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(57) Abrégé/Abstract:
The invention provides humanized antibodies, for example, those that that bind ICAM-1, methods of use and methods of producing the antibodies. Antibodies include sequences having a VH and VL domain selected from HumA, HumB, HumC, HumD, HumE, HumF, HumG, HumH, Huml, Hum40 and Hum50.

ABSTRACT

The invention provides humanized antibodies, for example, those that that bind ICAM-1, methods of use and methods of producing the antibodies. Antibodies include sequences having a VH and VL domain selected from HumA, HumB, HumC, HumD, HumE, HumF, HumG, HumH, HumI, Hum40 and Hum50.

Humanized Antibodies

FIELD OF THE INVENTION

The invention relates to humanized antibody compositions and methods of making and using humanized antibodies.

BACKGROUND

Monoclonal antibodies have become an important class of therapeutic proteins. However, foreign immunoglobulins used in humans can elicit an anti-globulin response which may interfere with therapy or cause allergic or immune complex hypersensitivity. To avoid this problem, a monoclonal antibody may be "humanized," and this is typically carried out by CDR grafting.

CDR's, also called hypervariable regions, are present in immunoglobulin light and heavy chains and are flanked by "framework" regions. CDR grafting was first described in Jones *et al.* ((1986) *Nature* 321:522-525). In this and later publications, the CDRs of three mouse antibodies were grafted onto the variable domain framework of the human immunoglobulin NEW (V_H) and REI (V_L). The resulting humanized antibodies had the same antigen specificity and a similar affinity as the parental murine monoclonal antibody (mAb) (Jones *et al. supra*; Verhoeyen *et al.* (1988) *Science* 239:1534-1536; Riechmann *et al.* (1988) *Nature* 332:323-327; U.S. Patent No. 5,225,539).

CDR grafting has been described by Queen and coworkers who reported the humanization of four murine monoclonal antibodies (Queen *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:10029-10033; Co *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:2869-2873; Co *et al.* (1992) *J. Immunol.* 148:1149-1154; and U.S. Patent Nos. 5,585,089; 5,693,761; and 5,693,762). Murine residues were inserted in the human framework in order to maintain affinity and, in each case the original antigen specificity was maintained. The affinities of the humanized antibodies ranged from 1/3 to 3 times of the parental unmodified murine antibodies.

SUMMARY

The invention provides humanized antibodies that bind ICAM-1. In one embodiment, the antibody has a V_H and V_L domain selected from: SEQ ID NO:1 and 3

(HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50).

Subsequences of antibodies that bind ICAM-1 are provided, for example, single chain, Fab, Fab' and $(Fab)_2$ fragments. In particular aspects, the humanized antibody has greater affinity for ICAM-1 than the parental (non-human) antibody. Varaint and modified forms of antibodies that bind ICAM-1 are also provided, for example, antibodies having a V_H and V_L domain selected from: SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50), having one or more amino acid substitutions, insertions or deletions.

The invention also provides humanized antibodies that bind ICAM-1 and inhibit pathogen infection of cells expressing ICAM-1. Such invention antibodies include antibodies that provide equal or greater protection from pathogen infection than parental (non-human) antibody. In particular aspects, a humanized antibody has a protective efficacy equal to or at least 2 times greater, 5 times greater, 10 times greater, 20 times greater, 30 times greater than the non-humanized antibody. In other aspects, the pathogen is human rhinovirus (HRV), coxackie A virus, respiratory syncytial virus (RSV), or malaria.

The humanized antibodies of the invention include intact immunoglobulin molecules, comprising 2 full-length heavy chains and 2 full-length light chains, for example, IgG, IgA, IgM, IgE, and IgD, and subsequences that inhibit pathogen infection. Particular subsequences include, for example, single chain, Fab, Fab' or $(Fab)_2$ fragment.

The humanized antibodies of the invention include multispecific or multifunctional antibodies. In one aspect, such an antibody is formed by linking a humanized antibody to one or more identical or different antibodies to form a multimer (e.g. using a linker). Antibody multimers include a homo- or hetero-dimer, trimer,

tetramer or any other higher order oligomer. Antibody multimers that include different antibodies are human, humanized or non-human. Multimeric forms include antibody oligomers that form via a multimerization domain (e.g. a human amino acid sequence) or a covalent bond. Antibody multimers that include a multimerization domain further include forms having a linker located between the multimerization domain and the antibody.

The invention additionally provides methods for producing humanized antibodies. In one embodiment, a method includes: selecting a human framework sequence as an acceptor, wherein said sequence has 50% or more identity (e.g., 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, 90-95%, or more identity) to a non-human donor antibody framework region; grafting a CDR from the donor non-human antibody (e.g., murine) onto the human framework; comparing the vernier zone residues of the human acceptor and the non-human donor framework regions; and maintaining one or more of the human acceptor residues in the vernier zone when the donor non-human and human residues are structurally or chemically similar, or substituting one or more of the vernier zone residues with a residue that is different from both the donor non-human vernier zone residue and acceptor human vernier zone residue if the donor non-human vernier zone residue is structurally or chemically dissimilar to the human residue, wherein the different residue is structurally or chemically similar to the donor non-human vernier zone residue. In additional embodiments, human framework acceptor sequences are selected from a consenus sequence, for example, from V_H domain subgroup I and subgroup II consensus sequences.

Nucleic acid sequences encoding humanized antibodies, subsequences and modified forms thereof (e.g., amino acid additions, deletions or substitutions) are also provided. Nucleic acid sequences further include expression cassettes in which nucleic acid encoding humanized antibodies are operably linked to an expression control element. Vectors and cells (prokaryotic and eukaryotic) that include the nucleic acids also are provided.

The invention further provides pharmaceutical compositions including humanized antibodies, subsequences, multimers, variants and modified forms, and nucleic acids

encoding them, and a pharmaceutically acceptable carrier. In particular aspects, the pharmaceutically acceptable carrier is compatible with inhalation or nasal delivery to a subject.

The invention further provides methods of inhibiting pathogen infection of a cell. In one embodiment, a method includes contacting a pathogen or a cell with an amount of a humanized antibody, subsequence, multimer, variant or modified form sufficient to inhibit pathogen infection of the cell. In one aspect, the cell expresses ICAM-1. In another aspect, the cell (e.g., epithelial cell) is present in a subject.

The invention also provides methods of inhibiting HRV infection of a cell. In one embodiment, a method includes contacting HRV or a cell susceptible to HRV infection with an amount of a humanized antibody, subsequence, multimer, variant or modified form effective to inhibit HRV infection of the cell (e.g., epithelial cell). In one aspect, the cell is present in a subject. In another aspect, the cell is present in a subject having or at risk of having asthma. In yet another aspect, the subject is a newborn or between the ages of 1 to 5, 5 to 10 or 10 to 18. In still another aspect, the antibody, subsequence, multimer, variant or modified form binds to an antigen present on the surface of the cell (e.g., ICAM-1). In various additional aspects, the humanized antibody is administered locally, via inhalation or intranasaly.

The invention also provides methods of inhibiting HRV infection, inhibiting HRV progression or treating HRV infection of a subject. In one embodiment, a method includes administering to a subject having or at risk of having HRV infection an amount of a humanized antibody, subsequence, multimer, variant or modified form effective to inhibit HRV infection, inhibit HRV progression or treat HRV infection of the subject. In one aspect, the subject has or is at risk of having asthma. In another aspect, the subject is a newborn or between the ages of 1 to 5, 5 to 10 or 10 to 18. In various additional aspects, the humanized antibody is administered locally, via inhalation or intranasaly.

The invention additionally provides methods of decreasing or inhibiting one or more symptoms of the common cold in a subject. In one embodiment, a method includes administering to a subject having a common cold an amount of a humanized antibody, subsequence, multimer, variant or modified form effective to decrease or inhibit one or

more symptoms of the common cold in the subject. In one aspect, the subject has or is at risk of having asthma. In another aspect, the subject is a newborn or between the ages of 1 to 5, 5 to 10 or 10 to 18. In various additional aspects, the humanized antibody is administered locally, via inhalation or intranasaly.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequences of murine 1A6 antibody heavy and light chain (SEQ ID NO:77 and 79) and human consensus sequence of heavy chain subgroup III (Hum3; SEQ ID NO:78) and light chain kappa subgroup I (HumκI; SEQ ID NO:80). Asterisks denote amino acid differences between human and mouse sequence. CDR amino acids as defined by Kabat and Chothia are in bold face.

Figure 2 shows the amino acid sequences of murine 1A6 antibody (SEQ ID NO:77), humanized 1A6 (HumB; SEQ ID NO:4) and human consensus sequences of heavy chain subgroup III (Hum3; (SEQ ID NO:78) and light chain kappa subgroup I (HumκI; (SEQ ID NO:80). Asterisks and bold face amino acids are as previously indicated.

Figure 3 shows the cDNA sequences of humanized scFVA (HumA) antibody (SEQ ID NO:2). Restriction sites are indicated by underlining; CCATGG Nco I site; GGATCC BamH I site; GTTAAC Hpa I site. Bold face amino acids are as previously indicated.

Figure 4 shows protection from HRV15 infection with mouse 1A6 scFv antibody (Ms1) and humanized 1A6 scFv antibodies HumA, HumB, HumC, HumD, HumF, HumH and HumI.

Figure 5 shows amino acid sequences of murine 1A6 V_H domain (SEQ ID NO:77) and human consensus sequences of V_H domain subgroup I (Hum1; (SEQ ID NO:82) and subgroup II (Hum2; SEQ ID NO:81). Bold face amino acids are as previously indicated.

Figure 6 shows the V_H domain amino acid sequences of murine 1A6 antibody (SEQ ID NO:77), humanized 1A6 (Hum40; SEQ ID NO:20) and human consensus

sequences of heavy chain subgroup II (Hum2; SEQ ID NO:81). Asterisks and bold face amino acids are as previously indicated.

Figure 7 shows the V_H domain amino acid sequences of murine 1A6 antibody (SEQ ID NO:77), humanized 1A6 (Hum50; SEQ ID NO:21) and human consensus sequences of heavy chain subgroup I (Hum1; SEQ ID NO:82). Asterisks and bold face amino acids are as previously indicated.

DETAILED DESCRIPTION

The present invention is based, at least in part, upon producing humanized antibodies. More particularly, complementarity determining region (CDR) from a non-human antibody are grafted into a human framework region. Following grafting, one or more amino acids of the antibody is mutated to a human amino acid or is mutated to a non-human amino acid having structural similarity to the amino acid it replaces. For example, mutating a murine amino acid to a human amino acid in a framework region or CDR of the grafted antibody can produce a humanized antibody having increased antigen binding affinity relative to the non-human or grafted antibody. Humanized antibodies are not immunogenic or are less immunogenic than non-human antibodies when administered to human subjects. Therefore, humanized antibodies are useful in a variety of therapeutic and diagnostic applications. For example, as exemplified herein, a humanized antibody of the invention protects cells from HRV infection, a virus that can cause the common cold, and other associated disorders (e.g. otitis media, bronchitis, sinusitis etc.).

Thus, in accordance with the invention, there are provided humanized antibodies. In one embodiment, a humanized antibody binds to ICAM-1. In one aspect, a humanized antibody that binds ICAM-1 protects against pathogen infection of cells expressing ICAM-1. In other aspects, a humanized antibody has a V_H and V_L domain selected from: SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50). In another embodiment, a humanized antibody has a greater or less

affinity for the antigen than the donor non-human antibody. In various aspects, affinities range from greater or less affinity for the antigen than either the donor or recombinant antibody. In particular aspects, humanized antibody has an antigen binding affinity 2- to 4-fold, 5-fold, 5- to 8-fold, 5- to 10-fold, 8- to 15-fold, 10- to 20-fold, 20- to 40-fold, 20-60-fold, 20- to 100-fold or greater than the parental antibody.

Human antibody sequence regions can be used for producing humanized antibodies of the invention. For example, a "consensus sequence," an antibody sequence having the most frequently occurring amino acid residues at particular positions in an antibody or an antibody region, may be used. As an example, human variable region domain sequences are described in Kabat (*Sequences of Proteins of Immunological Interest.* 4th Ed.US Department of Health and Human Services. Public Health Service (1987)). Sequences that are completely determined in the framework regions, 1-23, 35-49, and 57-88 in the light chains, and in the framework regions, 1-30, 36-49, and 66-94, in the heavy chains, are included in the survey. For the fourth framework region, 98-107 in the light chain and 103-113 in the heavy chain, residues that can be derived from the known J-minigene segments are surveyed.

At the end of the survey, the most frequently occurring residue at a given position is chosen as the residue in the consensus sequence. Consensus sequences may therefore be identified by surveying amino acid residues at each position of a plurality of antibodies; the most frequently occurring amino acid at a given position in the region of interest is a part of the consensus. In many instances, more than one residue will be found at high frequency at a given position. In such cases, if the amino acid that occurs at least one-fourth as frequently as the most frequently occurring the amino acid residue is considered a part of the consensus sequence.

The published consensus sequence of human V_H subgroup III is based on a survey of 22 known human V_H III sequences, the consensus sequence of human V_H subgroup I is based on 6 known human V_H I sequences, and the consensus sequence of human V_H subgroup II is based on 10 known sequences in the same group. The published consensus sequence of human V_L kappa-chain subgroup I, based on a survey of 30 known human kappa I sequences (Padlan (1994) *Mol. Immunol.* 31:169-217; Padlan (1991) *Mol.*

Immunol. 28:489-498). The human consensus sequences were previously used to humanize two antibodies (Carter *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:4285-4289; Presta *et al.* (1993) *J. Immunol.* 151: 2623-2632). These human V_H subgroup I, II and III sequences, and V_L -kappa subgroup I consensus sequences are selected as frameworks, respectively, to humanize mAb1A6 as described in Examples 1 and 8. Thus, consensus sequences known in the art, as exemplified for human V_H subgroup I, II and III or V_L -kappa subgroup I, are selected as acceptor frameworks for producing humanized antibody in accordance with the invention.

Any mouse, rat, guinea pig, goat, non-human primate (e.g., ape, chimpanzee, macaque, orangutan, etc.) or other non-human animal antibody may be used as a CDR donor for producing humanized antibody. Murine antibodies secreted by hybridoma cell lines can also be used. Donor CDRs are selected based upon the antigen to which the antibody binds. Thus, donor CDRs include sequences from antibodies that bind to pathogens, such as bacteria, viruses, protozoa and other microorganisms. Donor CDRs also include antibodies that bind to molecules to which the pathogens bind, for example, cell surface proteins (e.g., adhesion proteins, receptor proteins, immune-recognition/modulation proteins such as HLA, tumor associated antigens, etc.). In one particular example, the donor antibody is a mouse monoclonal antibody 1A6 (mAb1A6), which specifically binds to ICAM-1.

"Complementarity determining regions" or "CDRs" are among the sequences that can be grafted into framework sequences. CDRs refer to sequence regions that confer antibody specificity and affinity. CDRs are also generally known as supervariable regions or hypervariable loops. CDR regions of antibodies have been mapped and are defined as in Kabat (*Sequences of Proteins of Immunological Interest.* 4th Ed.US Department of Health and Human Services. Public Health Service (1987)) and Chothia and Lesk ((1987) J. Mol. Biol. 186:651-663)). In particular, for heavy chain, CDR1 is defined as H26-H35, CDR2 is H50-65 and CDR3 is H95-H102; for light chain, CDR 1 is L24-L34, CDR2 is L50-L56 and CDR3 is L89-L97. The amino acids are numbered according to the scheme described in Kabat (*Sequences of Proteins of Immunological Interest.* 4th Ed.US Department of Health and Human Services. Public Health Service (1987)). Variable region domains typically comprise the amino-terminal approximately

105-115 amino acids a of a naturally-occurring immunoglobulin chain (e.g., amino acids 1-110). Variable domains shorter or longer than these exemplary sequence lengths may also be used.

Thus, the invention provides humanized antibodies, methods of making the antibodies and methods of using the antibodies, including therapeutic and diagnostic methods. In one embodiment, a humanized antibody has increased affinity for the antigen relative to non-humanized antibody (e.g., less than 1.18×10^{-6} M in K_D against ICAM-1, less than 1×10^{-7} M in K_D, less than 5×10^{-7} M in K_D, less than 1×10^{-8} M in K_D, less than 5×10^{-8} M in K_D or less than 1×10^{-9} M in K_D). In various aspects, a humanized antibody includes a V_H and V_L domain selected from: SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50); and antigen binding subsequences thereof. In various additional aspects, an antibody subsequence comprises Fab, Fab', $(Fab')_2$, Fv, and single chain antibody (SCA), *e.g.*, scFv fragments.

The humanized antibodies of the invention also include antibody multimers. In various aspects, a multimer comprises a dimer, trimer, tetramer or other higher order oligomer. In other aspects, multimers comprise combinations of the same antibodies (homo-oligomers) and different antibodies (hetero-oligomers), the different antibodies being human, humanized or non-human.

The terms "protein," "polypeptide" and "peptide" are used interchangeably herein to refer to two or more covalently linked amino acids, also referred to as "residues," through an amide bond or equivalent. Polypeptides are of unlimited length and may be comprised of L- or D-amino acids as well as mixtures thereof. Amino acids may be linked by non-natural and non-amide chemical bonds including, for example, those formed with glutaraldehyde, N-hydoxysuccinimide esters, bifunctional maleimides, or N,N'-dicyclohexylcarbodiimide (DCC). Non-amide bonds include, for example, ketomethylene, aminomethylene, olefin, ether, thioether and the like (see, e.g., Spatola

(1983) in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp 267-357, "Peptide and Backbone Modifications," Marcel Decker, NY). Polypeptides may have one or more cyclic structures such as an end-to-end amide bond between the amino and carboxy- terminus of the molecule or intra- or inter-molecular disulfide bond. Polypeptides may be modified in vitro or *in vivo*, e.g., post-translationally modified to include, for example, sugar residues, phosphate groups, ubiquitin, fatty acids or lipids. Polypeptides further include amino acid structural and functional analogues, for example, peptidomimetics having synthetic or non-natural amino acids or amino acid analogues.

The term "antibody" refers to a protein that binds to other molecules (antigens) via heavy and light chain variable domains, V_H and V_L, respectively. Antibodies include IgG, IgD, IgA, IgM and IgE. The antibodies may be intact immunoglobulin molecules, two full length heavy chains linked by disulfide bonds to two full length light chains, as well as subsequences (i.e. fragments) of immunoglobulin molecules, with our without constant region, that bind to an epitope of an antigen, or subsequences thereof (i.e. fragments) of immunoglobulin molecules, with or without constant region, that bind to an epitope of an antigen. Antibodies may comprise full length heavy and light chain variable domains, V_H and V_L, individually or in any combination. For example, each of a a V_H and V_L domain selected from: SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50) are included individually and in any combination.

Polypeptide sequences can be made using recombinant DNA technology of polypeptide encoding nucleic acids via cell expression or in vitro translation, or chemical synthesis of polypeptide chains using methods known in the art. Antibodies according to the invention, including humanized sequences and subsequences can be expressed from recombinantly produced antibody-encoding nucleic acid (see, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1989; Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1999; Fitzgerald *et al.*, *J.A.C.S.* 117:11075 (1995); Gram *et al.*, *Proc. Natl. Acad. Sci. USA*

89:3576-80 (1992)). For example, as described in Example 3, cDNA encoding humanized antibody sequences can be expressed in bacteria in order to produce invention antibodies. Antibodies may also be produced by expressing encoding nucleic acids in mammalian, insect, and plant cells. Polypeptide sequences including antibodies can also be produced by a chemical synthesizer (see, e.g., Applied Biosystems, Foster City, CA).

As used herein, the term "subsequence" or "fragment" means a portion of the full length molecule. For example, a subsequence of an antibody is one or more amino acid less in length than full length polypeptide (e.g. one or more internal or terminal amino acid deletions from either amino or carboxy-termini). Subsequences therefore can be any length up to the full length molecule.

Specific examples of antibody subsequences include, for example, Fab, Fab', $(Fab')_2$, Fv, or single chain antibody (SCA) fragment (*e.g.*, scFv). Subsequences include portions which retain at least part of the function or activity of full length sequence. For example, an antibody subsequence will retain the ability to selectively bind to an antigen even though the binding affinity of the subsequence may be greater or less than the binding affinity of the full length antibody. Subsequences can comprise a portion of any of the invention humanized sequences, for example, a portion of V_H and V_L domain selected from: SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50).

Pepsin or papain digestion of whole antibodies can be used to generate antibody fragments. In particular, an Fab fragment consists of a monovalent antigen-binding fragment of an antibody molecule, and can be produced by digestion of a whole antibody molecule with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain. An $(Fab')_2$ fragment of an antibody can be obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. An Fab' fragment of an antibody molecule can be obtained from $(Fab')_2$ by reduction with a thiol reducing agent, which yields a molecule consisting of an intact

light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody molecule treated in this manner.

An Fv fragment is a fragment containing the variable region of a light chain V_L and the variable region of a heavy chain V_H expressed as two chains. The association may be non-covalent or may be covalent, such as a chemical cross-linking agent or an intermolecular disulfide bond (Inbar et al., (1972) *Proc. Natl. Acad Sci.* USA 69:2659; Sandhu (1992) *Crit. Rev. Biotech.* 12:437).

A single chain antibody ("SCA") is a genetically engineered or enzymatically digested antibody containing the variable region of a light chain V_L and the variable region of a heavy chain, optionally linked by a flexible linker, such as a polypeptide sequence, in either V_L-linker-V_H orientation or in V_H-linker-V_L orientation. Alternatively, a single chain Fv fragment can be produced by linking two variable domains via a disulfide linkage between two cysteine residues. Methods for producing scFv antibodies are described, for example, by Whitlow et al., (1991) In: Methods: A Companion to Methods in Enzymology 2:97; U.S. Patent No. 4,946,778; and Pack et al., (1993) *Bio/Technology* 11:1271.

Other methods of producing antibody subsequences, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, provided that the subsequences bind to the antigen to which the intact antibody binds.

As used herein, the term "bind" or "binding" means that the compositions referred to have affinity for each other. "Specific binding" is where the binding is selective between two molecules. A particular example of specific binding is that which occurs between an antibody and an antigen. Typically, specific binding can be distinguished from non-specific when the dissociation constant (K_D) is less than about 1×10^{-5} M or less than about 1×10^{-6} M or 1×10^{-7} M. Specific binding can be detected, for example, by ELISA, immunoprecipitation, coprecipitation, with or without chemical crosslinking, two-hybrid assays and the like. Appropriate controls can be used to distinguish between "specific" and "non-specific" binding.

Invention antibodies, including full length antibodies, subsequences (e.g., single chain forms) may be present as dimer, trimers, tetramers, pentamers, hexamers or any other higher order oligomer that retains at least a part of antigen binding activity of monomer. Multimers can comprise heteromeric or homomeric combinations of full length antibody, subsequences, unmodified or modified as set forth herein and known in the art. Antibody multimers are useful for increasing antigen avidity in comparison to monomer due to the multimer having multiple antigen binding sites. Antibody multimers are also useful for producing oligomeric (e.g., dimer, trimer, tertamer, etc.) combinations of different antibodies thereby producing compositions of antibodies that are multifunctional (e.g., bifunctional, trifunctional, tetrafunctional, etc.).

The term "multifunctional" means that the composition referred to has two or more activities or functions (e.g., antigen binding, enzyme activity, ligand or receptor binding, toxin, etc.). For example, an antibody that binds to a particular antigen which also has an attached polypeptide with enzyme activity (e.g., luciferase, acetyltransferase, galactosidase, peroxidase, etc.) is one particular example of a muiltifunctional antibody.

Multifunctional antibodies further include multispecific (e.g., bispecific, trispecific, tetraspecific, etc.) forms. The term "multispecific" means an antibody that binds to two or more different antigenic epitopes. The term "multispecific" means that the antibody contains two or more variable region sequences that bind to different epitopes. The different epitopes may be present on the same antigen or different antigens. For example, a multispecific antibody oligomer comprises a mixture of two or more antibodies each having different epitope binding specificity and which form a multimer. Multispecific antibodies may be comprised of individual antigen binding polypeptides each of which have distinct variable domains. For example, one of the antibodies may have two variable domains each of which recognizes a different epitope.

Candidate functions for multifunctional antibodies other than antigen binding and in addition to enzyme activity include, for example, detectable moieties such as radioisotopes and amino acid sequences (e.g., ^{35}S, ^{131}I, T7, immunoglobulin or polyhistidine tags, toxins (e.g., ricin, cholera, pertussis), cell surface proteins such as receptors, ligands (substrates, agonists and antagonists), adhesion proteins (e.g.,

streptavidin, avidin, lectins), growth factors, differentiative factors and chemotactic factors.

Multifunctional humanized antibodies can be produced through chemical crosslinking of the selected molecules (which have been produced by synthetic means or by expression of nucleic acid that encode the polypeptides) or through recombinant DNA technology combined with in vitro, or cellular expression of the polypeptide, and subsequent oligomerization. Multispecific antibodies can be similarly produced through recombinant technology and expression, fusion of hybridomas that produce antibodies with different epitopic specificities, or expression of multiple nucleic acid encoding antibody variable chains with different epitopic specificities in a single cell.

Antibodies may be either joined directly or indirectly through covalent or non-covalent binding, e.g. via a multimerization domain, to produce multimers. A "multimerization domain" mediates non-covalent protein-protein interactions. Specific examples include coiled-coil (e.g., leucine zipper structures) and alpha-helical protein sequences. Sequences that mediate protein-protein binding via Van der Waals' forces, hydrogen bonding or charge-charge bonds are also contemplated as multimerization domains. Additional examples include basic-helix-loop-helix domains and other protein sequences that mediate heteromeric or homomeric protein-protein interactions among nucleic acid binding proteins (e.g., DNA binding transcription factors, such as TAFs). One specific example of a multimerization domain is p53 residues 319 to 360 which mediate tetramer formation. Another example is human platelet factor 4, which self-assembles into tetramers. Yet another example is extracellular protein TSP4, a member of the thrombospondin family, which can form pentamers. Additional specific examples are the leucine zippers of jun, fos, and yeast protein GCN4.

Humanized antibodies may be directly linked to each other via a chemical cross linking agent or can be connected via a linker sequence (e.g., a peptide sequence) to form multimers. As used herein, "linker" or "spacer" refers to a molecule or group of molecules that connects two or more molecules to each other. A flexible linker allows rotation of the two molecules linked to each other to the extent that the molecules do not block each others function. For example, a linker such as an amino acid sequence attached to a humanized antibody which is itself attached to a multimerization domain,

allows the antibody to bind to antigen without significant steric interference from the multimers of the oligomer. Non-peptide linkers include chemical cross linking agents and polyethylene glycol.

One specific example of a peptide linker is an immunoglobulin hinge sequence. Additional specific examples are polylyisne, ploygluta mic acid and mixtures of randomized amino acid sequences. Linker amino acid sequences may be fully human, humanized or non-human amino acid sequences, unmodified or modified as set forth herein. The invention therefore further provides humanized antibodies that include a linker sequence. Linker sequences include, for example, sequences from about 2 to 10, 10 to 20, 10 to 30, 25 to 50, 30 to 60 and 50 to 75 amino acids in length.

Antibodies also include modified forms such as sequences having one or more amino acid substitutions, additions or deletions, provided the modification does not destroy function, e.g., does not destroy antigen binding activity; the antibody retains, at least in part, antigen binding activity. For example, a modified humanized antibody will retain, at least in part, affinity for the antigen to which unmodified antibody binds. The term "modification" therefore denotes an alteration of a molecule that does not destroy an activity of the modified molecule.

Modifications therefore include, for example, amino acid additions, insertions, deletions and substitutions. An example of an addition is where one or more amino acids are added to the N- or C-terminal end of a humanized antibody. An example of an insertion is where an amino acid is inserted into the sequence. An example of a deletion is where one or more amino acids are deleted from the N- or C-terminal end, or internally within the sequence.

The invention therefore also provides modified forms of the humanized antibodies, including one or more amino acid additions, insertions, deletions and substitutions. In one embodiment, a humanized antibody has one or more amino acid substitutions of a sequence set forth in SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and

20 (Hum40); and SEQ ID NO:5 and 21 (Hum50), provided that the substituted antibody is capable of antigen binding. In a particular aspect, one or more of the amino acid substitutions are conservative amino acid substitutions. In another aspect, the substitution comprises 1-3, 3-5 or 5-10 amino acids. In yet another aspect, the substitution is with a human amino acid. In still another aspect, the substitution is with a non-human amino acid which is structurally similar to the non-human residue, for example, where a non-human vernier zone amino acid of the framework acceptor (e.g., murine acceptor) is structurally dissimilar to the human counterpart vernier zone amino acid.

Exemplary amino acid substitutions include conservative amino acid substitutions. The term "conservative substitution" means the replacement of one amino acid by a biologically or chemically or structurally similar residue. Biologically similar means that the substitution is compatible with biological activity, e.g., for a humanized antibody, antigen binding. Structurally similar means that the amino acids have side chains with similar length, such as alanine, glycine and serine, or having similar size. Chemical similarity means that the residues have the same charge or are both hydrophilic or hydrophobic. Particular examples of conservative substitutions include the substitution of one hydrophobic residue, such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, serine for threonine, and the like.

Modifications also include derivatized sequences, for example, amino acids in which free amino groups form amine hydrochlorides, p-toluene sulfonyl groups, cabrobenzoxy groups; the free carboxy groups from salts, methyl and ethyl esters; free hydroxl groups that form O-acyl or O-alkyl derivatives, as well as naturally occurring amino acid derivatives, for example, 4-hydroxyproline, for proline, 5-hydroxylysine for lysine, homoserine for serine, ornithine for lysine, etc. Also included are modifications that confer covalent bonding, for example, a disulfide linkage between two cysteine residues thereby producing a cyclic polypeptide. Modifications can be produced using any of a variety of methods well known in the art (*e.g.*, PCR based sited-directed,

deletion and insertion mutagenesis, chemical modification and mutagenesis, cross-linking, etc.).

Modifications also include addition of functional entities such as tags (e.g., polyhistidine, T7, immunoglubulin, etc.), gold particles, covalently or non-covalently attached to the humanized antibodies or subsequences or multimers. Thus, the invention provides modified humanized antibodies having one or more activities (e.g., retain at least part of the antigen binding activity) of unmodified parent antibody. Modifications include radioactive or alternatively non-radioactive detectable labels attached to or incorporated into the molecule.

The term "identical" or "identity" means that two or more referenced entities are the same. Thus, where two nucleic acid sequences are identical, they have the same sequence. "Areas of identity" means that a portion of two or more referenced entities are the same. Thus, where two nucleic acid sequences are identical over one or more parts of their sequence, they share identity in these areas. The term "substantial identity" means that the identity is structurally or functionally significant. That is, the identity is such that the molecules are structurally identical or perform the same function (e.g., biological function) even though the molecules differ. Due to variation in the amount of sequence conservation between structurally and functionally related proteins, the amount of sequence identity for substantial identity will depend upon the type of region/domain and its function. For nucleic acid sequences, 50% sequence homology and above may constitute substantial homology. Substantial homology for proteins can be significantly less, for example, as little as 30% sequence homology, but typically is more, e.g., 50%, 60%, 75%, 85% or more.

The extent of identity between two sequences can be ascertained using various computer programs and mathematical algorithms known in the art. Such algorithms that calculate percent sequence identity (homology) generally account for sequence gaps and mismatches over the comparison region. For example, a BLAST (*e.g.*, BLAST 2.0) search algorithm (see, *e.g.*, Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10)

has exemplary search parameters as follows: Mismatch -2; gap open 5; gap extension 2. For polypeptide sequence

comparisons, a BLASTP algorithm is typically used in combination with a scoring matrix, such as PAM100, PAM 250, BLOSUM 62 and the like.

As used herein, the term "isolated," when used as a modifier of invention compositions (e.g., antibodies, subsequences, modified forms, multimers, nucleic acids encoding same, cells, vectors, etc.), means that the compositions are made by the hand of man and are separated from their naturally occurring *in vivo* environment. Generally, compositions so separated are substantially free of one or more materials with which they normally associate with in nature, for example, one or more protein, nucleic acid, lipid, carbohydrate, cell membrane. An "isolated" antibody can also be "substantially pure" when free of most or all of the materials with which they may normally associate with in nature. Thus, an isolated molecule that also is substantially pure does not include polypeptides or polynucleotides present among millions of other sequences, such as antibodies of an antibody library or nucleic acids in a genomic or cDNA library, for example. Purity can be at least about 60% or more by mass. The purity can also be about 70% or 80% or more, and can be greater, for example, 90% or more. Purity can be determined by any appropriate method, including, for example, UV spectroscopy, chromatography (*e.g.*, HPLC, gas phase), gel electrophoresis (*e.g.*, silver or coomassie staining) and sequence analysis (nucleic acid and peptide).

The invention additionally provides methods for producing humanized antibodies. In one embodiment, a method includes: selecting a human framework sequence as an acceptor, wherein said sequence has 50% or more identity (e.g., 50-55%, 55-60%, 60-65%, 65-70%, 70-75%, 75-80%, 80-85%, 85-90%, 90-95%, or more identity) to a non-human donor antibody framework region; grafting a CDR from the donor non-human antibody (e.g., murine) onto the human framework; comparing the vernier zone residues of the human acceptor and the non-human donor framework regions; and maintaining one or more of the human acceptor residues in the vernier zone when the donor non-human and human residues are structurally or chemically similar, or substituting one or more of the vernier zone residues with a residue that is different from both the donor non-human vernier zone residue and acceptor human vernier zone residue if the donor non-human vernier zone residue is structurally or chemically dissimilar to the human residue, wherein the different residue is structurally or chemically similar to the donor non-human

vernier zone residue. In other words, if the donor non-human vernier zone residue is structurally or chemically dissimilar to the acceptor human vernier zone residue, then this vernier zone residue is modified to a residue that is different from both the donor non-human vernier zone residue and the acceptor human vernier zone residue, yet structurally or chemically similar to the donor non-human vernier zone residue. In additional embodiments, human framework acceptor sequences are selected from consensus sequences, for example, from V_H domain subgroup I and subgroup II consensus sequenes.

The invention also provides nucleic acids encoding invention humanized antibodies, including high affinity humanized antibodies, subsequences, modified forms and multimers thereof. In various embodiments, a nucleic acid encodes a polypeptide set forth in SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50).

As used herein, a "nucleic acid" refers to at least two or more ribo- or deoxy-ribonucleic acid base pairs that are linked through a phosphoester bond or equivalent. Nucleic acids include polynucleotides and polynculeosides. Nucleic acids include single, double or triplex, circular or linear, molecules. A nucleic acid molecule may belong exclusively or in a mixture to any group of nucleotide-containing molecules, as exemplified by, but not limited to, the following groups of nucleic acid molecules: RNA, DNA, cDNA, genomic nucleic acids, non-genomic nucleic acids, naturally occurring and non naturally occurring nucleic acids and synthetic nucleic acids. This includes, by way of example, nucleic acids associated with any organelle, such as the mitochondria, ribosomal RNA, and nucleic acid molecules comprised chimerically of one or more components that are not naturally occurring along with naturally occurring components.

Additionally, a "nucleic acid molecule" may contain in part one or more non-nucleotide-based components as exemplified by, but not limited to, amino acids and

sugars. Thus, by way of example, but not limitation, a ribozyme that is in part nucleotide-based and in part protein-based is considered a "nucleic acid molecule."

Nucleic acids can be of any length. Nucleic acid lengths typically range from about 20 to 10 Kb, 10 to 5Kb, 1 to 5 Kb or less, 1000 to about 500 base pairs or less in length. Nucleic acids can also be shorter, for example, 100 to about 500 base pairs, or from about 12 to 25, 25 to 50, 50 to 100, 100 to 250, or about 250 to 500 base pairs in length.

As a result of the degeneracy of the genetic code, nucleic acids include sequences and subsequences degenerate with respect to nucleic acids that encode SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50), and subsequences thereof. Nucleic acids also include sequences complementary to a sequence that encodes SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50), and subsequences thereof. Nucleic acid subsequences have from about 15 to 25, 25 to 50 or 50 to 100 nucleotides. Such nucleic acids are useful for hybridization to detect the presence or an amount of humanized antibody in a sample (*in vitro*, cell, culture medium, tissue or organ, serum, in a subject, etc.).

The invention further includes nucleic acids that hybridize at high stringency to nucleic acids that encode SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50), subsequences thereof and nucleic acid sequences complementary thereto. Hybridizing nucleic acids are also useful for detecting the presence or an amount of humanized antibody in a sample.

The term "hybridize" refers to the binding between complementary nucleic acids. Sequences will generally have more than about 50% homology to a nucleic acid that encodes SEQ ID NO:1 and 3 (HumA); SEQ ID NO:4 and 5 (HumB); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50). The region between related sequences can extend over at least about 30 base pairs, or about 50 base pairs, or about 100 to 200 or more residues.

As is understood by those skilled in the art, the T_M (melting temperature) refers to the temperature at which binding between complementary sequences is no longer stable. For two sequences to bind, the temperature of a hybridization reaction must be less than the calculated T_M for the sequences. The T_M is influenced by the amount of sequence complementarity, length, composition (%GC), type of nucleic acid (RNA vs. DNA), and the amount of salt, detergent and other components in the reaction (*e.g.*, formamide). All of these factors are considered in establishing appropriate hybridization conditions (see, *e.g.*, the hybridization techniques and formula for calculating T_M described in Sambrook *et al.*, 1989, *supra*).

Typically, wash conditions are adjusted so as to attain the desired degree of hybridization stringency. Thus, hybridization stringency can be determined empirically, for example, by washing under particular conditions, *e.g.*, at low stringency conditions or high stringency conditions. Optimal conditions for selective hybridization will vary depending on the particular hybridization reaction involved. An example of high stringency hybridization conditions are as follows: 2X SSC/0.1% SDS at about 37°C or 42°C (hybridization conditions); 0.5X SSC/0.1% SDS at about room temperature (low stringency wash); 0.5X SSC/0.1% SDS at about 42°C (moderate stringency wash); and 0.1 X SSC/0.1% SDS at about 65°C (high stringency wash).

Nucleic acids of the invention can be produced using various standard cloning and chemical synthesis techniques. Such techniques include, but are not limited to: 1) nucleic acid amplification, *e.g.*, polymerase chain reaction (PCR), with genomic DNA or cDNA targets using primers (*e.g.*, a degenerate primer mixture) capable of annealing to

antibody sequence; 2) chemical synthesis of nucleic acid sequences which can then be cloned into a plasmid, propagated amplified and purified and; 3) computer searches of databases for related sequences. Purity of nucleic acids can be determined through sequencing, gel electrophoresis and the like.

The invention further provides expression cassettes comprising a nucleic acid encoding a humanized antibody operably linked to an expression control element. As used herein, the term "operably linked" refers to a physical or a functional relationship between the elements referred to that permit them to operate in their intended fashion. Thus, an expression control element "operably linked" to a nucleic acid means that the control element modulates transcription and as appropriate, translation of the transcript.

There need not be physical linkage to nucleic acid in order to control expression. Thus, physical linkage is not required for the elements to be operably linked. For example, a minimal element can be linked to a nucleic acid encoding a humanized antibody. A second element that controls expression of an operably linked nucleic acid encoding a protein that functions "in trans" to bind to the minimal element can influence expression of the humanized antibody. Because the second element regulates expression of humanized antibody, the second element is operably linked to the nucleic acid encoding the humanized antibody.

The term "expression control element" refers to nucleic acid that influences expression of an operably linked nucleic acid. Promoters and enhancers are particular non-limiting examples of expression control elements. A "promotor sequence" is a DNA regulatory region capable of initiating transcription of a downstream (3' direction) coding sequence. The promoter sequence includes a minimum number of bases necessary to initiate transcription. Enhancers also regulate gene expression but can function a distance from the transcription start site of the gene to which it is operably linked. Enhancers also function at either 5' or 3' ends of the gene, as well as within the gene (e.g., in introns or coding sequences).

An expression control element can confer expression in a manner that is "constitutive," such that transcription of the operably linked nucleic acid occurs without the presence of a signal or stimuli. Expression control elements can confer expression in

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A humanized antibody that binds ICAM-1, said antibody having a VH and VL domain selected from the group consisting of: SEQ ID NO:1 and 3 (HumA); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50).

2. The humanized antibody of claim 1, wherein (i) said VH domain has at least 85% sequence identity to said SEQ ID NO:1, and said VL domain has at least 85% sequence identity to said SEQ ID NO:3; (ii) said VH domain has at least 85% sequence identity to said SEQ ID NO:6, and said VL domain has at least 85% sequence identity to said SEQ ID NO:7; (iii) said VH domain has at least 85% sequence identity to said SEQ ID NO:8, and said VL domain has at least 85% sequence identity to said SEQ ID NO:9; (iv) said VH domain has at least 85% sequence identity to said SEQ ID NO:10, and said VL domain has at least 85% sequence identity to said SEQ ID NO:11; (v) said VH domain has at least 85% sequence identity to said SEQ ID NO:12, and said VL domain has at least 85% sequence identity to said SEQ ID NO:13; (vi) said VH domain has at least 85% sequence identity to said SEQ ID NO:14, and said VL domain has at least 85% sequence identity to said SEQ ID NO:15; (vii) said VH domain has at least 85% sequence identity to said SEQ ID NO:16, and said VL domain has at least 85% sequence identity to said SEQ ID NO:17; (viii) said VH domain has at least 85% sequence identity to said SEQ ID NO:18, and said VL domain has at least 85% sequence identity to said SEQ ID NO:19; (ix) said VH domain has at least 85% sequence identity to said SEQ ID NO:5, and said VL domain has at least 85% sequence identity to said SEQ ID NO:20; or (x) said VH domain has at least 85% sequence identity to said SEQ ID NO:5, and said VL domain has at least 85% sequence identity to said SEQ ID NO:21.

3. A subsequence of the antibody of claim 1 or 2, said antibody subsequence capable of binding an ICAM-1 epitope.

4. The humanized antibody of claim 1 or 2, wherein the antibody subsequence comprises a single chain, Fab, Fab' or $(Fab)_2$ fragment.

5. The humanized antibody of any one of claims 1 to 3, said antibody having one or more amino acid substitutions, provided that said antibody is capable of binding an ICAM-1 epitope.

6. A humanized antibody that binds ICAM-1 and inhibits pathogen infection of cells expressing ICAM-1.

7. The humanized antibody of claim 6, said antibody having a protective efficacy at least 2 times greater than the non-humanized antibody.

8. The humanized antibody of claim 6, said antibody having a protective efficacy at least 5 times greater than the non-humanized antibody.

9. The humanized antibody of claim 6, said antibody having a protective efficacy at least 10 times greater than the non-humanized antibody.

10. The humanized antibody of claim 6, said antibody having a protective efficacy at least 20 times greater than the non-humanized antibody.

11. The humanized antibody of claim 6, said antibody having a protective efficacy at least 30 times greater than the non-humanized antibody.

12. The humanized antibody of claim 6, wherein the pathogen is human rhinovirus (HRV).

13. The humanized antibody of claim 6, wherein the pathogen is coxsackie A virus, respiratory syncytial virus, or malaria.

14. The humanized antibody of claim 6, wherein the antibody is an intact immunoglobulin molecule comprising two full-length heavy chains and two full-length light chains.

15. The humanized antibody of claim 6, wherein the antibody is an antibody subsequence that binds to ICAM-1.

16. The humanized antibody of claim 15, wherein the antibody subsequence comprises a single chain, Fab, Fab' or $(Fab)_2$ fragment.

17. The humanized antibody of claim 6, wherein the antibody is multispecific or multifunctional.

18. The humanized antibody of claim 6, wherein the antibody is linked to one or more identical or different antibodies to form a multimer.

19. The humanized antibody of claim 18, wherein the multimer comprises a homodimer, heterodimer, trimer, or tetramer or pentamer.

20. The humanized antibody of claim 18, wherein the multimer is formed via a multimerization domain.

21. The humanized antibody of claim 20, wherein the multimerization domain comprises a human amino acid sequence.

22. The humanized antibody of claim 20, further comprising a linker located between the multimerization domain and the antibody.

23. A humanized antibody that inhibits human rhinovirus (HRV) infection of cells comprising a VH and VL domain selected from the group consisting of: SEQ ID NO:1 and 3 (HumA); SEQ ID NO:6 and 7 (HumC); SEQ ID NO:8 and 9 (HumD); SEQ ID NO:10 and 11 (HumE); SEQ ID NO:12 and 13 (HumF); SEQ ID NO:14 and 15 (HumG); SEQ ID NO:16 and 17 (HumH); and SEQ ID NO:18 and 19 (HumI); and SEQ ID NO:5 and 20 (Hum40); and SEQ ID NO:5 and 21 (Hum50); or a subsequence thereof.

24. The humanized antibody of claim 23, wherein (i) said VH domain has at least 85% sequence identity to said SEQ ID NO:1, and said VL domain has at least 85% sequence identity to said SEQ ID NO:3; (ii) said VH domain has at least 85% sequence identity to said SEQ ID NO:6, and said VL domain has at least 85% sequence identity to said SEQ ID NO:7; (iii) said VH domain has at least 85% sequence identity to said SEQ ID NO:8, and said VL domain has at least 85% sequence identity to said SEQ ID NO:9; (iv) said VH domain has at least 85% sequence identity to said SEQ ID NO:10, and said VL

domain has at least 85% sequence identity to said SEQ ID NO:11; (v) said VH domain has at least 85% sequence identity to said SEQ ID NO:12, and said VL domain has at least 85% sequence identity to said SEQ ID NO:13; (vi) said VH domain has at least 85% sequence identity to said SEQ ID NO:14, and said VL domain has at least 85% sequence identity to said SEQ ID NO:15; (vii) said VH domain has at least 85% sequence identity to said SEQ ID NO:16, and said VL domain has at least 85% sequence identity to said SEQ ID NO:17; (viii) said VH domain has at least 85% sequence identity to said SEQ ID NO:18, and said VL domain has at least 85% sequence identity to said SEQ ID NO:19; (ix) said VH domain has at least 85% sequence identity to said SEQ ID NO:5, and said VL domain has at least 85% sequence identity to said SEQ ID NO:20; or (x) said VH domain has at least 85% sequence identity to said SEQ ID NO:5, and said VL domain has at least 85% sequence identity to said SEQ ID NO:21.

25. The humanized antibody of claim 23 or 24, wherein the antibody is an immunoglobulin molecule comprising two full-length heavy chain polypeptides and two full-length light chain polypeptides.

26. The humanized antibody of claim 23 or 24, wherein the subsequence comprises a single chain, Fab, Fab' or $(Fab)_2$ fragment.

27. The humanized antibody of claim 23 or 24, wherein the antibody is linked with other identical or different antibodies to form a multimer.

28. The humanized antibody of claim 27, wherein the multimer comprises a homodimer, heterodimer, trimer, or tetramer.

29. The humanized antibody of claim 27, wherein the different antibodies are human, humanized or non-human.

30. A nucleic acid sequence encoding a humanized antibody of any one of claims 1, 2, 23 and 24, or a subsequence thereof.

31. An expression cassette comprising the nucleic acid sequence of claim 30 operably linked to an expression control element.

32. A vector comprising the nucleic acid sequence of claim 30.

33. The vector of claim 32, wherein the nucleic acid sequence is operably linked to an expression control element.

34. A cell comprising the nucleic acid sequence of claim 30.

35. The cell of claim 34, wherein the cell is prokaryotic or eukaryotic.

36. A pharmaceutical composition comprising a humanized antibody of any one of claims 1, 2, 6, 23 and 24, and a pharmaceutically acceptable carrier.

37. The pharmaceutical composition of claim 36, wherein the carrier is compatible with inhalation or nasal delivery to a subject.

38. Use of the humanized antibody of any one of claims 1 to 29 to inhibit pathogen infection of a cell.

39. Use of the humanized antibody of any one of claims 1 to 29 in the manufacture of a medicament to inhibit pathogen infection of a cell.

40. The use of claim 38 or 39, wherein the cell is present in a subject.

41. The use of claim 40, wherein the cell is an epithelial cell.

42. The use of claim 40, wherein the cell expresses ICAM-1.

43. Use of the humanized antibody of any one of claims 1 to 29 to inhibit HRV infection of a cell.

44. Use of the humanized antibody of any one of claims 1 to 29 in the manufacture of a medicament to inhibit HRV infection of a cell.

45. The use of claim 43 or 44, wherein the cell is present in a subject.

46. The use of claim 45, wherein the subject has or is at risk of having asthma.

47. The use of claim 43 or 44, wherein the antibody binds to an antigen present on the surface of the cell.

48. The use of claim 43 or 44, wherein the cell expresses ICAM-1.

49. The use of claim 43 or 44, wherein the cell is an epithelial cell.

50. The use of claim 43 or 44, wherein the humanized antibody is formulated for local administration.

51. The use of claim 43 or 44, wherein the humanized antibody is formulated for inhalation or intranasal administration.

52. Use of the humanized antibody of any one of claims 1 to 29 to inhibit HRV infection, in a subject.

53. Use of the humanized antibody of any one of claims 1 to 29 in the manufacture of a medicament to inhibit HRV infection in a subject.

54. Use of the humanized antibody of any one of claims 1 to 29 to inhibit HRV progression in a subject.

55. Use of the humanized antibody of any one of claims 1 to 29 in the manufacture of a medicament to inhibit HRV progression in a subject.

56. Use of the humanized antibody of any one of claims 1 to 29 to treat HRV infection in a subject.

57. Use of the humanized antibody of any one of claims 1 to 29 in the manufacture of a medicament to treat HRV infection in a subject.

58. The use of any one of claims 52 to 57, wherein the humanized antibody is formulated for administration locally.

59. The use of any one of claims 52 to 57, wherein the humanized antibody is formulated for inhalation or intranasal administration.

60. The use of any one of claims 52 to 57, wherein the subject has or is at risk of having asthma.

61. The use of any one of claims 52 to 57, wherein the subject is a newborn or between the ages of 1 to 5, 5 to 10 or 10 to 18.

62. Use of the humanized antibody of any one of claims 1 to 29 for decreasing or inhibiting one or more symptoms of the common cold in a subject.

63. Use of the humanized antibody of any one of claims 1 to 29 in the manufacture of a medicament for decreasing or inhibiting one or more symptoms of the common cold in a subject.

64. The use of claim 62 or 63, wherein the humanized antibody is formulated for local administration.

65. The use of claim 62 or 63, wherein the humanized antibody is formulated for inhalation or intranasal administration.

66. The use of claim 62 or 63, wherein the subject has or is at risk of having asthma.

67. The use of claim 62 or 63, wherein the subject is a newborn or between the ages of 1 to 5, 5 to 10 or 10 to 18.

68. A method for producing humanized antibody comprising:

a) selecting a human framework sequence as an acceptor, wherein said sequence has 50% or more identity to a non-human donor antibody framework region;

b) grafting a CDR from the donor non-human antibody onto the human framework;

c) comparing the vernier zone residues of the human acceptor and the non-human donor framework regions; and

d) maintaining one or more of the human acceptor residues in the vernier zone when the donor non-human and acceptor human residues are structurally or chemically

similar, or substituting one or more of the vernier zone residues with a residue that is different from both the donor non-human vernier zone residue and acceptor human vernier zone residue if the donor non-human vernier zone residue is structurally or chemically dissimilar to the human residue, wherein the different residue is structurally or chemically similar to the donor non-human vernier zone residue.

69. The method of claim 68, wherein the human framework acceptor sequences is selected from a VH domain subgroup I or subgroup II consensus sequence.

Figure 1

V_H Domain

```
                1          11         21         31         41
Mouse1A6        EVQLQQSGAE LVKPGASVKL SCTASGFNIK DTYIHWMKQR PEQGLEW I GR
                    **   **    *  * ***     *                * *  *  **      **
Hum3            EVQLVESGGG LVQPGGSLRL SCAASGFNFS ---------WVRQA PGKGLEWVA---

                51 a        61         71          81 abc              91
Mouse1A6        IDPANDNTIYD PKVQGKATMT ADTSS NTAYL QLSSLTSEDTAVY YCTT
                               ** ** * * *             **  **
Hum3            ------------------A DSVKGRF T IS RDDSKNTAYL QMNSLRAEDTAVY YCTT

                       103        111
Mouse1A6        SGYWFA YWGQGTLVT VSA
                                   *
Hum3            ----------------WGQGTLVT VSS
```

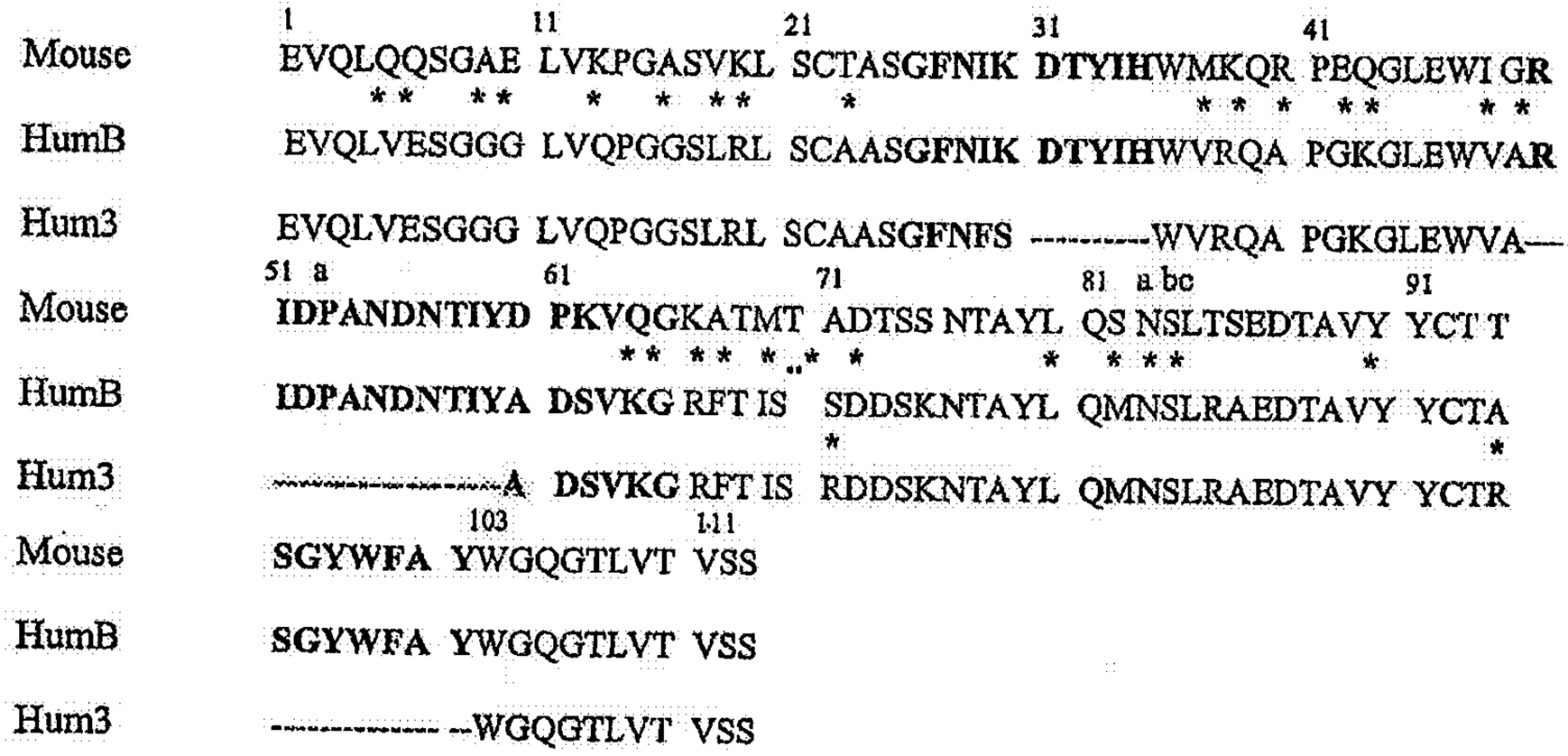
V_L Domain

```
                1          11         21         31         41         51
Mouse1A6        DIVLTQSPAT LSVTPGDSVS LSCRASQSIS NNLHWYQQKS HESPRLLIKH ASQ
                  **     **   ***   * * **                  * *** *    *
HumκI           DIQMTQSPSS LSASVGDRVT ITC------------ -------- WYQQKP GKAPKLLIY -- -----

                        61         71         81         91         101
Mouse1A6        SISG I PS RFSGSGSGTD FTLSINSVET EDFGMFFCQQ SNSWPYTFGG GTKLEIKR
                     *                      * *  ***   ****               *      *
HumκI           ----GVPS RFSGSGSGTD FTLTISSLQP EDFATYYC---- -------------FGQ GTKVEIKR
```

Figure 2

V_H Domain



V_L Domain

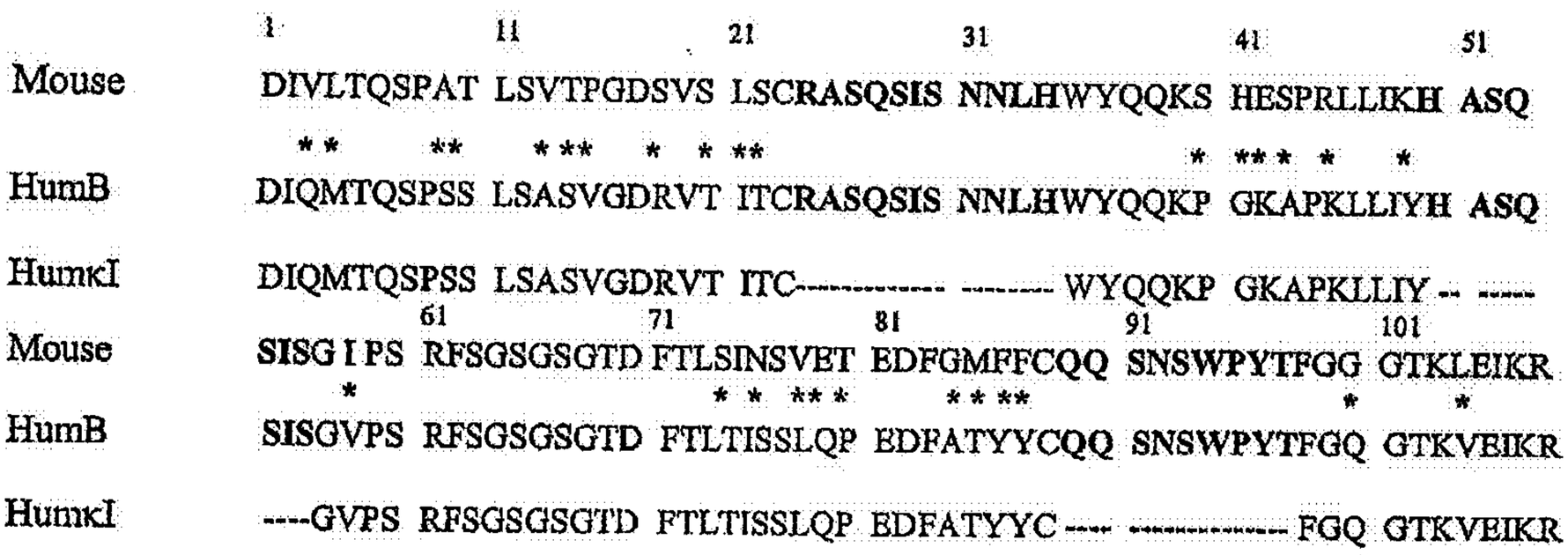


Figure 3

CGAACCATGGGCGATATCCAGATGACCCAATCTCCGTCTAGCCTGAGCGCCA
GTGTTGGTGATCGAGTTACCATTACTTGCCGCGCCAGCCAATCTATCAGTAAT
AATCTTCACTGGTATCAACAAAAACCGGGTAAAGCTCCGAAACTTCTTATCTA
TCACGCCTCTCAGAGCATTAGCGGCGTTCCGAGCCGCTTCTCTGGCTCTGGCT
CGGGCACGGACTTTACCCTTACCATCAGCTCTCTTCAGCCGGAAGACTTTGCC
ACCTATTATTGTCAGCAGTCTAATAGCTGGCCGTATACCTTCGGTCAAGGTAC
CAAGGTCGAGATTAAGCGCGGCGGTGGCGGTTCTGGTGGCGGTGGTAGCGGT
GGCGGTGGATCCGGTGGCGGTGGCAGCGAAGTTCAACTTGTTGAGTCTGGTG
GCGGTCTGGTTCAGCCGGGTGGCTCTCTGCGCCTGTCTTGCGCAGCAAGCGGT
TTCAACATTAAGGACACCTACATCCATTGGGTGAGGCAAGCTCCGGGTAAGG
GTCTGGAGTGGGTGGCACGTATCGACCCGGCAAACGACAACACCATTTACGC
TGACAGCGTGAAGGGCCGTTTTACTATTTCTAGCGACGACTCTAAGAACACCG
CGTACCTTCAGATGAACTCTCTGCGTGCCGAGGACACCGCCGTCTACTACTGC
ACGGACTCTGGCTACTGGTTTGCCTACTGGGGCCAGGGCACGCTTGTCACCGT
CTCTTCTGGTTAACCC

Figure 4

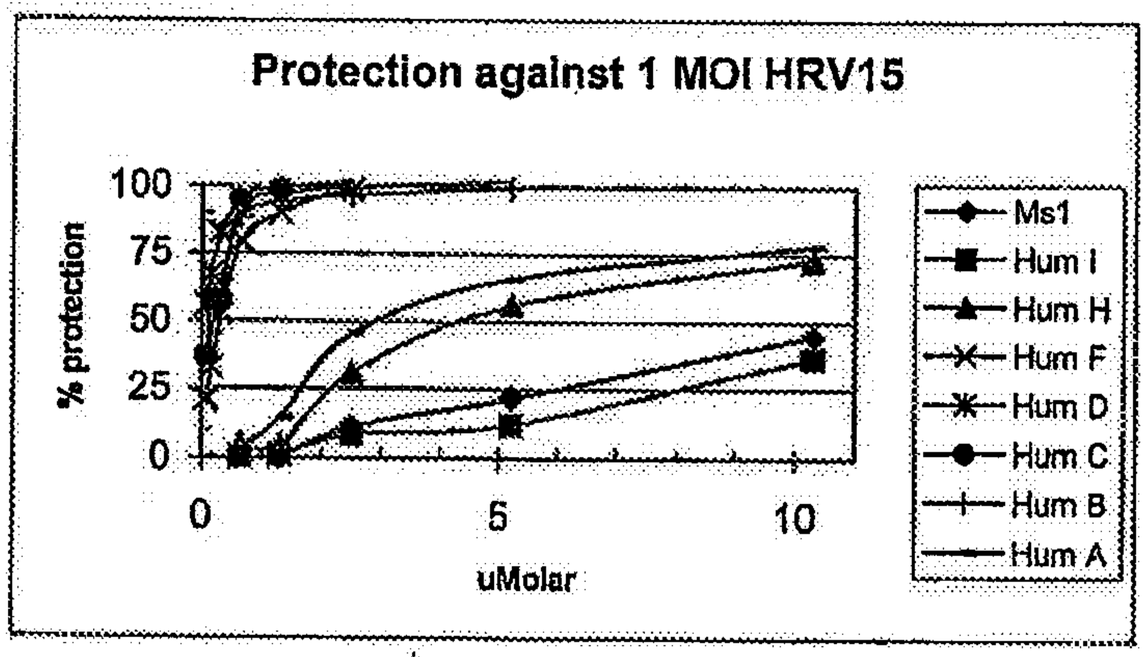

Protection against 1 MOI HRV15
100
75
50
25
0
% protection
0
5
10
uMolar
Ms1
Hum I
Hum H
Hum F
Hum D
Hum C
Hum B
Hum A

Figure 5

```
                            10                                 20
M1A6 VH:   E V Q L Q Q S G A E L V K P G A S V K L S C T A S
Hum2:      Q V Q L Q E S G P G L V K P S E T L T L T C T V S

Hum1:      Q V Q L V Q S G A E V K K P G A S V K V S C K A S

                   30                      40                      50
M1A6 VH:   G F N I K D T Y I H W M K Q R P E Q G L E W I G R
Hum2:      G F S I S           W I R Q P P G K G L E W I G
Hum1:      G G T F S           W V R Q A P G Q G L E W V G

                                                66        70
M1A6 VH:   I D P A N D N T I Y D P K V Q G K A T M T A D T S
Hum2:                                     R V T I T K D T S

Hum1:                                     R V T M T A D T S

                       80        a b c             90
M1A6 VH:   S N T A Y L Q L S S L T S E D T A V Y Y C T T S G
Hum2:      K N Q V S L N L N S V T A A D T A V Y Y C A A
Hum1:      T N T A Y M E L S S L R S E D T A V Y Y C A A

                     103         110
M1A6 VH:   Y W F A Y W G Q G T L V T V S S
Hum2:                W G Q G T L V T V S S
Hum1:                W G Q G T L V T V S S
```

Figure 6

V_H Domain

```
                1                  11                  21                  31                  41
Mouse           EVQLQQSGAE LVKPGASVKL SCTASGFNIK DTYIHWMKQR PEQGLEWIGR
                *     *   **      *****  *   *                 ***  **
Hum40           QVQLQESGPG LVKPSETLTL TCTVSGFNIK DTYIHWIRQP PGKGLEWIGR
Hum2            QVQLQESGPG LVKPSETLTL TCTVSGFSIS ----------WIRQP PGKGLEWIG---

                51 a               61                  71                81 a bc             91
Mouse           IDPANDNTIYD PKVQGKATMT ADTSSNTAYL QLSSLTSEDTAVY YCTT
                                    ** *   *   * **** *  * * **          * *
Hum40           IDPANDNTIYD PKVQG RVTIT SDTSKNQVSL NLNSVTAADTAVY YCAA
                                    *                                     *
Hum2            ---------------------------------- RVTIT KDTSKNQVSL NLNSVTAADTAVY YCAR

                                  103               111
Mouse           SGYWFA YWGQGTLVT VSA
                                                    *
Hum40           SGYWFA YWGQGTLVT VSS
Hum2            ------------ --WGQGTLVT VSS
```

Figure 7

V_H Domain

```
                 1                  11                 21                 31                 41
Mouse            EVQLQQSGAE LVKPGASVKL SCTASGFNIK DTYIHWMKQR PEQGLEW I GR
                 *    *     * *        *    *                * * *  *      *
Hum50            QVQLVQSGAE VKKPGASVKV SCKASGFNIK DTYIHWVRQA PGQGLEWVGR
Hum1             QVQLVQSGAE VKKPGASVKV SCKASGYTFS ----------WVRQA PGQGLEWVG---

                 51 a               61                 71                 81 a bc            91
Mouse            IDPANDNTIYD PKVQGKATMT ADTSSNTAYL QLSSLTSEDTAVY YCT T
                                    * *        *      * *     *              * *
Hum50            IDPANDNTIYD PKVQGRVTM T ADTSTNTAYM ELSSLRSEDTAVY YCAA
                                                                           *
Hum1             ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~RVT MT ADTSTNTAYM ELSSLRSEDTAVY YCAR

                                  103                111
Mouse            SGYWFA YWGQGTLVT VSA
                                                     *
Hum50            SGYWFA YWGQGTLVT VSS
Hum1             ---------- --WGQGTLVT VSS
```