HIGH AFFINITY ANTIBODIES TO HUMAN IL-6 RECEPTOR

Statement of Related Art

[0001] Interleukin-6 (IL-6) is a pleiotropic cytokine produced by immune and non-immune cells that plays a crucial role in regulation of immune response, acute-phase reactions, and hematopoiesis. It binds to soluble and cell membrane bound IL-6R (α chain) forming a binary complex and this complex is able to interact with cell membrane bound gp130 (β chain), induces formation of signaling complex comprising two each of IL-6, IL-6R, and gp130.

[0002] Antibodies to hIL-6R are described in US 5,670,373, 5,795,965, 5,817,790, 6,410,691, and EP 409 607B1. Therapeutic methods are described in US 5,888,510 and 6,723,319.

BRIEF SUMMARY OF THE INVENTION

[0003] In a first aspect, the invention provides human antibodies, preferably recombinant human antibodies, that specifically bind human interleukin-6 receptor (hIL-6R). These antibodies are characterized by binding to hIL-6R with high affinity and slow dissociation kinetics and by the ability to neutralize IL-6 activity. The antibodies can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to effect functionality, e.g., to eliminate residual effector functions (Reddy et al. (2000) J. Immunol. 164:1925-1933). In a preferred embodiment, the invention provides an antibody or antigen-binding fragment thereof, which binds human IL-6 receptor (SEQ ID NO:1) with a K_D of about 500 pM or less, as measured by surface plasmon resonance. In a more specific embodiment, the antibody or antigen-binding fragment has a K_D of less than 300 pM, or less than 200 pM, or even less than 100 pM. In various embodiments, the antibody or antigen-binding fragment thereof blocks hIL-6 activity with an IC_{50} of 250 pM or less, as measured by luciferase bioassay. In more specific embodiments, the antibody or antigen-binding fragment thereof exhibits an IC_{50} of 150 pM or less.

[0004] In related aspects, the antibody or antigen-binding fragment of the invention binds hIL-6R with an affinity at least 2-fold higher than it binds monkey IL-6R. In more preferred embodiments, the antibody or antigen-binding fragment binds hIL-6R protein (SEQ ID NO:1) with an affinity that is up to about 3-fold higher relative to its binding to monkey IL-6R (*Macaca fascicularis* extracellular domain shown in SEQ ID NO:251).

[0005] In one embodiment, the antibody or antigen-binding portion of the antibody of the invention comprises a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NO:3, 227, 19, 231, 35, 51, 67, 83, 99, 115, 131, 147, 239, 241, 163, 179, 235, 195 and 211, or substantially similar sequence thereof. In a more specific embodiment, the antibody or antigen-binding fragment thereof further comprises a light chain variable region (LCVR) selected from the group consisting of SEQ ID NO: 11, 229, 27, 233, 43, 59, 75, 91, 107, 123, 139, 155, 171, 187, 237, 203 and 219, or a substantially similar sequence thereof. In specific

embodiments, the antibody or antigen-binding fragment thereof comprise HCVR/LCVR pairs selected from the group consisting of SEQ ID NO:3/11; 227/229; 19/27; 231/233; 35/43; 51/59; 67/75; 83/91; 99/107; 115/123; 131/139; 147/155; 239/155; 241/155; 163/171; 179/187; 235/237; 195/203; and 211/219, or substantially similar sequences thereof.

[0006] In a second aspect, the invention provides isolated nucleic acid molecules that encode an antibody or antigen-binding fragment of an antibody of the invention. In one embodiment, the nucleic acid molecule of the invention encodes an antibody or fragment thereof comprising an HCVR as described above. In specific embodiments, the nucleic acid molecule encoding the HCVR is selected from the group consisting of SEQ ID NO:2, 226, 18, 230, 34, 50, 66, 82, 98, 114, 130, 146, 238, 240, 162, 178, 234, 194 and 210, or a substantially identical sequence thereof. In a related aspect, the invention provides an isolated nucleic acid molecule encoding an LCVR as described above. In specific embodiments, the nucleic acid molecule encoding the LCVR is a nucleotide sequence selected from the group consisting of SEQ ID NO: 10, 228, 26, 232, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 236, 202 and 218, or a substantially identical sequence thereof.

[0007] In a third aspect, the invention features an antibody or antigen-binding fragment, comprising a heavy chain complementary determining region 3 (CDR3) domain and a light chain CDR3 domain, wherein

the heavy chain CDR3 domain comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} - X^{19}$ (SEQ ID NO:247) wherein X^1 = Ala, X^2 = Lys, X^3 = Gly, X^4 = Arg, X^5 = Asp, X^6 = Ser or Ala, X^7 = Phe, X^8 = Asp; X^9 = Ile, X^{10} = Pro or absent, X^{11} = Phe or absent, X^{12} = Val or absent, X^{13} = Tyr or absent, X^{14} = Tyr or absent, X^{15} = Tyr or absent, X^{16} = Gly or absent, X^{17} = Met or absent, X^{18} = Asp or absent, and X^{19} = Val or absent; and

the light chain CDR3 domain comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9$ (SEQ ID NO:250) wherein X^1 = Gln, X^2 = Gln or His, X^3 = Ala, X^4 = Asn or Tyr, X^5 = Ser, X^6 = Phe, X^7 = Pro, X^8 = Pro, and X^9 = Thr.

[0008] In a more specific embodiment, the antibody or antigen-binding fragment further comprises

a heavy chain CDR1 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO:245) wherein X^1 = Gly or Arg, X^2 = Phe, X^3 = Thr, X^4 = Phe, X^5 = Asp, X^6 = Asp, X^7 = Tyr, and X^8 = Ala;

a heavy chain CDR2 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO:246) wherein X^1 = Ile or Val, X^2 = Ser, X^3 = Trp, X^4 = Asn, X^5 = Ser, X^6 = Gly, X^7 = Ser, and X^8 = Ile;

a light chain CDR1 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO:248), wherein X^1 = Gln, X^2 = Gly, X^3 = Ile, X^4 = Ser, X^5 = Ser, and X^6 = Trp; and

a light chain CDR2 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3$ (SEQ ID NO:249), wherein X^1 = Gly or Ala, X^2 = Ala, and X^3 = Ser.

[0009] In a fourth aspect, the invention features an antibody or antigen-binding fragment, comprising:

a heavy chain CDR3 domain selected from the group consisting of SEQ ID NO: 25, 153, 9, 185, 41, 57, 73, 89, 105, 121, 137, 169, 201 and 217; and

a light chain CDR3 domain selected from the group consisting of SEQ ID NO:33, 161, 17, 193, 49, 65, 81, 97, 113, 129, 145, 177, 209 and 225.

[0010] In a more specific embodiment, the antibody or antigen-binding fragment further comprises:

a heavy chain CDR1 domain selected from the group consisting of SEQ ID NO: 21, 149, 5, 181, 37, 53, 69, 85, 101, 117, 133, 165, 197, and 213;

a heavy chain CDR2 domain selected from the group consisting of SEQ ID NO: 23, 151, 7, 183, 39, 55, 71, 87, 103, 119, 135, 167, 199 and 215;

a light chain CDR1 domain selected from the group consisting of SEQ ID NO: 29, 157, 13, 189, 45, 61, 77, 93, 109, 125, 141, 173, 205 and 221; and

a light chain CDR2 domain selected from the group consisting of SEQ ID NO: 31, 159, 15, 191, 47, 63, 79, 95, 111, 127, 143, 175, 207 and 223.**

[0011] In specific embodiments, the antigen or antigen-binding fragment comprises heavy chain CDR sequences SEQ ID NO:21, 23, 25 and light chain CDR sequences SEQ ID NO:29, 31, 33; heavy chain CDR sequences SEQ ID NO:149, 151, 153 and light chain CDR sequences SEQ ID NO:157, 159, 161; heavy chain CDR sequences SEQ ID NO:5, 7, 9 and light chain SEQ ID NO: 13, 15, 17; and heavy chain CDR sequences SEQ ID NO: 181. 183, 185 and light chain CDR sequences SEQ ID NO:189, 191, 193.

[0012] In a fifth aspect, the invention features isolated nucleic acid molecules encoding an antibody or antigen-binding fragments of the invention, wherein the antibody or fragment thereof comprises

a heavy chain CDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:24, 152, 8, 184, 40, 56, 72, 88, 104, 120, 136, 168, 200 and 216; and

a light chain CDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:32, 160, 16, 192, 48, 64, 80, 96, 112, 128, 144, 176, 208 and 224; as well as substantially identical nucleic acid sequences thereof.

[0013] In a more specific embodiment, isolated nucleic acid molecules are provided encoding an antibody or antigen-binding fragment of the invention, wherein the antibody or fragment thereof comprises

a heavy chain CDR1 encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 20, 148, 4, 180, 36, 52, 68, 84, 100, 116, 132, 164, 196 and 212;

a heavy chain CDR2 domain encoded by a nucleotide sequence selected from the group

consisting of SEQ ID NO: 22, 150, 6, 182, 38, 54, 70, 86, 102, 118, 134, 166, 198 and 214;

a light chain CDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:28, 156, 12, 188, 44, 60, 76, 92, 108, 124, 140, 172, 204 and 220; and

a light chain CDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO:30, 158, 14, 190, 30, 46, 62, 78, 94, 110, 126, 142, 174, 206 and 222; as well as substantially identical nucleic acid sequences thereof.

[0014] The invention encompasses anti-hIL-6R antibodies or antigen-binding fragments thereof having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful, or an antibody lacking a fucose moiety on an oligosaccharide chain, for example, to increase antibody-dependent cellular cytotoxicity (ADCC) (see Shield et al. (2002) JBC 277:26733). In other applications, modification of a galactosylation can be made in order to modify complement-dependent cytotoxicity (CDC).

[0015] In further aspects, the invention provides recombinant expression vectors carrying the nucleic acid molecules of the invention, and host cells into which such vectors have been introduced, as are methods of making the antibodies or antigen-binding fragments of the invention obtained by culturing the host cells of the invention. The host cell may be a prokaryotic or eukaryotic cell, preferably the host cell is an *E. coli* cell or a mammalian cell, such as a CHO cell.

[0016] In a further aspect, the invention features a pharmaceutical composition comprising a human antibody or antigen-binding fragment of an antibody which specifically binds hIL-6R and a pharmaceutically acceptable carrier.

[0017] In further aspects, the invention features methods for inhibiting human IL-6 activity using an antibody, or antigen-binding portion thereof, of the invention. In one embodiment, the invention encompasses a therapeutic method comprising administering an antibody of the invention, or a fragment thereof, to a human subject suffering from a disorder which is treated or ameliorated by inhibition of IL-6 activity. The disorder can be, for example, arthritis, including chronic rheumatoid arthritis; inflammatory bowel diseases, including Crohn's disease and ulcerative colitis; systemic lupus erythematosus; and inflammatory diseases.

[0018] In further aspects, the invention provides the use of an antibody or antigen-binding fragment of an antibody as defined above in the manufacture of a medicament for use to attenuate or inhibit an IL-6-mediated disease or disorder in a human. In a related aspect, the invention provides an antibody or antigen-binding fragment of an antibody as defined above for use in the attenuation or inhibition of an IL-6-mediated disease or disorder in a human.

[0019] Other objects and advantages will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0020] Before the present methods are described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and

conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0021] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

[0022] The term "human IL6R" (hIL-6R), as used herein, is intended to refer to a human cytokine receptor that specifically binds interleukin-6 (IL-6). The extracellular domain of hIL-6R is shown in SEQ ID NO:1.

[0023] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain (CL1). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementary determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0024] The term "antigen-binding portion" of an antibody (or simply "antibody portion" or "antibody fragment"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hIL-6R). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL1 and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al. (1989) Nature 241:544-546), which consists of a VH domain; and (vi) an isolated complementary determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single contiguous chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also

intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies, are also encompassed (see e.g., Holliger et al. (1993) Proc. Natl. Acad Sci. USA 90:6444-6448).

[0025] A "neutralizing" or "blocking" antibody, as used herein, is intended to refer to an antibody whose binding to hIL-6R results in inhibition of the biological activity of hIL-6. This inhibition of the biological activity of hIL-6 can be assessed by measuring one or more indicators of hIL-6 biological activity known to the art, such as hIL-6-induced cellular activation and hIL-6 binding to hIL-6R (see examples below).

[0026] A "CDR" or complementary determining region is a region of hypervariability interspersed within regions that are more conserved, termed "framework regions" (FR). In different embodiments of the anti-hIL-6R antibody or fragment of the invention, the FRs may be identical to the human germline sequences, or may be naturally or artificially modified. A group of CDRs may be defined as an amino acid consensus sequence; for example, in one embodiment, the anti-hIL-6R antibody or antigen-binding fragment of the invention may be described as comprising a heavy chain CDR3 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18}$ - X^{19} (SEQ ID NO:247) wherein X^1 = Ala, X^2 = Lys, X^3 = Gly, X^4 = Arg, X^5 = Asp, X^6 = Ser or Ala, X^7 = Phe, X^8 = Asp; X^9 = Ile, X^{10} = Pro or absent, X^{11} = Phe or absent, X^{12} = Val or absent, X^{13} = Tyr or absent, X^{14} = Tyr or absent, X^{15} = Tyr or absent, X^{16} = Gly or absent, X^{17} = Met or absent, X^{18} = Asp or absent, and X^{19} = Val or absent; and a light chain CDR3 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9$ (SEQ ID NO:250) wherein X^1 = Gln, X^2 = Gln or His, X^3 = Ala, X^4 = Asn or Tyr, X^5 = Ser, X^6 = Phe, X^7 = Pro, X^8 = Pro, and X^9 = Thr.

[0027] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (Pharmacia Biosensor AB).

[0028] The term "epitope" is an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstances, an epitope may include moieties of saccharides, phosphoryl groups, or sufonyl groups on the antigen.

[0029] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%,

97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below.

[0030] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) Science 256: 1443 45. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0031] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database

containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. See, e.g., Altschul et al. (1990) J. Mol. Biol. 215: 403 410 and Altschul et al. (1997) Nucleic Acids Res. 25:3389 402.

Preparation of Human Antibodies

[0032] Methods for generating human antibodies include, for example, VelocImmune™ (Regeneron Pharmaceuticals), XenoMouse™ technology (Green et al. (1994) Nature Genetics 7:13-21; Abgenix), the "minilocus" approach, and phage display (and see, for example, US 5,545,807, US 6,787,637). The VelocImmune™ technology (US 6, 596,541) encompasses a method of generating a high specificity fully human antibody to a select antigen. This technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody. In specific embodiment, the cell is a CHO cell.

[0033] Antibodies may be therapeutically useful in blocking a ligand-receptor interaction or inhibiting receptor component interaction, rather than by killing cells through fixation of complement (complement-dependent cytotoxicity)(CDC) and participation antibody-dependent cell-mediated cytotoxicity (ADCC) The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0034] Human immunoglobulins can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via interchain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covantlently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification. The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. In fact, a single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, CH2 or CH3 region which may be desirable, for example, in production, to improve the yield of the

desired antibody form.

[0035] Antibodies of the invention are preferably prepared with the use of VelocImmune™ technology. A transgenic mouse in which the endogenous immunoglobulin heavy and light chain variable regions are replaced with the corresponding human variable regions is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0036] In one embodiment, the transgenic mouse comprises up to 18 functional human variable heavy chain genes and 12 functional human variable kappa light chain genes. In another embodiment, the transgenic mouse comprises up to 39 human variable heavy chain genes and 30 human variable kappa light chain genes. In yet another embodiment, the transgenic mouse comprises up to 80 human variable heavy chain genes and 40 human variable kappa light chain genes.

[0037] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_Ds of from about 10^{-9} through about 10^{-12} M, when measured by binding to antigen either immobilized on solid phase or in solution phase.

[0038] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As described below, the antibodies are characterized and selected for desirable characteristics, including binding affinity to hIL-6R, ability to block hIL-6, and/or selectivity for the human protein. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG4 or IgG1 (for example, SEQ ID NO:242, 243, 244). While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Epitope Mapping and Related Technologies

[0039] To screen for antibodies which bind to a particular epitope, a routine cross-blocking assay such as that described in Antibodies: A Laboratory Manual 1988 Cold Spring Harbor Laboratory, Harlow and Lane, eds., can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63), or peptide cleavage analysis as described in the examples below. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000)

Protein Science: 9: 487-496).

[0040] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (US Patent Application Publication No. 2004/0101920). Each category may reflect a unique epitope either distinctly different from or partially overlapping with an epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones with desired characteristics. MAP may be used to sort the hIL-6R antibodies of the invention into groups of antibodies binding different epitopes.

[0041] Agents useful for altering the structure of the immobilized antigen are enzymes, such as, for example proteolytic enzymes and chemical agents. The antigen protein may be immobilized on either biosensor chip surfaces or polystyrene beads. The latter can be processed with, for example, an assay such as a multiplex Luminex™ detection assay (Luminex Corp., TX). Because of the capacity of Luminex™ to handle multiplex analysis with up to 100 different types of beads, Luminex™ provides almost unlimited antigen surfaces with various modifications, resulting in improved resolution in antibody epitope profiling over a biosensor assay.

Therapeutic Administration and Formulations

[0042] The administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, Pa., 1975), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Powell et al. PDA (1998) J Pharm Sci Technol. 52:238-311 and the citations therein for additional information related to excipients and carriers well known to pharmaceutical chemists.

EXAMPLES

[0043] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to Human IL-6 Receptor.

[0044] Immunization of rodents can be done by any methods known in the art (see, for example, Harlow and Lane (1988) *supra*; Malik and Lillehoj, Antibody techniques: Academic Press, 1994, CA). In a preferred embodiment, hIL-6R antigen is administered directly to mice which comprise DNA loci encoding both human Ig heavy chain variable region and Kappa light chain variable region (VelocImmune™, Regeneron Pharmaceuticals, Inc.; US 6,596,541), with an adjuvant to stimulate the immune response. Such an adjuvant includes complete and incomplete Freund's adjuvant, MPL+TDM adjuvant system (Sigma), or RIBI (muramyl dipeptides) (see O'Hagan, Vaccine Adjuvant, by Human Press, 2000, NJ). Such an adjuvant can prevent rapid dispersal of polypeptide by sequestering the antigen in a local depot, and may contain factors that can stimulate host immune response. In one embodiment, hIL-6R is administered indirectly as DNA plasmid that contains hIL-6R gene and expresses hIL-6R using the host cellular protein expression machinery to produce antigen polypeptide *in vivo*. In both approaches, the immunization schedule requires several administrations spaced by a few weeks. The antibody immune response is monitored by standard antigen-specific immunoassay. When animals reached their maximum immune response, the antibody expressing B cells were harvested and fused with mouse myeloma cells to preserve their viability, forming hybridoma cells. To select functionally desirable monoclonal antibodies, conditioned media of the hybridoma cells or transfected cells were screened for specificity, antigen-binding affinity, and potency in blocking hIL-6 binding to hIL-6R (described below).

Example 2. Anti-hIL6R antibodies generated via direct isolation of splenocytes

[0045] DNA encoding VH and VL domains may be isolated directly from a single antigen positive B cell. Briefly, the hIL-6Rα immunized transgenic mouse was terminated and splenocytes were harvested. Red blood cells were removed by lysis followed by pelleting the harvested splenocytes. Resuspended splenocytes were first incubated with a cocktail of human IgG, FITC-anti-mFc, and biotin-IL6Ra for 1 hour. The stained cells were washed twice with PBS, then stained with a cocktail of human and rat IgG, APC-anti-mIgM, and SA-PE for one hour. The

stained cells were washed once with PBS and were analyzed by flow cytometry on a MoFlo (Cytomation). Each IgG positive, IgM negative, and antigen positive B cell was sorted and plated into a separate well on a 96-well plate. RT-PCR of antibody genes from these B cells was performed according to a method described by Wang et al. (2000) (J Immunol Methods 244:217–225). Briefly, cDNAs for each single B cell were synthesized via RT-PCR. Each resulting RT product was then split and transferred into two corresponding wells on two 96-well plates. One set of the resulting RT products was first amplified by PCR using a 5' degenerate primer specific for human IgG heavy chain variable region leader sequence and a 3' primer specific for mouse heavy chain constant region, to form an amplicon. The amplicon was then amplified again by PCR using a 5' degenerate primer set specific for framework 1 of human IgG heavy chain variable region sequence and a nested 3' primer specific for mouse heavy chain constant region. The other set of the resulting RT products was first amplified by PCR using a 5' degenerate primer specific for human kappa light chain variable region leader sequence and a 3' primer specific for mouse kappa light chain constant region to form an amplicon. The amplicon was then amplified again by PCR using a 5' degenerate primer set specific for framework 1 of human kappa light chain variable region sequence and a nested 3' primer specific for mouse kappa light chain constant region. The heavy chain and light chain PCR products were cloned into Sap I-linearized antibody vectors containing IgG1 heavy chain constant region and kappa light chain constant region, respectively. The heavy chain plasmid has a lox2272 site and a lox511 site flanking the heavy chain expression cassettes. In addition, immediately downstream of the lox2272 in the heavy chain plasmid there is a hygromycin-resistance gene that lacks a promoter and an initiating ATG. The hygromycin-resistance gene is also transcriptionally linked to a downstream eGFP gene via an IRES sequence. The light chain plasmid has a loxP site and lox2272 site flanking the light chain expression cassette. In addition, The light chain plasmid has a SV40 promoter immediately before an ATG at the lox2272 site, such that upon integration into an appropriate host cell the lox2272-proximal SV40 promoter and initiating ATG from the light chain plasmid is brought adjacent to the hygromycin-resistance gene in the heavy chain plasmid in the proper reading frame to allow transcription and translation of the hygromycin-resistance and eGFP genes. Purified recombinant plasmids having a heavy chain variable region sequence and plasmids having a light chain variable region sequence from the same B cell were then combined and transfected, together with a plasmid that expresses the Cre recombinase, into a modified CHO host cell line. The modified CHO host cell line contains, from 5' to 3', a loxP site, an eCFP, a lox2272 site, DsRed, and a lox511 site at a transcriptionally active locus. Consequently, the host CHO cell can be isolated by flow cytometry as a blue-positive, red-positive, and green-negative cell. When recombinant plasmids expressing heavy chain and light chain genes are transfected together with a plasmid expressing the Cre recombinase, site-specific recombination mediated by the Cre recombinase results in the integration of the antibody plasmids at the chromosomal locus containing the lox

sites and replacement of the eCFP and DsRed genes. Recombinants can then be isolated as blue-negative, red-negative, and green-positive cells by flow cytometry. Accordingly, CHO cells transfected with recombinant plasmids having a heavy chain variable region sequence and plasmids having a light chain variable region sequence from the same B cell were sorted by flow cytometry, and proper recombinants that show the blue-negative, red-negative, and green-positive phenotype were isolated, and stable recombinant antibody-expressing CHO cell lines were established from isolated clones.

Example 3. Antigen Binding Affinity Determination

[0046] *The K_D of the antigen binding to the selected antibodies described above were* determined by surface kinetics on a real-time biosensor surface plasmon resonance assay (BIAcore™). More specifically, the affinity of the antibodies for human IL-6R was measured using a BIAcore® 2000 or BIAcore® 3000. The antibody was captured on an anti-mouse IgG surface and exposed to various concentrations of recombinant hIL-6R protein either in monomeric or dimeric form. Kinetic analysis using BIAevaluation™ software was performed to obtain the association and dissociation rate constants.

[0047] Binding affinities of the antibodies to hIL-6R was also measured for either hybridoma-conditioned media or purified proteins by plate-based competition immunoassay. The antibody proteins were purified using Protein G affinity chromatography from hybridoma cell conditioning medium that was bovine IgG-depleted (Invitrogen). For the competition ELISA, briefly, constant *amounts of antibody at different levels were premixed with serial dilutions of antigen protein,* hIL-6R-hFc, ranging from 0 to 10 μg/ml, and incubated for two hours at room temperature to *reach pseudo-binding equilibrium between the antibody and antigen.* These solutions were then transferred to 96-well hIL-6R-hFc pre-coated plates to allow the free-antibody in the mixtures to bind to plate-coated hIL-6R-hFc. The plates were typically coated with 1 to 2 μg/ml hIL-6R-hFc protein in PBS solution overnight at 4°C followed by BSA nonspecific blocking. After washing off excess antibody in solution, plate-bound antibodies were detected with an HRP-conjugated goat anti-mouse IgG or IgA polyclonal antibody reagent and developed using either colorimetric or chemiluminescence substrates. The dependency of the signals on the concentrations of antigen in solution was analyzed with a 4-parameter fit analysis using Prism™ software (Graph Pad) and reported as IC_{50}. Competition immunoassay were also carried out using steady state solution phase Kinexa™ instrument (Sapidyne Inc.).

[0048] Results are shown in Table 1 (control: humanized monoclonal antibody to human IL-6R *(U.S. Patent No. 5,817,790 SEQ ID NO:69 and 71). Antibody (HCVR and LCVR amino acid* sequences): VQ8A9-6 (3, 11); VQ8F11-21 (19, 27); VV7G4-1 (35, 43); VV7G4-10 (51, 59) VV6C10-1 (67, 75); VV6C10-3 (83, 91); VV6C10-4 (99, 107); VV6F12-11 (115, 123); VV9A6-11 (131, 139); VV6A9-5 (147, 155), VV3D8-4 (163, 171); VV1G4-7 (179, 187); 248982-13-1-E5 (195, 203); 248982-13-2-A9 (211, 219). Monomer and dimer K_D determined by BIAcore™;

solution K_D by Kinexa™; IC_{50} by ELISA assays (n.d. = not determined).

Table 1. Antigen Binding Affinity

Antibody	K_D Monomer (nM)	K_D Dimer (nM)	Solution K_D Monomer (nM)	ELISA IC_{50} Dimer (nM)
VQ8A9-6	0.222	0.101	0.120	0.004
VQ8F11-21	0.067	0.023	0.009	0.008
VV3D8-4	2.410	0.172	1.910	0.013
VV6A9-5	0.097	0.146	0.032	0.005
VV1G4-7	0.225	0.070	0.197	0.041
VV6C10-1	0.267	0.032	2.050	0.010
VV6F12-11	n.d.	n.d	n.d	0.033
VV7G4-10	n.d.	n.d.	n.d.	1.980
VV9A6-11	n.d.	n.d.	n.d.	0.347
VV6C10-3	n.d.	n.d.	n.d.	0.009
248982-13-1-E5	0.987	0.785	n.d.	0.360
248982-13-2-A9	2.870	n.d.	n.d.	0.054
Control	1.790	n.d.	1.960	n.d.

Example 4. Neutralization of hIL-6 Activity

[0049] hIL-6 blocking activities of the anti-hIL-6R antibodies of the invention were screened by hIL-6 blocking immunoassays, *in vitro* hIL-6 dependent cell growth bioassays, and surface plasmon resonance (BIAcore™). The immunoassay was used to screen ability of the tested antibody to block hIL-6 binding to hIL-6R, and the *in vitro* bioassay was used to determine the potency of the antibodies in neutralizing hIL-6R-mediated cellular signal transduction.

[0050] For the immunoassay, hIL-6 recombinant protein was coated on a 96-well plate in PBS buffer overnight at 4°C. This plate was used to capture free hIL-6R-hFc from antibody sample solutions, and the amount of captured hIL-6R-hFc was quantified according to the standard curve. The sample solutions were composed of a constant amount of hIL-6R-hFc recombinant protein (100 pM) and varying amounts of antibody, either in crude hybridoma condition medium or as purified antibody protein, ranging from 0 to about 50 nM in serial dilutions. The antibody-antigen mixtures were incubated at room temperature for ~2 hours to allow antibody-antigen binding to reach equilibrium. The equilibrated sample solutions were then transferred to the hIL-6 coated plates for measurement of free hIL-6R-hFc. After 1 hour binding, the plate was washed and bound hIL-6R-hFc was detected using HRP-conjugated goat anti-hFc polyclonal antibodies (Jackson Immuno Research), and developed using TMB substrate (BD Pharmigen). IC_{50}s were determined as the amount of antibody required to reduce 50% of IL-6R-hFc detectable to plate bound hIL-6 ligand. Results are shown in the first column of Table 2.

[0051] Additionally, the ability of the test antibody to block hIL-6 binding to the hIL-6R receptor was determined using surface plasmon resonance. Purified antigen hIL-6R-hFc molecules were captured by goat anti-human IgG polyclonal antibodies immobilized on CM-5 surface through amine coupling to a density of 250 RU. hIL-6 solution (0.25ml, 50 nM) was injected over the receptor surface and bound hIL-6 recorded (first injection of IL-6). Bound hIL-6 was then removed with a pulse of 3 M $MgCl_2$ following by conditioning buffer. Anti-hIL6R antibody in hybridoma conditioned medium was injected over the captured receptor surface followed by second injection of hIL-6. The percent reduction in hL-6 binding resulting from preformed antibody and receptor complex was used as a score to define hIL-6 blockers from non-blockers (second column, Table 2).

Table 2. *Neutralization of hIL-6 Binding*

Antibody	hIL6R/hIL6 Binding Inhibition IC_{50} (nM)	hIL6/hIL6R Binding Inhibition (%)	XG-1 cell proliferation Inhibition IC_{50} (nM)	HepG2/Stat3 Luciferase activity IC_{50} (nM)
VQ8A9-6	0.39	68	0.40	0.097
VQ8F11-21	0.12	98	0.62	0.135
VV3D8-4	0.61	93	>100	n.d.
VV6A9-5	0.35	100	1.10	0.188
VV1G4-7	1.10	34	1.80	0.578
VV6C10-1	4.60	61	>6.90	n.d.
VV6F12-11	2.20	n.d.	n.d.	n.d.
VV7G4-10	13.00	n.d.	n.d.	n.d.
VV9A6-11	0.50	n.d.	n.d.	n.d.
VV6C10-3	0.06	n.d.	n.d.	n.d.
Control	2.20	91	1.50	0.854

[0052] The ability of hIL-6R antibodies to block hIL-6 activity *in vitro* was measured in the hIL-6-dependent myeloma line XG-1. XG-1 cells maintained in hIL-6-containing medium were washed twice with hIL-6-free media and cultured for ~24 hours in hIL-6-free medium to deplete residual hIL-6. The starved cells were then spun down and re-suspended in the medium at 4 x 10^5 cells per ml and plated 20,000 cells per well in a 96-well tissue culture plate. The purified antibody proteins were serially diluted in medium and added to the plated cells at concentrations ranging from 0 to 50 nM. Subsequently, recombinant hIL-6 was added to the wells to a final concentration of 8 pM. Cells were allowed to grow for ~72 hours at 37°C in a humidified 5% CO_2 incubator. At the end of growth period, live cells were measured using CCK-8 kit (Dojindo, Japan). IC_{50}s were determined as described above, and reported in the third column of Table 2.

[0053] The ability of hIL-6R antibodies to block hIL-6 activity was also measured *in vitro* in the

hIL-6-responsive human hepatoma cell line, HepG2. HepG2 cells were transfected with a reporter plasmid containing a STAT3 (Signal Transducer and activator of Transcription 3) response element linked to a luciferase gene. The transfected cells were trypsinized, spun down and re-suspended in the medium at approximately 2.5 x 10^5 cells per ml and plated at 20,000 cells per well in a 96-well tissue culture plate. The purified antibody proteins were serially diluted in medium and added to the plated cells at concentrations ranging from 0 to 100 nM. Subsequently, recombinant hIL-6 was added to the wells to a final concentration of 50 pM. The response was measured after incubating the cells for 6 hours at 37°C in a humidified 5% CO_2 incubator. Luciferase activity was measured with the Steady-Glo™ luciferase assay system (Promega). IC_{50}s were determined as described above, and reported in the fourth column of Table 2.

Example 5. Binding Epitope Diversity

[0054] An antibody binding competition immunoassay was performed using as a control humanized antibody to human IL-6R. Briefly, a 96-well immunosorbent plate was coated with 20 ng per well hIL-6R recombinant protein overnight at 4°C. After blocking non-specific binding with BSA, the hIL-6R binding sites on one half of the plate were saturated with binding of the control antibody by addition of 500 ng of the control per well, and to the other half of the plate was added binding buffer only. After three hours binding at room temperature, the purified antibodies were spiked in at a final concentration of 50 ng/ml with and without the preexisting control antibody in the well. After one hour of additional binding, the free antibody was washed away and the plate-bound antibody was detected with HRP-conjugated goat anti-mouse IgG or IgA, polyclonal antibody and the plate was developed using chromatic HRP substrates and absorbance at 450 nm was recorded. Percentage deductions of the binding of the anti-hIL6R antibodies by the presence of the control antibody are listed in Table 3 below. A similar experiment was conducted using surface plasmon resonance technology (Table 3). Both methods generated consistent results. Antibodies VQ8F11, VV3D8, VV6A9, VV6C10-1 bound epitopes overlapping with the control antibody; while antibodies VQ8A9, VV1G4, VV6F12, VV7G4, VV9A6, and VV6C10-3 appeared to bind distinct epitopes as antigen binding was not blocked by the control antibody. Partial competition may result from steric hindrance from the first antibody bound, even though epitopes may not be overlapping.

Table 3. Competition of Antigen Binding with Control Antibody

Antibody	BIAcore™ (% reduction)	Immunoassay (% reduction)
VQ8A9-6	26	3
VQ8F11-21	96	79
VV3D8-4	97	84

VV6A9-5	96	84
VV1G4-7	12	3
VV6C10-1	90	80
VV6F12-11	n.d.	3
VV7G4-10	n.d.	26
VV9A6-11	n.d.	18
VV6C10-3	n.d.	1

Example 6. Cross-species Binding Property

[0055] Four antibodies were tested for cross-reactivity to monkey IL-6R recombinant protein using BIAcore™ technology. Briefly, a biosensor chip on which goat anti-mouse Fc polyclonal antibody was immobilized was used to present anti-hIL-6R monoclonal antibodies to a density of about 75 RU. Recombinant human or monkey monomeric IL-6R protein (*Macaca fascicularis*, extracellular domain; SEQ ID NO:251), at a concentration range between 1.25 – 40 nM, was injected over the antibody surface. The binding of the receptor to the antibody and the dissociation of the bound complex were monitored in real-time. Both association rate constant (ka) and dissociate rate constant (kd) were obtained, and K_D calculated (Table 4).

Table 4. Comparison of Binding Affinity to Human and Monkey IL-6R

Antibody	Antigen	ka ($M^{-1}S^{-1}$)	kd (S^{-1})	K_D (nM)
Control	Human IL6R	1.74E+05	1.67E-04	0.963
	Monkey IL6R	1.44E+05	1.68E-04	1.170
VQ8F11-21	Human IL6R	8.51E+05	4.38E-05	0.051
	monkey IL6R	3.39E+05	4.86E-05	0.143
VV1G4-7	Human IL6R	2.57E+05	6.18E-05	0.240
	monkey IL6R	no binding		
VV6A9-5	Human IL6R	5.18E+05	8.41E-05	0.162
	monkey IL6R	5.00E+05	7.70E-05	0.154
VQ8A9-6	Human IL6R	7.32E+05	2.76E-04	0.377
	monkey IL6R	7.31E+05	4.16E-04	0.569

[0056] Among the four tested antibodies, VQ8F11, VV6A9, and VQ8A9 strongly reacted to monkey receptor with K_D values that differed by up to about 1.5- to about 3-fold from human receptor binding, respectively. VV1G4, which was not blocked by the control antibody (Table 3), showed no binding to monkey receptor despite strong binding to the human receptor with K_D of 241 pM.

Example 7. Effect of Constant Region on Binding Affinity

[0057] The binding affinity to monomeric hIL-6R of four antibodies having mouse IgG, human IgG1 or human IgG4 (wild-type and modified) were determined using BIAcore™ as described above except a goat anti-human Fc polyclonal antibody surface was used to capture hIgG antibodies. Monomeric hIL-6R was injected at concentrations of 12.5, 6.25, 3.12, and 1.56 nM. The ability of the antibodies to neutralize hIL-6-dependent HepG2/STAT3 signal transduction was also determined in a luciferase assay (IC_{50}). IC_{50}s for different IgG isotypes were similar, suggesting no effect of isotype on antibody affinity for antigen.

Table 5. Comparison of IgG Isotypes

Antibody	IgG	ka ($M^{-1}S^{-1}$)	kd (S^{-1})	K_D (nM)	IC_{50} (nM)
VQ8F11-21	hIgG1	6.22E+05	4.54E-05	0.073	0.150
	hIgG4	7.17E+05	5.22E-05	0.073	0.228
	mIgG2a	7.86E+05	5.27E-05	0.067	0.135
	modhIgG4	8.81E+05	4.705-05	0.053	0.249
VQ8A9-6	hIgG1	1.09E+06	2.60E-04	0.238	0.130
	hIgG4	1.17E+06	2.35E-04	0.201	0.185
	mIgG1	9.95E+05	2.21E-04	0.222	0.097
VV6A9-5	hIgG1	7.12E+05	8.87E-05	0.125	0.204
	hIgG4	5.67E+05	7.64E-05	0.135	0.343
	mIgG2a	7.72E+05	7.52E-05	0.097	0.188
VQ1G4-21	hIgG1	3.34E+05	7.92E-05	0.237	0.767
	hIgG4	2.73E+05	9.18E-05	0.336	0.528
	mIgG2a	3.41E+05	7.66E-05	0.225	0.578

Claims:

1. An antibody or antigen-binding fragment thereof, which specifically binds human interleukin-6 receptor (hIL-6R) with a K_D of about 500 pM or less, and 300 pM or less, as measured by surface plasmon resonance.

2. An antibody or antigen-binding fragment according to claim 1 which binds hIL-6R with an affinity at least 2-fold higher relative to its binding to monkey IL-6R.

3. An antibody or antigen-binding fragment according to claim 1 or 2, comprising a heavy chain complementary determining region 3 (CDR3) domain and a light chain CDR3 domain, wherein

the heavy chain CDR3 domain comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9 - X^{10} - X^{11} - X^{12} - X^{13} - X^{14} - X^{15} - X^{16} - X^{17} - X^{18} - X^{19}$ (SEQ ID NO:247) wherein X^1 = Ala, X^2 = Lys, X^3 = Gly, X^4 = Arg, X^5 = Asp, X^6 = Ser or Ala, X^7 = Phe, X^8 = Asp; X^9 = Ile, X^{10} = Pro or absent, X^{11} = Phe or absent, X^{12} = Val or absent, X^{13} = Tyr or absent, X^{14} = Tyr or absent, X^{15} = Tyr or absent, X^{16} = Gly or absent, X^{17} = Met or absent, X^{18} = Asp or absent, and X^{19} = Val or absent; and

the light chain CDR3 domain comprises an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8 - X^9$ (SEQ ID NO:250) wherein X^1 = Gln, X^2 = Gln or His, X^3 = Ala, X^4 = Asn or Tyr, X^5 = Ser, X^6 = Phe, X^7 = Pro, X^8 = Pro, and X^9 = Thr.

4. An antibody or antigen-binding fragment according to claim 3, further comprising

a heavy chain CDR1 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO:245) wherein X^1 = Gly or Arg, X^2 = Phe, X^3 = Thr, X^4 = Phe, X^5 = Asp, X^6 = Asp, X^7 = Tyr, and X^8 = Ala;

a heavy chain CDR2 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO:246) wherein X^1 = Ile or Val, X^2 = Ser, X^3 = Trp, X^4 = Asn, X^5 = Ser, X^6 = Gly, X^7 = Ser, and X^8 = Ile;

a light chain CDR1 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3 - X^4 - X^5 - X^6 - X^7 - X^8$ (SEQ ID NO:248), wherein X^1 = Gln, X^2 = Gly, X^3 = Ile, X^4 = Ser, X^5 = Ser, and X^6 = Trp; and

a light chain CDR2 domain comprising an amino acid sequence of the formula $X^1 - X^2 - X^3$ (SEQ ID NO:249), wherein X^1 = Gly or Ala, X^2 = Ala, and X^3 = Ser.

5. An antibody or antigen-binding fragment according to claim 1 or 2, comprising:

a heavy chain CDR3 domain selected from the group consisting of SEQ ID NO: 25, 153, 9, 185, 41, 57, 73, 89, 105, 121, 137, 169, 201 and 217; and

a light chain CDR3 domain selected from the group consisting of SEQ ID NO:33, 161, 17, 193, 49, 65, 81, 97, 113, 129, 145, 177, 209 and 225.

6. An antibody or antigen-binding fragment according to claim 5, further comprises:

a heavy chain CDR1 domain selected from the group consisting of SEQ ID NO: 21, 149, 5, 181, 37, 53, 69, 85, 101, 117, 133, 165, 197, and 213;

a heavy chain CDR2 domain selected from the group consisting of SEQ ID NO: 23, 151, 7, 183, 39, 55, 71, 87, 103, 119, 135, 167, 199 and 215;

a light chain CDR1 domain selected from the group consisting of SEQ ID NO: 29, 157, 13, 189, 45, 61, 77, 93, 109, 125, 141, 173, 205 and 221; and

a light chain CDR2 domain selected from the group consisting of SEQ ID NO: 31, 159, 15, 191, 47, 63, 79, 95, 111, 127, 143, 175, 207 and 223.

7. An antibody or antigen-binding fragment according to claim 5 or 6, wherein the heavy chain CDRs and the light chain CDRs comprise

SEQ ID NO:21, 23, 25 and SEQ ID NO:29, 31, 33;

SEQ ID NO:149, 151, 153 and SEQ ID NO:157, 159, 161;

SEQ ID NO:5, 7, 9 and SEQ ID NO: 13, 15, 17; and

SEQ ID NO: 181. 183, 185 and SEQ ID NO:189, 191, 193, respectively.

8. An antibody or antigen-binding fragment according to claim 1 or 2, comprising

a heavy chain variable region (HCVR) selected from the group consisting of SEQ ID NO:19, 147, 3, 179, 227, 231, 35, 51, 67, 83, 99, 115, 131, 239, 241, 163, 235, 195 and 211; and

a light chain variable region (LCVR) selected from the group consisting of SEQ ID NO: 27, 155, 11, 187, 229, 233, 43, 59, 75, 91, 107, 123, 139, 171, 237, 203 and 219.

9. An antibody or antigen-binding fragment according to claim 8, comprising HCVR/LCVR pairs selected from the group consisting of SEQ ID NO: 19/27; 147/155; 3/11; 179/187; 227/229; 231/233; 35/43; 51/59; 67/75; 83/91; 99/107; 115/123; 131/139; 239/155; 241/155; 163/171; 235/237; 195/203 and 211/219.

10. An isolated nucleic acid molecule encoding an antibody or antigen-binding fragment according to any one of the preceding claims.

11. A vector comprising the nucleotide sequence according to claim 10.

12. A host-vector system for the production of an antibody or antigen-binding fragment of an antibody which specifically binds human IL-6 receptor, comprising a vector according to claim 11, in a suitable host cell.

13. A host-vector system according to claim 12, wherein the host cell is a prokaryotic or eukaryotic cell selected from one of an *E. coli* or a CHO cell.

14. A method for producing an anti-IL-6R antibody or antigen-binding fragment thereof, comprising growing cells of a host-vector system according to claim 12 or 13 under conditions permitting production of the antibody or fragment thereof and recovering the antibody or fragment so produced.

15. Use of an antibody or antigen-binding fragment of an antibody according to any one of claims 1 to 9 in the manufacture of a medicament for use to attenuate or inhibit an IL-6-mediated disease or disorder in a human.

16. An antibody or antigen-binding fragment of an antibody according to any one of claims 1 to 9 for use in the attenuation or inhibition of an IL-6-mediated disease or disorder in a human.

17. A pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody according to any one of claims 1 to 9 and a pharmaceutically acceptable carrier or excipient.

18. A method of attenuating or inhibiting an IL-6-mediated disease or disorder in a human patient, the method comprising administering an effective amount of an antibody or antigen-binding fragment of an antibody according to any one of claims 1 to 9 to a human patient.

19. A method according to claim 18, wherein the IL-6-mediated disease or disorder is arthritis, such as chronic rheumatoid arthritis; an inflammatory bowel disease, such as Crohn's disease or ulcerative colitis; or systemic erythematosus.