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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 March 2011 (17.03.2011)

PCT

(10) International Publication Number
WO 2011/031986 A1

(51) International Patent Classification:
C07K 14/54 (2006.01) *G01N 33/68* (2006.01)
C07K 14/715 (2006.01)

(21) International Application Number:
PCT/US2010/048451

(22) International Filing Date:
10 September 2010 (10.09.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/241,265 10 September 2009 (10.09.2009) US
61/242,595 15 September 2009 (15.09.2009) 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, CL, 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, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2011/031986 A1

(54) **Title**: ASSAYS FOR SOLUBLE IL-15 RECEPTOR ALPHA

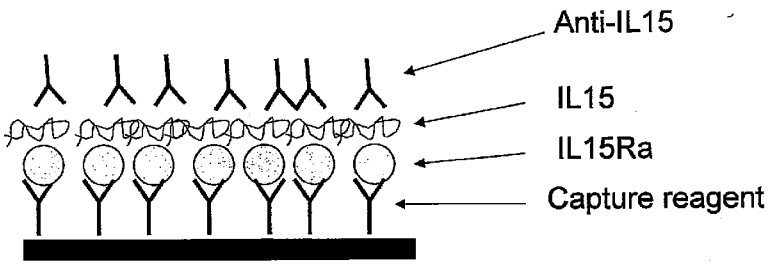


FIG. 1

(57) **Abstract**: The invention provides a sensitive and specific assay for quantifying low levels of soluble IL-15Rα in any mammalian sample containing or suspected of containing sIL-15Rα, including biological samples such as serum, plasma, tissue lysate, tumor lysate, or tumor cell culture supernatant. These methods are particularly suitable for the enzyme-linked immunosorbent assay (ELISA). The invention also provides methods for diagnosing or monitoring IL-15Rα-associated disorders including leukemias, lymphomas, autoimmune diseases, retroviral diseases, or LPS induced septic shock. The invention also provides methods for monitoring the effectiveness of a therapeutic treatment that increases the amount of sIL- 15Rα in a patient, particularly when the therapeutic treatment is LPS, interferon, IL- 15 or IL-15Rα.

ASSAYS FOR SOLUBLE IL-15 RECEPTOR ALPHA

FIELD OF THE INVENTION

The present invention generally relates to cytokine receptors, and more specifically to assays for detecting soluble interleukin-15 receptor alpha (IL-15Rα).

BACKGROUND OF THE INVENTION

Interleukin-15 (IL-15) is a proinflammatory cytokine that shares several biological functions with interleukin-2 (IL-2). As reported in the review by Waldmann, *The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design,* Nature Review Immunology 6:595-601 (2006), both cytokines stimulate the proliferation of T cells; induce the generation of cytotoxic T lymphocytes; stimulate B cell proliferation and immunoglobulin synthesis; and induce the generation and persistence of natural killer (NK) cells. The redundancy of function can be explained by the usage of a common IL-2Rβ/γ signaling complex, which is an intermediate affinity receptor for both IL-2 and IL-15.

In addition to this shared receptor, IL-15 also has a private receptor, IL-15Rα. IL-15Rα binds to IL-15 with far greater affinity for IL-15 than IL-2 has for its private receptor, IL-2Rα. Soluble IL-15Rα is believed to be released by activated T cells by proteolytic shedding and retains its high affinity for IL-15.

IL-15 also has functions that are distinct from, and at times competitive with, IL-2. These differences may be due, at least in part, to their private receptors. In addition, IL-15 is not a secreted molecule but acts as a cell surface molecule during an immunological synapse. IL-15 bound to IL-15R alpha on antigen presenting cells is presented in trans to target NK and CD8 T cells that only express the IL-2/IL-15 Receptor beta and common gamma chain of the multi-subunit receptor (Immunity. 2002:17(5):537-47). For example, IL-2 has a unique role in activation-induced cell death (AICD), a process by which fully activated T cells undergo programmed cell death in response to certain cell surface-expressed death receptors such as FAS or TNF receptors, and participates in the maintenance of peripheral $CD4^+$ $CD25^+$ regulatory T cells. Through these functions, IL-2 is involved in the elimination of self-reactive T cells, which have roles in the pathogenesis of autoimmune diseases.

In contrast, IL-15 is important for the maintenance of long-lasting, high-avidity T-cell responses to invading pathogens by supporting the survival of $CD8^+$ memory T cells and is also known to inhibit AICD. In studies involving knock-out mice, IL-$2^{-/-}$ and IL-$2R^{-/-}$ mice showed lymphoid proliferation associated with autoimmune disorders, while IL-$15^{-/-}$ and IL-$15R\alpha^{-/-}$ mice showed normal total T and B cell populations, but profound defects in NK cells, NK-T cells, intraepithelial lymphocytes and $CD8^+$ memory cells. Thus, IL-15 has potential as an immunotherapeutic agent for cancer treatment because of its critical role in the proliferation and activation of NK and $CD8^+$ T cells.

The deleterious effects of IL-15 overexpression have also been reported. According to U.S. Patent No. 7,329,405, the upregulation of IL-15 has been implicated in several disorders, including arthritides, connective tissue disorder, ophthalmological disorders, neurological disorders, gastrointestinal and hepatic disorders, allergic disorders such as asthma, hematologic disorders, skin disorders, pulmonary disorders, malignancies, transplantation-related disorders, endocrinologic disorders, vascular disorders, gynecological disorders and infectious diseases. IL-15 and IL-15Rα are co-expressed in association with a number of autoimmune disorders, including rheumatoid arthritis, psoriasis, inflammatory bowel disease, multiple sclerosis, chronic liver disease, refractory celiac syndrome, a disease associated with the development of enteropathy associated CD8 T-cell lymphoma.

The ability to accurately measure soluble IL-15Rα has clinical implications. For example, sIL-15Rα can be used as a surrogate marker to determine the efficacy of cancer therapy agents or IL-15 receptor-inducing agents (such as LPS and interferon), or to identify patients who would be good candidates for certain IL-15 therapies.

However, the ability to measure soluble IL-15Rα depends on the availability of sensitive and specific assays. Various radioimmunoassays have been used to detect IL-15Rα. For example, Mortier et al., *Natural, Proteolytic Release of a Soluble Form of Human IL-15 Receptor α-Chain that Behaves as a Specific, High Affinity IL-15 Antagonist*, J. Immunology 173:1681-1688 (2004), describes the measurement of sIL-15Rα using a sandwich radioimmunoassay in which a polyclonal goat anti-human IL-15Rα antibody was used as the capture antibody and a radio-iodinated monoclonal anti-human IL-15Rα antibody was used as the detection antibody. An assay sensitivity of about 1 pM was achieved using a recombinant IL-15Rα-IL-2 fusion protein as the assay standard.

Similarly, Badoual et al., *The Soluble α Chain of Interleukin-15 Receptor: A Proinflammatory Molecule Associated with Tumor Progression in Head and Neck Cancer*, Cancer Res. 68(10)3907-3914 (2008), reports using the same radioimmunoassay procedure as Mortier et al. (2004), *supra*, using a recombinant sIL-15Rα-IL-2 fusion protein containing the entire sIL-15Rα extracellular domain. Previously, Mortier et al., *soluble Interleukin-15 Receptor α (IL-15Rα)-sushi as a Selective and Potent Agonist of IL-15 Action through IL-15Rβ/γ*, J. Biol. Chem. 281(3):1612-1619 (2006) found that the fusion protein consisting of the IL-15Rα sushi domain bound to IL-15 with a 10-fold lower affinity compared with the full length IL-15Rα extracellular domain.

Enzyme-linked immunosorbent assays (ELISA) for murine soluble IL-15Rα have been developed, but lack the desired sensitivity. A sandwich ELISA with a detection limit of 20 pg/ml of recombinant IL-15Rα in cell culture media is reported in Budagian et al., *Natural Soluble Interleukin-15Rα is Generated by Cleavage That Involves the Tumor Necrosis Factor-α-converting Enzyme (TACE/ADAM17),* J. Biol. Chem. 279(39):40368-40375. In the Budagian ELISA, IL-15-IgG was used as the capture protein, with biotinylated anti-mouse IL-15Rα antibodies used for detection. Serial dilutions of murine recombinant IL-15Rα were used as assay standards. Mortier et al., *IL-15Rα Chaperones IL-15 to Stable Dendritic Cell Membrane Complexes that Activate NK Cells via Trans Presentation*, J. Exp. Med. 205(5):1213-1225 (2008) reports the development of a complex ELISA in which an murine anti-IL-15 antibody is used as the capture antibody to detect murine IL-15 when complexed to IL-15Rα, while an anti-IL-15Rα antibody was used as the detection antibody. This complex ELISA was used to measure the levels of murine IL-15Rα-IL-15 complexes in cell lysates. The authors claim to detect picomolar levels of these complexes without data or specific details.

An ELISA DuoSet for murine IL-15Rα is also commercially available from R&D Systems (Minneapolis, MN). The R&D IL-15Rα DuoSet product literature describes a typical indirect sandwich ELISA in which a goat anti-mouse IL-15Rα antibody is used as the capture antibody to bind sIL-15Rα in a sample, which in turn is bound by biotinylated goat anti-mouse IL-15Rα antibody as the detection antibody. A recombinant mouse IL-15Rα/Fc chimeric protein is used as the assay standard, with no specified detection limit.

Thus, a need exists for a sensitive assay that can measure low levels of soluble IL-15Rα in a sample compared to existing immunoassays and preferably without the use of potentially harmful reagents, such as radioisotopes. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

One aspect of the present invention relates to the novel discovery of a sensitive and specific assay for quantifying low levels of soluble IL-15Rα in a sample. The method is generally accomplished by the steps of:

(a) contacting a sample containing or suspected of containing soluble IL-15Rα with a capture reagent that selectively binds to IL-15Rα to form a first complex;

(b) contacting the first complex with IL-15 to form a second complex comprising the capture reagent, IL-15Rα and IL-15;

(c) contacting the second complex with a detectable ligand that selectively binds to IL-15; and

(d) measuring an amount of soluble IL-15Rα in the sample.

The methods of the present invention can be used on any sample containing or suspected of containing sIL-15Rα. Assay samples can include biological samples such as serum, plasma, tissue lysate, tumor lysate, or tumor cell culture supernatant. Biological samples can be obtained from a mammal, including humans.

In one embodiment of the method, the capture reagent that selectively binds sIL-15Rα is preferably an anti-IL-15Rα antibody, including monoclonal and polyclonal antibodies. Although IL-15 can be obtained from any source, human IL-15 is particularly useful to assay samples containing human sIL-15Rα. The detectable ligand is preferably an anti-IL-15 antibody. Suitable anit-IL-15 antibodies are monoclonal or polyclonal antibodies that selectively bind IL-15, but not IL-15Rα or the capture reagent. The detectable ligand can be directly or indirectly labeled. For example, the ligand can be directly labeled with a colorimetric reagent, or indirectly labeled using biotin and avidin or streptavidin-alkaline phosphatase as the detection means. The methods of the present invention are particularly suitable for the enzyme-linked immunosorbent assay (ELISA) format.

In another embodiment of the present invention, soluble IL-15Rα (sIL-15Rα) in a sample is quantified with the use of a standard curve produced using pre-determined quantities of an IL-15Rα protein. Preferably, the IL-15Rα is an IL-15Rα-IL-2 chimeric protein having full length extracellular domains of IL-15Rα and IL-2. A novel IL-15Rα-IL-2 chimeric protein having an amino acid sequence of SEQ ID NO:6 is also provided. The methods of the present invention are capable of quantifying less than about 20 pg/ml of sIL-15Rα in a sample, preferably less than about 7 pg/ml, and more preferably at least about 1 pg/ml.

The present invention further provides kits for carrying out the present methods. The kits contain a capture reagent that selectively binds sIL-15Rα, IL-15, a detectable ligand that selectively binds IL-15 and a means for measuring the amount of sIL-15Rα in the sample. The kits can also include a reference standard, a standard curve for IL-15Rα and any ancillary reagents and components for carrying out the methods of the present invention.

Another aspect of the invention relates to methods for diagnosing or monitoring IL-15Rα-associated disorders in a patient by measuring the amount of sIL-15Rα according to the methods of the present invention and comparing the amount with a control. IL-15Rα-associated disorders can be leukemia (e.g., adult T cell leukemia, T-cell large granular lymphocyte (LGL) leukemia, Natural killer cell leukemia), lymphoma (e.g., adult T cell leukemia/lymphoma), autoimmune disease (e.g., type I diabetes, celiac disease, HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), rheumatoid arthritis or multiple sclerosis), retroviral disease, or LPS induced septic shock.

A further aspect of the invention relates to methods for monitoring the effectiveness of a therapeutic treatment that increases the amount of sIL-15Rα in a patient, comprising measuring the amount of soluble IL-15Rα in a sample from the patient. An increase in the amount of sIL-15Rα, compared to a control amount of IL-15Rα, indicates the treatment is effective. These methods are particularly useful when therapeutic treatment is LPS, interferon, IL-15 or IL-15Rα.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates the basic assay format of the present invention.

Fig. 2 shows typical data obtained for an IL-15Rα standard curve using the IL-15Rα-IL2 chimeric protein as the reference standard.

Fig. 3 shows the neutralizing activity of sIL-15Rα-IL2 chimeric protein on IL-15 in CTLL-2 cell assays. Fig. 3A shows that the chimeric protein maintains the biologic activity of IL2 in the assays. Fig. 3B shows that IL-15Rα-IL2 blocks the biologic activity of IL-15 when bound to IL-15.

Fig. 4 shows that IL-15Rα was detected in the supernatant of (A) PT18 and (B) 293T cells transfected with wildtype IL-15Rα and/or soluble IL-15Rα.

Fig. 5 shows that the soluble IL-15Rα expressed by transfected cells was not detected by Western blot because the soluble IL-15Rα was secreted into the supernatant as shown in Fig. 3.

Fig. 6 shows that sIL-15Rα spiked into normal human serum has similar sensitivity as sIL-15Rα spiked into reagent diluents.

Fig. 7 shows elevated serum level of sIL-15Rα in type I diabetes patients (mean = 6.18 pM) compared to normal patients (mean = 1.05 pM).

Fig. 8 shows elevated serum level of sIL-15Rα in Adult T cell leukemia (mean = 5.22 pM) compared to normal subjects (mean = 1.05 pM).

Fig. 9 shows elevated serum level of sIL-15Rα in T-cell large granular lymphocyte leukemia (mean = 11.12 pM) compared to normal subjects (mean=1.05 pM).

Fig. 10 shows elevated serum level of sIL-15Rα in HTLV-I-associated myelopathy/tropical spastic paraparesis (mean = 4.57 pM) and Natural killer cell leukemia (n=1; about 16 pM) compared to normal subjects (mean = 1.05 pM).

Fig. 11 shows elevated serum level of sIL-15Rα in celiac patients (mean = 4.37 pM) compared to normal subjects (mean = 1.05 pM).

Fig. 12 shows the serum level of sIL-15Rα in patients with multiple sclerosis treated with β-interferon (mean =2.18 pM) and without treatment (mean = 2.32 pM) compared to normal subjects (mean = 1.05 pM).

Fig. 13 shows the serum level of sIL-15Rα in patients with rheumatoid arthritis (mean = 2.06 pM) compared to normal subjects (mean = 1.05 pM).

Fig. 14 shows that the serum level of sIL-15Rα is not elevated in CSF and other neuroimmunological disorders.

Fig. 15 shows the DNA and amino acid sequences for the full IL15Rα-IL2 chimeric protein.

DETAILED DESCRIPTION OF THE INVENTION

The present invention generally relates to assays that are sensitive and specific for soluble IL-15Rα. In describing the present invention, it is to be understood that the terminology used herein is for the purpose of describing particular embodiments and is not intended to be limited to such embodiments. For example, as used herein, the singular forms of "a", "an" and "the" also include the plural, and vice versa, unless expressly limited otherwise by the context. The terms "having," "containing," "including" and the like are used interchangeably to mean "comprising," "consisting essentially of" or "consisting of" as those patent terms of art are typically defined. In addition, each publication, patent or other reference disclosed herein is hereby incorporated by reference in its entirety.

The methods of the present invention are based, in part, on the discovery that lower amounts of IL-15Rα in a sample can be measured (i.e., improved assay sensitivity) using IL-15 as a component of the assay. Figure 1 illustrates the basic assay format of the present invention. Generally, the methods of the present invention can be accomplished by:

(a) contacting a sample containing or suspected of containing soluble IL-15Rα with a capture reagent that selectively binds to IL-15Rα to form a first complex;

(b) contacting the first complex with IL-15 to form a second complex comprising the capture reagent, IL-15Rα and IL-15;

(c) contacting the second complex with a detectable ligand that selectively binds to IL-15; and

(d) measuring an amount of soluble IL-15Rα in the sample.

Although the assays of the present invention can be carried out using a variety of methods known in the art, including, without limitation, Western blot, immunoblot, enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA), or immunohistochemical analysis, the ELISA format is particularly useful for its simplicity and use of non-hazardous materials. Therefore, when the ELISA format is used to describe the present invention, it is done so solely for illustrative purposes. Those skilled in the art can readily determine, without undue experimentation, appropriate steps and reagents for the other assay formats based on the teachings provided herein.

In accordance with the present invention, a sample first comes into contact with the capture reagent for a sufficient period of time to allow soluble IL-15Rα to bind to the capture reagent. As used herein, the term "capture reagent" refers to any reagent capable of forming a complex with IL-15Rα. Accordingly, a capture reagent includes, but is not limited to, a protein, peptide or other antigen binding partner that selectively binds to IL-15Rα or a mixture thereof. As used herein, the term "selectively binds to" or the like refers to the specific binding of a target protein (e.g., IL-15Rα) to an antigen binding partner (e.g., an antibody, antibody fragment or other non-antibody binding partner), wherein the level of binding, as measured by any standard assay known to those skilled in the art (e.g., an immunoassay), is statistically and significantly higher than the background control for the assay or for other non-target proteins.

As used herein, the term "antigen" generally refers to any portion of a protein or peptide, cellular composition, organism or other moiety that elicits an antigen-specific immune response (humoral and/or cellular immune response). For example, an antigen can be IL-15Rα or any functional fragment thereof having an epitope capable of being selectively bound by a capture reagent. An epitope can be defined by both the amino acid residues involved in the selective binding and by its three dimensional conformation. Therefore, an epitope can be included in peptides as small as 4-6 amino acid residues or in larger segments of non-contiguous amino acids that are brought together by protein folding into a three dimensional conformation.

Particularly useful capture reagents include antibodies. As used herein, the term "antibody" includes polyclonal and monoclonal antibodies, and any functional equivalents to whole antibodies that selectively bind to IL-15Rα. Functional equivalents of whole antibodies, such as antigen binding fragments in which one or more antibody domains are truncated or absent (e.g., Fv, Fab, Fab' or $F(ab)_2$ fragments), as well as genetically-engineered antibodies or antigen binding fragments are also included in the term "antibodies". Antibodies useful in the present invention can be produced by any means or obtained from any source known to those skilled in the art. Suitable anti-IL-15Rα antibodies are also commercially available.

Preferably, the capture reagents of the present invention are polyclonal or monoclonal antibodies. Polyclonal antibodies include a population of different antibodies directed

against different epitopes of a target antigen. The production of polyclonal antibodies is well known in the art and typically involves the immunization of a suitable experimental animal, for example, a rodent, rabbit, sheep and the like, with a desired target antigen (e.g., IL-15Rα). An effective amount of the antigen is injected into the animal to induce antibody production, sometimes repeatedly over a period of time, until the animal is producing antibodies against the target antigen. Serum, ascites or other bodily fluid or tissue known to contain antibodies is collected from the animal and can be used. Such antibodies can also be purified to varying degrees.

In contrast to polyclonal antibodies, monoclonal antibodies are highly specific for a single epitope of a target antigen. Methods for producing monoclonal antibodies are well known in the art, including the method of Kohler and Milstein, *Nature* 256:495-497 (1975), in which B lymphocytes are recovered from the spleen of an immunized animal and fused with myeloma cells to obtain a population of hybridoma cells capable of continued growth in cell culture medium. Alternatively, monoclonal antibodies can be made by recombinant DNA methods, such as described in U.S. Patent No. 5,591,630, or isolated from phage antibody libraries using the techniques as described, for example, in Clarkson et al., Nature 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222:581-597 (1991).

Genetically-engineered antibodies include those produced by standard recombinant DNA techniques involving the manipulation and re-expression of DNA encoding antibody variable and/or constant regions. The hypervariable region of an antibody refers to the amino acid residues from the complementarity-determining region ("CDR") that is responsible for antigen binding. Framework amino acid residues are located in the non-hypervariable region of the variable domain. Examples of genetically-engineered antibodies include chimeric antibodies in which the V_H and/or V_L domains of the antibody come from a different source to the remainder of the antibody, and CDR grafted antibodies in which at least one CDR sequence and, optionally, at least one variable region framework amino acid, are derived from one source and the remaining portions of the variable and the constant regions are derived from a different source. Construction of chimeric and CDR-grafted antibodies are well known in the art and are described, for example, in U.S. Patent No. 5, 859,205.

The capture reagents can also be non-antibody binding partners or antigen binding peptides that have been designed to selectively bind to IL-15Rα. The design of non-antibody

binding partners are generally described in Beste et al., *Proc. Natl. Acad. Sci.*, 96:1898-1903 (1993).

In one embodiment of the present invention, the capture reagent is immobilized on a solid support or carrier that is essentially insoluble in an aqueous solution. Suitable solid supports and carriers include, without limitation, those described in U.S. Patent Publication No. 2008/0227119, such as beads, plates, tubes, sheets, porous matrices made of polyethylene, polypropylene, polystyrene, filter paper, agarose, cross-linked dextran or other polysaccharides. For an ELISA, a multi-well microtiter plate is particularly useful to analyze several samples at one time.

The capture antibody is attached to the solid support by any means known to those skilled in the art, including the means described in U.S. Patent Publication No. 2008/0227119 for covalent, non-covalent and physical attachment. The concentration of the capture reagents can be determined by the concentration range of interest and is typically determined experimentally to maximize the sensitivity of the assay for soluble IL-15Rα. The concentration range for an anti-IL-15Rα antibody is typically about .05 ug/ml to about 50 ug/ml, preferably about .05 ug/ml to about 20 ug/ml, and more preferably about 10 ug/ml. Multi-well plates, typically used in ELISAs, can be coated with the capture reagent diluted in a suitable dilution buffer, such as 0.05 M sodium carbonate at a pH between 8-12, preferably between 9-10, and usually incubated between 2 hours to overnight at 4°– 25° C. The coated wells can be used immediately or stored for a long period of time before used in the present assays.

If desired, the coated plates can be washed one or more times with a suitable washing buffer known to those skilled in the art prior to being treated with a blocking agent. A blocking agent binds non-specifically to and saturates the binding sites on a solid support to prevent unwanted binding of the soluble IL-15Rα to sites other than on the capture reagent. Suitable blocking agents include, for example, bovine serum albumin (BSA), gelatin, egg albumin, casein and non-fat milk. The blocking treatment is usually done from about 25°-37° C for about 1-4 hours or overnight at about 0°-4° C. After blocking, the coated wells are preferably washed with washing buffer to remove any excess blocking agent.

The sample to be analyzed can then be added to the immobilized capture reagent. As used herein, the terms “sample” and “test sample” are used interchangeably to refer to a

sample of any type that contains or is suspected of containing soluble IL-15Rα. A test sample can be obtained from a natural source or synthetically produced. A sample can therefore include cell culture supernatant, biological sample, buffer or other media that contains or is suspected of containing IL-15Rα. The sample can include a reference standard for obtaining a standard curve for accurately measuring the quantity of IL-15Rα. Biological samples suitable for use as samples in the present methods include, without limitation, blood, serum, plasma, tissue lysates, tumor lysates and the like that are obtained from a test subject. The terms "test subject" and "patient" are used interchangeably herein and refer to any mammal capable of expressing IL-15Rα, for example, humans, non-human primates, domestic, research or farm animals such as rodents, dogs, cats, horses, sheep, pigs, and cows. The methods of the present invention are particularly useful when the test subject is a human.

If desired, the sample can be diluted in a suitable sample buffer, which can be readily selected by those skilled in the art. Suitable sample buffers include, without limitation, borate, phosphate, carbonate, Tris-HCL and Tris-phosphate. A suitable sample buffer can also be selected to maintain a desired pH during the incubation in order to maximize the binding between the capture reagent and any soluble IL-15Rα in the sample. Preferably, the buffer has a pH in the range of about 6-9. For ELISAs, a pH in a range from about 7-8 is particularly useful, and preferably about 7.4. Other reagents can be included in the sample buffers, including, for example, bovine serum albumin, stabilizers, preservatives, detergents, surfactants and the like.

The sample can be incubated with the immobilized capture reagent at a fairly constant temperature ranging from about 0° -40° C. The incubation time will primarily depend on the incubation temperature, with time decreasing as temperature increases. For example, a sample can be incubated overnight at 4° C, but only about 1-3 hours at room temperature to maximize sensitivity and to minimize disassociation. In addition, the incubation time can be increased if a protease inhibitor is added to prevent proteases in a biological sample from degrading IL-15Rα. Suitable protease inhibitors are well known to those skilled in the art.

After incubation, a measurable amount of soluble IL-15Rα will be complexed to the capture reagents if present in the sample. Prior to the addition of IL-15, the sample is optionally washed with a suitable washing buffer one or more times to separate bound IL-15Rα from the remaining sample. A cross-linking agent can be added, if desired, to allow

the bound IL-15Rα to be covalently attached to the capture reagent to inhibit disassociation during the remaining steps of the assay.

Next, IL-15 is added to the assay. As discussed above, in biological conditions, IL-15 is not a secreted molecule but acts as a cell surface molecule during an immunological synapse. This is due to the high affinity binding of IL-15 to its private receptor IL-15Rα ($Ka=1\text{-}2x10^{-11}/M$, EMBO Journal 1995:14:3654-3663) . Accordingly, the addition of IL-15 is considered an important step to improve assay sensitivity, particularly for ELISAs. Currently known or available ELISAs for soluble IL-15Rα have only attained a sensitivity of about 20 pg/ml, compared to the present methods, which are able to achieve a significantly improved sensitivity as discussed below. Currently known radioimmunoassays have achieved a sensitivity of about 1 pM, but only with the use of hazardous reagents, such as radioisotopes.

As used to describe the present methods and in the claims, the term "IL-15" includes wild-type IL-15 and functional equivalents of IL-15. As used herein, the term "functional equivalents" of IL-15 includes homologues, variants, muteins and active fragments of IL-15 having similar or increased affinity for IL-15Rα compared to wild-type IL-15.

Those skilled in the art can readily determine, without undue experimentation, a suitable IL-15 for use in the methods of the present invention. For example, to quantify soluble IL-15Rα in a biological sample obtained from a human subject, human IL-15 is preferred, with recombinant human IL-15 being most preferred. Methods for obtaining or producing IL-15 are well known in the art, including, without limitation, isolating IL-15 from a naturally-occurring source, by recombinant DNA methods, or by chemical synthesis. Examples of suitable IL-15s and methods of making them for use in the present methods are described in U.S. Patent Publication No. 2008/0255039.

If desired, the IL-15 can be diluted in a suitable buffer solution as described above. Such buffer solutions can include suitable amounts of other reagents, such as BSA, detergents, stabilizers, preservatives, surfactants and the like to increase the binding of IL-15 to IL-15Rα bound to the capture reagent. Useful concentrations of IL-15 can be determined by the concentration range of interest and is typically determined experimentally to maximize the sensitivity of the assay for IL-15Rα. For example, the concentration of IL-15 is in the range of about 50 ng/ml to about 500 ng/ml in an ELISA, with a concentration of about 200

ng/ml being preferred when recombinant human IL-15 is used. The IL-15 is incubated at a temperature and for a period of time sufficient for the IL-15 to bind the IL-15Rα bound to the immobilized capture reagent, thus forming a complex of IL-15, IL-15Rα and capture reagent (referred to herein as the "second complex"). Generally, the IL-15 is incubated at a temperature between about 20° -40° C, preferably about 37° C, for a period of about 1-3 hours, preferably about 1 hour.

If desired, the wells are again washed one or more times with a suitable wash buffer as described above to remove any unbound IL-15. The wash buffer is removed completely before proceeding to the next step.

After the optional washings, a detectable ligand is added to the wells. As used herein, the term "detectable ligand" refers to a protein, peptide or other antigen binding partner that selectively binds to IL-15 or a mixture thereof and is capable of being directly or indirectly detectable. Ideally, useful ligands should not bind IL-15Rα or the capture reagent. Particularly useful ligands of the present invention include polyclonal and monoclonal antibodies specific for IL-15. The methods of obtaining and making anti-IL-15Rα antibodies as described above are generally applicable for obtaining anti-IL-15 antibodies. Additional anti-IL-15 antibodies and various methods of making the same are also described in U.S. Patent No. 7,329,405.

The ligands can also be non-antibody binding partners or antigen binding peptides that have been designed or selected to selectively bind to IL-15. The design of non-antibody binding partners are generally described in Best et al., *supra*.

The ligands can be made detectable by the use of a label that does not interfere with the binding of IL-15Rα to the capture reagent, the binding of IL-15Rα to IL-15 or the binding of IL-15 to the detectable ligand. Suitable labels for use in the present invention are well known to those skilled in the immunoassay art, including labels that can be detected directly, such as fluorochrome and chemiluminscent labels for example, or indirectly, such as enzymes that must be reacted or derivatized to be detected. For RIAs of the present invention, suitable radioisotope labels and methods of using them as labels are well known in the art.

Additional examples of useful labels are set forth in U.S. Patent Publication No. 2008/0227119, which include fluorophores such as rare earth chelates or fluorescein and its

derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, luciferin, 2,3-dihydrophthalazinediones, horseradish perioxidase (HRP), alkaline phosphatase, β-galactosidase, gluconamylase, lysozyme, saccharide oxidases such as glucose oxidase, galactose oxidase and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that uses hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperioxidase or microperioxidase, biotin/avidin, biotin/streptavidin, biotin/Streptavidin-β-galactosidase, spin labels, bacteriophage labels, stable free radicals. In one embodiment, the ligand has a fluorometric label. In a colorimetric embodiment, the ligand is biotinylated and the detection means is streptavidin alkaline phosphatase and 4-nitrophenyl phosphate disodium salt in a diethanolamine buffer. Methods for attaching labels to the ligands are well known in the art. For example, the ligands can be coupled to the fluorescent, chemiluminescent and enzyme labels using coupling agents such as dialdehydes, carbodiimides, dimaleimides, bis-imidates, bis-diazotized benzidine and the like as described in U.S. Patent Publication No. 2008/0227119 and the references cited therein.

The detectable ligand is contacted (i.e., incubated) with the second complex at a temperature and for a period of time that are dependent on the ligand and label used. Those skilled in the art can readily determine, without undue experimentation, the appropriate temperature and period of time for such incubation. Generally, the incubation temperature is between about 20°-40° C and the incubation period is about 1 hour or more to amplify the signal. For example, a temperature of about 37° C with an incubation period of about 1 hour are preferred when streptavidine alkaline phosphatase and 4-nitrophenyl phosphate are used as the detection means. Excess or unbound labeled ligand is preferably removed by one or more washings with a suitable wash buffer as described above.

In the final step of the present methods, the amount of once soluble IL-15Rα, but now sandwiched between the capture reagent and IL-15, which in turn is bound to the detectable ligand, is measured. The amount of soluble IL-15Rα is quantified by comparing the results with a standard curve obtained by conducting the same immunoassay with known concentrations of a IL-15Rα reference standard. An IL-15Rα standard curve can be used, for example, to compare the level of soluble IL-15Rα in a sample taken from a patient with that of a normal individual. Suitable IL-15Rα reference standards include isolated and purified

IL-15Rα, fusion proteins in which IL-15Rα is fused to a second protein or polypeptide, or chimeric IL-15Rα proteins in which a suitable protein or peptide can be linked by recombinant technology to increase the sensitivity of the assay. Suitable reference standards for the assays of the present invention can be readily determined by those skilled in the art without undue experimentation, including, for example, the IL-15Rα proteins described in U.S. Patent Publication Nos. 2009/0018316 and 2008/0255039.

The ability to measure low concentrations of soluble IL-15Rα is a particularly useful advantage of the present invention. For example, picomolar amounts may distinguish a patient suffering from a certain disorder associated with an increase or decrease of soluble IL-15Rα compared to a normal individual.

In a further aspect of the present invention, the inventors discovered that the sensitivity of the present assays can be improved significantly using an IL-15Rα-IL-2 chimeric protein containing the full length extracellular domain of IL-15Rα as the reference standard compared with a commercially available soluble IL-15Rα-Ig fusion protein in which exon 3 has been deleted from IL-15Rα. The deletion of exon 3 has been shown to reduce the affinity of the IL-15Rα to IL-15. Figure 2 shows a typical standard curve using a novel IL-15Rα-IL-2 chimeric protein as the reference standard. This chimeric protein was produced in accordance with Example 2 below. Depending on the reference standard used, the methods of the present invention are capable of accurately measuring at least about 20 pg/ml, at least about 19 pg/ml, at least about 18 pg/ml, at least about 17 pg/ml, at least about 16 pg/ml, at least about 15 pg/ml, at least about 14 pg/ml, at least about 13 pg/ml, at least about 12 pg/ml, at least about 11 pg/ml, at least about 10 pg/ml, at least about 9 pg/ml, at least about 8 pg/ml, at least about 7 pg/ml, at least about 6 pg/ml, at least about 5 pg/ml, at least about 4 pg/ml, at least about 3 pg/ml, at least about 2 pg/ml or at least about 1 pg/ml of soluble IL-15Rα in a sample. The sensitivity of the present assays is comparable to commercially available ELISAs for other cytokines and cytokine receptors, such as IL-2 (7 pg/ml; R& D Systems) and sIL-2Rα (10 pg/ml; R& D Systems).

The present invention further provides kits containing at least the essential components for carrying out the present methods. As such, the kits minimally include: (a) a capture reagent; (b) IL-15; and (c) a detectable ligand, all of which are described more fully

above. Preferably, the kits also include reference standards and/or a standard curve for IL-15Rα.

In another embodiment, the kits can also include a solid support for the capture reagents, in which the capture reagents are provided separately or immobilized on the solid support. The kits can additionally include detection reagents for detecting the ligand. For example, if the label is an enzyme, the kit can include substrates and any desired cofactors required by the enzyme. If the label is biotin, the kit can include an avidin or an avidin conjugate (e.g., streptavidin conjugated to alkaline phosphatase).

The kits can also include any or all of the other reagents used in the methods of the present invention, including, among others, washing buffers, incubation buffers, stabilizers, preservatives, and detergents. Such reagents can be in solution or can be lyophilized for later reconstitution (e.g., at the time of performing the assay).

The methods and kits of the present invention can be used to diagnose or monitor patients with various IL-15Rα-associated diseases. Therefore, the present invention further provides methods for diagnosing or monitoring a patient with an IL-15Rα-associated disorder by:

(a) contacting a sample from the patient with a capture reagent that selectively binds to IL-15Rα to form a first complex;

(b) contacting the first complex with IL-15 to form a second complex comprising the capture reagent, IL-15Rα and IL-15;

(c) contacting the second complex with a detectable ligand that selectively binds to IL-15; and

(d) measuring the amount of soluble IL-15Rα in the sample, wherein an increased amount of soluble IL-15Rα in the sample, compared to a negative control, indicates the patient is susceptible to or has the IL-15Rα-associated disease.

All the conditions, reagents and steps described in relation to the assay methods described above are applicable to the methods for diagnosing or monitoring a patient with an IL-15Rα-associated disorder. Such disorders include leukemia, lymphoma, autoimmune diseases, retroviral diseases, multiple sclerosis and lipopolysaccharide (LPS)-induced septic shock. As used herein, the term “leukemia” includes, without limitation, adult T cell leukemia, T-cell large granular lymphocyte (LGL) leukemia and natural killer cell leukemia.

"Autoimmune diseases" include, for example, type I diabetes, HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), celiac disease, rheumatoid arthritis, inflammatory bowel disease. Retroviral diseases include, for example, HTLV and HIV and multiple sclerosis. The methods can also be used to monitor the effectiveness of IL-15 therapy or vaccination with IL-15 or IL-15Rα.

Soluble IL-15Rα can also be used as a surrogate marker for the effectiveness of LPS and interferon treatment. As such, methods for monitoring the effectiveness of LPS or interferon treatment are provided in which the amount of soluble IL-15Rα is measured in a patient sample using the assays of the present invention, wherein the detection of an increased amount of soluble IL-15Rα in the sample, as compared to a control level of soluble IL-15Rα, indicates the treatment is effective in inducing immunity since LPS and interferon are known to induce expression of IL-15Rα.

For the diagnostic and monitoring methods of the present invention, those skilled in the art can readily determine appropriate controls to use for comparison purposes without undue experimentation. For example, knowing whether an IL-15Rα-associated disorder involves an increase or decrease of soluble IL-15Rα in a patient, one skilled in the art should be able to readily identify an appropriate control (e.g., a sample from a normal patient as a negative control or from a patient known to have the disorder as a positive control).

The following examples are provided for the purpose of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

This example illustrates an ELISA for detecting soluble IL-15Rα in a sample.

Reagents and equipment

Polyclonal anti-human IL15Rα (AF247, R&D Systems, Minneapolis, MN) was used as capture antibody. Human IL15 was obtained from Peprotech Inc. (Rocky Hill, NJ; catalog No.200-15. Anti-IL15 antibody was purified from mouse ascites (Harlan Bioproducts for Science Inc, Madison, WI), which were made from anti-human IL15 hybridoma (M111, Genzyme, Cambridge, MA) and then labeled with biotin as described below. ImmunoPure™ Streptavidin-alkaline phosphatase conjugated was purchased from Pierce Biotechnology (Rockford, IL). The substrate 4--Nitrophenyl phosphate disodium salt dexahydrate (N9389,

Sigma, St. Louis, MO) was dissolved at 1mg/ml into diethanolamine buffer (1M Diethanolamine containing 0.5mM $MgCl_2$, pH9.8). Standards and Samples were diluted into 1X reagent diluents (DY995, R&D Systems). 1X wash buffer was made from wash buffer concentrate (WA126, R&D Systems). BSA was purchased from sigma (A3803) and was dissolved into PBS (Invitrogen) at 3% (g/V). The plate was read at 405nm on SpectraMax 340PC (Molecular Device, Sunnyvale, CA). SoftMax was used for data analysis.

Preparation of biotin labeled monoclonal anti-human IL15 antibody

Monoclonal anti-IL15 was prepared in PBS and the concentration was measured using BCA™ protein assay kit (Pierce Biotechnology). The volume of 10mM biotin reagent solution needed to add to the reaction was calculated by following the instructions provided with sulfo-NHS-LC-Biotin (21335, Pierce Biotechnology). Immediately before use, a 10mM biotin solution was prepared using ultrapure water. The calculated volume of 10mM biotin solution was added to the protein solution and incubate on ice for 2 hours. The protein solution was then dialyzed against PBS for 2 days to remove the free biotin. The concentration of biotin labeled anti-IL15 antibody was then measured using BCA™ protein assay kit.

Assay procedure

1. Coat the plate (DY990, R&D system) with 100ul of 10 ug/ml Polyclonal anti-human IL15Rα (AF247, R&D system) at 37°C for 3hours.
2. Wash 4 times with 1X wash buffer (soak the plate with wash buffer for 30sec between each wash). Completely remove the wash buffer.
3. Block the plate with 200ul 3%BSA at 37°C for 2 hours.
4. Wash 4 times with 1X wash buffer (soak the plate with wash buffer for 30 seconds between each wash). Completely remove the wash buffer.
5. Add 100ul standards or samples and incubated at 4°C overnight (17-18hours).
6. Wash 4 times with 1X wash buffer (soak the plate with wash buffer for 30sec between each wash). Completely remove the wash buffer.
7. Add 100ul 200ng/ml recombinant human IL15 (dissolved in 3% BSA buffer) and incubate at 37°C for 1hour.
8. Wash 4 times with 1X wash buffer (soak the plate with wash buffer for 30sec between each wash). Completely remove the wash buffer.

9. Add 100ul 2ug/ml Biotin labeled anti-hIL15 antibody (prepared in 3% BSA buffer) and incubate at 37°C for 1 hour.
10. Wash 4 times with 1X wash buffer (soak the plate with wash buffer for 30sec between each wash). Completely remove the wash buffer.
11. Add 100ul 0.5ug/ml (prepared in PBS) Streptavidin-alkaline phosphatase conjugated and incubate at 37°C for 1hour.
12. 30 minutes before the end of incubation, prepare substrate reagent by dissolve 4-Nitrophenyl phosphate disodium salt into Diethanolamine buffer at 1mg/ml, incubate at 37°C and protect from light.
13. At the end of incubation, wash the ELISA plate 4 times with 1X wash buffer (soak the plate with wash buffer for 30sec between each wash). Completely remove the wash buffer.
14. Add 100ul prepared substrate reagent into ELISA plate, incubate at 37°C degree for 30 to 40minutes, protect from light.
15. At the end of incubation, immediately read the plate at wavelength 405nm.

Example 2

This example describes the production of the sIL15Rα -IL2 chimeric protein.

The sIL15Rα coding region including preprolactin signal peptide and Kozak sequence were amplified using primers 5'ATTGAATTCGCCGCCACCATGGACAGC3' (SEQ ID NO:1) (sIL15Rα sense primer) and 5' GACCTGCAGAGTGGTGTCGCTGTGGCCC 3' (SEQ ID NO:2)(sIL15Rα anti-sense primer). IL-2 coding region was amplified using primers 5' AGCCTGCAGATGTACAGGATGCAACTCC 3' (SEQ ID NO:3)(IL-2 sense primer) and 5'GATGGATCCTCAAGTTAGTGTTGAGATGATGC3'(SEQ ID NO:4)(IL-2 anti-sense primer). The sIL15Rα-IL2 coding region was then amplified with sIL-15Rα sense primer and IL-2 anti-sense primer using sIL15Rα and IL2 coding regions as a template. The PCR amplified sIL15Rα-IL2 coding region and the expression plasmid pEF-neo were then digested with EcoRI and BamH1 and then ligated using T4 DNA ligase (Roche Molecular Biochemicals, Indianapolis, IN). DH5α competent cells (Invitrogen, Carlsbad, CA) were transformed with the ligation mixture and transformants containing the pEF-neo-sIL15Rα-IL2 construct were selected. To express sIL15Rα-IL2 chimeric protein, 293T cells were

transfected with pEF-neo-sIL15Rα-IL2. Culture supernatants were collected 72 hours after transfection by centrifugation and then filtered through 0.22um filter (Millipore, Billerica, MA).

The DNA sequence of the full IL15Rα-IL2 chimeric protein was determined to be as follows:

```
atggacagcaaaggttcgtcgcagaaagcagggtcccgcctgctcctgctgctggtggtg
tcaaatctactcttgtgccagggtgtggtctccaccacgcgtatcacgtgccctcccccc
atgtccgtggaacacgcagacatctgggtcaagagctacagcttgtactccagggagcgg
tacatttgtaactctggtttcaagcgtaaagccggcacgtccagcctgacggagtgcgtg
ttgaacaaggccacgaatgtcgcccactggacaacccccagtctcaaatgcattagagac
cctgccctggttcaccaaaggccagcgccaccctccacagtaacgacggcaggggtgacc
ccacagccagagagcctctccccttctggaaaagagcccgcagcttcatctcccagctca
aacaacacagcggccacaacagcagctattgtcccgggctcccagctgatgccttcaaaa
tcaccttccacaggaaccacagagataagcagtcatgagtcctcccacggcacccctct
cagacaacagccaagaactgggaactcacagcatccgcctcccaccagccgccaggtgtg
tatccacagggccacagcgacaccactctgcagatgtacaggatgcaactcctgtcttgc
attgcactaagtcttgcacttgtcacaaacagtgcacctacttcaagttctacaaagaaa
acacagctacaactggagcatttactgctggatttacagatgattttgaatggaattaat
aattacaagaatcccaaactcaccaggatgctcacatttaagttttacatgcccaagaag
gccacagaactgaaacatcttcagtgtctagaagaagaactcaaacctctggaggaagtg
ctaaatttagctcaaagcaaaaactttcacttaagacccagggacttaatcagcaatatc
aacgtaatagttctggaactaaagggatctgaaacaacattcatgtgtgaatatgctgat
gagacagcaaccattgtagaatttctgaacagatggattacctttgtcaaagcatcatc
tcaacactgacttga (SEQ ID NO:5)
```

The amino acid sequence of the full IL15Rα-IL2 chimeric protein was determined to be as follows:

```
M  D  S  K  G  S  S  Q  K  A  G  S  R  L  L  L  L  L  V  V
S  N  L  L  L  C  Q  G  V  V  S  T  T  R  I  T  C  P  P  P
M  S  V  E  H  A  D  I  W  V  K  S  Y  S  L  Y  S  R  E  R
Y  I  C  N  S  G  F  K  R  K  A  G  T  S  S  L  T  E  C  V
L  N  K  A  T  N  V  A  H  W  T  T  P  S  L  K  C  I  R  D
P  A  L  V  H  Q  R  P  A  P  P  S  T  V  T  T  A  G  V  T
P  Q  P  E  S  L  S  P  S  G  K  E  P  A  A  S  S  P  S  S
N  N  T  A  A  T  T  A  A  I  V  P  G  S  Q  L  M  P  S  K
S  P  S  T  G  T  T  E  I  S  S  H  E  S  S  H  G  T  P  S
Q  T  T  A  K  N  W  E  L  T  A  S  A  S  H  Q  P  P  G  V
Y  P  Q  G  H  S  D  T  T  L  Q  M  Y  R  M  Q  L  L  S  C
I  A  L  S  L  A  L  V  T  N  S  A  P  T  S  S  S  T  K  K
T  Q  L  Q  L  E  H  L  L  L  D  L  Q  M  I  L  N  G  I  N
N  Y  K  N  P  K  L  T  R  M  L  T  F  K  F  Y  M  P  K  K
A  T  E  L  K  H  L  Q  C  L  E  E  E  L  K  P  L  E  E  V
L  N  L  A  Q  S  K  N  F  H  L  R  P  R  D  L  I  S  N  I
N  V  I  V  L  E  L  K  G  S  E  T  T  F  M  C  E  Y  A  D
E  T  A  T  I  V  E  F  L  N  R  W  I  T  F  C  Q  S  I  I
S  T  L  T  *  (SEQ ID NO:6)
```

Example 3

This example provides the minimum detectable dose of sIL15Rα attained by ELISA.

Standard Curve

The concentration of sIL15Rα-IL2 made from culture supernatant of 293T cell transfectants was measured using human IL-2 ELISA (R&D Systems) and converted to molar concentration. sIL15Rα-IL2 was diluted into 1X reagent diluents to obtain 60pM sIL15Rα-IL2. Two fold series dilutions were made in 1X reagent diluents to obtain 30pM, 15pM, 7.5pM, 3.75pM, 1.875pM, 0.9375pM sIL15Rα-IL2. 1x reagent diluents were used as controls. Duplicates of 100ul different concentrations of sIL15Rα-IL2 and reagent control were assayed to obtain a standard curve as shown in Figure 2. Data used to obtain the standard curve is shown in Table 1.

Table 1

pM	OD	Average	Corrected
0	0.121 0.114	0.117	---
0.938	0.156 0.157	0.156	0.039
1.875	0.205 0.224	0.215	0.097
3.75	0.305 0.322	0.314	0.196
7.5	0.513 0.515	0.514	0.397
15	1.007 0.958	0.983	0.865
30	1.892 1.787	1.840	1.722
60	3.571 3.536	3.554	3.436

Minimum Detectable Dose

The minimum detectable dose of sIL-15Rα is typically less than about 0.3 pM (equals 7 pg/ml). The molecular weight of sIL-15Rα is 21 KD. The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration as shown in Table 2.

Table 2

20 zero standard replicates OD405				Average	SD	Average + 2SD	Calculated sIL-15Rα level of average + 2SD
0.11	**0.11**	**0.112**	**0.11**				
0.112	**0.112**	**0.112**	**0.11**				
0.112	**0.109**	**0.111**	**0.113**	**0.1139**	**0.0068**	**0.1275**	**0.301pM**
0.112	**0.109**	**0.117**	**0.11**				
0.107	**0.134**	**0.117**	**0.122**				
0.136	**0.114**	**0.105**	**0.111**				
0.132	**0.119**	**0.112**	**0.113**				
0.11	**0.112**	**0.123**	**0.112**				
0.109	**0.115**	**0.11**	**0.11**				
0.11	**0.115**	**0.114**	**0.113**				

Example 4

This Example illustrates that the IL-15Rα-IL-2 chimeric protein is biologically active.

CTLL-2 cells were cultured with RPMI1640 plus 10%FBS, 200U/ml recombinant human IL-2 (Tecin ^{TM}, NCI BRB preclinical repository) and 55uM *2*-mercaptoethanol (Invitrogen, Carlsbad, CA). On the day of proliferation, CTLL-2 cells were washed 4 times with PBS, then resuspended in RPMI1640 plus 10%FBS at concentration of 10^6 cells/ml and use 100ul cells each well for proliferation.

To measure the biological activity of sIL15Rα-IL2, different concentrations of sIL15Rα-IL2 were added with or without 10ug/ml monoclonal antibody to human IL2 (MAB202, R&D Systems) and cultured for 20 hours at 37°C. 1uCi ^{3}H thymidine (GE Healthcare Life Sciences, Piscataway, NJ) was added to each well 4 hours before harvesting and the cells were harvested on TomTec 96 well cell harvester (TomTec, Hamden, CT).

To show the neutralizing activity of sIL15Rα-IL2 on IL15, different concentration of recombinant human IL15 (200-15, Peprotech Inc) or human IL15 with same molar concentration as sIL15Rα-IL2 and 10ug/ml monoclonal anti-IL2(MAB202, R&D system) were added to washed CTLL-2 cells. The cells were cultured for 20 hours at 37°C. 1uCi ^{3}H thymidine was added to each well 4 hours before harvesting and the cells were harvested on TomTec 96 well cell harvester. The results are shown in Figure 3. As shown in Figure 3A, IL-15Rα-IL2 maintains the biological activity of IL2 in the CTLL-2 assay. Figure 3B shows that IL-15Rα-IL2 blocks the biological activity of IL-15 through binding to IL-15.

Example 5

This example shows that expression of soluble IL-15Rα can be detected in the supernatants of transfected cells using the method of the present invention.

PT18 and PT18IL15Rα cells were cultured in RPMI1640 plus 10%FBS and 10ng/ml murine recombinant IL-3 (R&D system) as reported (Tagaya Y et al, *EMBO J. 1996, 15(18): 4928–493*). Culture supernatants from PT18 and PT18IL15Rα were collected by centrifugation when the cells grow confluent and filtered through 0.22um filter (Millipore). Duplicates of 100ul Undiluted or diluted (in 1X reagent diluents) culture supernatant were used to measure the level of sIL15Rα.

293T cells (ATCC) were cultured in RPMI1640 plus 10%FBS. To transfect 293T cells, split cells 1:4 the day before transfection. The transfection was performed according to the instructions of Fugene6 Transfection Reagent (Roche Molecular Biochemicals). For 10cm dish, dilute 10ug pEF-neo, pEF-neo-WTIL15Rα or pEF-neo-sIL15Rα construct into 100ul Opti-MEMI medium (Invitrogen). Dilute 30ul of Fugene6 into 100ul Opti-MEMI medium. Mix together diluted DNA constructs and fugene6 solution, incubate at room temperature for 30minutes and then add to 293T cells. Culture supernatants were collected 72 hours after transfection by centrifugation and filtered through 0.22um filter (Millipore). Duplicates of 100ul Undiluted or diluted (in 1X reagent diluents) culture supernatant were used to measure the level of sIL15Rα in accordance with the general assay procedure of Example 1. Figure 4 shows that sIL-15Rα was detected by ELISA in the supernatants of transfected cells expressing IL-15Rα.

Example 6

This example describes the Western blot analysis of cells transfected with IL-15Rα.

PT18, PT18IL15Rα, 293T cells and 293T cells transfected with wild type IL15Rα and soluble IL15Rα were solubilized at 4°C in RIPA lysis buffer (50mM Tris-CL, pH7.4, 0.5% sodium deoxycholate, 1% Nonidet P-40, 150mM NaCl, 66-µg/ml aprotinin, 100-µg/ml phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate). Cell lysates (50 µg) were resolved by electrophoresis on SDS-polyacrylamide (4-12%) gels and transferred to polyvinylidine difluoride membranes. After blocking of the membranes in 5% skim milk and 0.05% Tween 20 in Tris-buffered saline, the blots were incubated with goat polyclonal antibody to IL15Rα (SC-5526, Santa Cruz Biotechnology). After several washings with Tris-buffered saline plus 0.1% Tween 20, the blots were then incubated with HRP

conjugated mouse anti-goat antibody(SC-2354, Santa Cruz Biotechnology, Santa Cruz, CA). After several washes, the protein bands recognized by the antibodies were visualized with an enhanced chemiluminescence Western blotting detection system (RPN2132, GE Healthcare). The results of the Western blot analysis are provided in Figure 5. Soluble IL-15Rα expressed by these transfected cells was not detected by Western blot because sIL-15Rα was secreted into the supernatant as demonstrated by ELISA in Example 5.

Example 7

This example describes the results of a comparison between normal human serum and reagent diluents spiked with sIL-15Rα.

Two-fold series dilutions of 293T sIL15Rα transfection supernatant were prepared in 1X reagent diluents. Similarly, the same 2-fold series dilutions of 293T sIL15Rα supernatant were prepared in normal human AB serum (Gemini Bio-Products, West Sacramento, CA) diluted 1:3 into 1X reagent diluents. Duplicates of 100ul 2-fold series dilutions of sIL15Rα prepared in 1X regent diluents or normal human AB serum were assayed follow the general assay protocol of Example 1. The results are shown in Figure 6, which suggest the assay can be used to measure sIL-15Rα in human serum.

Example 8

This example shows the levels of s sIL-15Rα in various serum samples from patients afflicted with various disorders.

Serum samples were diluted 1:3 into 1X reagent diluents and duplicates of 100ul diluted serum samples were assayed follow the general assay procedure of Example 1. Serum samples from normal donors were collected from NIH blood bank (transfusion medicine, NIH).

Serum samples from type I diabetes patients were obtained from ProMedDx (Norton, MA). The results of Figure 7 show elevated levels of sIL-15Rα in serum type I diabetes patients compared to normal individuals.

Serum samples from Adult T cell leukemia patients, T-cell large granular lymphocyte (LGL) leukemia patients, HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), Natural killer cell leukemia (NKL) were collected by metabolism branch, NCI/NIH. The results for Adult T cell leukemia patients are shown in Figure 8, and the results for LGL leukemia patients, HAM/TSP patients and NKL patients are shown in

Figures 9 and 10, respectively.

Serum samples from celiac patients were provided by Dr. Bana Jabri, University of Chicago, with the results shown in Figure 11.

Serum samples from multiple sclerosis (MS) and rheumatoid arthritis patients were purchased from Bioserve, with the results shown in Figures 12 and 13, respectively. As shown in Figure 12, there was no significant difference between the β-interferon treated MS patients and the untreated MS patients.

CSF and serum samples from neuroimmunological disorders were collected by Dr. Bibi Bielekova (NINDS/NIH). The results of the CSF and neuroimmunological disorders are shown in Figure 14.

While various embodiments of the present invention have been described herein, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. Such modifications and adaptations are within the scope and spirit of the present invention as set forth in the claims.

What is claimed is:

1. A method to measure soluble interleukin-15 receptor alpha (IL-15Rα) in a sample, comprising the steps of:

(a) contacting the sample with a capture reagent that selectively binds to IL-15Rα to form a first complex;

(b) contacting the first complex with IL-15 to form a second complex comprising the first ligand, IL-15Rα and IL-15;

(c) contacting the second complex with a detectable ligand that selectively binds to IL-15; and

(d) measuring the amount of IL-15Rα in the sample.

2. The method of Claim 1, wherein step (d) further comprises using a standard curve for IL-15Rα to measure the amount of soluble IL-15Rα in said sample.

3. The method of Claim 2, wherein said method is an ELISA.

4. The method of Claim 2, wherein said method is capable of measuring at least about twenty (20) picograms/mL of soluble IL-15Rα in the sample.

5. The method of Claim 2, wherein said method is capable of measuring at least about seven (7) picograms/mL of soluble IL-15Rα in the sample.

6. The method of Claim 2, wherein a purified IL-15Rα protein is used to obtain said standard curve.

7. The method of Claim 6, wherein said purified protein is an IL-15Rα-IL-2 chimeric protein.

8. The method of Claim 1, wherein said sample is a biological sample.

9. The method of Claim 8, wherein said biological sample is obtained from a mammal.

10. The method of Claim 9, wherein said mammal is a human.

11. The method of Claim 8, wherein said biological sample is serum, plasma, tissue lysate, tumor lysate, or tumor cell culture supernatant.

12. The method of Claim 10, wherein the IL-15 is human IL-15.

13. The method of Claim 1, wherein the capture reagent is an anti- IL-15Rα antibody.

14. The method of Claim 13, wherein the anti- IL-15Rα antibody isa polyclonal

antibody.

15. The method of Claim 13, wherein the IL-15Rα antibody is AF247.

16. The method of Claim 1, wherein the detectable ligand is an anti-IL-15 antibody.

17. The method of Claim 16, wherein the anti- IL-15 antibody is a monoclonal antibody.

18. The method of Claim 16, wherein the anti-IL-15 antibody is labeled with a colorimetric reagent.

19. The method of Claim 16, wherein the anti-IL-15 antibody is biotinylated and the detection means is avidin or streptavidin-alkaline phosphatase .

20. A kit for measuring soluble IL-15Rα in a sample comprising:

(a) a capture reagent that selectively binds IL-15Rα;

(b) IL-15;

(c) a detectable ligand that selectively binds IL-15; and

(d) a means for measuring the amount of soluble IL-15Rα in said sample.

21. The kit of Claim 20, wherein the capture ligand is an anti- IL-15Rα antibody.

22. The kit of Claim 20, wherein the means for measuring the amount of soluble IL-15Rα in the sample comprises a purified IL-15Rα protein for use as a standard.

23. The kit of Claim 22, wherein said purified IL-15Rα protein is a chimeric protein.

24. The kit of Claim 23, wherein said chimeric protein is an IL-15Rα-IL-2 chimeric protein.

25. The kit of Claim 20, wherein said means for measuring further comprises reagents for detecting the detectable ligand.

26. A method for diagnosing or monitoring IL-15Rα-associated disorders in a patient, comprising the steps of:

(a) contacting a biological sample obtained from the patient with a capture reagent that selectively binds to IL-15Rα to form a first complex;

(b) contacting the first complex with IL-15 to form a second complex comprising the first ligand, IL-15Rα and IL-15;

(c) contacting the second complex with a detectable ligand that selectively

binds to IL-15; and

(d) measuring the amount of IL-15Rα in the sample, wherein an increase in the amount of IL-15Rα in the patient, as compared to a negative control, indicates the patient is susceptible to or has an IL-15Rα-associated disorder.

27. The method of Claim 26, wherein said IL-15Rα-associated disorder is selected from the group consisting of leukemia, lymphoma, autoimmune disease, retroviral disease, and LPS induced septic shock.

28. The method of Claim 27, wherein said leukemia is adult T cell leukemia, T-cell large granular lymphocyte (LGL) leukemia, or natural killer cell leukemia .

29. The method of Claim 27, wherein said lymphoma is adult T cell leukemia/lymphoma.

30. The method of Claim 27, wherein said autoimmune disease is type I diabetes, celiac disease, HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), rheumatoid arthritis or multiple sclerosis.

31. A method for monitoring the effectiveness of a therapeutic treatment that increases the amount of sIL-15Rα in a patient, comprising measuring the amount of soluble IL-15Rα in a patient sample, wherein an increased or decreased amount of soluble IL-15Rα in the sample, as compared to a control level of soluble IL-15Rα, indicates said treatment is effective or ineffective.

32. The method of claim 31, wherein said therapeutic treatment is LPS, interferon, IL-15, or IL-15Rα.

33. An sIL-15Rα-IL-2 chimeric protein having an amino acid sequence of SEQ ID NO:6.

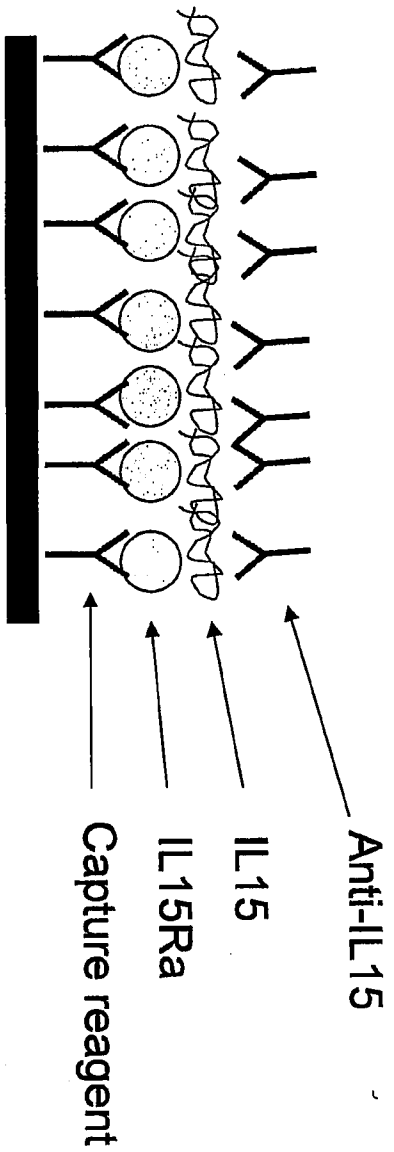


FIG. 1

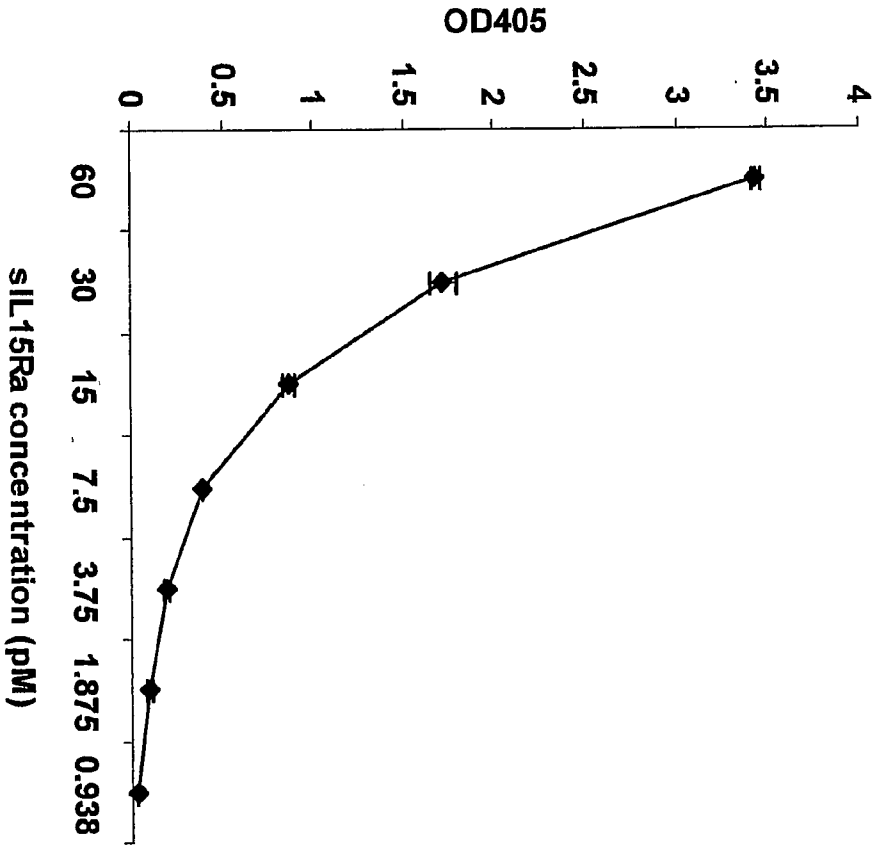
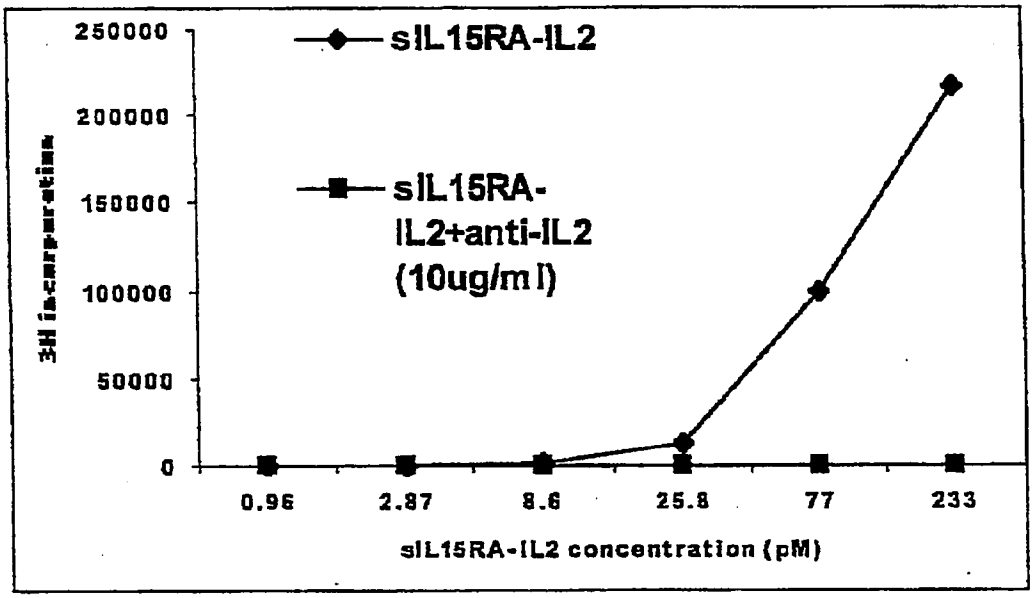

OD405
0
0.5
1
1.5
2
2.5
3
3.5
4
sIL15Ra concentration (pM)
60
30
15
7.5
3.75
1.875
0.938

Fig. 2

SHEET 3/15



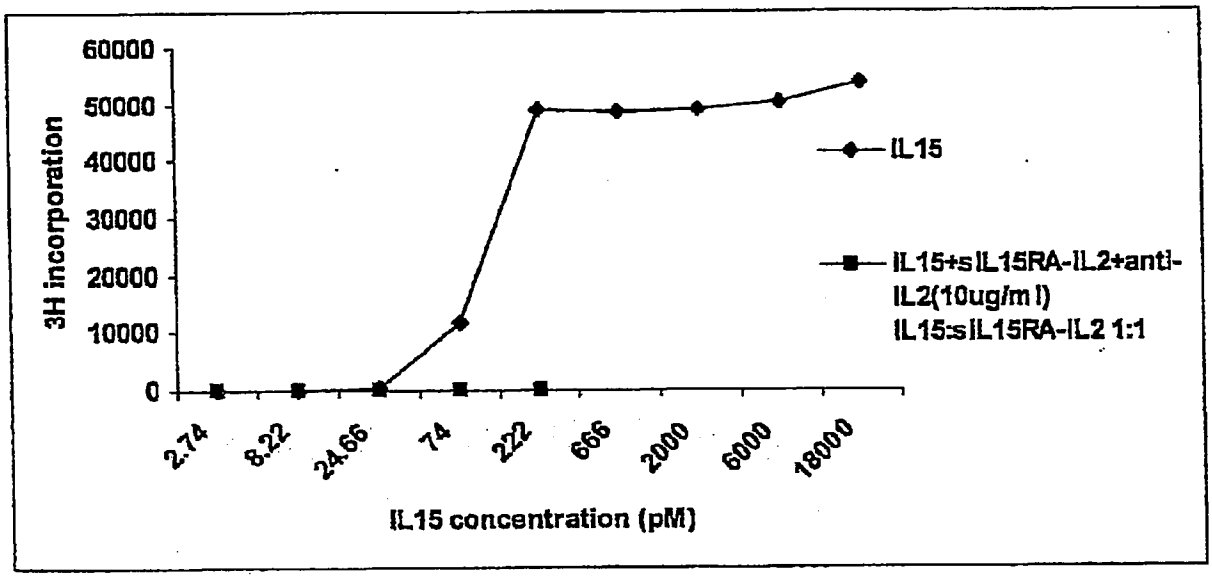
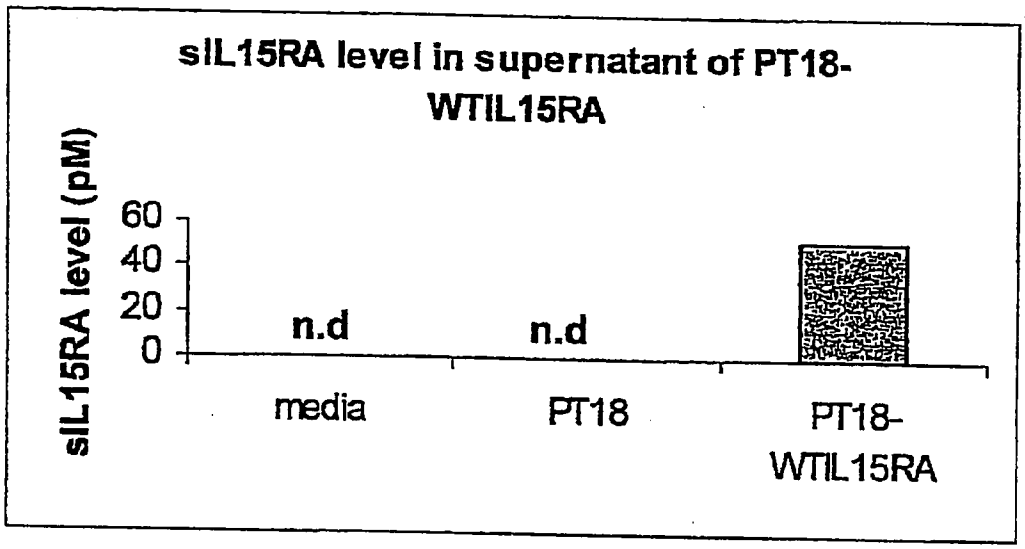
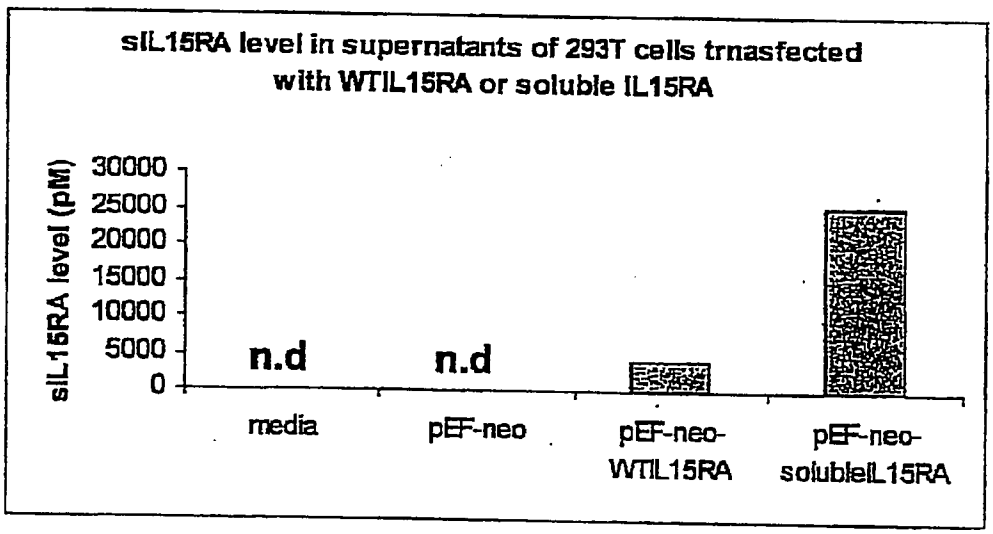


Fig. 3

A



B



n.d : Non detectable

Fig. 4

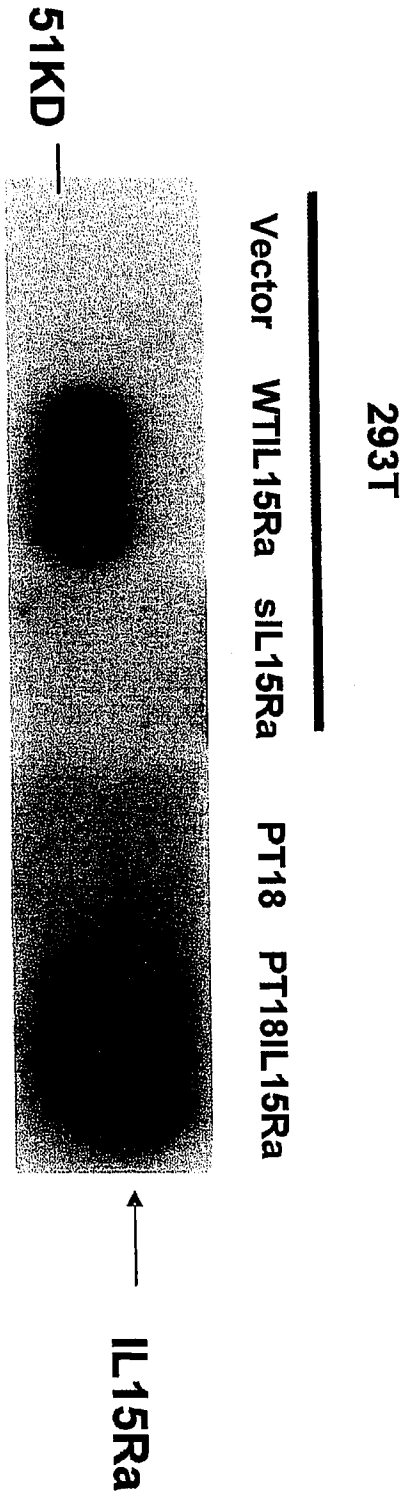


Fig. 5

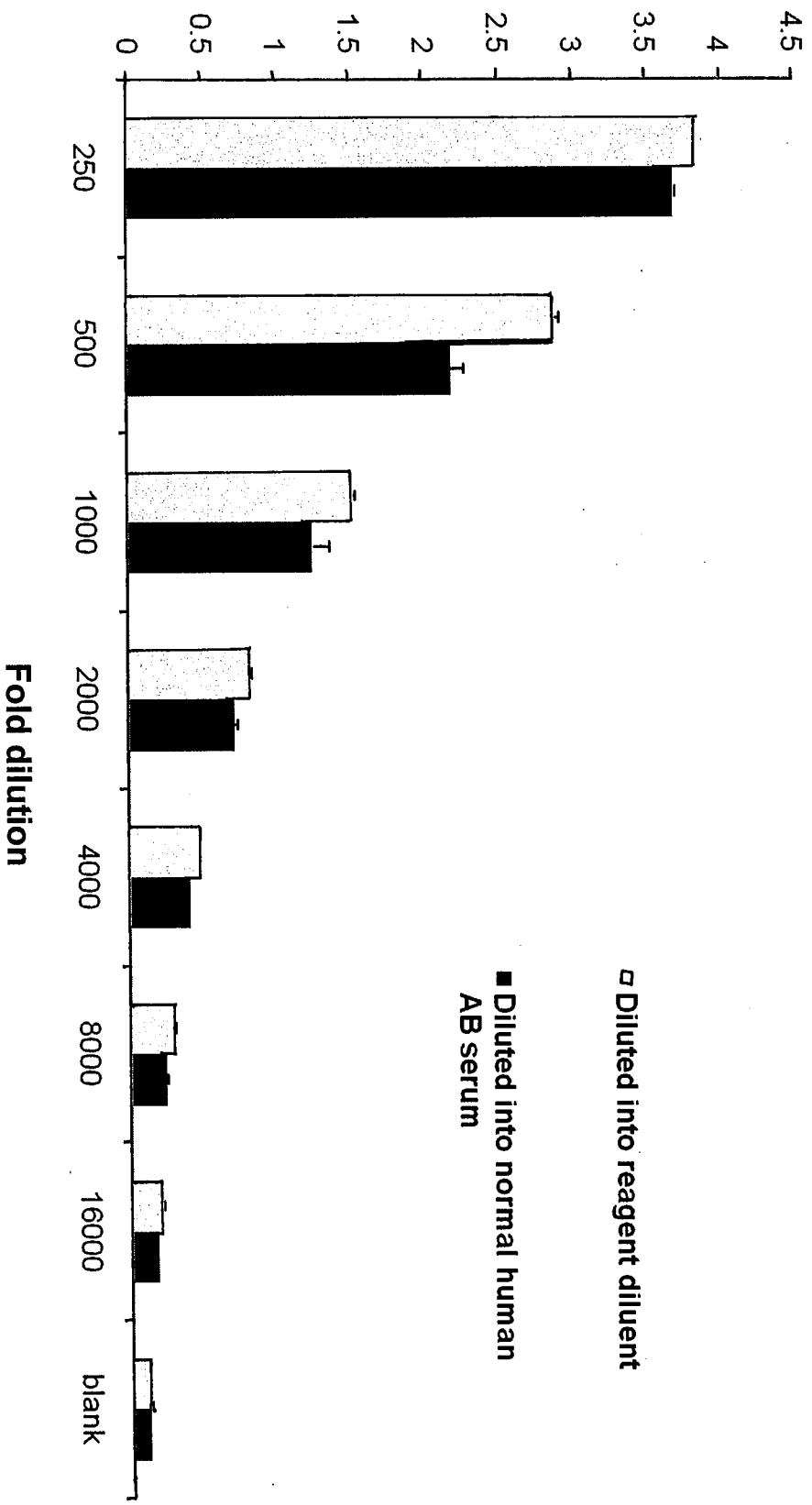

OD405
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
250
500
1000
2000
4000
8000
16000
blank
Fold dilution
Diluted into reagent diluent
Diluted into normal human AB serum

Fig. 6

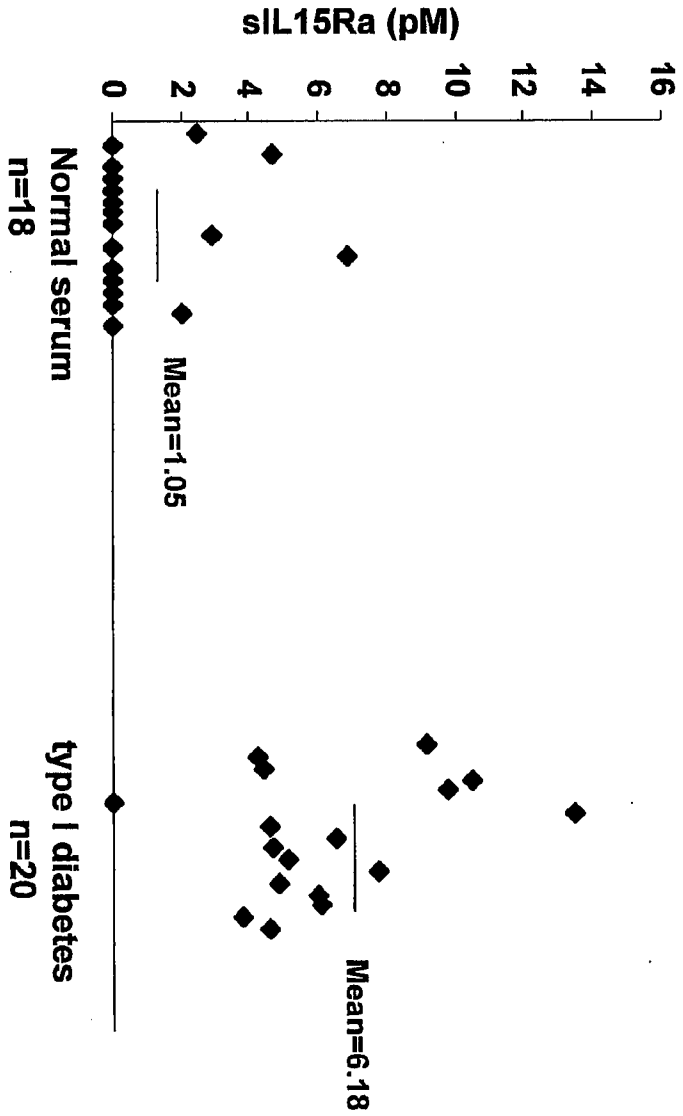

sIL15Ra (pM)
0
2
4
6
8
10
12
14
16
Normal serum
n=18
Mean=1.05
type I diabetes
n=20
Mean=6.18

Fig. 7

SHEET 8/15

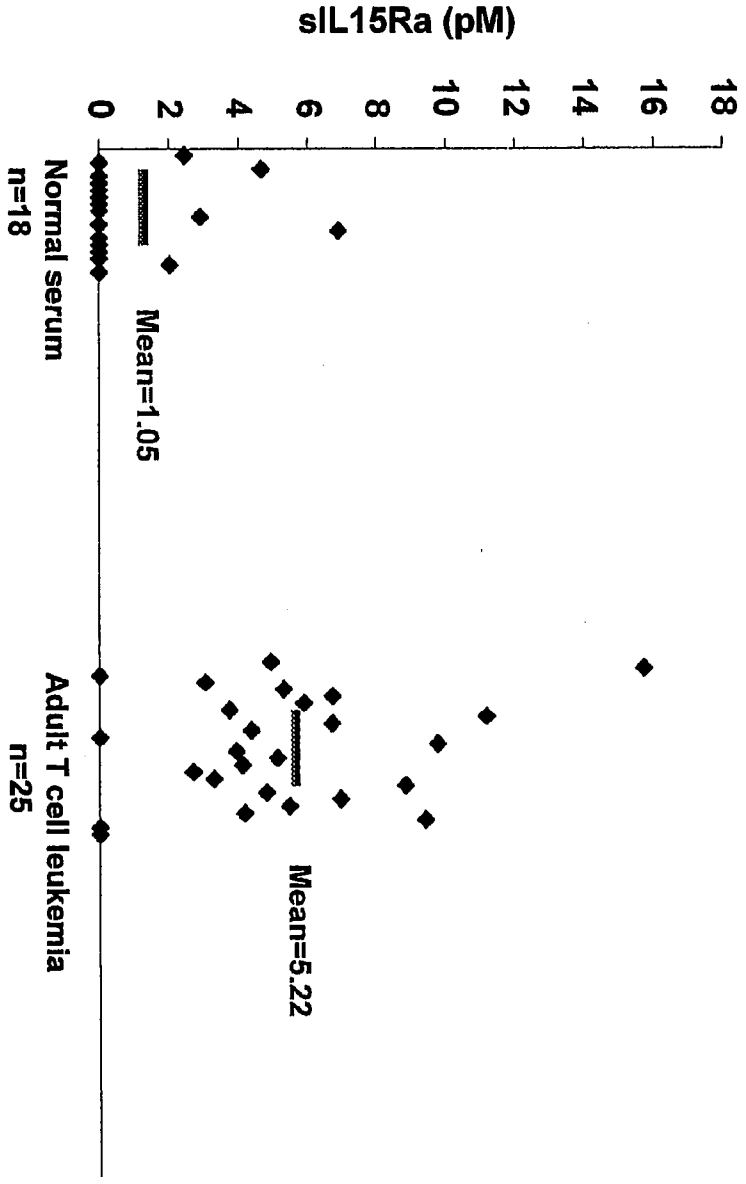


Fig. 8

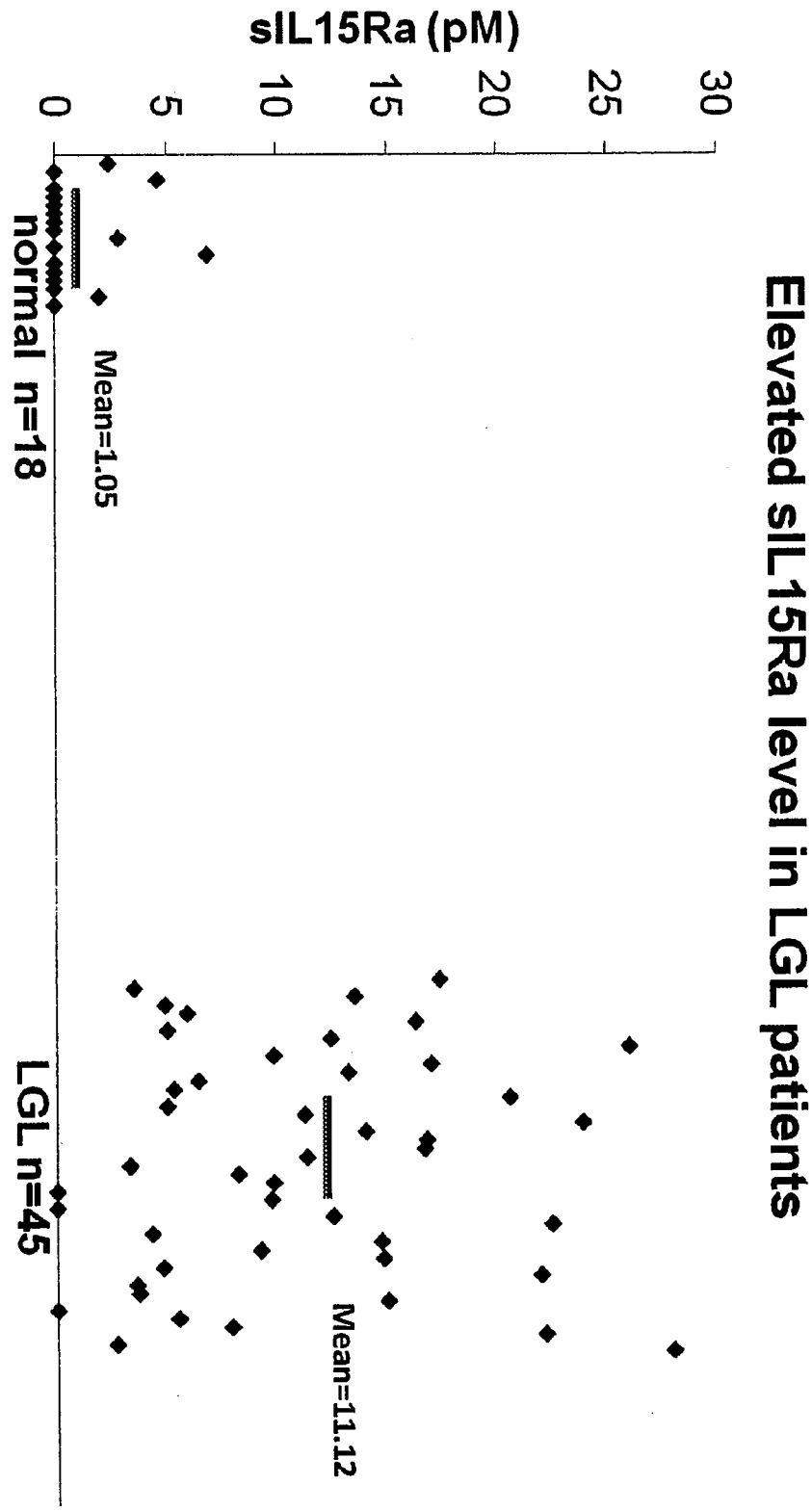

Elevated sIL15Ra level in LGL patients
sIL15Ra (pM)
0
5
10
15
20
25
30
normal n=18
Mean=1.05
LGL n=45
Mean=11.12

Fig.9

SHEET 10/15

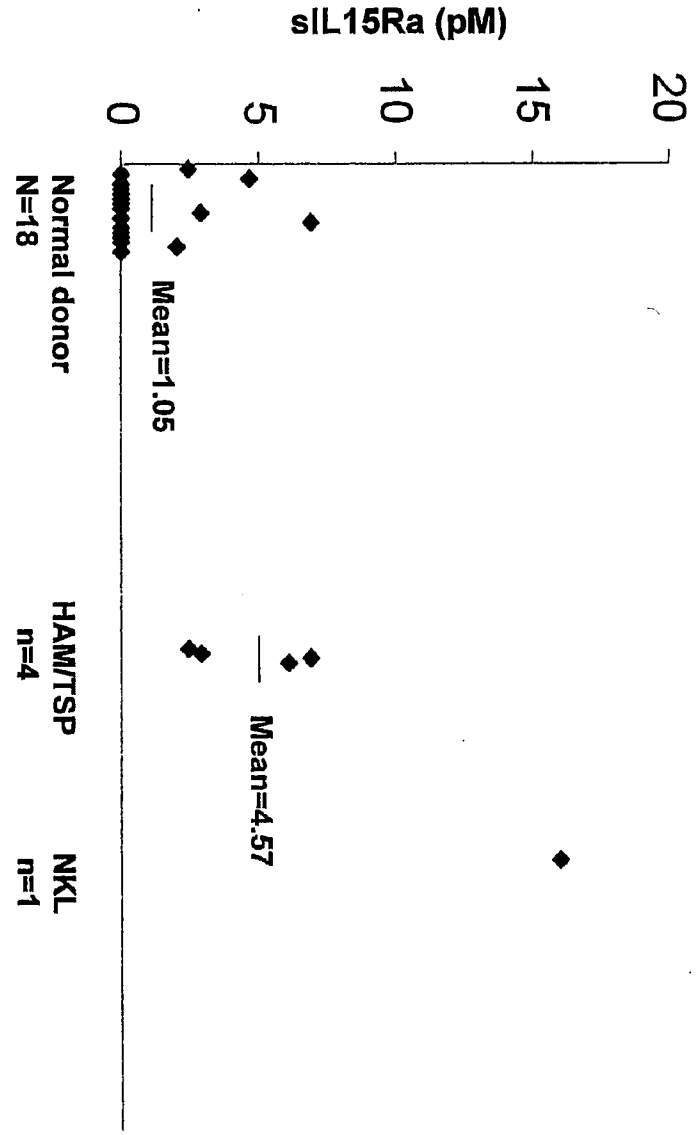
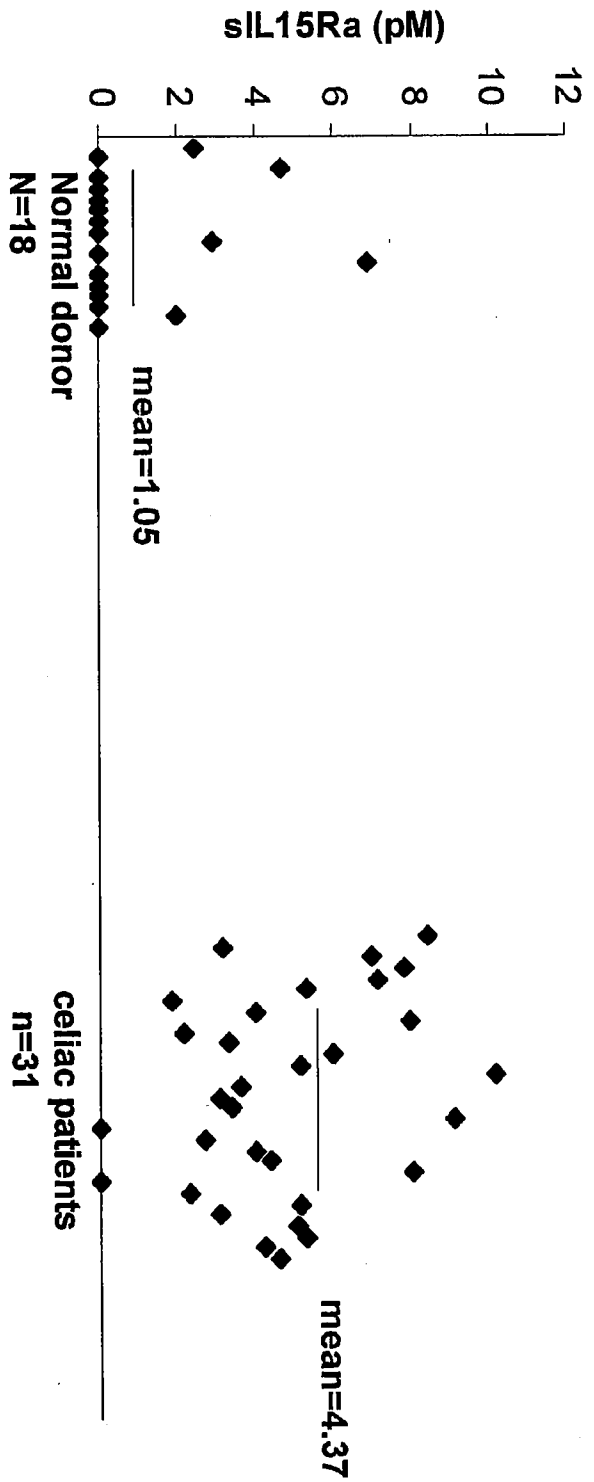


Fig. 10



sIL15Ra level in celiac patients

Fig. 11

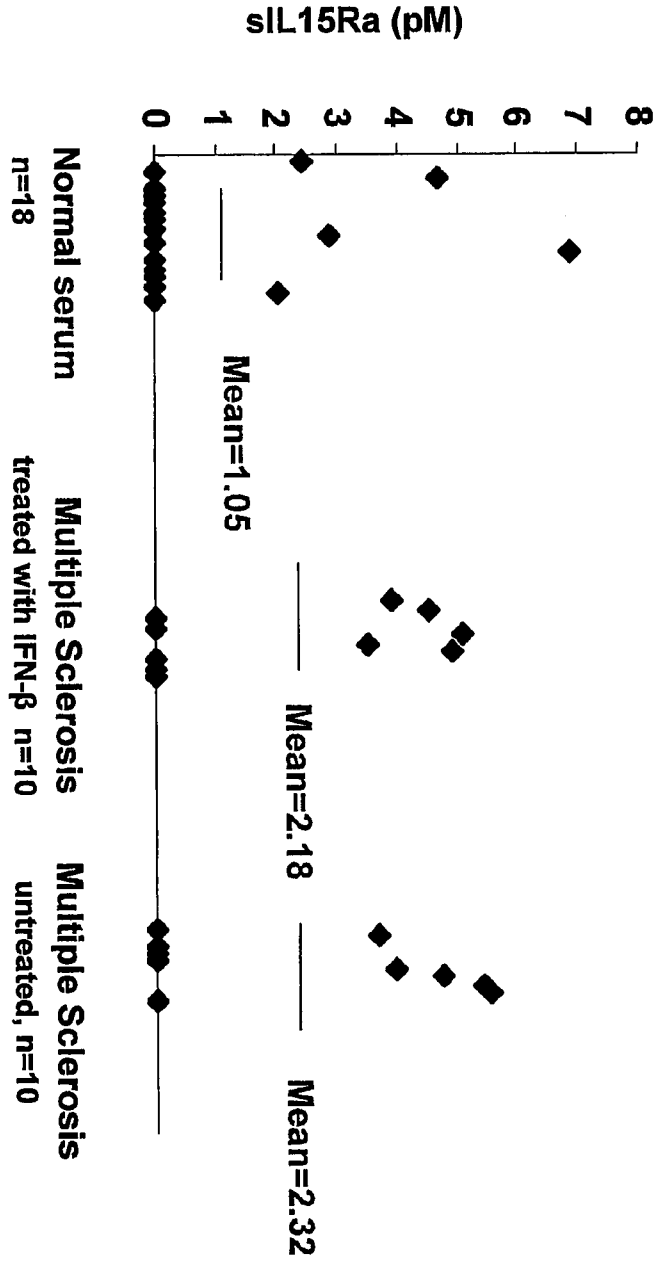

sIL15Ra level in Multiple Sclerosis
sIL15Ra (pM)
0
1
2
3
4
5
6
7
8
Mean=1.05
Mean=2.18
Mean=2.32
Normal serum
n=18
Multiple Sclerosis
treated with IFN-β n=10
Multiple Sclerosis
untreated, n=10

Fig. 12

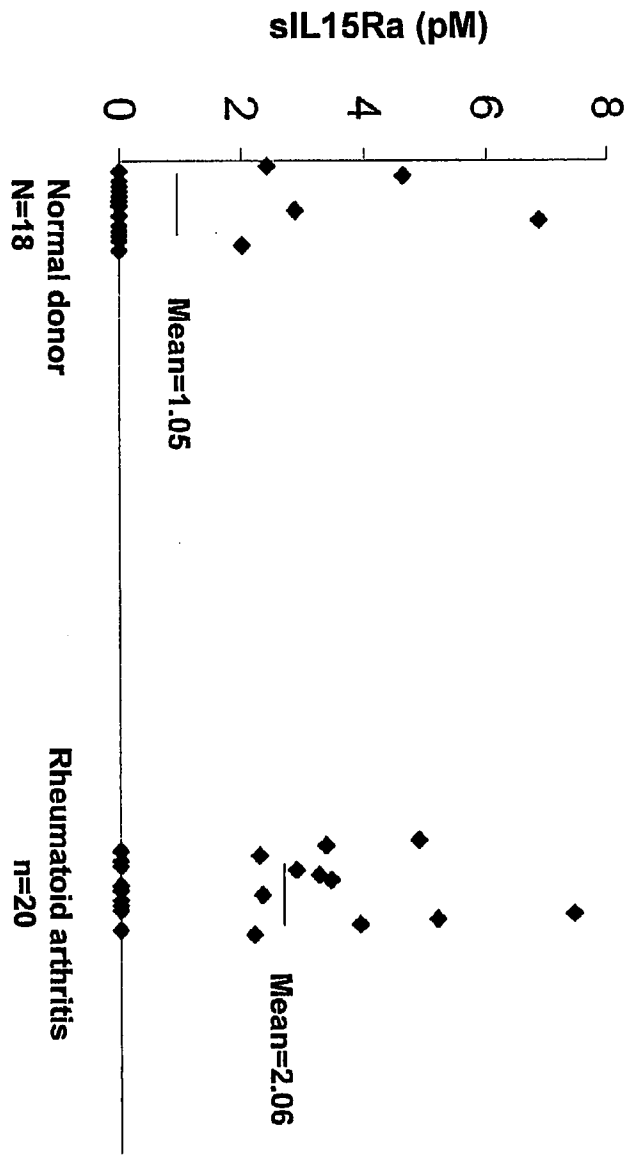

sIL15Ra (pM)
0
2
4
6
8
Normal donor
N=18
Mean=1.05
Rheumatoid arthritis
n=20
Mean=2.06

Fig. 13

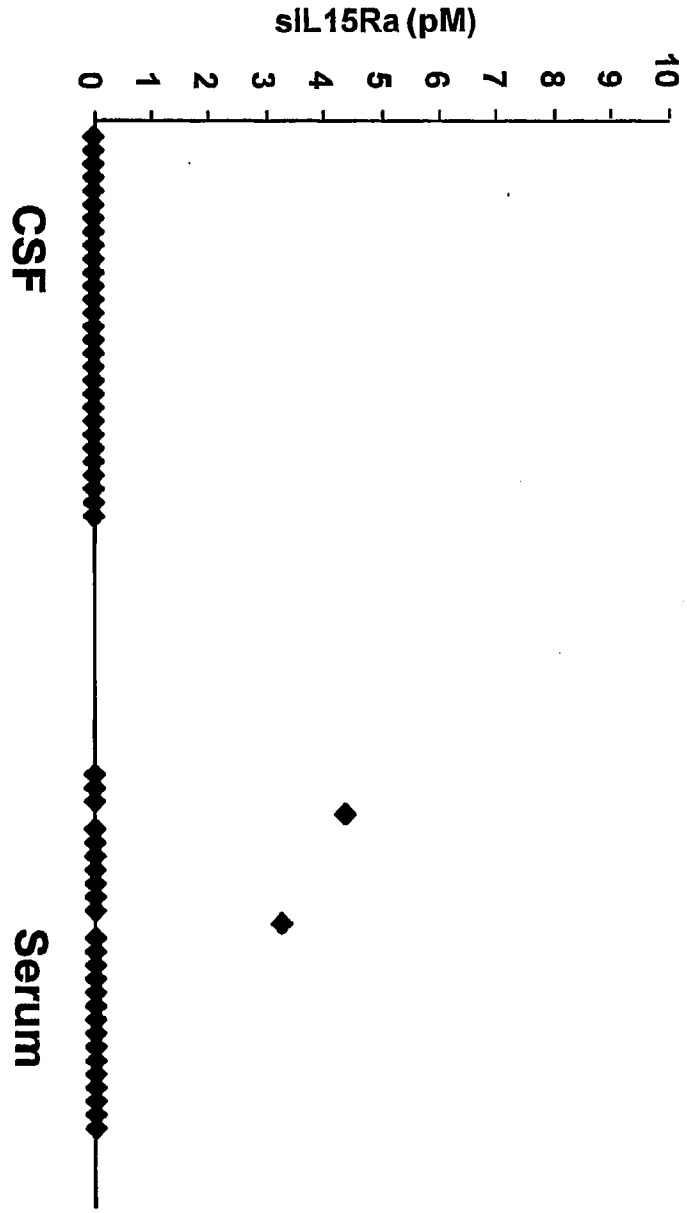


Fig. 14

```
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tcaaatctactcttgtgccagggtgtggtctccaccacgcGtatcacgtgccctcccccc
S  N  L  L  L  C  Q  G  V  V  S  T  T  R  I  T  C  P  P  P

atgtccgtggaacacgcagacatctgggtcaagagctacagcttgtactccagggagcgg
M  S  V  E  H  A  D  I  W  V  K  S  Y  S  L  Y  S  R  E  R

tacatttgtaactctggtttcaagcgtaaagccggcacgtccagcctgacggagtgcgtg
Y  I  C  N  S  G  F  K  R  K  A  G  T  S  S  L  T  E  C  V

ttgaacaaggccacgaatgtcgcccactggacaacccccagtctcaaatgcattagagac
L  N  K  A  T  N  V  A  H  W  T  T  P  S  L  K  C  I  R  D

cctgccctggttcaccaaaggccagcgccaccctccacagtaacgacggcaggggtgacc
P  A  L  V  H  Q  R  P  A  P  P  S  T  V  T  T  A  G  V  T

ccacagccagagagcctctccccttctggaaaagagcccgcagcttcatctcccagctca
P  Q  P  E  S  L  S  P  S  G  K  E  P  A  A  S  S  P  S  S

aacaacacagcggccacaacagcagctattgtcccgggctcccagctgatgccttcaaaa
N  N  T  A  A  T  T  A  A  I  V  P  G  S  Q  L  M  P  S  K

tcaccttccacaggaaccacagagataagcagtcatgagtcctcccacggcacccctct
S  P  S  T  G  T  T  E  I  S  S  H  E  S  S  H  G  T  P  S

cagacaacagccaagaactgggaactcacagcatccgcctcccaccagccgccaggtgtg
Q  T  T  A  K  N  W  E  L  T  A  S  A  S  H  Q  P  P  G  V

tatccacagggccacagcgacaccactCTGCAGatgtacaggatgcaactcctgtcttgc
Y  P  Q  G  H  S  D  T  T  L  Q  M  Y  R  M  Q  L  L  S  C

attgcactaagtcttgcacttgtcacaaacagtgcacctacttcaagttctacaaagaaa
I  A  L  S  L  A  L  V  T  N  S  A  P  T  S  S  S  T  K  K

acacagctacaactggagcatttactgctggatttacagatgattttgaatggaattaat
T  Q  L  Q  L  E  H  L  L  L  D  L  Q  M  I  L  N  G  I  N

aattacaagaatcccaaactcaccaggatgctcacatttaagtttacatgcccaagaag
N  Y  K  N  P  K  L  T  R  M  L  T  F  K  F  Y  M  P  K  K

gccacagaactgaaacatcttcagtgtctagaagaagaactcaaacctctggaggaagtg
A  T  E  L  K  H  L  Q  C  L  E  E  E  L  K  P  L  E  E  V

ctaaatttagctcaaagcaaaaactttcacttaagacccagggacttaatcagcaatatc
L  N  L  A  Q  S  K  N  F  H  L  R  P  R  D  L  I  S  N  I

aacgtaatagttctggaactaaagggatctgaaacaacattcatgtgtgaatatgctgat
N  V  I  V  L  E  L  K  G  S  E  T  T  F  M  C  E  Y  A  D

gagacagcaaccattgtagaatttctgaacagatggattacctttgtcaaagcatcatc
E  T  A  T  I  V  E  F  L  N  R  W  I  T  F  C  Q  S  I  I

tcaacactgacttga
S  T  L  T  *
```

Fig. 15

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/048451

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/54 C07K14/715 G01N33/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BERNARD J ET AL: "Identification of an interleukin-15alpha receptor-binding site on human interleukin-15" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, INC, US LNKD- DOI:10.1074/JBC.M312458200, vol. 279, no. 23, 4 June 2004 (2004-06-04) , pages 24313-24322, XP002286785 ISSN: 0021-9258	33
Y	page 24314, right-hand column, paragraph 4	23,24
	-/--	

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 October 2010	18/11/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wiesner, Martina

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/048451

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORTIER ERWAN ET AL: "IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation" THE JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, US LNKD-DOI:10.1084/JEM.20071913, vol. 205, no. 5, 12 May 2008 (2008-05-12), pages 1213-1225, XP002571967 ISSN: 0022-1007 cited in the application	20-22, 25,31,32
Y	page 1214, left-hand column, paragraph 4; figure 3 page 1223, right-hand column, paragraph 2 page 1216, left-hand column, last paragraph - page 1217, left-hand column, paragraph 1	1-19,23, 24
X	& MORTIER ERWAN ET AL: "IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation" THE JOURNAL OF EXPERIMENTAL MEDICINE, ROCKEFELLER UNIVERSITY PRESS, US LNKD-DOI:10.1084/JEM.20071913, vol. 205, no. 5, 12 May 2008 (2008-05-12), XP002607608 Retrieved from the Internet: URL:http://jem.rupress.org/content/suppl/2008/05/04/jem.20071913.DC1/1.html> [retrieved on 2010-10-26]	20-22, 25,31,32
Y	sublementary figurfigure S1	1-19,23, 24

Y	RUCHATZ H ET AL: "SOLUBLE IL-15 RECEPTOR ALPHA-CHAIN ADMINISTRATION PREVENTS MURINE COLLAGEN-INDUCED ARTHRITIS: A ROLE FOR IL-15 IN DEVELOPMENT OF ANTIGEN-INDUCED IMMUNOPATHOLOGY" JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 160, 1 January 1998 (1998-01-01), pages 5654-5660, XP002941876 ISSN: 0022-1767 cited in the application page 5655, left-hand column, paragraph 1; figure 1	1-19, 26-30

	-/--	

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/048451

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BADOUAL C ET AL: "The soluble alpha chain of interleukin-15 receptor: a proinflammatory molecule associated with tumor progression in head and neck cancer" CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER REREARCH, US LNKD-DOI:10.1158/0008-5472.CAN-07-6842, vol. 68, no. 10, 15 May 2008 (2008-05-15), pages 3907-3914, XP002488938 ISSN: 0008-5472 cited in the application page 3912, left-hand column, paragraph 2; figure 1	26-30
Y	MORTIER ERWAN ET AL: "Natural, proteolytic release of a soluble form of human IL-15 receptor alpha-chain that behaves as a specific, high affinity IL-15 antagonist" JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 173, no. 3, 1 August 2004 (2004-08-01), pages 1681-1688, XP002445753 ISSN: 0022-1767 cited in the application page 1682, right-hand column, paragraphs 4,5	1-19,24
Y	BULANOVA E ET AL: "Soluble interleukin (IL)-15R[alpha] is generated by alternative splicing or proteolytic cleavage and forms functional complexes with IL-15" JOURNAL OF BIOLOGICAL CHEMISTRY 20070504 AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY INC. US, vol. 282, no. 18, 4 May 2007 (2007-05-04), pages 13167-13179, XP002607607 DOI: DOI:10.1074/JBC.M610036200 page 13169, right-hand column, paragraphs 2,3	1-19

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/048451

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MATSUMOTO M ET AL: "On-column refolding and characterization of soluble human interleukin-15 receptor alpha-chain produced in Escherichia coli" PROTEIN EXPRESSION AND PURIFICATION, ACADEMIC PRESS, SAN DIEGO, CA LNKD-DOI:10.1016/S1046-5928(03)00143-8, vol. 31, no. 1, 1 September 2003 (2003-09-01), pages 64-71, XP004454317 ISSN: 1046-5928 page 66, right-hand column, last paragraph - page 67, left-hand column, paragraph first ----	1-19
X,P	BOUCHAUD G ET AL: "Interleukin-15 and Its Soluble Receptor Mediate the Response to Infliximab in Patients With Crohn's Disease" GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, vol. 138, no. 7, 1 June 2010 (2010-06-01), pages 2378-2387, XP027094499 ISSN: 0016-5085 [retrieved on 2010-02-23] the whole document ----	31