgG from degradation and allows for IgG transport through a cell layer and down a concentration gradient of IgG. In addition, it extends IgG half-life by recycling IgG back into the circulation, thus extending its half-life.

[599] Materials and Methods: Two plates are set up with IL-17A/IL-23 bispecific antagonists and control antibodies: one to wash at pH 6.0 and one to wash at pH 7.4. Day 1: Two Nunc Maxisorp 96 well elisa plates (cat # 44-2404) are coated with 300ng/well NeutrAvidin (Pierce Chemical Co. cat. # 31000) made up in 100mM NaHC03, pH 9.3. Plates are incubated at 4°C overnight. Day 2: The plates are washed 5 times with 0.1% Tween-20/PBS (PBST). The plates are then blocked with 250ul/well of blocking buffer containing 0.8% NaCl, 0.02% KCL, 0.102% Na2HPO4, 0.02% KH2PO4, 1% BSA, 0.05% Polysorbate, 0.05% Proclin 300 pH 7.2, for one hour at room temperature. The plates are then washed 2 times with PBST. Each well is then coated with 150 ng of biotinylated FCRN protein (produced in-house) diluted in PBST + 1% BSA. Plates are incubated at room temperature for one hour. IL-17A/IL-23 bispecific antagonists and control antibodies (Herceptin, for example) are diluted in 100mM NaPO4, 0.05% Tween 20 (v/v), + 0.1% BSA adjusted to pH 6.0 (pH 6.0 buffer) at concentrations ranging from 150 mM to 0.0185 mM. Samples are tested in duplicate at a volume of 50ul/well of each concentration. pH 6.0 buffer only is run as a control to determine the background levels on each plate. Plates are incubated at room temperature for two hours. After the binding step, each plate is washed in separate buffers: one plate is washed with 250 ul/well of pH 6.0 buffer, and one plate is washed with 250 ul/well of 100mM NaPO4, 0.05% Tween 20 (v/v), 0.1% BSA adjusted to pH 7.4 (pH 7.4 buffer). Plates are incubated in wash buffers at room temperature for a total of one hour with a wash step performed every twenty minutes. Following the wash steps, the bound antibody is detected with 100 ul/well of HRP goat anti-human IgG F(ab)2 fragment FC gamma specific secondary antibody (Jackson Immunoresearch Cat. #109-036-098). The secondary antibody is diluted 1:5,000 in the pH 6.0 buffer, and the incubation is done for one hour at room temperature. Plates are then washed 5 times with PBST. Finally, 100 ul of TMB (TMBW-1000-01, BioFX Laboratories) is added to each well, and the plates are developed at room temperature for approximately three minutes. At this point, 100 ul/well of stop buffer (STPR-100-01, BioFX Laboratories) is added to quench the reaction. The plates are read on a spectrophotometer at a wave length of 450/570nm. OD values are examined to compare binding patterns at pH 6.0 and release patterns at pH 7.4. Results indicate that C-terminal fusion (i.e., BiscFv-Fc and BiAb) does not alter FcRn binding at pH 6.0.

[600] From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

Claims

What is claimed is:

1. An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising

a) a light chain variable region comprising:

i) a LCDR1 consisting of the amino acid sequence of SEQ ID NO: 12;

ii) a LCDR2 consisting of the amino acid sequence of SEQ ID NO: 13; and

iii) a LCDR3 consisting of the amino acid sequence of SEQ ID NO: 14; and

b) a heavy chain variable region comprising:

i) a HCDR1 consisting of the amino acid sequence of SEQ ID NO: 15;

ii) a HCDR2 consisting of the amino acid sequence of SEQ ID NO: 16; and

iii) a HCDR3 consisting of the amino acid sequence selected from the group consisting of

1) the amino acid sequence of SEQ ID NO: 17;
2) the amino acid sequence of SEQ ID NO: 18; and
3) the amino acid sequence of SEQ ID NO:19.

2. The antibody of claim 1, wherein said HCDR3 amino acid consists of the amino acid sequence of SEQ ID NO: 17.

3. The antibody of claim 1, wherein said HCDR3 amino acid consists of the amino acid sequence of SEQ ID NO: 18.

4. The antibody of claim 1, wherein said HCDR3 amino acid consists of the amino acid sequence of SEQ ID NO: 19.

5. The isolated antibody of claim 1, wherein

LCDR1 has the amino acid sequence of SEQ ID NO: 30;

LCDR2 has the amino acid sequence of SEQ ID NO: 26;

LCDR3 has the amino acid sequence of SEQ ID NO: 31;

HCDR1 has the amino acid sequence of SEQ ID NO: 34;

HCDR2 has the amino acid sequence of SEQ ID NO: 35; and

HCDR3 has the amino acid sequence of SEQ ID NO: 18.

6. The isolated antibody of claim 5, wherein said antibody has the ATCC Patent Deposit Designation PTA-8819.

7. An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a variable light domain comprising LCDRs LCDR1, LCDR2, and LCDR3 and a variable heavy domain comprising HCDRs HCDR1, HCDR2, and HCDR3, wherein the set of LCDRs has three, two, or one amino acid substitutions and the set of HCDRs has three or fewer amino acid substitutions in which

LCDR1 has the amino acid sequence of SEQ ID NO: 30;

LCDR2 has the amino acid sequence of SEQ ID NO: 26;

LCDR3 has the amino acid sequence of SEQ ID NO: 31;

HCDR1 has the amino acid sequence of SEQ ID NO: 34;

HCDR2 has the amino acid sequence of SEQ ID NO: 35; and

HCDR3 has the amino acid sequence of SEQ ID NO: 18.

8. An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising

a) a light chain variable region comprising:

i) a LCDR1 consisting of the amino acid sequence of SEQ ID NO: 72;

ii) a LCDR2 consisting of the amino acid sequence of SEQ ID NO: 73; and

iii) a LCDR3 consisting of the amino acid sequence of SEQ ID NO: 74; and

b) a heavy chain variable region comprising:

i) a HCDR1 consisting of the amino acid sequence of SEQ ID NO: 75;

ii) a HCDR2 consisting of the amino acid sequence of SEQ ID NO: 76; and

iii) a HCDR3 consisting of the amino acid sequence selected from the group consisting of

1) the amino acid sequence of SEQ ID NO: 17;

2) the amino acid sequence of SEQ ID NO: 18;

3) the amino acid sequence of SEQ ID NO:19;

4) the amino acid sequence of SEQ ID NO: 77;

5) the amino acid sequence of SEQ ID NO: 78; and

6) the amino acid sequence of SEQ ID NO: 79.

9. The antibody of claim 8, wherein said HCDR3 amino acid consists of the amino acid sequence of SEQ ID NO: 19.

10. The antibody of claim 1, wherein

LCDR1 has the amino acid sequence of SEQ ID NO: 25;

LCDR2 has the amino acid sequence of SEQ ID NO: 26;

LCDR3 has the amino acid sequence of SEQ ID NO: 27;

HCDR1 has the amino acid sequence of SEQ ID NO: 28;

HCDR2 has the amino acid sequence of SEQ ID NO: 29; and

HCDR3 has the amino acid sequence of SEQ ID NO: 19.

11. The antibody of claim 10, wherein said antibody has the ATCC Patent Deposit Designation PTA-8821.

12. An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a variable light domain comprising LCDRs LCDR1, LCDR2, and LCDR3 and a variable heavy domain comprising HCDRs HCDR1, HCDR2, and HCDR3, wherein the set of LCDRs has three, two, or one amino acid substitutions and the set of HCDRs has three or fewer amino acid substitutions in which

LCDR1 has the amino acid sequence of SEQ ID NO: 25;

LCDR2 has the amino acid sequence of SEQ ID NO: 26;

LCDR3 has the amino acid sequence of SEQ ID NO: 27;

HCDR1 has the amino acid sequence of SEQ ID NO: 28;

HCDR2 has the amino acid sequence of SEQ ID NO: 29; and

HCDR3 has the amino acid sequence of SEQ ID NO: 19.

13. An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a first polypeptide having at least 90% sequence identity to the variable light region of an antibody entity and comprising a second polypeptide having at least 90% sequence identity to the variable heavy region of said antibody entity, wherein the antibody entity comprises a variable light region and a variable heavy region selected from the group consisting of:

a) the variable light region consisting of the amino acid sequence of SEQ ID NO: 932 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 933;

b) the variable light region consisting of the amino acid sequence of SEQ ID NO: 1192 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 1193; and

c) the variable light region consisting of the amino acid sequence of SEQ ID NO: 1194 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 1195.

14. The antibody of claim 13, wherein said antibody is a bispecific antibody that also binds IL-23A (SEQ ID NO: 4).

15. The antibody of claim 14, wherein the antibody comprises the variable light region of SEQ ID NO: 1904 and the variable heavy region of SEQ ID NO: 1905.

16. The antibody of claim 14, wherein the antibody comprises the variable light region of SEQ ID NO: 1982 and the variable heavy region of SEQ ID NO: 1983.

17. The antibody of claim 14 wherein said antibody neutralizes the activity of IL-17A (SEQ ID NO: 2).

18 An isolated antibody that specifically binds IL-23A (SEQ ID NO: 4) comprising

a) a light chain variable region comprising:

i) a LCDR1 consisting of the amino acid sequence of SEQ ID NO: 224;

ii) a LCDR2 consisting of the amino acid sequence of SEQ ID NO: 225; and

iii) a LCDR3 consisting of the amino acid sequence of SEQ ID NO: 226; and

b) a heavy chain variable region comprising:

i) a HCDR1 consisting of the amino acid sequence of SEQ ID NO: 227;

ii) a HCDR2 consisting of the amino acid sequence of SEQ ID NO: 228; and

iii) a HCDR3 consisting of the amino acid sequence of SEQ ID NO: 252.

19. The antibody of claim 18, wherein

LCDR1 has the amino acid sequence of SEQ ID NO: 2105;

LCDR2 has the amino acid sequence of SEQ ID NO: 2106;

LCDR3 has the amino acid sequence of SEQ ID NO: 2107;

HCDR1 has the amino acid sequence of SEQ ID NO: 2108;

HCDR2 has the amino acid sequence of SEQ ID NO: 2109; and

HCDR3 has the amino acid sequence of SEQ ID NO: 2110.

20. The antibody of claim 19, wherein said antibody has the ATCC Patent Deposit Designation PTA-8820.

21. An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a variable light domain comprising LCDRs LCDR1, LCDR2, and LCDR3 and a variable heavy domain comprising HCDRs HCDR1, HCDR2, and HCDR3, wherein the set of LCDRs has three, two, or one amino acid substitutions and the set of HCDRs has three or fewer amino acid substitutions in which

LCDR1 has the amino acid sequence of SEQ ID NO: 2105;

LCDR2 has the amino acid sequence of SEQ ID NO: 2106;

LCDR3 has the amino acid sequence of SEQ ID NO: 2107;

HCDR1 has the amino acid sequence of SEQ ID NO: 2108;

HCDR2 has the amino acid sequence of SEQ ID NO: 2109; and

HCDR3 has the amino acid sequence of SEQ ID NO: 2110.

22. The isolated of claim 7, 12, or 21, wherein the antibody has two or one amino acid substitutions in the set of LCDRs.

23. The isolated antibody of claim 7, 12, or 21, wherein the antibody has two or one amino acid substitutions in the set of HCDRs.

24, An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2) comprising a first polypeptide having at least 90% sequence identity to the variable light region of an antibody entity and comprising a second polypeptide having at least 90% sequence identity to the variable heavy region of said antibody entity, wherein the antibody entity comprises a variable light region and a variable heavy region selected from the group consisting of:

a) the variable light region consisting of the amino acid sequence of SEQ ID NO: 1934 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 1935;

b) the variable light region consisting of the amino acid sequence of SEQ ID NO: 1982 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 1983; and

c) the variable light region consisting of the amino acid sequence of SEQ ID NO: 2014 and the variable heavy region consisting of the amino acid sequence of SEQ ID NO: 2015.

25. The antibody of claim 13 or 24, wherein the first polypeptide has at least 95% sequence identity with the framework regions of the variable light region.

26. The antibody of claim 13 or 24, wherein the first polypeptide has at least 95% sequence identity with the framework region of the variable light region.

27. The antibody of claim 13 or 24, wherein the first polypeptide has at least 95% sequence identity with the variable light region.

28. The antibody of claim 13 or 24, wherein the first polypeptide has at least 97% sequence identity with the variable light region.

29. The antibody of claim 13 or 24, wherein the first polypeptide has at least 98% sequence identity with the variable light region.

30. The antibody of claim 13 or 24, wherein the first polypeptide has at least 99% sequence identity with the variable light region.

31. The antibody of claim 13 or 24, wherein the second polypeptide has at least 95% sequence identity with the framework regions of the variable heavy region.

32. The antibody of claim 13 or 24, wherein the second polypeptide has at least 95% sequence identity with the variable heavy region.

33. The antibody of claim 13 or 24, wherein the second polypeptide has at least 96% sequence identity with the variable heavy region.

34. The antibody of claim 13 or 24, wherein the second polypeptide has at least 97% sequence identity with the variable heavy region.

35. The antibody of claim 13 or 24, wherein the second polypeptide has at least 98% sequence identity with the variable heavy region.

36. The antibody of claim 13 or 24, wherein the first polypeptide has at least 99% sequence identity with the variable heavy region.

37. A bispecific antibody comprising the anti-IL-17A antibody of claim 24, wherein the antibody comprises the variable light region of SEQ ID NO: 1192 and the variable heavy region of SEQ ID NO: 1193.

38. The antibody of claim 37, wherein the bispecific antibody binds the heterodimer formed by IL-17A (SEQ ID NO: 2) with IL-17F (SEQ ID NO: 6).

39. The antibody of claim 37, wherein the antibody comprises the variable light region of SEQ ID NO: 1194 and the variable heavy region of SEQ ID NO: 1195.

40. The antibody of claim 39, wherein the bispecific antibody bind the heterodimer formed by IL-17A (SEQ ID NO: 2) with IL-17F (SEQ ID NO: 6).

41. The antibody of claim 37 wherein said antibody neutralizes the activity of IL-23A (SEQ ID NO: 4).

42. An isolated bispecific antibody that specifically binds to IL-17A (SEQ ID NO: 2) and to IL-23A (SEQ ID NO: 6) comprising an anti-IL-17A antibody and an anti-IL-23A antibody, wherein the anti-IL-17A antibody comprises a first variable light region, which comprises a set of first LCDRs, and comprising a first variable heavy region, which comprises a set of first HCDRs , wherein said set of first LCDRs and said set of first HCDRs are selected from the group consisting of:

a) a first LCDR1 consisting of the amino acid sequence of SEQ ID NO: 25, and a first LCDR2 consisting of the amino acid sequence of SEQ ID NO: 26, and a first LCDR3 consisting of the amino acid sequence of SEQ ID NO: 27, a first HCDR1 consisting of the amino acid sequence of SEQ ID NO: 28, a first HCDR2 consisting of the amino acid sequence of SEQ ID NO: 29, and a first HCDR3 consisting of the amino acid sequence of SEQ ID NO: 19;

b) a first LCDR1 consisting of the amino acid sequence of SEQ ID NO: 30, and a first LCDR2 consisting of the amino acid sequence of SEQ ID NO: 26, and a first LCDR3 consisting of the amino acid sequence of SEQ ID NO: 31, a first HCDR1 consisting of the amino acid sequence of SEQ ID NO: 32, a first HCDR2 consisting of the amino acid sequence of SEQ ID NO: 33, and a first HCDR3 consisting of the amino acid sequence of SEQ ID NO: 18; and

c) a first LCDR1 consisting of the amino acid sequence of SEQ ID NO: 30, and a first LCDR2 consisting of the amino acid sequence of SEQ ID NO: 26, and a first LCDR3 consisting of the amino acid sequence of SEQ ID NO: 31, a first HCDR1 consisting of the amino acid sequence of SEQ ID NO: 34, a first HCDR2 consisting of the amino acid sequence of SEQ ID NO: 35, and a first HCDR3 consisting of the amino acid sequence of SEQ ID NO: 18;

and wherein the anti-IL-23A antibody comprises a second variable light region, which comprises a set of second LCDRs, and comprising a second variable heavy region, which comprises a set of second HCDRs , wherein said set of second LCDRs and said set of second HCDRs are selected from the group consisting of:

a) a second LCDR1 consisting of the amino acid sequence of SEQ ID NO: 2086, and a second LCDR2 consisting of the amino acid sequence of SEQ ID NO: 2087, and a second LCDR3 consisting of the amino acid sequence of SEQ ID NO: 2088, a second HCDR1 consisting of the amino acid sequence of SEQ ID NO: 2089, a second HCDR2 consisting of the amino acid sequence of SEQ ID NO: 2092, and a second HCDR3 consisting of the amino acid sequence of SEQ ID NO: 2091;

b) a second LCDR1 consisting of the amino acid sequence of SEQ ID NO: 2093, and a second LCDR2 consisting of the amino acid sequence of SEQ ID NO: 2094, and a second LCDR3 consisting of the amino acid sequence of SEQ ID NO: 2095, a second HCDR1 consisting of the amino acid sequence of SEQ ID NO: 2096, a second HCDR2 consisting of

the amino acid sequence of SEQ ID NO: 2097, and a second HCDR3 consisting of the amino acid sequence of SEQ ID NO: 2098;

c) a second LCDR1 consisting of the amino acid sequence of SEQ ID NO: 2099, and a second LCDR2 consisting of the amino acid sequence of SEQ ID NO: 2100, and a second LCDR3 consisting of the amino acid sequence of SEQ ID NO: 2101, a second HCDR1 consisting of the amino acid sequence of SEQ ID NO: 2102, a second HCDR2 consisting of the amino acid sequence of SEQ ID NO: 2103, and a second HCDR3 consisting of the amino acid sequence of SEQ ID NO: 2104; and

d) a second LCDR1 consisting of the amino acid sequence of SEQ ID NO: 2105, and a second LCDR2 consisting of the amino acid sequence of SEQ ID NO: 2106, and a second LCDR3 consisting of the amino acid sequence of SEQ ID NO: 2107, a second HCDR1 consisting of the amino acid sequence of SEQ ID NO: 2108, a second HCDR2 consisting of the amino acid sequence of SEQ ID NO: 2109, and a second HCDR3 consisting of the amino acid sequence of SEQ ID NO: 2110.

43. The bispecific antibody of claim 42, wherein the anti-IL-17A antibody and the anti-IL-23A antibody are covalently linked via a linker.

44. The bispecific antibody of claim 42, wherein t the anti-IL-17A antibody and the anti-IL-23A antibody are linked via a peptide linker.

45. The bispecific antibody of claim 42, wherein the anti-IL-17A and anti-IL-23A antibodies are single chain Fc fragments covalently linked to form a tandem single chain Fv (tascFV) or a bispecific single chain Fv (biscFv).

46. The bispecific antibody of claim 45, comprising a pharmaceutically acceptable carrier.

47. The bispecific antibody of claim 42, wherein the anti-IL-17A antibody binds the heterodimer formed by IL-17A (SEQ ID NO: 2) and IL-17F (SEQ ID NO: 6).

48. The bispecific antibody of claim 42, wherein the anti-IL-17A antibody neutralizes the activity of IL-17A (SEQ ID NO: 2) and wherein the anti-IL-23A antibody neutralizes the activity of IL-23A (SEQ ID NO: 4).

49. The bispecific antibody of claim 42, wherein said bispecific antibody comprises an immunoglobulin heavy chain constant region.

50. The bispecific antibody of claim 49, wherein said immunoglobulin heavy chain constant region is an Fc fragment.

51. The bispecific antibody of claim 49, wherein said Fc fragment comprises a Fc region modified to reduce or eliminate one or more effector functions.

52. The bispecific antibody of claim 42, comprising PEG.

53. A polynucleotide encoding the bispecific molecule of claim 42.

54. An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2), wherein the antibody binds at least one amino acid of SEQ ID NO: 2222.

55. The antibody of claim 54, wherein the antibody specifically binds at least one amino acid of SEQ ID NO:2221.

56. An isolated antibody that specifically binds IL-17A (SEQ ID NO: 2), wherein the antibody is capable of binding an immobilized peptide consisting of the amino acid sequence of TNTNPKRSSDYYNRSTSPW (SEQ ID NO: 2222).

57. The antibody of claim 56, wherein the antibody is additionally capable of binding an immobilized peptide consisting of the amino acid sequence of NLNIHNRNTNTNPKR, (SEQ ID NO: 2221).

58. An isolated antibody that specifically binds IL-23A (SEQ ID NO: 4), wherein the antibody binds at least one amino acid from the peptide sequence of LQRIHQGLIFYEKLLGSDIFTGE (SEQ ID NO: 2236), and at least one amino acid from the peptide sequence of SLLPDSPVGQLHASLLGLSQLLQPEG (SEQ ID NO: 2237) , and at least one amino acid from the peptide sequence of WETQQIPSLSPSQPWQRLL (SEQ ID NO: 2238).

59. An isolated antibody that specifically binds IL-23A (SEQ ID NO: 4), wherein the antibody is capable of binding an immobilized peptide consisting of the amino acid sequence of LQRIHQGLIFYEKLLGSDIFTGE (SEQ ID NO: 2236) , and capable of binding an immobilized amino acid sequence of LLPDSPVGQLHASLLGLSQLLQPEG (SEQ ID NO: 2237), and capable of binding an immobilized peptide consisting of the amino acid sequence of WETQQIPSLSPSQPWQRLL (SEQ ID NO: 2238).

60. An anti-IL-17A antibody comprising the antibody deposited with ATCC as clone c631.1 HL scFv_pARB13 in zGold5 strain (MVE #228) given ATCC Patent Deposit Designation PTA-8819).

61. An anti-IL-17A antibody comprising the antibody deposited with ATCC as clone c632.1 LH scFv_pARB13 in DH10B strain (MVE #488) given ATCC Patent Deposit Designation PTA-8821).

62. An anti-IL-23p19 antibody comprising the antibody deposited with ATCC as clone c472.2 LH scFv_pARB13 in DH10B strain (MVE # 263) given ATCC Patent Deposit Designation PTA-8820).

63. A method for inhibiting IL-17A production by T cells comprising treating said T cells with the anti-23A antibody of claim 15.

64. A method for inhibiting IL-17A production by T cells comprising treating said T cells with the bispecific antibody of claim 42.

65. A method for inhibiting IL-17A production by T cells comprising treating said T cells with the anti-17A antibody of claim 1.

66. A method for treating a disease characterized by elevated expression of IL-17A, IL-17F, or IL-23 in a mammalian subject, comprising administering to said subject the bispecific antibody of claim 42.

67. A method for inhibiting or reducing relapse in multiple sclerosis or in inflammatory bowel disease in a mammalian subject, comprising administering to said subject the bispecific antibody of claim 42.

68. A method for treating multiple sclerosis or in inflammatory bowel disease in a mammalian subject, comprising administering to said subject the bispecific antibody of claim 42.

69. A method for inhibiting or reducing relapse in multiple sclerosis or in inflammatory bowel disease in a mammalian subject, comprising co-administering to said subject the anti-IL-17A antibody of claim 1 with the anti-IL-23A antibody of claim 15.

70. The method of claim 96, wherein the anti-IL-17A antibody and the anti-IL-23A antibody are administered in one entity.

71. An isolated bispecific antibody comprising the amino acid sequence selected from the group consisting of:

a) the amino acid sequence of SEQ ID NO: 2160;

b) the amino acid sequence of SEQ ID NO: 2151;

c) the amino acid sequence of SEQ ID NO: 2152;

d) the amino acid sequence of SEQ ID NO: 2138; and

e) the amino acid sequence of SEQ ID NO: 2149.

72. The antibody of any previous claim, wherein said antibody binds the heterodimer formed by IL-17A (SEQ ID NO: 2) and IL-17F (SEQ ID NO: 6).

73. The antibody of any previous claim, wherein said antibody comprises a single chain Fv fragment (scFv).

74. The antibody of any previous claim, wherein said antibody is a chimeric antibody.

75. The antibody of any previous claim, wherein said antibody is a bispecific antibody that also binds IL-23A (SEQ ID NO: 4)

					LC						HC			HC	
			218				222								230
wt	|Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro|
Fc4	| .	.	Arg	.	Ser	.	.	.	.	.	.	.	.	.	. |
Fc5	| .	.	.	.	Ser	.	.	.	.	.	.	.	.	.	. |
Fc6	| .	.	.	.	Ser	.	.	.	.	.	.	.	.	.	. |
Fc7	| .	.	.	.	.	.	.	.	.	.	.	.	.	.	. |
	|						<- hinge ->								|

				234	235		237								245
wt	|Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro
Fc4	| .	.	.	Ala	Glu	.	Ala	.	.	.	.	.	.	.	.
Fc5	| .	.	.	Ala	Glu	.	Ala	.	.	.	.	.	.	.	.
Fc6	| .	.	.	Ala	Glu	.	Ala	.	.	.	.	.	.	.	.
Fc7	| .	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	|CH2	->													

															260
wt	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

															275
wt	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

															290
wt	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

							297								305
wt	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	Gln	.	.	.	.	.	.	.	.

Fig. 1A

																320
wt	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

										330	**331**					**335**
wt	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr
Fc4	.	.	.	.	.	.	.	.	.	Ser	Ser	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	Ser	Ser	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	Ser	Ser	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

																350
wt	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

<- CH2|CH3 ->

						356		**358**								**365**
wt	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

																380
wt	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

																395
wt	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Fig. 1B

410																
wt	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys		
Leu																
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

425																
wt	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser		
Cys																
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

						431										
440																
wt	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys		
Ser																
Fc4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Fc7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

							446									
wt	Leu	Ser	Leu	Ser	Pro	Gly	Lys	***								
Fc4	.	.	.	.	.	.	.	.								
Fc5	.	.	.	.	.	.	.	.								
Fc6	.	.	.	.	.	.	***									
Fc7	.	.	.	.	.	.	.	.								

Fig. 1C

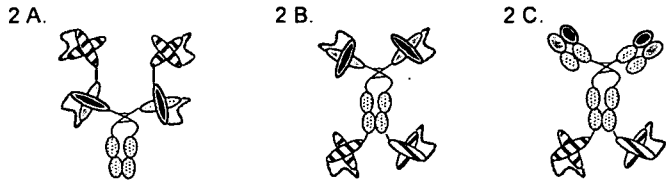


Figure 2 Diagrams of tetravalent, bispecific Fc fusion and Mab formats with specificity for targets "X" and "Y." The scFv against target X is indicated by a striped fill, scFv against target Y is indicated by a gray fill and the Ig constant domains are indicated by stippled fill. A) tascFv-Fc, tandem single chain Fc fusion, B) biscFv-Fc, bi-single chain Fv Fc fusion, C) BiAb, monoclonal antibody with single chain Fv fused to the carboxyl terminus.

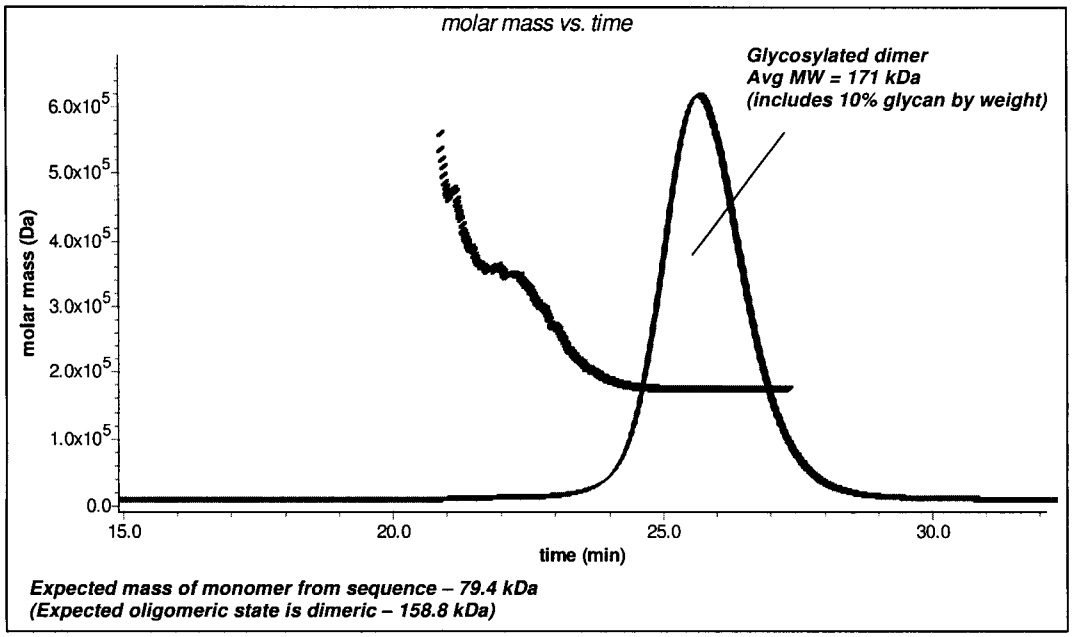


Figure 3

BsAb Characterization

Figures. 4a and 4b. Combination of IL-17A+IL23p19 mAbs significantly improves EAE symptoms in mice and prevents relapse. Treatment with suboptimal concentrations of anti-mouse IL-17A+IL-23p19 mAbs significantly reduced disease scores ($p = 0.002$, 2-way ANOVA) in the PLP EAE model of relapsing-remitting MS, compared to mice treated with vehicle (PBS). Importantly, the mAb combination completely prevented disease relapse. N = 13 mice per group.

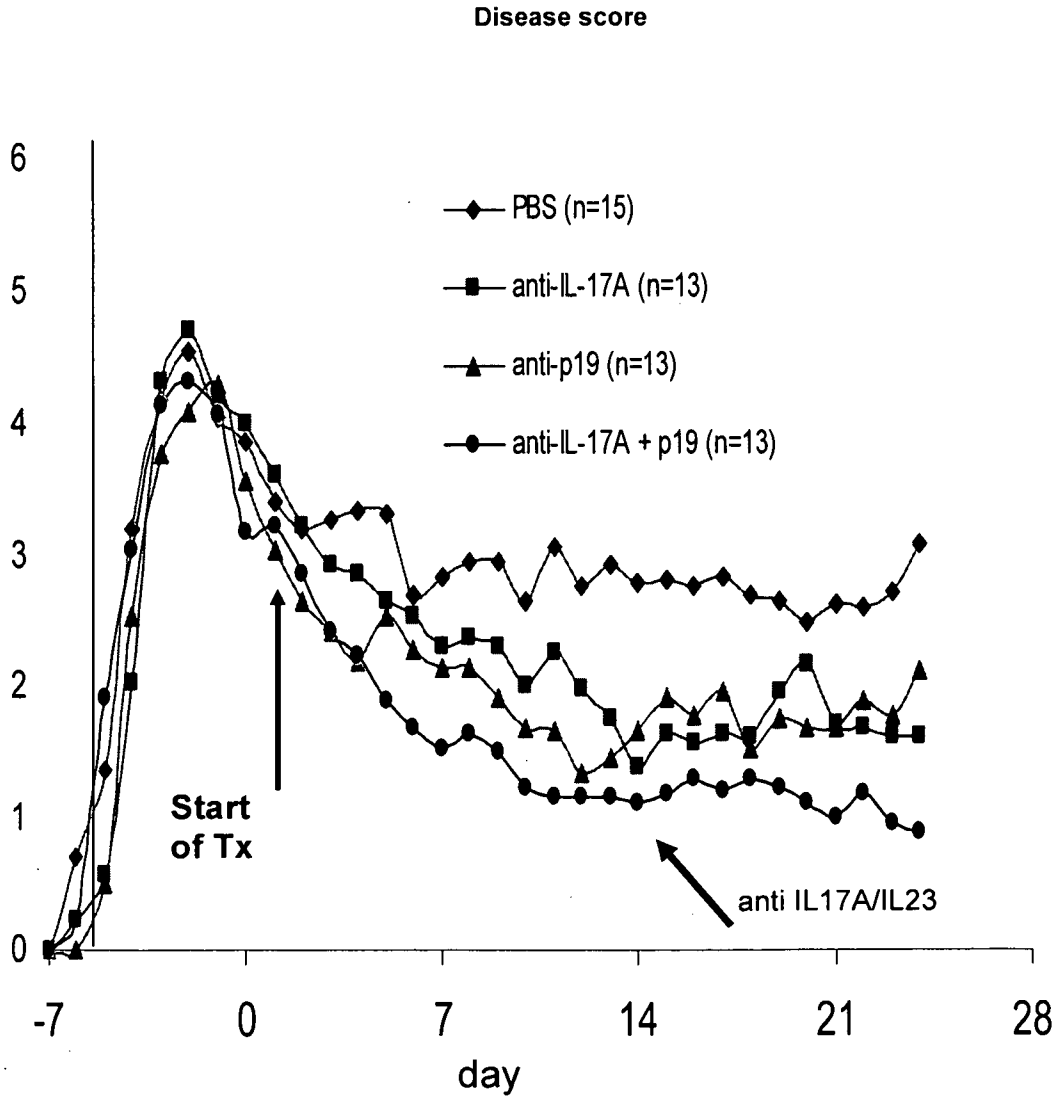


Figure 4a

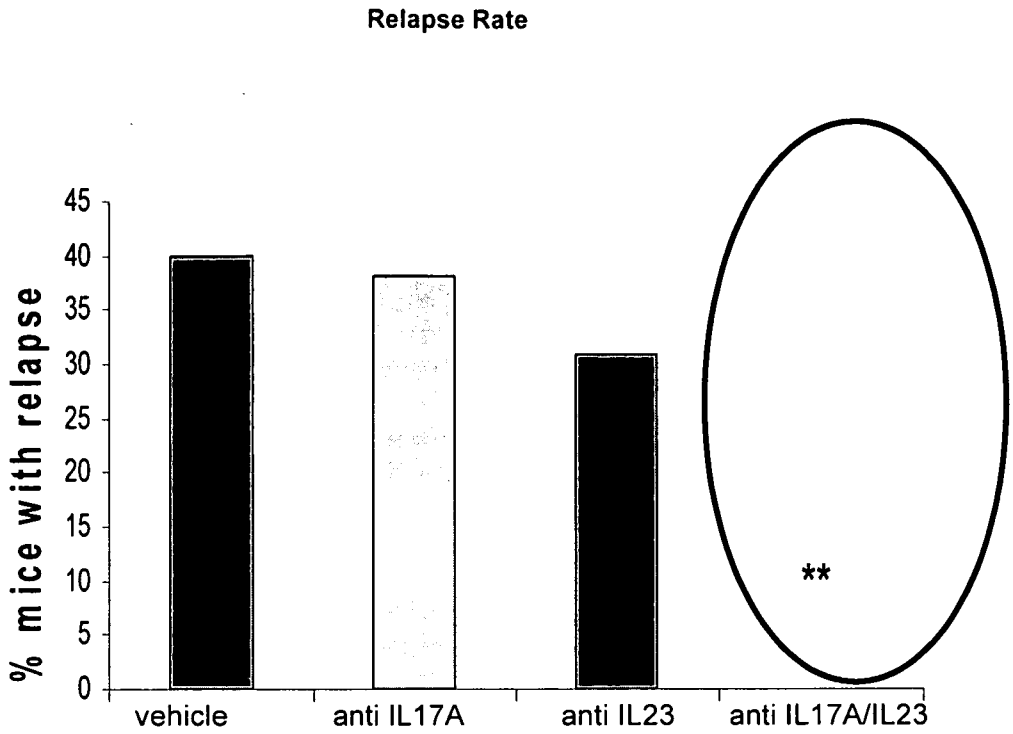

Relapse Rate
% mice with relapse
45
40
35
30
25
20
15
10
5
0
**
vehicle
anti IL17A
anti IL23
anti IL17A/IL23

Figure 4b

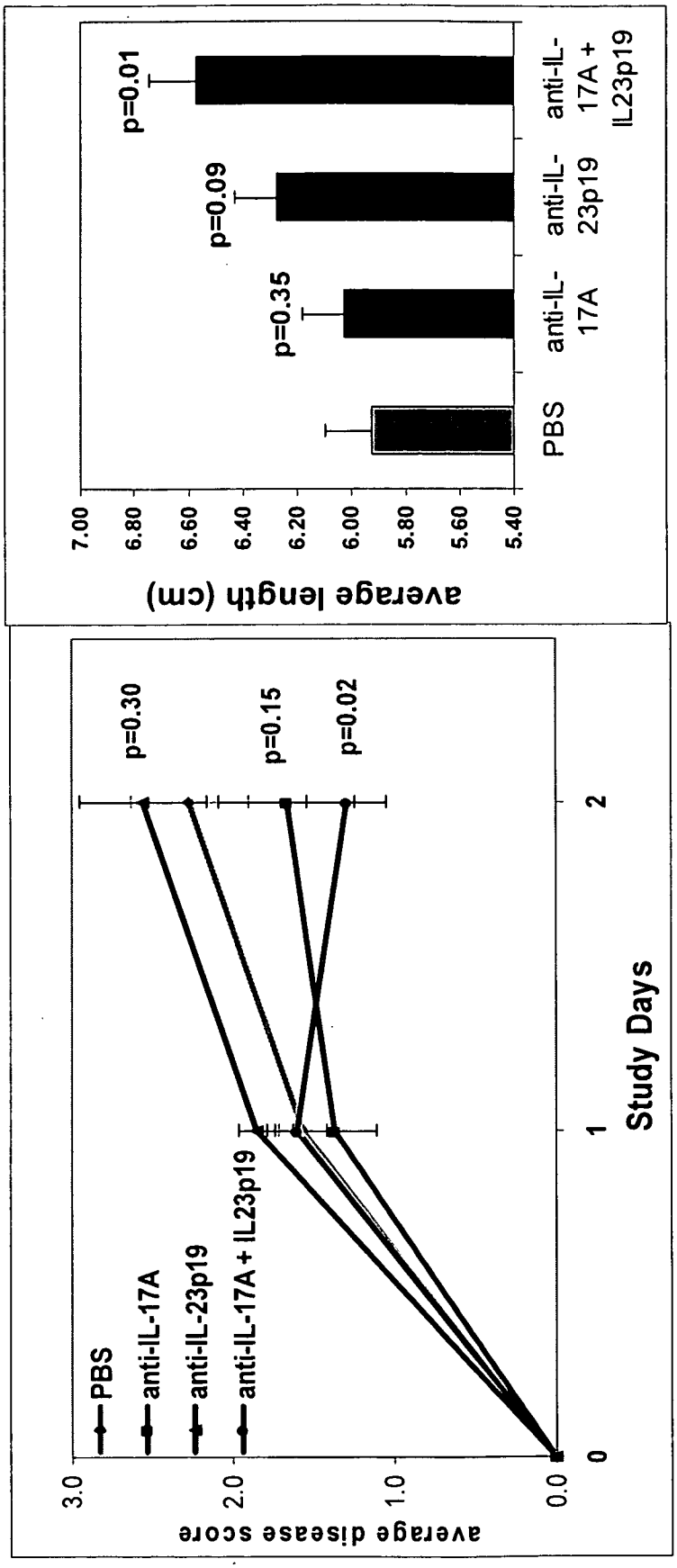


Fig. 5. Combination of IL-17A+IL-23p19 mAbs significantly improves colitis symptoms in mice. Treatment with suboptimal concentrations of anti-mouse IL-17A+IL-23p19 mAbs from day -5 of the oxazalone colitis (OXC) model to day 1, resulted in a significant reduction in disease score (weight loss, stool consistency and blood in stool) vs. oxazalone-mice treated with PBS. [mean ± SEM]. The mAb combination also alleviated colitis-mediated colon shortening observed at day 2 of the model compared to OXC mide treated with PBS [mean ± SEM]. N = 8/group.

Fig. 6. All three formats of IL-23p19/IL-17A bsAbs neutralize hIL-23-mediated STAT3 phosphorylation in human PHA T cell blasts. hIL-23 induces a dose-dependent increase in pSTAT3 activity (left panel) which is neutralized with bsAbs, IL-23p19 scFv, and to a lesser extent, soluble IL-23R (right panel). There is no neutralization with the IL-17A scFv alone (right panel), and no neutralization against hIL-12 or murine ligands (data not shown). BsAbs were able to neutralize cynomolgus IL-23-mediated activity to a similar extent (data not shown).

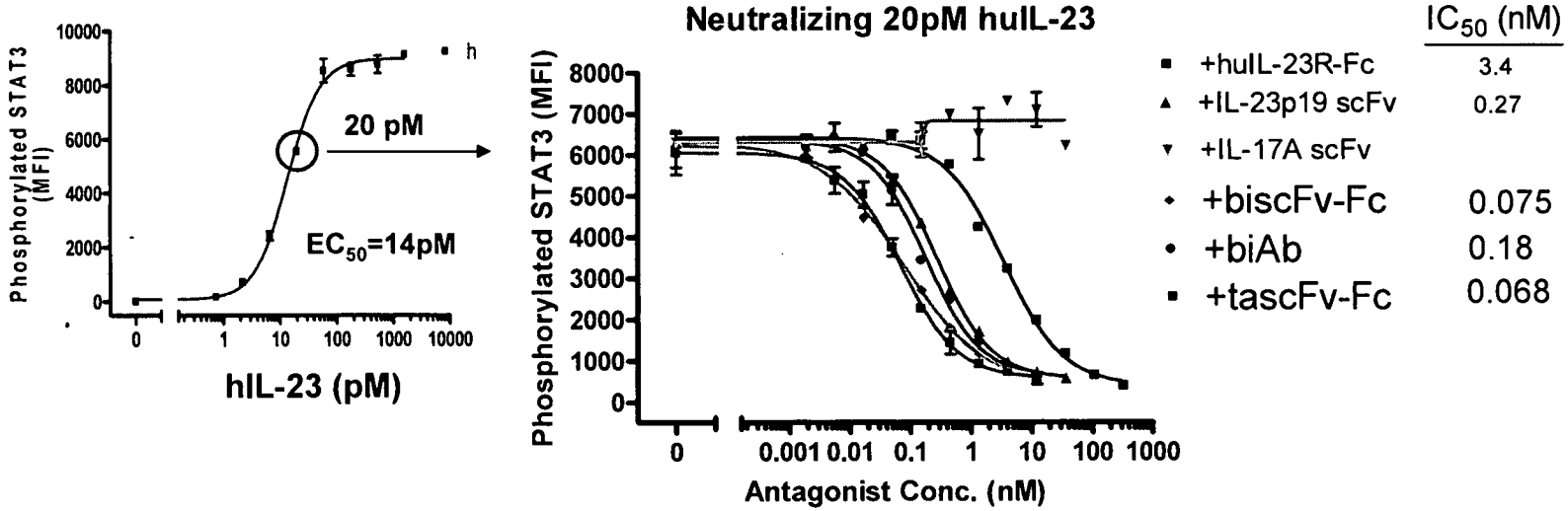


Fig. 7. All three formats of IL-23p19/IL-17A bsAbs neutralize hIL-23-mediated STAT3 phosphorylation in human PHA T cell blasts in the presence of high concentrations of hIL-17A. BsAbs were pre-incubated with hIL-17A (30 nM) for 30 min @37°C prior to 15 min pSTAT3 assay.

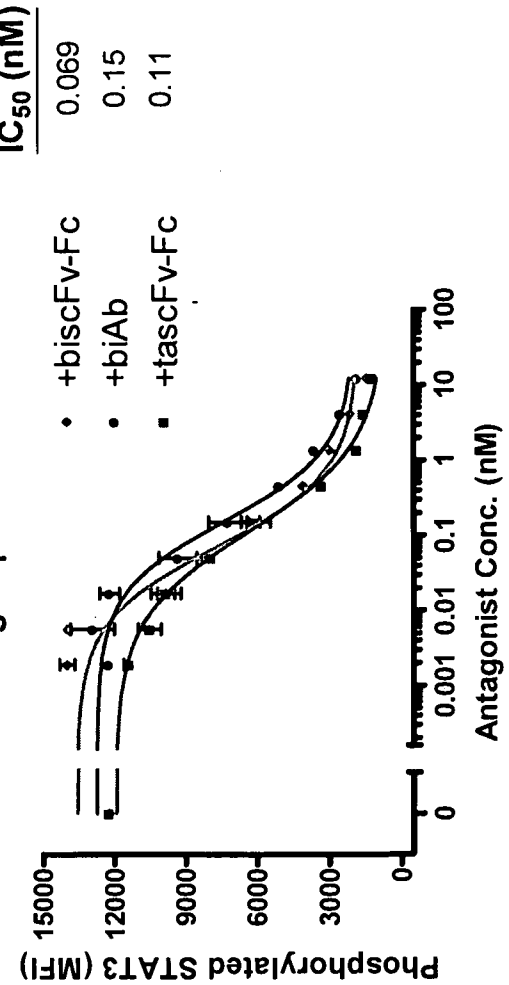
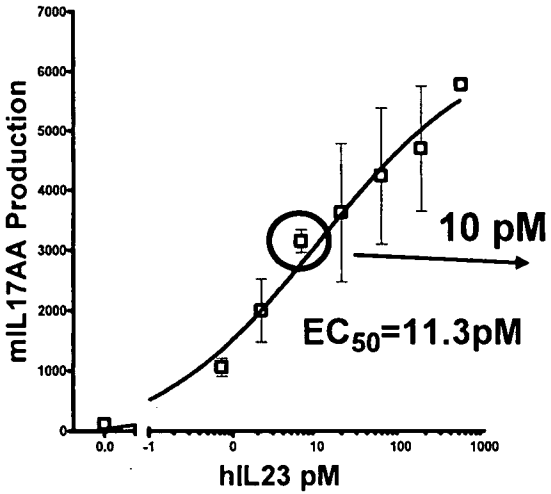
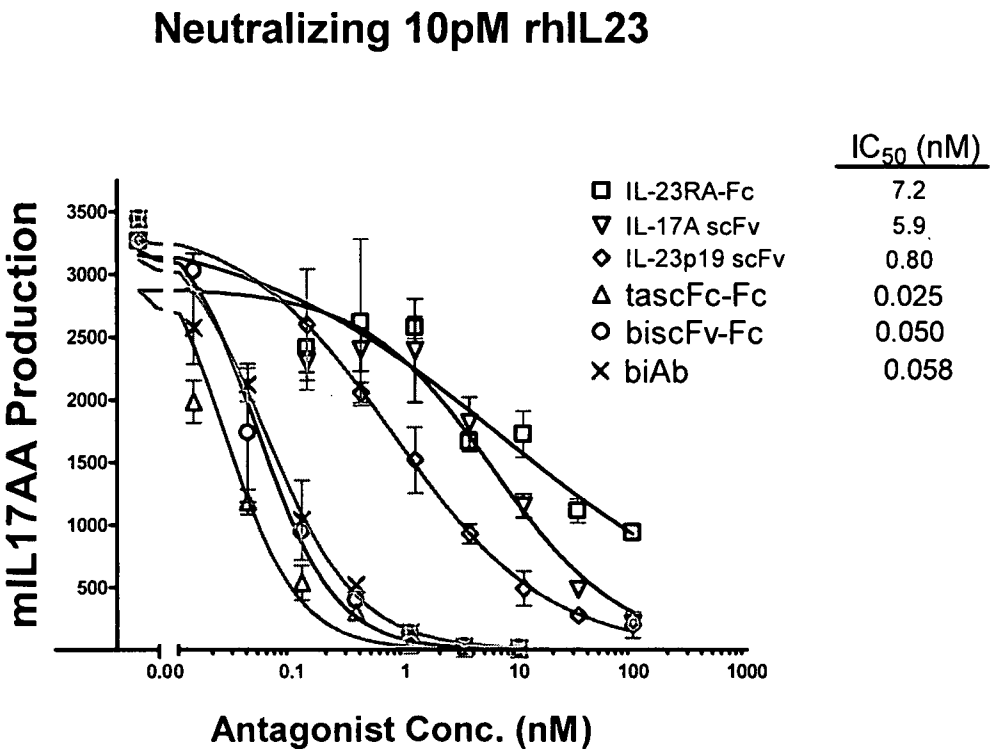


Fig. 8. All three formats of IL-23p19/IL-17A bsAbs neutralize hIL-23-mediated production of mIL-17A in murine splenocytes. hIL-23 induces a dose-dependent increase in mIL-17A production in 1° murine splenocytes (left panel) which is neutralized with bsAbs, IL-23p19 scFv, and to a lesser extent, soluble IL-23R (right panel). Similar trends were observed for neutralizing hIL-23 mediated production of IL-22 and IFN-g (data not shown).





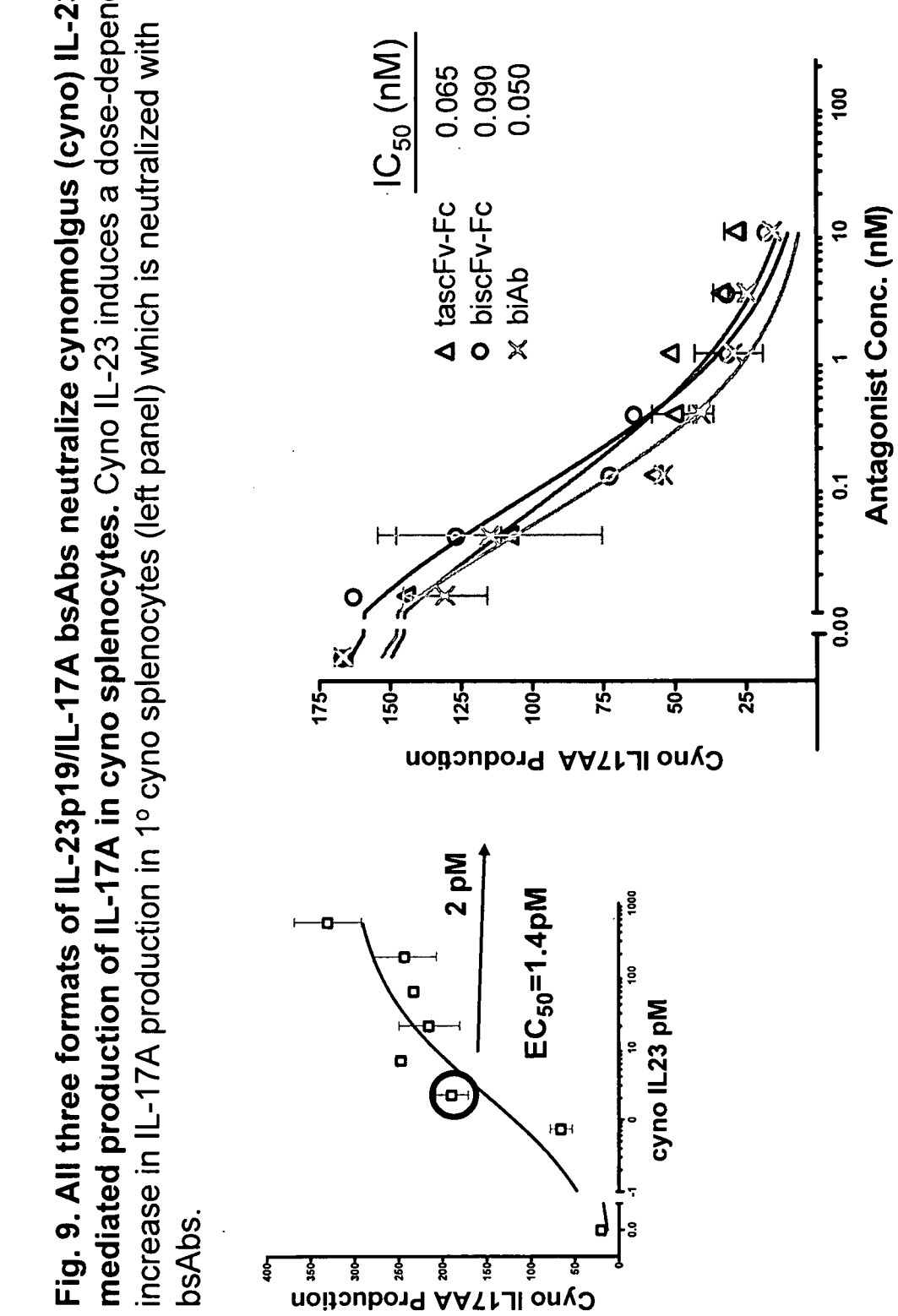


Fig. 9. All three formats of IL-23p19/IL-17A bsAbs neutralize cynomolgus (cyno) IL-23-mediated production of IL-17A in cyno splenocytes. Cyno IL-23 induces a dose-dependent increase in IL-17A production in 1° cyno splenocytes (left panel) which is neutralized with bsAbs.

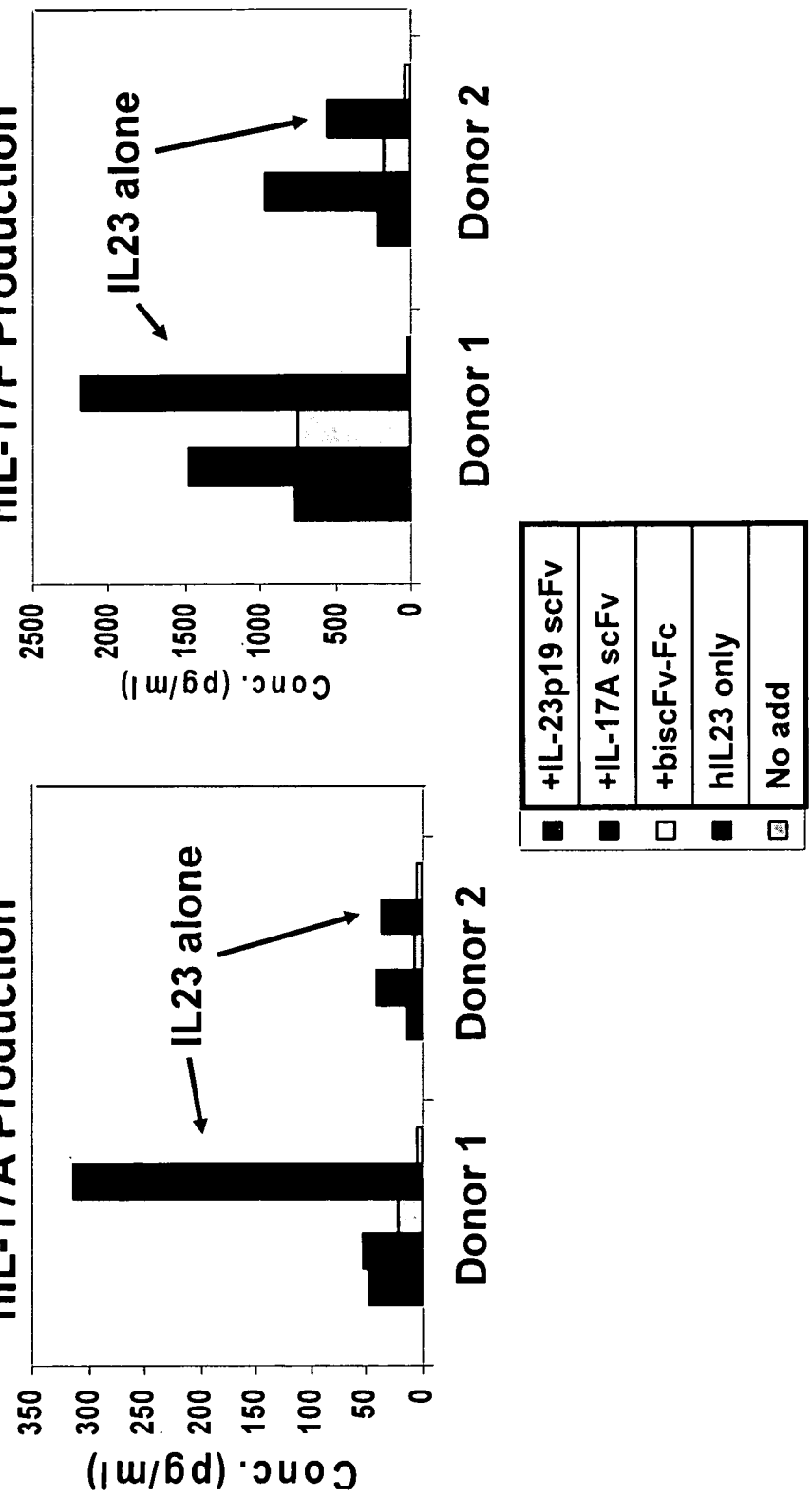
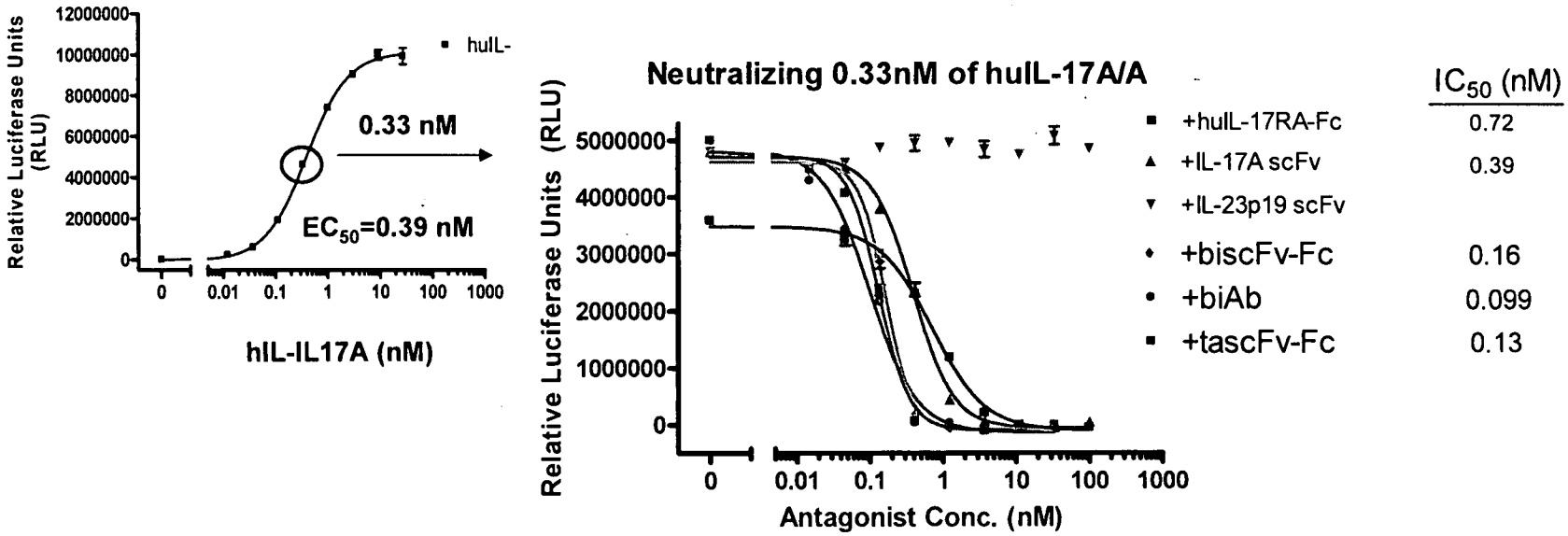


Fig. 10. IL-23p19/IL-17A biscFv-Fc neutralizes hIL-23-mediated production of IL-17A and IL-17F in human NK cells. hIL-23 induces IL-17A, IL-17F and IL-17A/F production in 1° hu NK cells which is neutralized with bsAbs and IL23p19 scFv, and to a lesser extent, IL-17A scFv.

Fig. 11. All three formats of IL-23p19/IL-17A bsAbs neutralize hIL-17A-mediated NFκB signaling. hIL-17A induces a dose-dependent increase in luciferase in NIH3T3 cells transfected with an NFκB promotor (left panel) which is neutralized with bsAbs, IL-17A scFv, and to a lesser extent, soluble IL-17RA (right panel). There is no neutralization with the IL-23p19 scFv alone (right panel), and no neutralization of the bsAbs against hIL-17F or murine ligands (data not shown). BsAbs were able to neutralize cynomolgus IL-17A-mediated activity to a similar extent (data not shown).



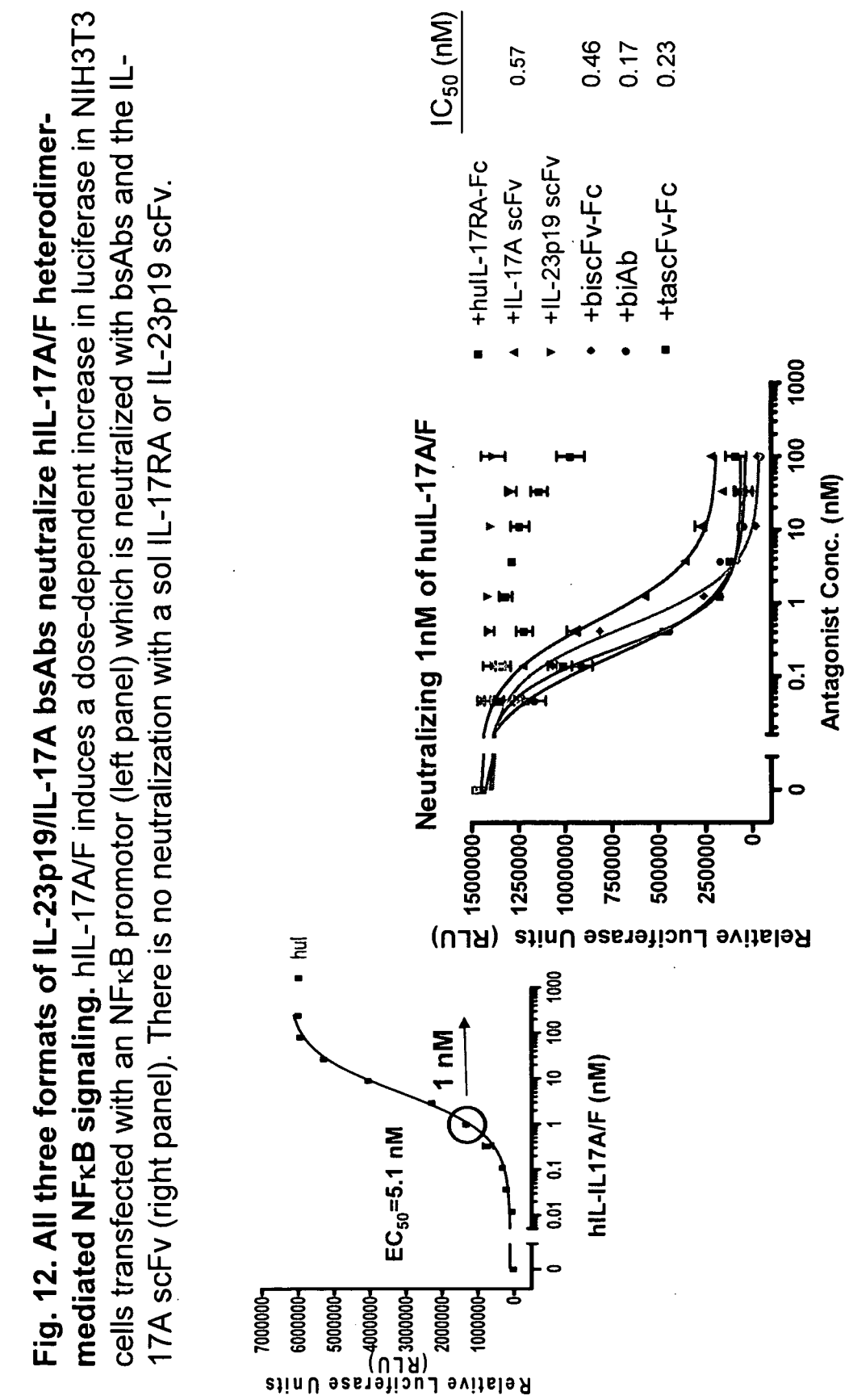


Fig. 12. All three formats of IL-23p19/IL-17A bsAbs neutralize hIL-17A/F heterodimer-mediated NFκB signaling. hIL-17A/F induces a dose-dependent increase in luciferase in NIH3T3 cells transfected with an NFκB promotor (left panel) which is neutralized with bsAbs and the IL-17A scFv (right panel). There is no neutralization with a sol IL-17RA or IL-23p19 scFv.

Fig. 13. All three formats of IL-23p19/IL-17A bsAbs neutralize hIL-17A-mediated NFκB signaling in the presence of high concentrations of hIL-23. BsAbs were pre-incubated with hIL-23 (30 nM) for 30 min @37°C prior to 4 h luciferase assay.

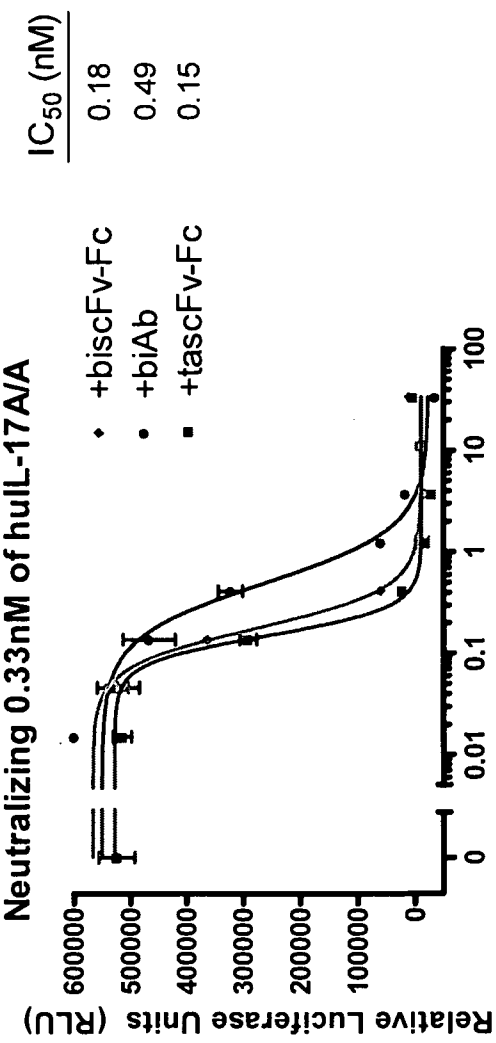
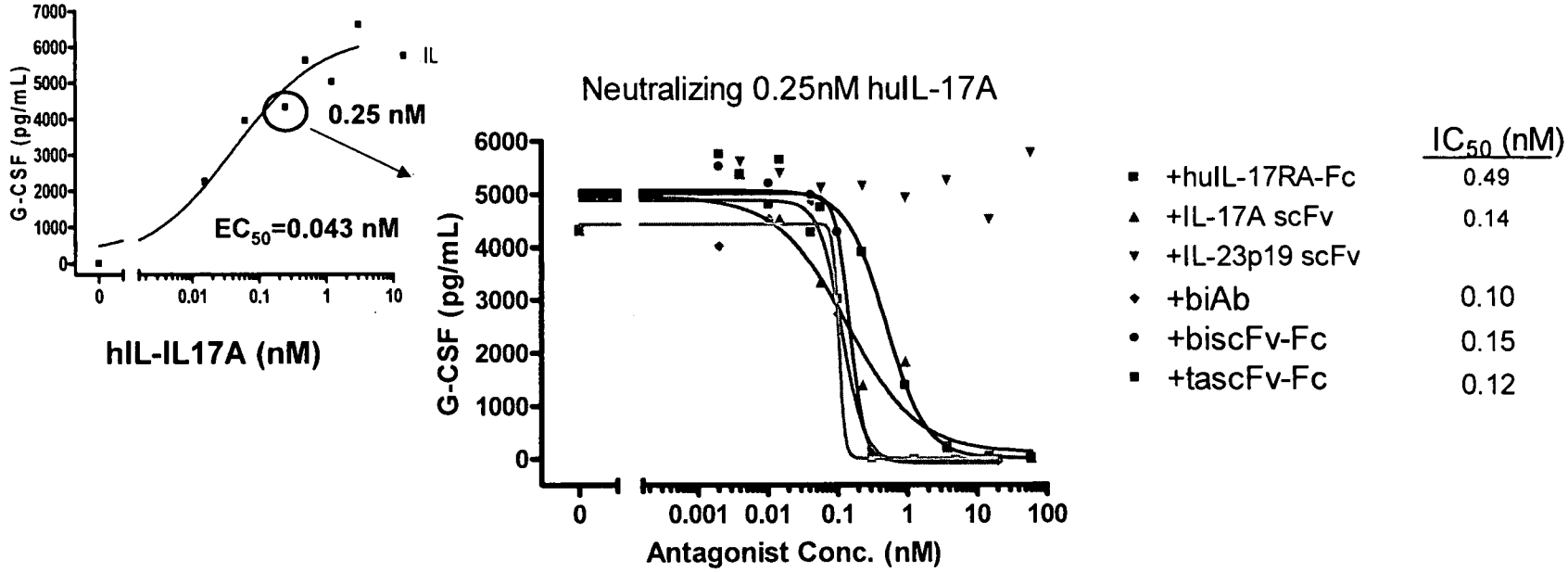


Fig. 14. All three formats of IL-23p19/IL-17A bsAbs neutralize hIL-17A-mediated G-CSF production in 1° human epithelial cells. hIL-17A induces a dose-dependent increase in G-CSF production in small airway epithelial cells (24 h) which is neutralized with bsAbs, IL-17A scFv, and to a lesser extent, sol IL-17RA (right panel). There is no neutralization with IL-23p19 scFv alone (right panel), and no neutralization of bsAbs against hIL-17F or murine ligands (data not shown). Similar trends were observed for neutralizing hIL-17A mediated production of IL-6 and IL-8 (data not shown).



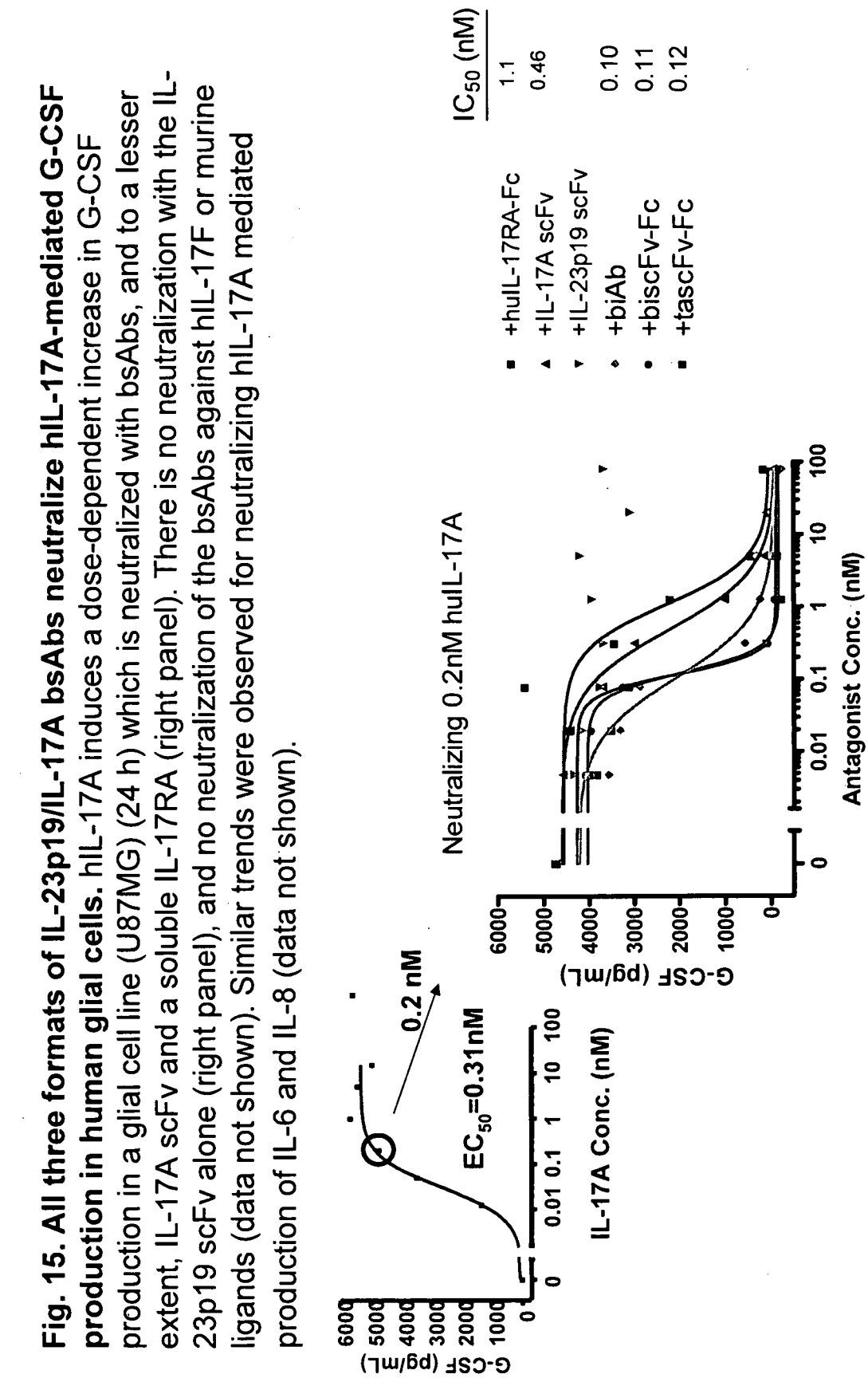


Fig. 15. All three formats of IL-23p19/IL-17A bsAbs neutralize hIL-17A-mediated G-CSF production in human glial cells. hIL-17A induces a dose-dependent increase in G-CSF production in a glial cell line (U87MG) (24 h) which is neutralized with bsAbs, and to a lesser extent, IL-17A scFv and a soluble IL-17RA (right panel). There is no neutralization with the IL-23p19 scFv alone (right panel), and no neutralization of the bsAbs against hIL-17F or murine ligands (data not shown). Similar trends were observed for neutralizing hIL-17A mediated production of IL-6 and IL-8 (data not shown).

Fig. 16. BsAbs are able to simultaneously co-bind targets without any effect on binding ability. Using surface plasmon resonance (Biacore), each bsAb was captured onto a CM4 chip via an anti-human IgG Fc-γ specific Ab. Saturating concentrations of hIL-17A (100 nM) and hIL-23 (250 nM) were allowed to bind in series to each bsAb. There was no evidence of reduced co-binding of IL-17A and IL-23. Shown below is a biscFv-Fc; identical results were obtained with tascFv-Fc and biAb molecules.