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(54) **Title:** ANTI-IL-6 ANTIBODIES PREVENTING THE BINDING OF IL-6 COMPLEXED WITH IL-6RALPHA TO GP130

(57) **Abstract:** The present invention provides anti IL-6 antibodies including an antibody variable region that prevents IL-6 from binding to gp130. The present invention also provides compositions and methods for treating IL-6 related diseases based on the IL-6 antagonists of the invention.

ANTI-IL-6 ANTIBODIES PREVENTING THE BINDING OF IL-6 COMPLEXED WITH IL-6RALPHA TO GP130

FIELD OF THE INVENTION

[0001] This invention relates to antagonists of interleukin-6 (IL-6) that are useful in suppressing IL-6 signaling pathway and in treating IL-6 related diseases. In particular, this invention relates to anti-IL-6 antibodies that block the interaction between IL-6 and gp130 and can be successfully used for the treatment of IL6-triggered diseases, preferably cancer and autoimmune diseases.

BACKGROUND OF THE INVENTION

[0002] Interleukin-6 (IL-6) is involved in several diseases, including many cancers and autoimmune diseases. Interleukin-6 is secreted by many advanced cancers, such as hormone-independent prostate cancer, and is believed to be a growth factor for such cancers. In addition, the secretion of IL-6 by cancer cells is believed to cause cachexia, the wasting syndrome characteristic of advanced cancers. Therefore, inhibition of IL-6 action would be useful in treatment of such cancers.

[0003] IL-6 also plays a key role in B cell development. Autoimmune diseases with a significant antibody component, such as rheumatoid arthritis, could be treated by inhibition of IL-6. Disorders involving proliferation of B cells, such as multiple myeloma and B cell lymphoma, could also be treated by inhibition of IL-6 activity.

[0004] In addition, IL-6 plays an important role in bone remodeling by promoting bone resorption. Inhibitors of IL-6 activity would have the effect of reducing bone resorption and could be used to treat osteoporosis.

[0005] When IL-6 is produced as part of a disease or disorder, it is often complexed with a soluble IL-6Ralpha subunit and is often secreted from cells in the form of such a complex. As a result, it is often not useful to treat a patient with an antibody or other inhibitor that blocks the interaction between IL-6 and IL-6Ralpha, because such an antibody or inhibitor can have no effect on a pre-formed complex. Therefore, there is a need in the art for improved treatment of IL-6-mediated diseases.

CONFIRMATION COPY

SUMMARY OF THE INVENTION

[0006] The present invention provides improved compositions and methods for treatment of IL-6-mediated diseases, in particular, cancers and autoimmune diseases that involve IL-6 over activation. Specifically, the present invention provides a novel IL-6 antagonist that effectively blocks the interaction between IL-6 and gp130, in particular, an IL-6 antagonist that prevents the pre-formed IL-6 and IL-6Ralpha complex from binding to gp130. In addition, the present invention provides methods of generating a novel IL-6 antagonist that blocks the interaction between IL-6 and gp130.

[0007] Thus, in one aspect, the present invention provides an isolated IL-6 antagonist that prevents IL-6 complexed with IL-6Ralpha from binding to gp130. In one embodiment, the isolated IL-6 antagonist contains an antibody variable region and an Fc region derived from a human antibody. In alternative embodiments, the Fc region suitable for the invention may be derived from an antibody obtained from a mouse, a rat, a cow, a dog, a chicken, a horse, a fish, a monkey, or other non-human species. In a preferred embodiment, the antibody variable region binds to a region on IL-6 such that the binding sterically blocks the interaction between IL-6 and gp130.

[0008] In some embodiments, the invention provides an isolated IL-6 antagonist comprising an antibody variable region that includes a heavy chain CDR1 containing the amino acid sequence $FX_1FSX_2X_3WMX_4$ (SEQ ID NO:1). X_1, X_2, X_3 or X_4 may be any amino acid. Preferably, X_1 is Thr, Ser, Ala or Cys; X_2 is Asn or Asp; X_3 is Tyr or Ala; and X_4 is Asn or Asp. In particular, the heavy chain CDR1 may contain one of the following amino acid sequences: FTFSNYWMN (SEQ ID NO:2), FSFSNYWMN (SEQ ID NO:3), or FTFSDAWMD (SEQ ID NO:4).

[0009] In some embodiments, the invention provides an isolated IL-6 antagonist comprising an antibody variable region that includes a heavy chain CDR2 containing the amino acid sequence $EIRX_1X_2X_3NX_4X_5AX_6X_7YAESVKG$ (SEQ ID NO:5). X_1, X_2, X_3, X_4, X_5, X_6 or X_7 may be any amino acid. Preferably, X_1 is Leu or Ser; X_2 is Lys or Thr; X_3 is Ser or Ala; X_4 is Asn or Lys; X_5 is Tyr, Gly, Gln or His; X_6 is Thr or Ile; and X_7 is His or Tyr. In particular, the antibody variable region includes a heavy chain CDR2 containing one of the following amino acid sequences: EIRLKSNNYATHYAESVKG (SEQ ID NO:6),

EIRLKSNKGATHYAESVKG (SEQ ID NO:7), EIRLTSNKQAIYYAESVKG (SEQ ID NO:8), or EIRSKANNHATYYAESVKG (SEQ ID NO:9).

[0010] In some embodiments, the invention provides an isolated IL-6 antagonist comprising an antibody variable region that includes a heavy chain CDR3 containing the amino acid sequence $X_1X_2X_3X_4GX_5X_6X_7X_8$ (SEQ ID NO:10). X_1, X_2, X_3, X_4, X_5, X_6, X_7 or X_8 may be any amino acid or a peptide bond. Preferably, X_1 is Glu, Leu or Pro; X_2 is Asp, Leu, Phe or Thr; X3 is Tyr or Leu; X_4 is Tyr or Asp; X_5 is Tyr or Ala; X_6 is Pro, Met or a peptide bond; X_7 is Asp or Leu; and X_8 is Tyr or His. In particular, the antibody variable region includes a heavy chain CDR3 containing one of the following amino acid sequences: EDYYGYPDY (SEQ ID NO:11), LLYDGYLH (SEQ ID NO:12), LFYDGYLH (SEQ ID NO:13), or PTLYGAMDY (SEQ ID NO:14).

[0011] In some embodiments, the invention provides an isolated IL-6 antagonist comprising an antibody variable region that includes a light chain CDR1 containing the amino acid sequence $RASESVX_1NX_2GISFM$ (SEQ ID NO:15). X_1 or X_2 may be any amino acid. Preferably, X_1 is Asp, Gly or His; and X_2 is Phe or Tyr. In particular, the antibody variable region includes a light chain CDR1 containing one of the following amino acid sequences: RASESVDNFGISFM (SEQ ID NO:16), RASESVGNFGISFM (SEQ ID NO:17), RASESVHNFGISFM (SEQ ID NO:18), or RASESVDNYGISFM (SEQ ID NO:19).

[0012] In some embodiments, the invention provides an isolated IL-6 antagonist comprising an antibody variable region that includes a light chain CDR2 containing the amino acid sequence XASNQGS (SEQ ID NO:20). X may be any amino acid. Preferably, X is Ala, Val or Thr. In particular, the antibody variable region includes a light chain CDR2 containing one of the following amino acid sequences: TASNQGS (SEQ ID NO:21), VASNQGS (SEQ ID NO:22), or AASNQGS (SEQ ID NO:23).

[0013] In some embodiments, the invention provides an isolated IL-6 antagonist comprising an antibody variable region that includes a light chain CDR3 containing the amino acid sequence $QQX_1KEX_2PX_3T$ (SEQ ID NO:24). X_1, X_2 or X_3 may be any amino acid. Preferably, X_1 is Ser or Gly; X_2 is Val or Ile; and X_3 is Trp or Tyr. In particular, the antibody variable region includes a light chain CDR3 containing one of the following amino acid sequences:

QQSKEVPWT (SEQ ID NO:25), QQSKEVPYT (SEQ ID NO:26), QQSKEIPWT (SEQ ID NO:27), or QQGKEVPWT (SEQ ID NO:28).

[0014] In a preferred embodiment, the IL-6 antagonist of the present invention is an antibody or a fragment thereof.

[0015] In some embodiments, the antibody includes a light chain containing one of the following amino acid sequences: SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, or SEQ ID NO:32. Alternatively, the antibody includes a light chain containing an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to any one of the above-identified sequences, for example, SEQ ID NO:31 (Mab#471).

[0016] In other embodiments, the antibody includes a heavy chain containing one of the following amino acid sequences: SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36. Alternatively, the antibody includes a heavy chain containing an amino acid sequence at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identical to any one of the above-identified sequences, for example, SEQ ID NO:35 (Mab#471).

[0017] The present invention also provides nucleic acids encoding the IL-6 antagonist as described in various embodiments above. In particular, the present invention provides nucleic acids encoding the light chain and/or the heavy chain of the anti-IL-6 antibodies described above.

[0018] In another aspect, the present invention provides an isolated IL-6 antagonist including an antibody variable region that binds to an epitope on IL-6 containing an amino acid selected from the group consisting of Leu19, Arg24, Lys27, Arg30, Tyr31, Asp34 and Trp157 such that the binding sterically blocks the interaction between IL-6 and gp130.

[0019] Typically, the IL-6 antagonist includes an Fc moiety. Preferably, the Fc moiety is derived from a human antibody. In a more preferred embodiment, all of the constant regions in the IL-6 antagonist of the invention are derived from a human antibody. In alternative embodiments, the Fc region suitable for the invention may be derived from an antibody obtained from a mouse, a rat, a cow, a dog, a chicken, a horse, a fish, a monkey, or other non-human species.

[0020] In another aspect, the present invention provides a method for treating a disease in a subject by administering to the subject the isolated IL-6 antagonist of the invention as described above. Specifically, the present

invention provides a method for treating a disease or a symptom in a subject by administering a protein including an antibody V region that binds to IL-6 and sterically blocks its interaction with gp130. Such proteins include, but are not limited to, antibodies, antibody fragments that lack various constant regions, minibodies, scFv proteins, antibody fusion proteins. Preferably, the antibody V region that binds to IL-6 and sterically blocks its interaction with gp130 does not sterically block the interaction between IL-6 and the IL-6 receptor alpha subunit. The method of the present invention is particularly useful in treating diseases, disorders, and side effects that involve IL-6, such as, for example, autoimmune diseases including, but not limited to, rheumatoid arthritis, Sjogren's syndrome, multiple sclerosis, systemic lupus erythematosus, Graves' disease, Hashimoto's disease, and Castleman's disease, acute and chronic inflammation, and osteoporosis and other disorders involving loss of bone mass, and cancers including, but not limited to, hormone-independent prostate cancer, B-cell proliferative disorders such as B cell non-Hodgkin's lymphoma, and advanced cancers of kidney, breast, colon, lung, brain, and other tissues.

[0021] In yet another aspect, the present invention provides a method of generating an IL-6 antagonist described in various embodiments above. In particular, the present invention provides a method of generating an IL-6 antagonist by (a) first generating antibodies against a complex of IL-6 and IL-6Ralpha by immunizing an animal with a composition including IL-6 and IL-6Ralpha, and (b) identifying an antibody that inhibits the interaction between gp130 and IL-6. The IL-6 and IL-6Ralpha may be in a fusion protein configuration to facilitate formation of the IL-6/IL-6Ralpha complex. In a preferred embodiment, the composition also includes an additional moiety that facilitates antigen presentation, such as an Fc moiety.

[0022] In yet another aspect, the present invention provides an antibody specific for a complex of IL-6 and IL-6Ralpha and capable of preventing IL-6 binding to gp130.

[0023] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various

changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

[0024] In summary the invention relates to:

- A corresponding anti-IL6 antibody or a fragment thereof comprising an antibody variable region, wherein the antibody variable region binds to an epitope on IL-6 in a way that the binding sterically blocks the interaction between IL-6 and gp130 on the surface of a diseased cell.
- A corresponding anti-IL6 antibody, wherein said binding prevents that IL-6 complexed with IL-6Ralpha binds to gp130 but does not sterically block the interaction between IL-6 and IL-6Ralph.
- A corresponding anti-IL6 antibody, wherein said epitope on IL-6 comprises an amino acid selected from the group consisting of Leu19, Arg24, Lys27, Arg30, Tyr31, Asp34 and Trp157.
- A corresponding anti-IL6 antibody, wherein the antibody variable region comprises a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of
  FTFSNYWMN (SEQ ID NO:2),
  FSFSNYWMN (SEQ ID NO:3), and
  FTFSDAWMD (SEQ ID NO:4).
- A corresponding anti-IL6 antibody, wherein the antibody variable region comprises a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of
  EIRLKSNNYATHYAESVKG (SEQ ID NO:6),
  EIRLKSNKGATHYAESVKG (SEQ ID NO:7),
  EIRLTSNKQAIYYAESVKG (SEQ ID NO:8), and
  EIRSKANNHATYYAESVKG (SEQ ID NO:9).
- A corresponding anti-IL6 antibody, wherein the antibody variable region comprises a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of
  EDYYGYPDY (SEQ ID NO:11),
  LLYDGYLH (SEQ ID NO:12),
  LFYDGYLH (SEQ ID NO:13), and
  PTLYGAMDY (SEQ ID NO:14).

- A corresponding anti-IL6 antibody, wherein the antibody variable region comprises a light chain CDR1 comprising an amino acid sequence selected from the group consisting of
RASESVDNFGISFM (SEQ ID NO:16),
RASESVGNFGISFM (SEQ ID NO:17),
RASESVHNFGISFM (SEQ ID NO:18), and
RASESVDNYGISFM (SEQ ID NO:19).
- A corresponding anti-IL6 antibody, wherein the antibody variable region comprises a light chain CDR2 comprising an amino acid sequence selected from the group consisting of
TASNQGS (SEQ ID NO:21),
VASNQGS (SEQ ID NO:22), and
AASNQGS (SEQ ID NO:23).
- A corresponding anti-IL6 antibody, wherein the antibody variable region comprises a light chain CDR3 comprising an amino acid sequence selected from the group consisting of
QQSKEVPWT (SEQ ID NO:25),
QQSKEVPYT (SEQ ID NO:26),
QQSKEIPWT (SEQ ID NO:27), and
QQGKEVPWT (SEQ ID NO:28).
- A corresponding anti-IL6 antibody comprising
a heavy chain CDR1 of
SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, and
a heavy chain CDR2 of
SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; or SEQ ID NO:9; and
a heavy chain CDR3 of
SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13 or SEQ ID NO:14.
- A corresponding anti-IL6 antibody comprising
a light chain CDR1 of
SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, or SEQ ID NO:19, and
a light chain CDR2 of
SEQ ID NO:21, SEQ ID NO:22, or SEQ ID NO:23, and
a light chain CDR3 of
SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 or SEQ ID NO:28.

- A corresponding anti-IL6 antibody comprising
  a heavy chain CDR1 of
  SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, and
  a heavy chain CDR2 of
  SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; or SEQ ID NO:9; and
  a heavy chain CDR3 of
  SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13 or SEQ ID NO:14,
  and
  a light chain CDR1 of
  SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, or SEQ ID NO:19, and
  a light chain CDR2 of
  SEQ ID NO:21, SEQ ID NO:22, or SEQ ID NO:23, and
  a light chain CDR3 of
  SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 or SEQ ID NO:28.
- A corresponding anti-IL6 antibody of any further comprising an antibody constant region.
- A corresponding anti-IL6 antibody comprising an Fc moiety.
- A corresponding anti-IL6 antibody, wherein the Fc moiety is human.
- A corresponding anti-IL6 antibody, wherein the antibody comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32.
- A corresponding anti-IL6 antibody, wherein the antibody comprises a light chain amino acid sequence of SEQ ID NO:31, or a sequence that is at least 90% identical to SEQ ID NO:31.
- A corresponding anti-IL6 antibody, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of
  SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36.
- A corresponding anti-IL6 antibody, wherein the antibody comprises a heavy chain comprising an amino acid sequence of SEQ ID NO:35, or a sequence that is at least 90% identical to SEQ ID NO:35.

- A corresponding anti-IL6 antibody comprising a heavy chain comprising an amino acid sequence of SEQ ID NO:35 and a light chain comprising an amino acid sequence of SEQ ID NO:31.
- A corresponding anti-IL6 antibody comprising a heavy chain comprising an amino acid sequence of SEQ ID NO:34 and a light chain comprising an amino acid sequence of SEQ ID NO:30.
- A corresponding anti-IL6 antibody comprising a heavy chain comprising an amino acid sequence of SEQ ID NO:36 and a light chain comprising an amino acid sequence of SEQ ID NO:32.
- A corresponding anti-IL6 antibodycomprising a heavy chain comprising an amino acid sequence of SEQ ID NO:33 and a light chain comprising an amino acid sequence of SEQ ID NO:29.
- A nucleic acid molecule encoding an antibodyas specidied above and below.
- A pharmaceutical composition suitable for the treatment of an IL6 triggered disease comprising in a pharmacologically effective amount an anti-IL6 antibody as specified above and below, optionally together with a pharmaceutically acceptable carrier, diluent or excipient.
- A use of an anti-IL6 antibody as specified above and below for the manufacture of a medicament for the treatment of cancer or an autoimmune disease.
- A corresponding use, wherein the disease is triggered by IL6 or IL6 complexed with IL-6Ralpha.
- A fusion protein comprising a Fc moiety of an antibody, IL6Ralpha and IL6, preferably wherein Fc derives from a non-human mammal, such as mouse, and IL-6Ralpha and IL6 derive from human origin.
- A corresponding fusion protein, wherein IL6Ralpha is fused to the C-terminus of the Fc moiety, and IL6 is fused to the C-terminus of IL6Ralpha.
- A use of a fusion protein as specified for the manufacture of an anti-IL6 antibody obtained by immunizing a mammal with said fusion protein, wherein the antibody screened has the following properties:
  (i) the variable region of said antibody binds to an epitope on IL-6,
  (ii) sterically blocks the interaction between IL-6 and gp130 on the surface

of a diseased cell, and

(ii) prevents that IL-6 complexed with IL-6Ralpha binds to gp130, but does not sterically block the interaction between IL-6 and IL-6Ralpha.

- A corresponding use, wherein the antibody screened is an antibodyas specified above and below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1A depicts undesired activation of gp130 on diseased cells or other target cells resulting from IL-6 overproduction.

[0026] Figure 1B depicts an IL-6 antagonist that sterically blocks the interaction between IL-6 and gp130 and whose binding to IL-6 is not affected by pre-bound sIL-6Ralpha.

[0027] Figure 2 shows an alignment of exemplary antibody V region sequences of the present invention. Positions of variation among the sequences are indicated with arrows. The CDRs are boxed.

[0028] Figure 3 is a schematic depiction of exemplary protein embodiments used in the present invention.

[0029] Figure 4 shows an experimental result reflecting the binding of exemplary antibodies of the invention to Fc-IL6Ralpha-IL6.

[0030] Figure 5 shows an experimental result reflecting the binding of exemplary antibodies of the invention to IL-6.

[0031] Figure 6 shows an experimental result reflecting the binding of exemplary antibodies of the invention to non-covalently complexed IL-6 and IL-6Ralpha.

[0032] Figure 7 shows an experimental result reflecting the binding of exemplary antibodies of the invention to IL-6Ralpha alone.

[0033] Figure 8 shows an experimental result illustrating that the exemplary antibodies of the invention inhibit the interaction between Fc-IL6Ralpha-IL6 and gp130.

[0034] Figure 9 shows an experimental result illustrating the ability to block haptoglobin release from HepG2 cells stimulated with an Fc-IL6Ralpha-IL6 complex by exemplary antibodies of the invention.

[0035] Figure 10A depicts experimental results reflecting that the Fc-IL6Ralpha-IL6 fusion protein stimulates proliferation of A431 human epithelial carcinoma cells.

[0036] Figures 10B-1 and 10B-2 depict experimental results reflecting that the exemplary antibodies of the invention inhibit proliferation of A431 human epithelial carcinoma cells stimulated by the Fc-IL6Ralpha-IL6 fusion protein.

[0037] Figure 11 illustrates pharmacokinetic properties of exemplary antibodies of the invention.

[0038] Figure 12 shows an experimental result reflecting *in vivo* inhibition of haptoglobin secretion by exemplary antibodies of the invention, using procedures described in Example 6.

[0039] Figure 13 shows an experimental result reflecting inhibition of lung metastasis by exemplary antibodies of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0040] The present invention provides a novel IL-6 antagonist that effectively blocks the interaction between IL-6 and gp130, thereby suppressing IL-6 signaling in the presence of preformed IL-6/IL-6Ralpha complexes. As discussed above, since IL-6 is typically complexed with IL-6Ralpha when produced as part of a disease or a disorder, the present invention thus achieves better therapeutic effects compared to existing antibodies that block the interaction between IL-6 and the IL-6Ralpha (van Zaanen *et al.*, (1996) J. Clin. Invest., 98(6):1441-8).

[0041] In one particular embodiment, the present invention provides an IL-6 antagonist containing an antibody variable region that binds to a region on IL-6 such that the binding sterically blocks the interaction between IL-6 and gp130. By "sterically block" is meant the means of blocking an interaction between first and second proteins by a third protein's binding to the first protein. The binding between the first and the third proteins prevents the second protein from binding to the first protein due to unfavorable van der Waals or electrostatic interactions between the second and third proteins.

[0042] The present invention also provides compositions and methods for treatment of diseases, disorders and side effects involving IL-6 based on the IL-6 antagonist of the invention.

[0043] Various aspects of the invention are described in further detail in the following subsections. The use of subsections is not meant to limit the invention. Each subsection may apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise. As used in this disclosure, the term "comprise" and variations of the term, such as "comprising" and "comprises," are not intended to exclude other additives, components, integers or steps.

IL-6 and Its Interaction with IL-6Ralpha and gp130

[0044] IL-6 signaling is understood to occur by its interaction with IL-6 receptor alpha (IL-6Ralpha) subunit and gp130, a transmembrane receptor protein that transduces the signals from IL-6 to STAT3, which then activates transcription of various genes. The structure of an extracellular portion of the signaling complex has been determined (Boulanger *et al.*, (2003), Science, 300:2101, the teachings of which are hereby incorporated by reference). This structure indicates that the signaling complex contains two copies of IL-6, two copies of the IL-6Ralpha subunit, and two copies of gp130. The structure analysis also indicates that IL-6 binds to the gp130 receptor through three conserved epitopes known as sites I, II, and III. IL-6 must first form a complex with IL-6Ralpha through site I. Site II is a composite epitope formed by the binary complex of IL-6 and IL-6Ralpha, which interacts with cytokine binding region CHR and D2D3 of gp130. Subsequently, site III interacts with the gp130 immunoglobulin-like activation domain (D1 or IGD) to form the competent signaling hexamer complex (Boulanger *et al.*, (2003) Science, 300:2101, the teachings of which are hereby incorporated by reference). The site I binding epitope of IL-6 is localized to the A and D helices and interacts with IL-6Ralpha. The remaining four unique protein-protein interfaces in the hexamer can be separated into two composite sites, sites II and III. Studies of the three dimensional structure of the hexamer complex revealed that a number of residues of IL-6 participate in and important to the interaction between IL-6 and

gp130. Such residues include, but are not limited to, Leu19, Arg24, Lys27, Arg30, Tyr31, Asp34 and Trp157.

[0045] Thus, the effect of formation of the hexamer complex is to dimerize gp130 and juxtapose its intracellular domains, so that signaling continues at the intracellular level. The IL-6Ralpha subunit does not have an intracellular domain, and serves only to stabilize the complex. Mammalian IL-6 is not capable of binding to gp130 in the absence of IL-6Ralpha. The canonical IL-6Ralpha subunit has three extracellular domains and a transmembrane region that anchors the IL-6Ralpha subunit to the membrane of the expressing cell. "Soluble IL-6Ralpha" or "sIL-6Ralpha" is meant a protein having the extracellular portion of the IL-6Ralpha subunit but lacking the transmembrane segment. The sIL-6Ralpha variant protein, lacking the transmembrane segment, may be generated by translation of an alternatively spliced mRNA encoding IL-6Ralpha, or by proteolytic cleavage of the membrane-bound form of IL-6Ralpha.

[0046] sIL-6Ralpha is present in the serum and may also be secreted by the same cell that expresses IL-6. IL-6 forms a complex with sIL-6Ralpha. Once the IL-6/sIL-6Ralpha complex has formed, it is reasonably stable and cannot be bound by an antibody that competes with the IL-6/sIL-6Ralpha interaction. The IL-6/sIL-6Ralpha complex has a significantly extended serum half-life compared to IL-6 alone. Only certain cells in the body, such as B cells, have both IL-6Ralpha and gp130, while many additional cells have only gp130. The IL-6/sIL-6Ralpha complex can bind to cells having only gp130 and stimulate signal transduction. Consequently, undesired activation of gp130 on many cells may result from IL-6 overproduction (Figure 1A). Therefore, antibodies or other molecules that sterically block the interaction between IL-6 and gp130 may be particularly useful in suppressing undesired IL-6 signaling (see Figure 1B).

IL-6 Antagonists Sterically Blocking the Interaction with gp130

[0047] Thus, as shown in Figure 1B, the present invention contemplates an IL-6 antagonist that sterically blocks the interaction with gp130 and whose binding to IL-6 is not affected by pre-bound sIL-6Ralpha. "IL-6 antagonists" of the present invention include antibodies or fragments thereof; functional equivalents of antibodies; modified antibodies, such as, single chain antibodies, chimeric

antibodies; or other proteins or molecules capable of binding to or associating with IL-6 or IL-6/IL-6Ralpha complex to sterically hinder binding to gp130.

[0048] In a preferred embodiment, the IL-6 antagonist of the invention contains an antibody variable region that binds to a region or an epitope on IL-6 or IL-6/IL-6Ralpha complex such that the binding sterically blocks the interaction between IL-6 and gp130. More preferably, the IL-6 antagonist of the invention is an antibody. For example, the binding of the antibody variable region to any regions or epitopes directly participating in the interaction between the IL-6/IL-6Ralpha complex and gp130 is sufficient to sterically interfere with binding to gp130. In addition, the binding to any regions or epitopes adjacent to those directly participating in the interaction may also be sufficient to sterically block the interaction between IL-6/IL-6Ralpha and gp130. Such regions or epitopes may exist on IL-6, IL-6Ralpha, or as composite sites only formed by the complex of IL-6 and IL-6Ralpha. In particular, suitable epitopes to which binding may sterically block the interaction between IL-6 and gp130 include, but are not limited to, any epitopes including at least one of the following amino acids: Leu19, Arg24, Lys27, Arg30, Tyr31, Asp34 and Trp157 of human IL-6.

Generation of Antibodies Sterically Blocking the Interaction Between IL-6 and gp130

[0049] Thus, one important feature of the invention is the isolation of antibodies that bind to IL-6/IL-6Ralpha complex and sterically block the IL-6/gp130 interaction. Such antibodies may be polyclonal or monoclonal. According to the invention, such antibodies may be generated by the following method. In a first step, a mouse, rat, rabbit, or other mammal is immunized with a protein composition comprising IL-6 and IL-6Ralpha. It is preferable to have the IL-6 and IL-6Ralpha forming a complex. To facilitate the formation of the complex, IL-6 and IL-6Ralpha may be covalently bound by, for example, chemical cross-linking or by connection through a polypeptide linker. Without wishing to be bound by theory, the goal of such an immunization is that the only exposed surfaces of IL-6 are either surfaces bound by gp130 or are non-neutralizing surfaces. Antibodies that are sterically blocked by IL-6Ralpha should not arise. Particularly preferred immunogens include covalently linked IL-6 and soluble IL-6Ralpha, which may be made by binding IL-6 and IL-6Ralpha *in vitro* and then

treating with a chemical crosslinking agent according to standard procedures, or by expressing IL-6 and soluble IL-6Ralpha as a fusion protein, preferably attached by a linker, for example as described by Peters *et al.* (J. Immunol., (1998) 161:3575-81, the teaching of which are hereby incorporated by reference). According to the invention, typically, an immunogen fusion protein including IL-6 and IL-6Ralpha also includes an additional moiety that facilitates antigen presentation, such as an Fc region. The protein composition may be administered to a mammal with or without adjuvant according to any of a variety of standard methods. The composition may be administered only once, but is preferably administered more than once according to standard boosting schedules.

[0050] As a second step, polyclonal antiserum is harvested from the immunized mammal. The polyclonal serum may be used directly, or may be affinity-purified according to standard methods. Alternatively, the process of generating monoclonal antibodies is initiated. Antibody-producing cells are removed from the immunized animal, for example by surgical removal of the spleen or withdrawal of PBMCs and subsequent sorting. Potential antibody-producing cells are then immortalized by fusion with an immortalized cell line according to standard procedures, cloned into microtiter wells, and screening for production of antibodies that bind to the immunogen.

[0051] In another embodiment, as a second step, a display library is generated by isolation of appropriate cells from non-immunized or immunized animals followed by isolation of nucleic acids encoding antibody V regions, insertion of the V region-encoding nucleic acids into phages, yeast, bacteria, or other replicable genetic display systems. The library members are then screened for their capability to bind to IL-6/IL-6Ralpha complexes.

[0052] In some embodiment, as a third step, the antibodies produced by monoclonal cell lines or the antibody V regions selected from the library are optionally subjected to secondary screens as follows. Specifically, the antibody clones or the antibody V regions are tested for the ability to bind to IL-6 alone, to bind to IL-6Ralpha alone, to bind to IL-6/IL-6Ralpha complex, and for the ability to inhibit the interaction between an IL-6/IL-6Ralpha complex and gp130. From the results of these tests, the antibodies or antibody V regions can be classified into several groups: neutralizing antibodies that bind to IL-6 and block the interaction

with gp130, antibodies that bind to IL-6Ralpha, non-neutralizing antibodies that bind to IL-6, neutralizing antibodies that bind to IL-6/IL-6Ralpha complex but not to IL-6 or IL-6Ralpha alone and block the interaction with gp130, and neutralizing antibodies that bind to IL-6 and block the interaction with IL-6Ralpha. Antibodies of each class except the last are expected. Such binding and signaling assays are well known in the art of protein biochemistry and signal transduction, and specific embodiments are further detailed in the Examples.

Identification of Antibodies not Significantly Extending Serum Half-Life of IL-6

[0053] One undesired effect of anti-IL-6 antibodies is that they often prolong the serum half-life of IL-6. The molecular weight of IL-6 is about 25,000 Daltons, which is well below the renal clearance threshold of 50,000 Daltons, while the molecular weight of an antibody-IL-6 complex is over 150,000 Daltons. The formation of anti-IL-6 antibody/antigen complexes generally has the effect of lengthening the serum half-life of IL-6 because the molecular weight of the complex is greater than the renal clearance threshold. Thus, the present invention also provides methods to identify antibodies that bind to IL-6 or IL-6/IL-6Ralpha complex but do not significantly extend the serum half-life of IL-6.

[0054] The method involves, as a first step, the isolation of a panel of anti-IL-6 antibodies. As a second step, the antibodies are then tested for their effect on IL-6 serum half-life, for example as follows. IL-6 is administered to an animal such as a rodent. It is convenient to use a labeled form of IL-6, such as radioactive IL-6. An antibody to be tested is also administered to the same animal, preferably by a different route of administration. As a negative control, PBS is administered in place of either the antibody or the IL-6. Serum samples are obtained at various times after administration of the proteins, and tested for levels of IL-6 and the antibody according to standard techniques. For example, radiolabelled, iodinated IL-6 may be quantitated using a radiation counter, and the antibody may be detected by an ELISA method based on IL-6 capture.

[0055] It is contemplated that some antibodies have an extended pharmacokinetic profile compared to others, and some antibodies cause an enhancement of the pharmacokinetics of IL-6 while other antibodies cause only a moderate extension or essentially none at all. Depending on the particular application, one class of preferred antibodies of the invention are those that

themselves have a favorable pharmacokinetic profile, but do not significantly extend the pharmacokinetic profile of IL-6.

Sequences of Antibody Variable Regions

[0056] The sequences of antibody V regions identified according to the methods described above are characterized using standard sequencing methods known in the art. An exemplary method is described in detail in Example 4. Exemplary sequences of antibody heavy chain and light chain V regions that bind to IL-6 and block its interaction with gp130 are shown in Figure 2. Figure 2 also illustrates an alignment of the antibody V region sequences identified according to the present invention. Position variation among the sequences are indicated with arrows. The CDR regions are boxed.

[0057] The V regions of the invention may be configured with human constant regions to form a chimeric antibody. An exemplary chimeric antibody may include VH and VL regions described in Figure 2 and constant regions derived from IgG1, IgG2, IgG3, IgG4, IgA, IgD, or IgM. Alternatively, the antibodies of the invention may be expressed with hybrid isotype constant regions, as described in PCT publication WO 02/072605, the disclosures of which are hereby incorporated by reference. The V regions of the invention may also be configured as Fab moieties, "minibodies" lacking the CH2 domain, or as single-chain Fv moieties. These latter configurations are smaller than whole antibodies and have enhanced diffusion characteristics, which may be useful in situations requiring efficient tissue penetration, such as in treatment of tumors that secrete IL-6.

Expression

[0058] The antibodies and proteins containing antibody variable regions of the invention are preferably expressed in mammalian cells, such as NS/0 cells, CHO cells, SP2/0 cells, BHK cells, or other mammalian cells. The expression of antibodies in mammalian cells is well known in the art of protein engineering. The antibodies may also be expressed in plant cells such as corn or tobacco, insect cells such as via a baculovirus vector, fungal cells such as *S. cerevisiae* or *Pichia pastoris*, or in bacterial cells, which are most useful in expressing smaller configurations such as single-chain Fv molecules.

Administration

[0059] The IL-6 antagonists of the invention are used in the treatment of a variety of diseases and disorders involving expression of IL-6. Such diseases and disorders include, but are not limited to, cancers such as hormone-independent prostate cancer, B-cell proliferative disorders such as B cell non-Hodgkin's lymphoma, and advanced cancers of kidney, breast, colon, lung, brain, and other tissues; antibody-driven autoimmune disorders such as rheumatoid arthritis, myasthenia gravis, systemic lupus erythematosis, and other autoimmune diseases; or osteoporosis. The molecules of the invention may also be used to treat cachexia in cancer patients, which often results from overproduction of IL-6 and IL-6Ralpha by tumors.

[0060] The antagonists of the invention may cause a target-related side effect of immunosuppression, particularly inhibition of antibody formation. When a patient is receiving an antibody of the invention, it is often useful to supplement the treatment with a prophylatic anti-infective agent. Such prophylactic treatments are well known in the art of treating immunosuppressed patients.

[0061] An antagonist of the invention is typically administered by infusion, but may also be administered by subcutaneous, intradermal, intramuscular, or intraperitoneal injection, inhalation, or oral administration. For a 70 kilogram adult human, a dose in the range of about 50 to 2000 milligrams is preferred, with a dose in the range of 100-800 milligrams more preferred, and a dose of about 300-600 milligrams is most preferred.

[0062] The precise dose may be adjusted on a patient-by-patient basis. For example, when treating a solid tumor in a patient, the effectiveness of a given dose may be evaluated as follows. At various points after administration of an antibody of the invention, biopsies of the tumor are withdrawn and tested for gp130 activation, for example by immunostaining with an appropriate anti-phosphotyrosine antibody. The goal is to have continuous, essentially complete inhibition of gp130 activation in the tumor. If inhibition of gp130 activation is not complete, the dose may be increased or the dosage frequency may be increased.

[0063] It should be understood that the above-described embodiments and the following examples are given by way of illustration, not limitation. Various changes and modifications within the scope of the present invention will become apparent to those skilled in the art from the present description.

EXAMPLES

Example 1. Expression of an IL-6/IL-6Ralpha complex for use as an antigen

[0064] To generate antibodies that bind to a surface of IL-6 that interferes with its binding to gp130 and is accessible in an IL-6/IL-6Ralpha complex, a fusion protein comprising an Fc domain, the extracellular domains of IL-6Ralpha, and IL-6 was expressed from a plasmid termed pdCs-Fc-IL6Ralpha-IL6. The fusion protein is referred to herein as Fc-IL6Ralpha-IL6. The Fc domain was derived from mouse $IgG_{\gamma 2a}$, and the IL-6Ralpha and IL-6 were based on the human sequences. The sequence of this protein and the DNA encoding this protein are shown below.

Protein sequence of Fc-IL6Ralpha-IL6 (SEQ ID NO:37):

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQ
ISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIER
TISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNT
EPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGSgdddddk
l**PPEEPQLSCFRKSPLSNVVCEWGPRSTPSLTTKAVLLVRKFQNSPAEDFQEPCQYSQES**
QKFSCQLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCGILQPDPPANITVTAVARNP
RWLSVTWQDPHSWNSSFYRLRFELRYRAERSKTFTTWMVKDLQHHCVIHDAWSGLRHVVQ
LRAQEEFGQGEWSEWSPEAMGTPWTESRSPPArggggsggggsvepvppg*EDSKDVAAPH*
RQPLTSSERIDKQIRYILDGISALRKETCNKSNMCESSKEALAENNLNLPKMAEKDGCFQ
SGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQARAVQMSTKVLIQFLQKKAKNLDA
ITTPDPTTNASLLTKLQAQNQWLQDMTTHLILRSFKEFLQSSLRALRQM

Underlined sequence: murine Fc IgG_{γ}
Lower case sequence: linker containing enterokinase cleavage site
Bold sequence: human IL6Ralpha
Lower case sequence underlined: linker
Italic sequence: human IL6

DNA sequence encoding mature Fc-IL6Ralpha-IL6 (SEQ ID NO:38):

GAGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATGCCCAGCACCTAACCTC
TTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCC
CTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAG
ATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAG
GATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATG
AGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCGAGAGA
ACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCA
GAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCCT
GAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACT
GAACCAGTCCTGGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAG
AAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAAT
CACCACACGACTAAGAGCTTCTCCCGGACCCCGGGTTCAGGGGATGACGATGACGATAAG
CTTCCCCCCGAGGAGCCCCAGCTCTCCTGCTTCCGGAAGAGCCCCCTCAGCAATGTTGTT
TGTGAGTGGGGTCCTCGGAGCACCCCATCCCTGACGACAAAGGCTGTGCTCTTGGTGAGG
AAGTTTCAGAACAGTCCGGCCGAAGACTTCCAGGAGCCGTGCCAGTATTCCCAGGAGTCC
CAGAAGTTCTCCTGCCAGTTAGCAGTCCCGGAGGGAGACAGCTCTTTCTACATAGTGTCC
ATGTGCGTCGCCAGTAGTGTCGGGAGCAAGTTCAGCAAAACTCAAACCTTTCAGGGTTGT
GGAATCTTGCAGCCTGATCCGCCTGCCAACATCACAGTCACTGCCGTGGCCAGAAACCCC
CGCTGGCTCAGTGTCACCTGGCAAGACCCCCACTCCTGGAACTCATCTTTCTACAGACTA
CGGTTTGAGCTCAGATATCGGGCTGAACGGTCAAAGACATTCACAACATGGATGGTCAAG
GACCTCCAGCATCACTGTGTCATCCACGACGCCTGGAGCGGCCTGAGGCACGTGGTGCAG
CTTCGTGCCCAGGAGGAGTTCGGGCAAGGCGAGTGGAGCGAGTGGAGCCCGGAGGCCATG
GGCACGCCTTGGACAGAATCCAGGAGTCCTCCAGCTAGAGGGGGCGGGGGCAGTGGGGGC
GGGGGCAGTGTAGAACCGGTACCCCCAGGAGAAGATTCCAAAGATGTAGCTGCCCCACAC
AGACAGCCACTCACCTCTTCAGAACGAATTGACAAACAAATTCGGTACATCCTCGACGGC
ATCTCAGCCCTGAGAAAGGAGACATGTAACAAGAGTAACATGTGTGAAAGCAGCAAAGAG
GCACTGGCAGAAAACAACCTGAACCTTCCAAAGATGGCTGAAAAAGATGGATGCTTCCAA
TCTGGATTCAATGAGGAGACTTGCCTGGTGAAAATCATCACTGGTCTTTTGGAGTTTGAG
GTATACCTAGAGTACCTCCAGAACAGATTTGAGAGTAGTGAGGAACAAGCCAGAGCTGTG
CAGATGAGTACAAAAGTCCTGATCCAGTTCCTGCAGAAAAAGGCAAAGAATCTAGATGCA
ATAACCACCCCTGACCCAACCACAAATGCCAGCCTGCTGACGAAGCTGCAGGCACAGAAC

CAGTGGCTGCAGGACATGACAACTCATCTCATTCTGCGCAGCTTTAAGGAGTTCCTGCAG
TCCAGCCTGAGGGCTCTTCGGCAAATGTAG

[0065] For rapid analysis of protein expression to characterize the Fc-IL6Ralpha-IL6 fusion protein product, the plasmid pdCs-Fc-IL6Ralpha-IL6 was introduced into human embryonic kidney HEK 293 cells (ATCC# CRL-1573) by transient transfection using lipofectamine (Invitrogen).

[0066] To obtain stably transfected clones which express Fc-IL6Ralpha-IL6, the appropriate plasmid DNA was introduced into the mouse myeloma NS/0 cells by electroporation. NS/0 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine and penicillin/streptomycin. About $5x10^6$ cells were washed once with PBS and resuspended in 0.5 ml PBS. 10 µg of linearized plasmid DNA were then incubated with the cells in a Gene Pulser® Cuvette (0.4 cm electrode gap, BioRad) on ice for 10 min. Electroporation was performed using a Gene Pulser® (BioRad, Hercules, CA) with settings at 0.25 V and 500 µF. Cells were allowed to recover for 10 min on ice, after which they were resuspended in growth medium and plated onto two 96 well plates. Stably transfected clones were selected by their growth in the presence of 100 nM methotrexate (MTX), which was added to the growth medium two days post-transfection. The cells were fed every 3 days for two to three more times, and MTX-resistant clones appeared in 2 to 3 weeks. Supernatants from clones were assayed by anti-Fc ELISA to identify high producers. High producing clones were isolated and propagated in growth medium containing 100 nM MTX. The growth medium typically used was H-SFM or CD medium (Life Technologies).

[0067] The Fc-IL6Ralpha-IL6 fusion protein was subsequently captured from the medium for further analysis. For routine characterization by gel electrophoresis, the Fc-IL6Ralpha-IL6 fusion proteins secreted into the medium was captured on Protein A Sepharose® beads (Repligen, Cambridge, MA) and then eluted by boiling the sample in protein sample buffer, with or without a reducing agent such as β-mercaptoethanol. The samples were analyzed by SDS-PAGE and the protein bands were visualized by Coomassie staining.

[0068] It will be recognized by those skilled in the art that any of a variety of proteins could be used as alternatives to the Fc-IL6Ralpha-IL6 protein

described above. For example, other configurations of IL-6Ralpha and IL-6 could be used, such as IL-6Ralpha-IL-6-Fc, albumin-IL-6Ralpha-IL-6, cytokine-IL-6Ralpha-IL-6, where the cytokine is chosen to stimulate an immune response against the IL-6/IL-6Ralpha complex. Proteins comprising a cytokine, an Fc moiety, and an IL-6Ralpha/IL-6 complex may also be used, for example, according to the methods of Gillies *et al.* (WO01/07081, the disclosures of which are hereby incorporated by reference). Finally, IL-6 and IL-6Ralpha may be produced separately, chemically cross-linked, and used as an antigen. When an Fc moiety and/or a secondary cytokine moiety are used, it is generally advantageous that these moieties be from the animal that is being immunized, such as a mouse.

[0069] In preparation for the characterization of the antibodies described below, DNAs encoding Fc-IL6 and Fc-IL6Ralpha were constructed similarly as described above. The corresponding proteins were purified similarly as described above. In addition, human IL-6, human IL-6Ralpha, and human gp130-Fc were purchased from R&D Systems, Inc. for use in certain experiments below. Schematic depictions of these proteins used in the experiments are shown in Figure 3. The protein and DNA sequences of relevant constructs are provided as follows.

Mature Fc-IL6Ralpha (SEQ ID NO:39)

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQ
ISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIER
TISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNT
EPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGSGDDDDDK
LPPEEPQLSCFRKSPLSNVVCEWGPRSTPSLTTKAVLLVRKFQNSPAEDFQEPCQYSQES
QKFSCQLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCGILQPDPPANITVTAVARNP
RWLSVTWQDPHSWNSSFYRLRFELRYRAERSKTFTTWMVKDLQHHCVIHDAWSGLRHVVQ
LRAQEEFGQGEWSEWSPEAMGTPWTESRSPPA

DNA encoding mature Fc-IL6Ralpha (SEQ ID NO:40)

GAGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATGCCCAGCACCTAACCTC
TTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCC
CTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAG
ATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAG
GATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATG
AGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCGAGAGA
ACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCA
GAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCCT
GAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACT
GAACCAGTCCTGGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAG
AAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAAT
CACCACACGACTAAGAGCTTCTCCCGGACCCCGGGTTCAGGGGATGACGATGACGATAAG
CTTCCCCCCGAGGAGCCCCAGCTCTCCTGCTTCCGGAAGAGCCCCCTCAGCAATGTTGTT
TGTGAGTGGGGTCCTCGGAGCACCCCATCCCTGACGACAAAGGCTGTGCTCTTGGTGAGG
AAGTTTCAGAACAGTCCGGCCGAAGACTTCCAGGAGCCGTGCCAGTATTCCCAGGAGTCC
CAGAAGTTCTCCTGCCAGTTAGCAGTCCCGGAGGGAGACAGCTCTTTCTACATAGTGTCC
ATGTGCGTCGCCAGTAGTGTCGGGAGCAAGTTCAGCAAAACTCAAACCTTTCAGGGTTGT
GGAATCTTGCAGCCTGATCCGCCTGCCAACATCACAGTCACTGCCGTGGCCAGAAACCCC
CGCTGGCTCAGTGTCACCTGGCAAGACCCCCACTCCTGGAACTCATCTTTCTACAGACTA
CGGTTTGAGCTCAGATATCGGGCTGAACGGTCAAAGACATTCACAACATGGATGGTCAAG
GACCTCCAGCATCACTGTGTCATCCACGACGCCTGGAGCGGCCTGAGGCACGTGGTGCAG
CTTCGTGCCCAGGAGGAGTTCGGGCAAGGCGAGTGGAGCGAGTGGAGCCCGGAGGCCATG
GGCACGCCTTGGACAGAATCCAGGAGTCCTCCAGCTTAG

Mature Fc-IL6 (SEQ ID NO:41)

EPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQ
ISWFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIER
TISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNT
EPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGKEDSKDVA
APHRQPLTSSERIDKQIRYILDGISALRKETCNKSNMCESSKEALAENNLNLPKMAEKDG

CFQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQARAVQMSTKVLIQFLQKKAKN
LDAITTPDPTTNASLLTKLQAQNQWLQDMTTHLILRSFKEFLQSSLRALRQM

DNA encoding mature Fc-IL6 (SEQ ID NO:42)

GAGCCCAGAGGGCCCACAATCAAGCCCTGTCCTCCATGCAAATGCCCAGCACCTAACCTC
TTGGGTGGACCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATGATCTCC
CTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGATGTCCAG
ATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAGAGAG
GATTACAACAGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGACTGGATG
AGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGACCTCCCAGCGCCCATCGAGAGA
ACCATCTCAAAACCCAAAGGGTCAGTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCA
GAAGAAGAGATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTCATGCCT
GAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACAGAGCTAAACTACAAGAACACT
GAACCAGTCCTGGACTCTGATGGTTCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAG
AAGAACTGGGTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTGCACAAT
CACCACACGACTAAGAGCTTCTCCCGGACCCCGGGTAAAGAAGATTCCAAAGATGTAGCT
GCCCCACACAGACAGCCACTCACCTCTTCAGAACGAATTGACAAACAAATTCGGTACATC
CTCGACGGCATCTCAGCCCTGAGAAAGGAGACATGTAACAAGAGTAACATGTGTGAAAGC
AGCAAAGAGGCACTGGCAGAAAACAACCTGAACCTTCCAAAGATGGCTGAAAAAGATGGA
TGCTTCCAATCTGGATTCAATGAGGAGACTTGCCTGGTGAAAATCATCACTGGTCTTTTG
GAGTTTGAGGTATACCTAGAGTACCTCCAGAACAGATTTGAGAGTAGTGAGGAACAAGCC
AGAGCTGTGCAGATGAGTACAAAAGTCCTGATCCAGTTCCTGCAGAAAAAGGCAAAGAAT
CTAGATGCAATAACCACCCCTGACCCAACCACAAATGCCAGCCTGCTGACGAAGCTGCAG
GCACAGAACCAGTGGCTGCAGGACATGACAACTCATCTCATTCTGCGCAGCTTTAAGGAG
TTCCTGCAGTCCAGCCTGAGGGCTCTTCGGCAAATGTAG

Example 2. Immunization with an IL-6/IL-6Ralpha complex.

[0070] Twenty mice (Balb/C) were immunized with the Fc-IL6Ralpha-IL6 protein produced as described in Example 1, and monoclonal antibodies to this protein were produced according to a modification of the method of Kohler and Milstein (1975) (Nature, 256:495-7). Specifically, 1 microgram of Fc-IL6Ralpha-IL6 was injected subcutaneously with 100 microliters of complete Freund's adjuvant. Injections were repeated 14 days later, using 1 microgram of protein

injected intraperitoneally with 100 microliters of incomplete Freund's adjuvant. 24 days after the first injection, mice were boosted with 1 microgram of Fc-IL6Ralpha-IL6 protein in 100 microliters of PBS intravenously. Three days later mice were sacrificed and spleens excised, and spleen cells cultured according to standard procedures. 435 million spleen cells from two mice with strong polyclonal anti-IL6Ralpha/IL6 responses were fused with 175 million NS/0 cells at a ratio of 2.4 spleen cells to 1 NS/0 cell. Immortalized B cell/NS/0 cell hybrids were produced according to standard procedures, and hybridomas were then screened for the production of antibodies against the Fc-IL6Ralpha-IL6 fusion protein using ELISA Technology.

Example 3. Screening for antibodies that block the interaction between the IL-6/IL-6Ralpha complex and gp130

Binding

[0071] Positive clones from Example 2 were further tested as follows. The isotype of the antibody was determined, and IgM-based clones were not characterized further. IgG-based monoclonal antibodies were tested for their ability to bind to either IL-6 or IL-6Ralpha using immobilized Fc-IL6 and Fc-IL6Ralpha according to standard procedures. Some clones bound to IL-6, some bound to IL-6Ralpha, and some bound to neither proteins, suggesting that these monoclonals might recognize some portion of the linker or might recognize composite epitopes consisting both IL-6 and IL-6Ralpha moieties.

[0072] Exemplary results of the binding characteristics of typical antibodies of the invention are shown in Figure 4 (binding to Fc-IL6Ralpha-IL6), Figure 5 (binding to IL-6), Figure 6 (binding to non-covalently complexed IL-6 and IL-6Ralpha), and Figure 7 (IL-6Ralpha alone). The results indicate that, for this set of antibodies, binding to IL-6 and covalently linked IL-6 and IL-6Ralpha was similar, binding to a non-covalent IL-6/IL-6Ralpha complex gave a less strong signal (possibly because of dissociation of IL-6 from IL-6R during washing steps), and binding to IL-6Ralpha alone could not be detected.

Competition tests

[0073] To further characterize the antibodies that recognize IL-6, the following competition tests were performed. First, the ability of the monoclonal antibodies to inhibit the interaction between Fc-IL6Ralpha-IL6 and gp130-Fc was

tested. The inhibition assays were performed based on the methods described by Scheller *et al.* J. Immuno. Methods, 291:93-100 (2004), the teachings of which are hereby incorporated by reference. Four antibodies, named Mab#195, Mab#309, Mab#471, and Mab#476 were identified that blocked this interaction. Second, inhibition of the interaction between Fc-IL6 and Fc-IL6Ralpha by the antibodies was tested. None of the antibodies were found to inhibit this interaction, which was expected based on the fact that the antibodies derived from an immunization with Fc-IL6Ralpha-IL6 and underwent an initial screen for binding to Fc-IL6Ralpha-IL6.

[0074] Typical results illustrating the inhibition of the interaction between Fc-IL6Ralpha-IL6 and gp130-Fc are shown in Figure 8.

Cell-based assay

[0075] Antibodies Mab#195, Mab#309, Mab#471, and Mab#476 were tested for their ability to block haptoglobin release from HepG2 cells stimulated with an Fc-IL6Ralpha-IL6 complex. Haptoglobin is a protein secreted by liver cells during inflammatory states. HepG2 cells are a liver cell line. The release of haptoglobin from HepG2 cells provides a convenient bioassay for the activity of IL-6Ralpha/IL-6 complexes.

[0076] The assay was performed as follows. HepG2 cells were plated at 0.1×10^6 cells per well in a 96-well plate, and allowed to grow in DMEM supplemented with 10% Fetal Bovine Serum (FBS) medium overnight. Cells were then washed with PBS, and incubated in starving medium, DMEM without FBS, for 1 hour at 37 °C. The cells were then incubated in stimulating medium, DMEM (without FBS) in the presence of 8 ng/ml Fc-IL6Ralpha-IL6 complex, and various concentrations of test antibody supplemented for 22 hours. Supernatants were withdrawn and the levels of haptoglobin were determined by an ELISA for haptoglobin detection. A standard ELISA procedure was followed, using a goat anti-human haptoglobin antibody (Sigma #H5015) for capture, a mouse anti-human haptoglobin antibody (US Biological #H1820-05) as a primary and an anti-mouse IgG-HRP antibody (Promega #W402B) as a secondary antibody. Typical results are shown in Figure 9.

Biacore analysis

[0077] The binding of antibodies Mab#195, Mab#309, Mab#471, and Mab#476 to IL-6 was quantitatively characterized using a Biacore machine. The antibodies were immobilized on a chip; IL-6 protein was passed over the chip, and on-rates and off-rates were measured. The following results were obtained.

Parameter	mAb#195	mAb#309	mAb#471	mAb#476
k_a (1/Ms)	2.4×10^6	8×10^5	1.8×10^6	$2.5\text{-}2.6 \times 10^6$
k_d (1/s)	4.8×10^{-3}	1.1×10^{-4}	1.4×10^{-5}	$1.2\text{-}2.6 \times 10^{-4}$
K_D (pM)	2000	135	7.5	47-106

Inhibition of proliferation of cancer cell lines.

[0078] The antibodies of the invention were tested for their ability to inhibit the proliferation of A431 cells and LP-1 cells. Inhibition of the proliferation of LP-1 cells is described in Example 8. Inhibition of proliferation of A431 was measured as follows. On day 1, cells were plated in 96-well plates at 25,000 cells/well in DMEM containing 10% FBS, with 200 microliters per well. On day 3, cells were washed once with 200 microliters of PBS. Cells were starved for one hour at 37 °C in 100 microliters of DMEM.

[0079] On day 3, dilutions of anti-IL-6 antibodies were prepared in 96 U-bottom plates in DMEM containing IL6Ralpha-IL6-His6 (33 ng/ml), with all proteins prepared as 2X dilution because they were later transferred to the plates containing the cells. Controls included DMEM, DMEM-1% FBS, and IL6Ralpha-IL6-His6 in 0%FBS. The plates were incubated with the dilutions of proteins for 1 h at 37 °C, after which 100 microliters of protein mixes were transferred to the starved cells.

[0080] On day 5, cells in each well were washed twice with 200 microliters of PBS, and then 100 microliters of a solution to measure acid phosphatase. The solution was 0.1 M sodium acetate pH 5.5, 0.1% Triton X-100, 2.5 mg/ml paranitrophenylphosphate. The plates were incubated at 37 °C for 1 hour, after which the reaction was stopped with 100 microliters of 0.1 N NaOH, and the plate was read at 410 nm..

[0081] Typical results were illustrated in Figures 10A, 10B-1 and 10B-2. As shown in Figures 10B-1 and 10B-2, the antibodies of the invention inhibit the proliferation of A431 human epithelial carcinoma cells seen with stimulation by IL-6/IL-6Ralpha fusion protein. A typical result of proliferation of A431 cells stimulated by IL-6/IL-6Ralpha fusion protein is shown in Figure 10A.

Example 4. V region sequences of antibodies that bind to IL-6 and block interaction with gp130.

[0082] The V region sequences of monoclonal antibodies Mab#195, Mab#309, Mab#471, and Mab#476 were determined according to standard procedures. mRNA of each hybridoma clones were purified using the Dynabeads Direct mRNA kit (Dynal) following the manufacturer's instructions. Reverse-Transcription PCR (RT-PCR) was performed to obtain cDNA using the BD SMART™ cDNA synthesis kit (BD Clontech) according to manufacturer manual. Two successive PCRs were performed using the cDNA as template, nested oligonucleotides and the polymerase KOD (EMD Biosciences) as instructed by the manufacturer. The 3' oligonucleotides were specific to amplify VH and Vk of a mouse IgGγ1 antibody while the 5' oligonucleotides were nested oligonucleotides from the BD SMART™ cDNA synthesis kit (sequences added to the 5' during the RT-PCR).

[0083] For example, heavy chain and light chain V regions were obtained by PCR amplification using a constant region primer and a V region primer with the oligonucleotides sequences and conditions indicated below.

VH amplification:

PCR#1

5' Oligonucleotide #1: 5' ACAACGCAGAGTACGCGG 3' (SEQ ID NO:43)

3' Oligonucleotide #1: 5' AGGAGAGCTGGGAAGGTGTG 3' (SEQ ID NO:44)

PCR#2

5' Oligonucleotide#2: 5' ACAACGCAGAGTACGCGG 3' (SEQ ID NO:45)

3' Oligonucleotide #2: 5' TAGCCCTTGACCAGGCATCCC 3' (SEQ ID NO:46)

Vk amplification:

PCR#1

5' Oligonucleotide #1: 5' ACAACGCAGAGTACGCGG 3' (SEQ ID NO:47)

3' Oligonucleotide #1: 5' CTGCCATCAATCTTCCACTTGAC 3' (SEQ ID NO:48)

PCR#2

5' Oligonucleotide#2: 5' CATCCTCTCTTCCAGCTCTC 3' (SEQ ID NO:49)

3' Oligonucleotide #2: 5' CTGAGGCACCTCCAGATG 3' (SEQ ID NO:50)

PCR #1	2min	94°C		PCR #2	2min	94°C
	30sec	90°C			30sec	90°C
	30sec	65°C	x30		30sec	65°C X40
	30 sec	72°C			30 sec	72°C
		72 °C				72 °C

[0084] PCR products were purified from agarose gel using the QIAquick gel extraction kit (QIAGEN) and subcloned into the TOPO blunt pCR4 vector (Invitrogen) for sequencing. Sequences were obtained using primers T7 and T3 and standard sequencing procedures.

[0085] The light chain and heavy chain sequences including the V region sequences of Mab#195, Mab#309, Mab#471, and Mab#476 are shown below.

MAb #195 VH mature region (SEQ ID NO:33)

EVKLEESGGGLVQPGGSMKLSCVASGFTFSNYWMNWVRQSPEKGLEWVAEIRLKSNNYAT
HYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYCTREDYYGYPDYWGQGTTLTVSS

MAb #195 VK mature region (SEQ ID NO:29)

DIVLTQSPASLAVSLGQRATISCRASESVDNFGISFMNWFQQKPGQPPKLLIYVASNQGS
GVPARFSGSGSGTDFSLNIHPMEEDDTAMYFCQQSKEVPWTFGGGTKLEIK

MAb #309 VH mature region (SEQ ID NO:34)

EVKLEESGGGLVQPGGSMKLSCVASGFTFSNYWMNWVRQSPEKGLEWVAEIRLKSNKGAT
HYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYCASLLYDGYLHWGQGTLVTVSA

MAb #309 VK mature region (SEQ ID NO:30)

DIVLTQSPASLAVSLGQRATISCRASESVGNFGISFMNWFQQKPGQPPKLLIYTASNQGS
GVPARFSGSGSGTDFSLNIHPMEEDDSAMYFCQQSKEVPWTFGGGTKLEIK

MAb #471 VH mature region (SEQ ID NO:35)

EVKFEESGGGLVQPGGSMKLSCVASGFSFSNYWMNWVRQSPEKGLEWVAEIRLTSNKQAI
YYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYCASLFYDGYLHWGQGTLVTVSA

MAb #471 VK mature region (SEQ ID NO:31)

DIVLTQSPASLAVSLGQRATISCRASESVGNFGISFMNWFQQKPGQPPKLLIYTASNQGS
GVPARFSGSGSGTDFSLNIHPMEEDDSAMYFCQQSKEIPWTFGGGTKLEIK

MAb #476 VH mature region (SEQ ID NO:36)

EVKLEESGGGLVQPGGSMKLSCAASGFTFSDAWMDWVRQSPEKGLEWVAEIRSKANNHAT
YYAESVKGRFTISRDDSKSSVYLQMNSLRAEDTGIYYCTTPTLYGAMDYWGQGTSVTVSA

MAb #476 VK mature region (SEQ ID NO:32)

DIVLTQSPASLAVSLGQRATISCRASESVHNFGISFMNWFQQKPGQPPKLLIYTASNQGS
GVPARFSGSGSGTDFSLNIHPVEEDDTAMYFCQQGKEVPWTFGGGTKLEIK

DNA encoding mAb #195 VH mature region (SEQ ID NO:51)

GAAGTGAAGCTTGAGGAGTCTGGAGGAGGCTTGGTGCAACCTGGAGGATCCATGAAACTC
TCCTGTGTTGCCTCTGGATTCACTTTCAGTAACTACTGGATGAACTGGGTCCGCCAGTCT
CCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATTAGATTGAAATCTAATAATTATGCAACA
CATTATGCGGAGTCTGTGAAAGGGAGGTTCACCATCTCAAGAGATGATTCCAAAAGTAGT
GTCTACCTGCAAATGAACAACTTAAGAGCTGAAGACACTGGCATTTATTACTGTACCAGG
GAGGACTACTACGGCTACCCTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA

DNA encoding mAb #195 VK mature region (SEQ ID NO:52)

GACATTGTGCTGACCCAGTCTCCAGCTTCTTTGGCTGTGTCTCTAGGTCAGAGGGCCACC
ATCTCCTGCAGAGCCAGCGAAAGTGTTGATAATTTTGGCATTAGTTTTATGAACTGGTTC
CAACAGAAACCTGGACAGCCACCCAAACTCCTCATCTATGTTGCATCCAACCAAGGATCC
GGGGTCCCTGCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTTCAGCCTCAACATCCAT
CCTATGGAGGAGGATGATACTGCAATGTATTTCTGTCAGCAAAGTAAGGAGGTTCCGTGG
ACGTTCGGTGGAGGCACCAAGCTGGAAATCAAA

DNA encoding mAb #309 VH mature region (SEQ ID NO:53)

GAAGTGAAACTTGAGGAGTCTGGAGGAGGCTTGGTTCAACCTGGAGGATCCATGAAACTC
TCCTGTGTTGCCTCTGGATTCACTTTCAGTAACTACTGGATGAACTGGGTCCGCCAGTCT
CCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATTAGACTGAAATCTAATAAGGGTGCAACA
CATTATGCGGAGTCTGTGAAAGGGAGGTTCACCATCTCAAGGGATGATTCCAAAAGTAGT
GTCTACCTGCAAATGAACAACTTAAGAGCTGAAGACACTGGCATTTATTACTGTGCCAGC
CTTTTGTATGATGGTTACTTACATTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA

DNA encoding mAb #309 VK mature region (SEQ ID NO:54)

GACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACC
ATCTCCTGCAGAGCCAGCGAAAGTGTTGGTAATTTTGGCATTAGTTTTATGAATTGGTTC
CAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATACTGCATCCAACCAAGGATCC
GGGGTCCCTGCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTTCAGCCTCAACATCCAT
CCTATGGAGGAGGATGATTCTGCAATGTATTTCTGTCAGCAAAGTAAGGAGGTTCCGTGG
ACGTTCGGTGGAGGCACCAAACTGGAAATCAAA

DNA encoding mAb #471 VH mature region (SEQ ID NO:55)

GAAGTGAAGTTTGAGGAGTCTGGAGGAGGCTTGGTGCAACCGGGAGGATCCATGAAACTC
TCCTGTGTTGCCTCTGGATTCAGTTTCAGTAACTACTGGATGAACTGGGTCCGCCAGTCT
CCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATTAGATTGACATCTAATAAGCAGGCAATA
TATTATGCGGAGTCTGTGAAAGGGAGATTCACCATCTCAAGAGATGATTCCAAAAGTAGT
GTCTACCTGCAAATGAACAACCTAAGAGCTGAAGACACTGGCATTTATTACTGTGCCAGC
CTTTTCTATGATGGTTACTTACATTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA

DNA encoding mAb #471 VK mature region (SEQ ID NO:56)

GACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGGCCACC
ATCTCCTGCAGAGCCAGCGAAAGTGTTGGTAATTTTGGCATTAGTTTTATGAACTGGTTC
CAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATACTGCATCCAACCAAGGATCC
GGGGTCCCTGCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTTCAGCCTCAACATCCAT
CCTATGGAGGAGGATGATTCTGCAATGTATTTCTGTCAGCAAAGTAAGGAGATTCCGTGG
ACGTTCGGTGGAGGCACCAAACTGGAAATCAAA

DNA encoding mAb #476 VH mature region (SEQ ID NO:57)

GAAGTGAAGCTTGAGGAGTCTGGAGGAGGCTTGGTGCAACCTGGAGGATCCATGAAACTC
TCTTGTGCTGCCTCTGGATTCACTTTTAGTGACGCCTGGATGGACTGGGTCCGCCAGTCT
CCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATTAGAAGTAAAGCTAATAATCATGCAACA

TACTATGCTGAGTCTGTGAAAGGGAGGTTCACCATCTCAAGAGATGATTCCAAAAGTAGT
GTCTACCTGCAAATGAACAGCCTAAGAGCTGAAGACACTGGCATTTATTACTGTACGACC
CCTACTCTCTATGGCGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCTGCA

DNA encoding mAb #476 VK mature region (SEQ ID NO:58)

GACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTTGGGCAGAGGGCCACC
ATCTCCTGCAGAGCCAGCGAAAGTGTTCATAATTTTGGCATTAGCTTTATGAACTGGTTC
CAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATACTGCATCCAACCAAGGATCC
GGGGTCCCTGCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTTCAGCCTCAACATCCAT
CCTGTGGAAGAGGATGATACTGCAATGTATTTCTGTCAACAAGGTAAGGAGGTTCCGTGG
ACGTTCGGTGGAGGCACCAAGCTGGAAATCAAAC

[0086] The sequences of the V regions were aligned as shown in Figure 2. Position variations among the sequences are indicated with arrows. The CDR regions are boxed. Based on the alignment, it is apparent that each antibody represents an independent isolate, and that the antibodies are similar to each other. Antibodies 309 and 471 are closely related, with only one substitution in the light chain, an Ile/Val substitution at position 98; and only six substitutions in the heavy chain. These antibodies may derive from an original IgM clone that diversified through somatic mutation and thus might not be truly independent.

[0087] Antibodies 195 and 476 are more similar to each other than to antibodies 309 and 471. Antibodies 195 and 476 differ at 5 positions in the light chain and 18 positions in the heavy chain. Analysis of the CDR3 in the heavy chain of the antibodies 195 and 476 suggested that these chains were formed by independent V-D-J joining events and thus represent antibodies deriving from independent IgM parents. Thus, the antibody sequences in Figure 2 represent at least 3 independent selections of antibodies that bind to human IL-6 and block the interaction with gp130.

Example 5. Pharmacokinetic properties of antibodies that bind to IL-6 and block interaction with gp130.

[0088] The serum half-life of antibodies 195 and 476 in mice was determined. Antibodies 195 and 476 were labeled with ^{125}I according to standard procedures. About 25 micrograms of labeled antibody protein was injected

intravenously into Balb/C mice, and blood samples were withdrawn at various times including 12, 24, 48, and 72 hours after injection. Levels of radioactivity in whole blood samples were determined. Based on this analysis, the elimination half-life was about 5 days for each antibody. Exemplary Data are shown in Figure 11.

Example 6. *In vivo* inhibition of haptoglobin secretion

[0089] The antibodies of the invention were selected specifically for their ability to inhibit the binding of IL-6 to the receptor subunit gp130. In this experiment, using an assay measuring haptoglobin secretion induced by administration of a soluble IL-6Ralpha/IL-6 complex, the ability of antibody Mab#471 and a commercial anti IL-6 antibody to block activation of a gp130–dependent pathway *in vivo* were compared.

[0090] On day 0, nine-week old female Balb/C mice (n=3 per treatment group) were injected intraperitoneally with 100 µg of either antibody Mab#471 or an anti-IL-6 commercial antibody from R&D Systems (R&D Systems MAb #206) in a 200 µl volume. Mice in positive and negative control groups received 200 µl PBS. After 24 hours, mice in the experimental and positive control groups were administered 4 µg muFc-IL6Ralpha-IL6 intra-peritoneally in a 200 µl volume to induce haptoglobin secretion, and mice in the negative control group were administered 200 µl PBS. At 0, 8, and 24 hours after treatment, approximately 100 µl of blood was obtained from each mouse by retro-orbital bleeding, and the plasma fraction was isolated. Haptoglobin concentration in the plasma fraction was determined using a murine haptoglobin ELISA kit (Immunology Consultants Laboratory, Inc., Newberg, OR, Cat # E90HPT), following manufacturer's instructions.

[0091] As shown in Figure 12, haptoglobin levels in antibody Mab#471-treated mice were significantly less, about 30% of the level seen in the positive control group at 24 hours, and also significantly lower than in mice treated with the commercial antibody MAb #206. The increased levels of haptoglobin seen in mice of the negative control group were likely due to irritation caused by repeated retro-orbital bleeding procedure. Thus, the actual inhibition of haptoglobin secretion specifically caused by Fc-IL6Ralpha-IL6 is even greater than 70%, when the background of haptoglobin secretion seen in the control mice is

subtracted. These results demonstrate that the antibody of the invention, *e.g.*, antibody #471, which binds to IL-6 and blocks its interaction with the gp130 receptor subunit, is effective at inhibiting gp130-dependent signaling pathways that can be activated by a pre-formed IL-6Ralpha/IL-6 complex.

Example 7. Anti-tumor activity *in vivo.*

[0092] Antibodies Mab#195, Mab#309, Mab#471 and Mab#476 were tested for anti-tumor activity *in vivo*. A lung metastasis model was set up in SCID mice using PC3-MM2 cells which secrete IL-6 and for which IL-6 is a growth factor. About 2.0 x 10^6 PC3-MM2 cells were injected *i.v.* into each mouse. After 11 days the mice were treated with five daily doses of 5 µg/ml of the monoclonal antibodies Mab#195, Mab#309, Mab#471 and Mab#476. Typical results are shown in Figure 13. As illustrated in Figure 13, antibodies Mab#195, Mab#309, Mab#471 and Mab#476 inhibit lung metastases in mice.

[0093] Another experiment was done using antibody treatment after only 5 days post cell injection, instead of the usual 10 days. Similar results were obtained.

Example 8. Construction of anti-IL-6 antibodies with human constant regions.

[0094] A chimeric antibody against IL-6 that includes the V regions from antibody Mab#471 and human constant regions was constructed by techniques as described in U.S. Patent No. 6,969,517, to Gillies *et al.*, the teachings of which are hereby incorporated by reference. DNA sequences encoding the V regions of Mab#471 were obtained by PCR amplification using the following oligonuceotides. The lowercase regions are for adapters and the uppercase regions are V region specific.

VL forward (with Afl II site): cttaagcGACATTGTGCTGACCCAATC
(SEQ ID NO:59)
VL reverse (with Bgl II site): agatctacttacgTTTGATTTCCAGTTTGGTGCC
(SEQ ID NO:60)
VH forward (with Afl II site): cttaagcGAAGTGAAGTTTGAGGAGTC
(SEQ ID NO:61)

VH reverse (with Hind III site): aagcttacttaccTGCAGAGACAGTGACCAG (SEQ ID NO:62)

[0095] The resulting mouse-derived sequences were inserted into an antibody expression vector as described in U.S. Patent No. 6,969,517, Example 3, to generate a expression plasmid encoding a chimeric antibody with a human kappa light chain and a human IgG1 heavy chain.

[0096] In order to obtain stably transfected human cell clones, the plasmid DNA was introduced into the mouse myeloma NS/0 cells by electroporation as described below. NS/0 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. About $5x10^6$ cells were washed once with PBS and re-suspended in 0.5 ml phosphate buffer solution (PBS). Ten µg of linearized plasmid DNA was then incubated with the cells in a Gene Pulser® Cuvette (0.4 cm electrode gap, BioRad) for 10 minutes on ice. Electroporation was performed using a Gene Pulser® (BioRad) with settings at 0.25 V and 500 µF Cells were allowed to recover for 10 minutes on ice, after which they were resuspended in growth medium and then plated onto two 96-well plates. Stably transfected clones were selected by growth in the presence of 100 nM methotrexate (MTX), which was introduced two days post-transfection. The cells were fed every 3 days for two to three more times, and MTX-resistant clones appeared in 2 to 3 weeks. Supernatants from clones were assayed by anti-human Fc ELISA to identify high producers (Gillies *et al.* (1989) J. Immunol. Methods, 125:191). High producing clones were isolated and propagated in growth medium containing 100 nM MTX.

[0097] To confirm that the chimeric 471 antibody retained the desired properties, this molecule was tested for inhibition of haptoglobin production *in vitro*, as described in Example 6 above; for inhibition of proliferation of LP-1 myeloma cells; and for inhibition of haptoglobin production in vivo, as described in Example 6 above. As controls, the anti-IL-6 antibodies Mab 206 (R&D Systems; Minneapolis, Minnesota) and CNTO-328 (Zaki M.H. *et al.*, Int. J. Cancer, (2004) 111:592-5) were used.

[0098] Effects of chimeric 471 antibody (Ch anti IL-6 #471) produced from a stably transfected cell line on haptoglobin secretion are indicated in the table

below. Similar results were obtained from chimeric 471 antibody produced from transiently transfected cells.

	Haptoglobin Inhibition		
	IC50(ng/ml)		
	AVG	SD	Repetitions
Ch anti IL-6 # 471 + IL-6	1.89	0.42	2
MAb 206+ IL-6	16.08	12.48	2
Ch anti IL-6 CNTO-328 + IL-6	5.19	0.88	2
Ch anti IL-6 # 471 + IL-6Rα-IL-6	1.31	0.57	3
MAb 206+ IL-6Rα-IL-6	4.80	2.64	3
Ch anti IL-6 CNTO-328 + IL-6Rα-IL-6	>333	0.00	3

[0099] The results indicate that the chimeric antibody 471 is effective at inhibiting the function of both IL-6 and the IL-6/IL-6Ralpha fusion protein. In contrast, Mab#206 is relatively ineffective at inhibiting haptoglobin secretion stimulated by either IL-6 or IL-6/IL-6Ralpha fusion protein, and CNTO-328 shows a profound defect in inhibition of haptoglobin secretion stimulated by IL-6/IL-6Ralpha fusion protein.

[00100] The chimeric antibody 471 was also tested for its ability to inhibit proliferation of LP-1 myeloma cells. LP-1 is a human myeloma cell line whose proliferation can be stimulated by IL-6.

[00101] The LP-1 cell proliferation assay was performed as follows. LP-1 cells were purchased from the DSMZ (cat # ACC 41) (Georgii-Hemming P. *et al.* Blood (1996) 88:2250). Cells were cultured in 20% FBS and then starved for 3 days in 1% FBS media before the proliferation assay. After starvation, the cells were washed three times and diluted into 0.5% FBS containing media. Anti-IL-6 antibodies were diluted and incubated in the plate with either 0.005 ng/ml IL-6 or 0.05 ng/ml Fc-IL6Ralpha-IL6 fusion protein stimulation for one hour at 37 °C 5% CO_2. Then about 100,000 cells in 100µl were added to wells of a 96 well plate with 100 µl of diluted proteins plus stimulation, incubated for 56 hours, and then

^{3}H Thymidine was added for the last 16 hours. The cells were then harvested from the wells with water onto glass microfiber filter plates and radioactivity was measured by liquid scintillation counting.

[00102] The table below shows typical results with chimeric 471 antibody produced from a stably transfected cell line. Similar results were obtained from chimeric 471 antibody produced from transiently transfected cells.

	LP-1 Proliferation		
	IC50(ng/ml)		
	AVG	SD	Repetitions
Ch anti IL-6 # 471 + IL-6	5.15	2.27	3
MAb 206+ IL-6	341.87	234.58	3
Ch anti IL-6 CNTO-328 + IL-6	10.07	1.42	3
Ch anti IL-6 # 471 + IL-6Rα-IL-6	0.39	0.36	3
MAb 206+ IL-6Rα-IL-6	7.77	8.08	3
Ch anti IL-6 CNTO-328 + IL-6Rα-IL-6	1608	539	3

[00103] The results indicate that the chimeric 471 antibody is effective at inhibiting LP-1 proliferation stimulated by both IL-6 and the IL-6/IL-6Ralpha fusion protein. In contrast, Mab#206 is ineffective at inhibiting LP-1 proliferation stimulated by IL-6, and CNTO-328 shows a profound defect in inhibition of LP-1 proliferation stimulated by IL-6/IL-6Ralpha fusion protein.

[00104] The inhibitory effects of chimeric 471 antibody and various control antibodies on haptoglobin secretion *in vivo* were also tested as described in Example 6.

The following results were obtained.

	0 hours ug/ml Hapatoglobin		8 Hours ug/ml Hapatoglobin		24 Hours ug/ml Hapatoglobin		8 Hours % Inhi-bition	24 hours % Inhi-bition	TTEST VS PBS	
	AVG	SD	AVG	SD	AVG	SD			8 Hrs	24 Hrs
No treatment	0.8	0.1	0.8	0.9	4.2	4.7				
PBS	0.6	0.1	24	17	113	170				
Fc-IL6Rα-IL6	0.8	0.1	463	128	994	125				
Ch 471	1.0	0.2	83	61	214	176	**82**	**78**	0.01	0.00
CNTO 328	0.8	0.2	406	58	1027	91	12	-3	0.52	0.73
R&O MAB206	0.6	0.3	297	71	672	58	36	32	0.12	0.02
Anti-CD19 chB4	0.8	0.1	487	138	1335	346	-5	-34	0.86	0.18

[00105] These results indicate that chimeric 471 antibody (referred to as Ch 471 in the above table) strongly blocks haptoglobin secretion stimulated by an IL-6/IL-Ralpha complex, while control antibodies such as Mab#206 and CNTO-328 are less effective or ineffective at inhibition of haptoglobin secretion.

Example 9. Treatment of a human patient with antibodies and methods of the invention.

[00106] The anti-IL-6 antibodies of the invention are used to treat human diseases and disorders as follows. In general, the preferred method of administration is by *i.v.* infusion or *i.v.* injection, although subcutaneous injection, inhalation, oral delivery, and other methods are also possible. Administration about once every 2, 3 or 4 weeks is used, although the frequency of administration may vary depending on the needs of the patient. A typical dose is about 100 to 800 mgs for an adult human. Treated patients are monitored for signs of infection that may result from immunosuppression.

[00107] For example, a patient with Castleman's disease is treated with chimeric 471 antibody of the invention about once every two weeks at a dose of about 8 mg/kg, with administration by drip infusion.

[00108] A patient with rheumatoid arthritis is treated with chimeric 471 antibody about once every four weeks at a dose of about 8 mg/kg, with administration by drip infusion. Progression of joing destruction is found to be significantly inhibited by monotherapy, even when compared to disease-modifying anti-rheumatic drugs.

[0100] A patient with Crohn's disease is treated with chimeric 471 antibody about once every four weeks at a dose of about 8 mg/kg, with administration by drip infusion.

[0101] A patient with multiple myeloma is treated with chimeric 471 antibody about once every three weeks at a dose of about 8 mg/kg, with administration by drip infusion. Treatment with chimeric 471 is combined with a standard-of-care treatment for multiple myeloma as determined by a physician as appropriate for the patient.

[0102] A patient with advanced metastatic prostate cancer, with a history of treatment by conventional chemotherapy, is treated with chimeric 471 antibody about once every three weeks at a dose of about 8 mg/kg, with administration by drip infusion. Treatment with chimeric 471 antibody is combined with a standard-of-care treatment for prostate cancer as determined by a physician as appropriate for the patient. Non-steroidal anti-inflammatory drugs, for example Naproxen™ are also prescribed. As a result of prior chemotherapy, the patient has depressed white cells and low levels of naïve T cells. The patient is monitored particularly closely for infection resulting from immunosuppression and is given prophylactic antibiotics. It is found that the treatment has a positive effect on cachexia-type symptoms, such as bone loss.

[0103] A patient with hormone-refractory breast cancer is treated with chimeric 471 antibody about once every three weeks at a dose of about 8 mg/kg by drip infusion. Treatment with chimeric 471 antibody is combined with a standard-of-care treatment for advanced breast cancer as determined by a physician as appropriate for the patient. Non-steroidal anti-inflammatory drugs, for example Naproxen™ are also prescribed.

[0104] In an alternative treatment strategy, a patient with advanced hormone-refractory prostate cancer or advanced hormone-refractory breast cancer is treated with chimeric 471 antibody about once every three weeks at a dose of about 8 mg/kg, in combination with an immunocytokine such as KS-IL2.

These two agents may be co-administered by drip infusion. Prior to the treatment, the patient is dosed with an immunostimulatory amount of cyclophosphamide. Non-steroidal anti-inflammatory drugs, for example Naproxen™ are also prescribed. Without wishing to be bound by theory, the combination of an anti-IL6 antibody of the invention and an immunocytokine such as KS-IL2 is particularly effective, in part because IL-6 causes a suppression of IL-12 signaling and TH1 responses, and the antibodies of the invention reverse this inhibition.

[0105] A patient with a B cell lymphoma is treated is treated with chimeric 471 antibody about once every three weeks at a dose of about 8 mg/kg, optionally in combination with an antibody such as Rituxan™ at about 375 milligrams per square meter of body surface area, which is administered every week. Alternatively, in the case of a patient with refractory lymphoma, treatment with chimeric 471 antibody is combined with a radioimmunoconjugate such as Bexxar™ or Zevalin™.

Patent Claims:

1. An anti-IL6 antibody or a fragment thereof comprising an antibody variable region, wherein the antibody variable region binds to an epitope on IL-6 in a way that the binding sterically blocks the interaction between IL-6 and gp130 on the surface of a diseased cell.

2. An anti-IL6 antibody of claim 1, wherein said binding prevents that IL-6 complexed with IL-6Ralpha binds to gp130 but does not sterically block the interaction between IL-6 and IL-6Ralph.

3. An anti-IL6 antibody of claim 1 or 2, wherein said epitope on IL-6 comprises an amino acid selected from the group consisting of Leu19, Arg24, Lys27, Arg30, Tyr31, Asp34 and Trp157.

4. An anti-IL6 antibody of any of the claims 1 – 3, wherein the antibody variable region comprises a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of
FTFSNYWMN (SEQ ID NO:2),
FSFSNYWMN (SEQ ID NO:3), and
FTFSDAWMD (SEQ ID NO:4).

5. An anti-IL6 antibody of any of the claims 1 – 3, wherein the antibody variable region comprises a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of
EIRLKSNNYATHYAESVKG (SEQ ID NO:6),
EIRLKSNKGATHYAESVKG (SEQ ID NO:7),
EIRLTSNKQAIYYAESVKG (SEQ ID NO:8), and
EIRSKANNHATYYAESVKG (SEQ ID NO:9).

6. An anti-IL6 antibody of any of the claims 1 – 3, wherein the antibody variable region comprises a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of
EDYYGYPDY (SEQ ID NO:11),

LLYDGYLH (SEQ ID NO:12),
LFYDGYLH (SEQ ID NO:13), and
PTLYGAMDY (SEQ ID NO:14).

7. An anti-IL6 antibody of any of the claims 1 – 3, wherein the antibody variable region comprises a light chain CDR1 comprising an amino acid sequence selected from the group consisting of
RASESVDNFGISFM (SEQ ID NO:16),
RASESVGNFGISFM (SEQ ID NO:17),
RASESVHNFGISFM (SEQ ID NO:18), and
RASESVDNYGISFM (SEQ ID NO:19).

8. An anti-IL6 antibody of any of the claims 1 – 3, wherein the antibody variable region comprises a light chain CDR2 comprising an amino acid sequence selected from the group consisting of
TASNQGS (SEQ ID NO:21),
VASNQGS (SEQ ID NO:22), and
AASNQGS (SEQ ID NO:23).

9. An anti-IL6 antibody of any of the claims 1 – 3, wherein the antibody variable region comprises a light chain CDR3 comprising an amino acid sequence selected from the group consisting of
QQSKEVPWT (SEQ ID NO:25),
QQSKEVPYT (SEQ ID NO:26),
QQSKEIPWT (SEQ ID NO:27), and
QQGKEVPWT (SEQ ID NO:28).

10. An anti-IL6 antibody of any of the claims 4 – 6 comprising
a heavy chain CDR1 of
SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, and
a heavy chain CDR2 of
SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; or SEQ ID NO:9; and
a heavy chain CDR3 of
SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13 or SEQ ID NO:14.

11. An anti-IL6 antibody of any of the claims 7 – 9 comprising
a light chain CDR1 of
SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, or SEQ ID NO:19, and
a light chain CDR2 of
SEQ ID NO:21, SEQ ID NO:22, or SEQ ID NO:23, and
a light chain CDR3 of
SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 or SEQ ID NO:28.

12. An anti-IL6 antibody of claim 10 or 11 comprising
a heavy chain CDR1 of
SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4, and
a heavy chain CDR2 of
SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; or SEQ ID NO:9; and
a heavy chain CDR3 of
SEQ ID NO:11; SEQ ID NO:12; SEQ ID NO:13 or SEQ ID NO:14,
and
a light chain CDR1 of
SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, or SEQ ID NO:19, and
a light chain CDR2 of
SEQ ID NO:21, SEQ ID NO:22, or SEQ ID NO:23, and
a light chain CDR3 of
SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 or SEQ ID NO:28.

13. An anti-IL6 antibody of any of the claims 1 – 12 further comprising an antibody constant region.

14. An anti-IL6 antibody of claim 13 comprising an Fc moiety.

15. An anti-IL6 antibody of claim 14, wherein the Fc moiety is human.

16. An anti-IL6 antibody of any of the claims 13 – 15, wherein the antibody comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32.

17. An anti-IL6 antibody of claim 16, wherein the antibody comprises a light chain amino acid sequence of SEQ ID NO:31, or a sequence that is at least 90% identical to SEQ ID NO:31.

18. An anti-IL6 antibody of any of the claims 13 – 15, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of
SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36.

19. An anti-IL6 antibody of claim 18, wherein the antibody comprises a heavy chain comprising an amino acid sequence of SEQ ID NO:35, or a sequence that is at least 90% identical to SEQ ID NO:35.

20. An anti-IL6 antibody of any of the claims 16 – 19 comprising a heavy chain comprising an amino acid sequence of SEQ ID NO:35 and a light chain comprising an amino acid sequence of SEQ ID NO:31.

21. An anti-IL6 antibody of any of the claims 16 – 19 comprising a heavy chain comprising an amino acid sequence of SEQ ID NO:34 and a light chain comprising an amino acid sequence of SEQ ID NO:30.

22. An anti-IL6 antibody of any of the claims 16 – 19 comprising a heavy chain comprising an amino acid sequence of SEQ ID NO:36 and a light chain comprising an amino acid sequence of SEQ ID NO:32.

23. An anti-IL6 antibody of any of the claims 16 – 19 comprising a heavy chain comprising an amino acid sequence of SEQ ID NO:33 and a light chain comprising an amino acid sequence of SEQ ID NO:29.

24. A nucleic acid molecule encoding an antibody of any of the claims 1 – 23.

25. A pharmaceutical composition suitable for the treatment of an IL6 triggered disease comprising in a pharmacologically effective amount an anti-IL6

antibody according to any of the claims 1 – 23 optionally together with a pharmaceutically acceptable carrier, diluent or excipient.

26. Use of an anti-IL6 antibody according to any of the claims 1 – 23 for the manufacture of a medicament for the treatment of cancer or an autoimmune disease.

27. Use of claim 26, wherein the disease is triggered by IL6 or IL6 complexed with IL-6Ralpha.

28. A fusion protein comprising
(i) a Fc moiety of an antibody,
(ii) IL6Ralpha and
(iii) IL6.

29. A fusion protein of claim 28, wherein IL6Ralpha is fused to the C-terminus of the Fc moiety, and IL6 is fused to the C-terminus of IL6Ralpha.

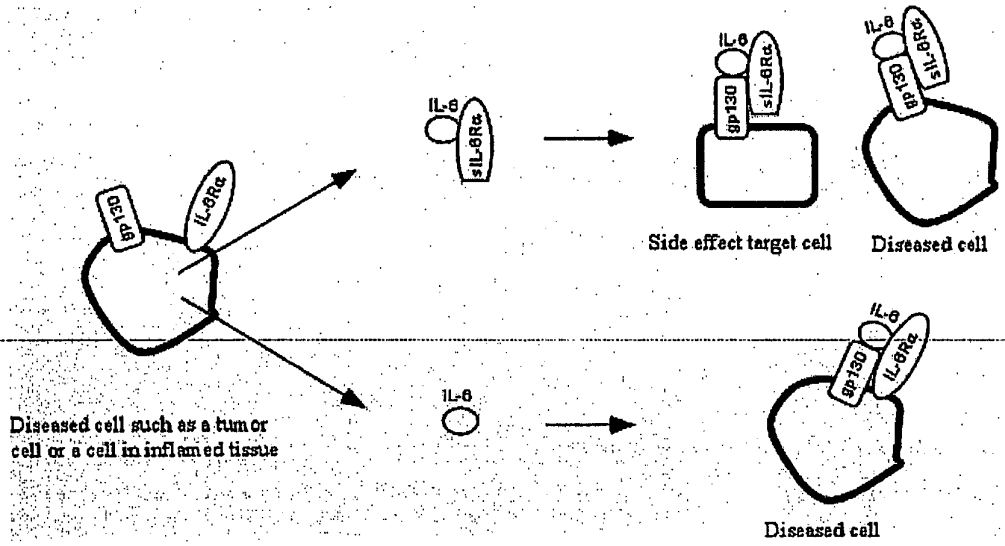
30. A fusion protein of claim 28 or 29, wherein the Fc moiety is murine and IL6Ralpha and IL6 is human.

31. Use of a fusion protein of any of the claims 28 – 30 for the manufacture of an anti-IL6 antibody obtained by immunizing a mammal with said fusion protein, wherein the antibody screened has the following properties:
(i) the variable region of said antibody binds to an epitope on IL-6,
(ii) sterically blocks the interaction between IL-6 and gp130 on the surface of a diseased cell, and
(ii) prevents that IL-6 complexed with IL-6Ralpha binds to gp130, but does not sterically block the interaction between IL-6 and IL-6Ralpha.

32. Use of claim 31, wherein the antibody screened is an antibody of any of the claims 1 – 23.

Figure 1. The method of treatment of the invention.

A. No treatment



B. Treatment with an antibody of the invention

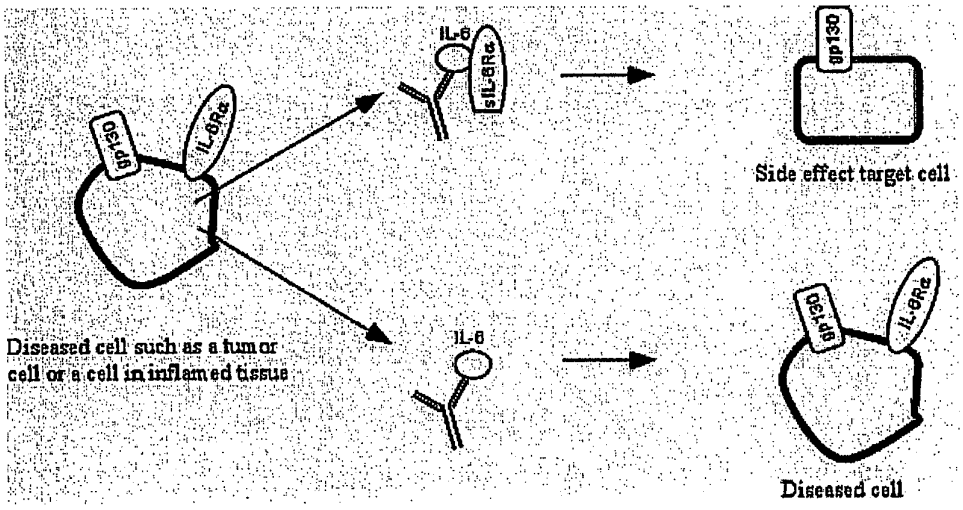
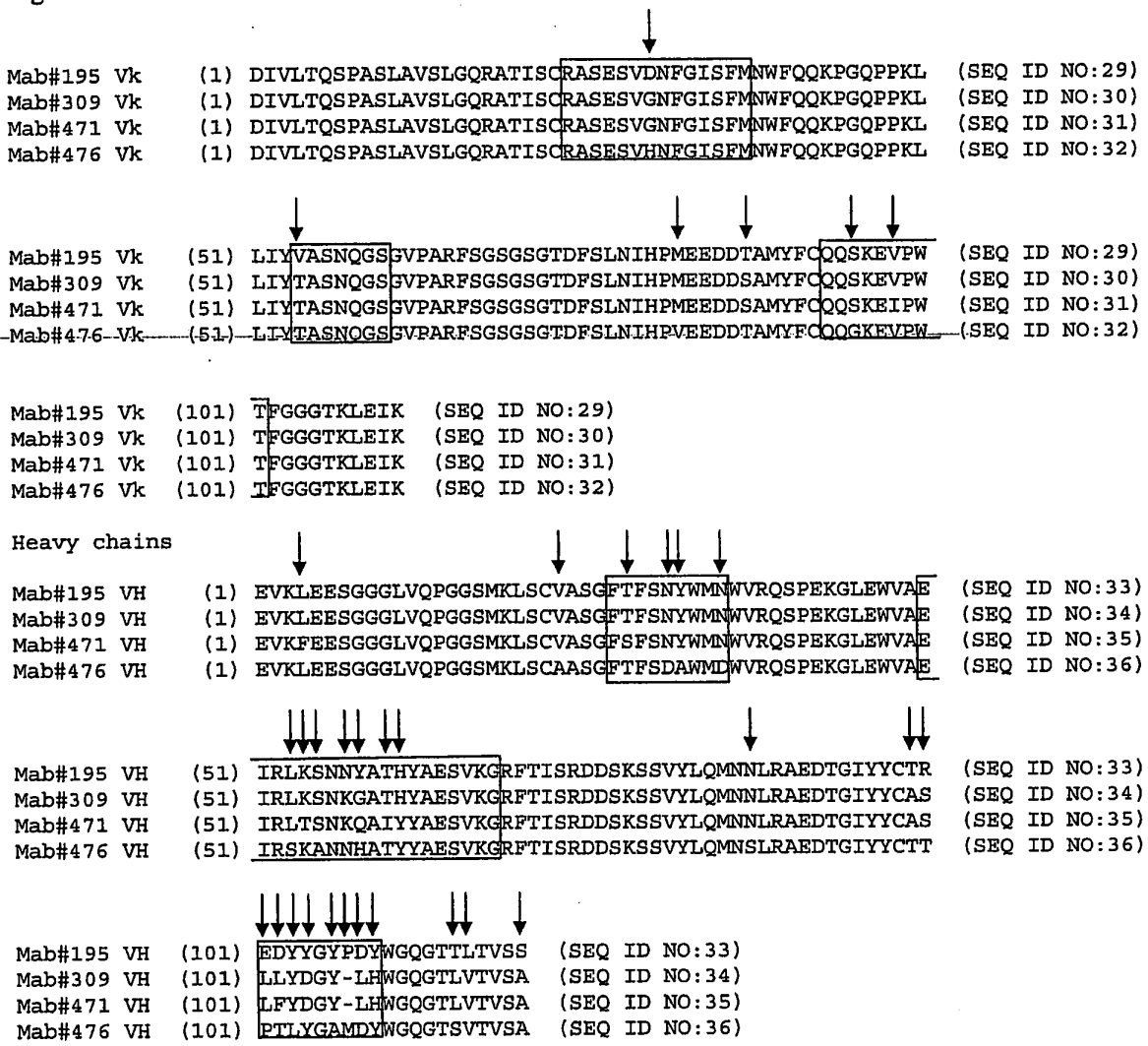


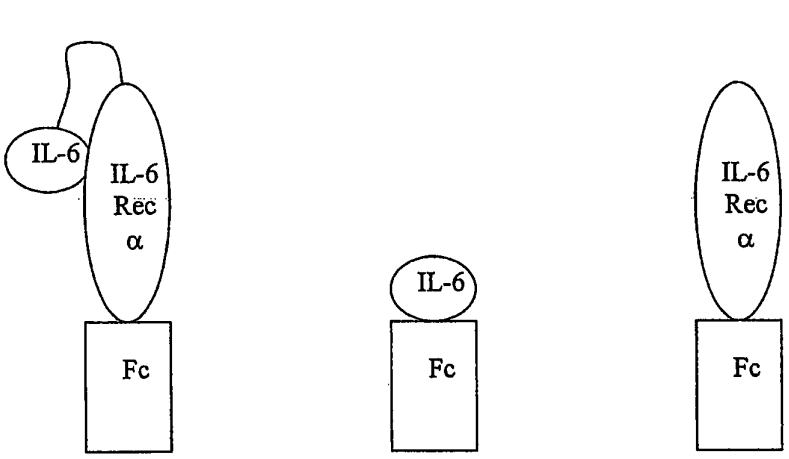
Figure 2. Alignment of antibody V region sequences that bind to IL-6 and block interaction with gp130. Positions variation among the sequences are indicated with arrows. The CDRs are boxed.

Light chains



Heavy chains

Figure 3. Schematic depiction of the proteins used herein.



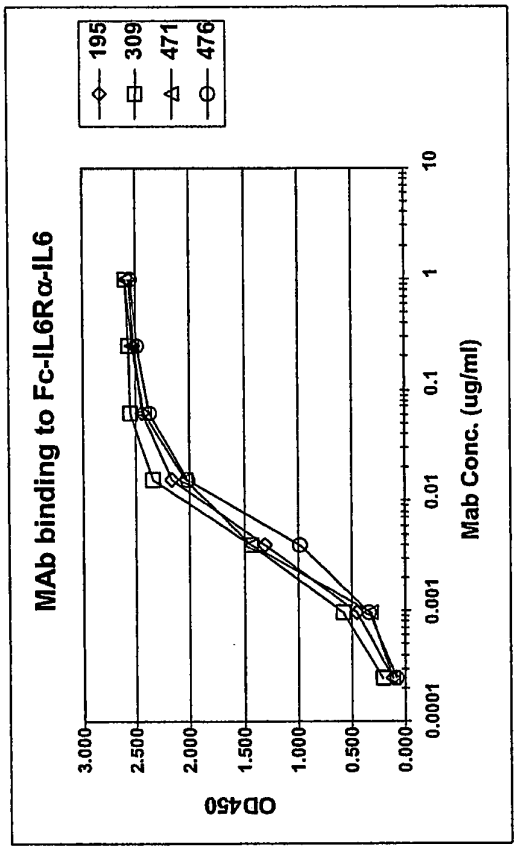

MAb binding to Fc-IL6Rα-IL6
195
309
471
476
OD450
3.000
2.500
2.000
1.500
1.000
0.500
0.000
0.0001
0.001
0.01
0.1
1
10
Mab Conc. (ug/ml)

Figure 4

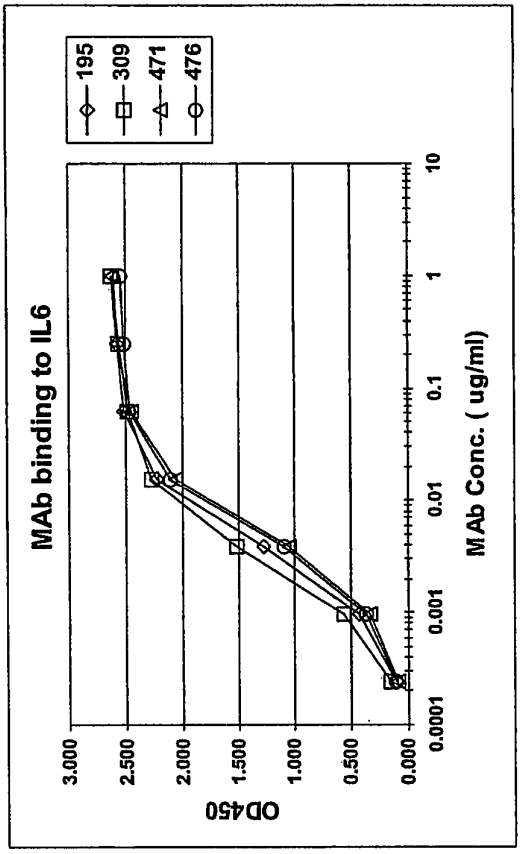

MAb binding to IL6
195
309
471
476
OD450
3.000
2.500
2.000
1.500
1.000
0.500
0.000
0.0001
0.001
0.01
0.1
1
10
MAb Conc. (ug/ml)

Figure 5

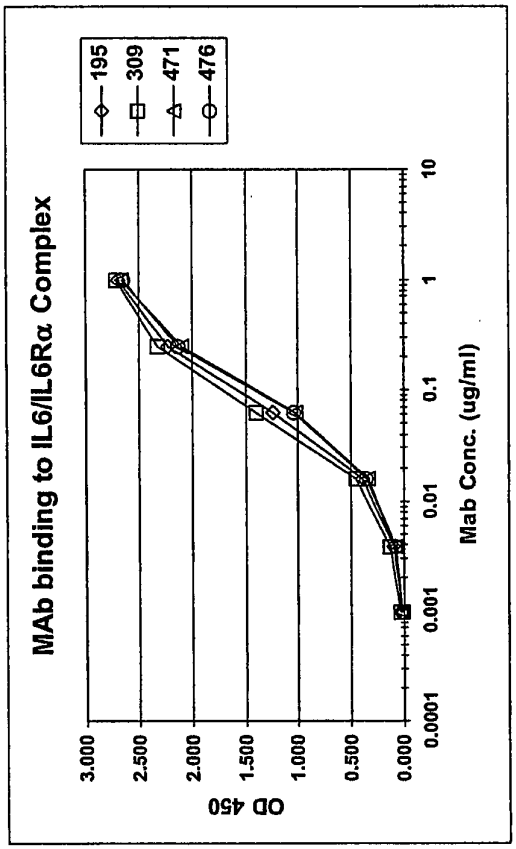

MAb binding to IL6/IL6Rα Complex
195
309
471
476
OD 450
3.000
2.500
2.000
1.500
1.000
0.500
0.000
0.0001
0.001
0.01
0.1
1
10
Mab Conc. (ug/ml)

Figure 6

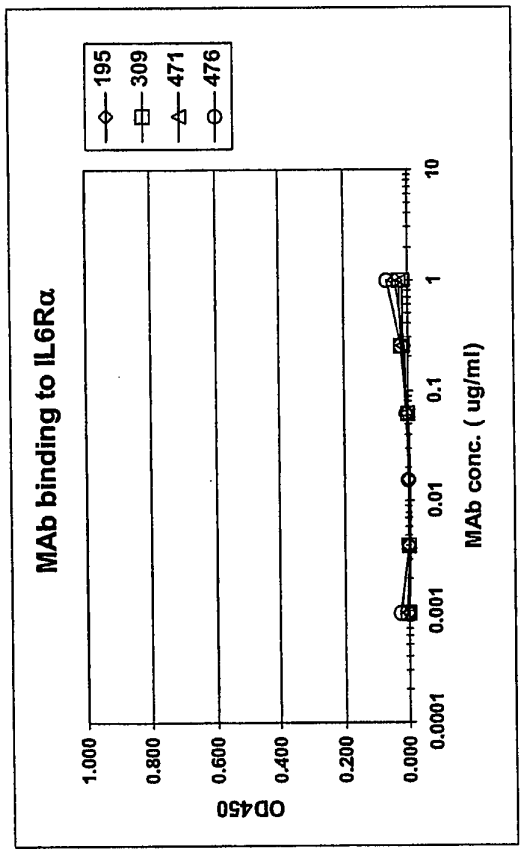

MAb binding to IL6Rα
195
309
471
476
OD450
1.000
0.800
0.600
0.400
0.200
0.000
0.0001
0.001
0.01
0.1
1
10
MAb conc. (ug/ml)

Figure 7

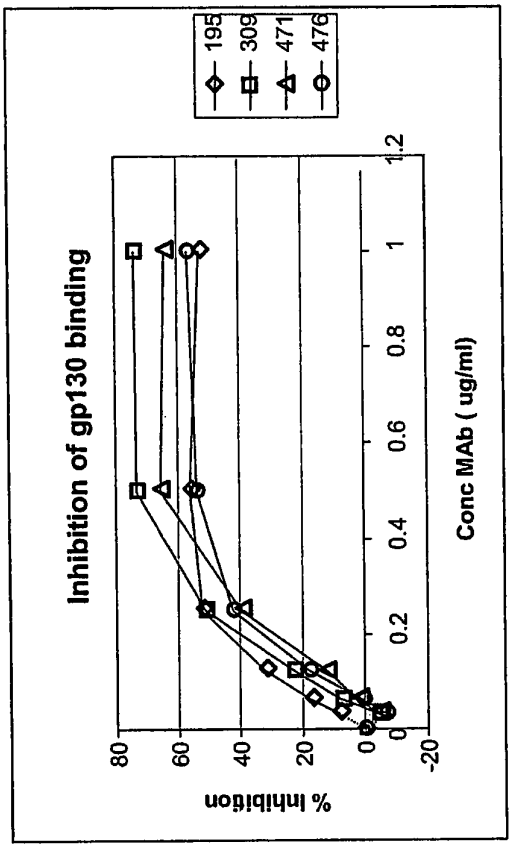

Inhibition of gp130 binding
% Inhibition
Conc MAb (ug/ml)
195
309
471
476
80
60
40
20
0
-20
0
0.2
0.4
0.6
0.8
1
1.2

Figure 8

Figure 9

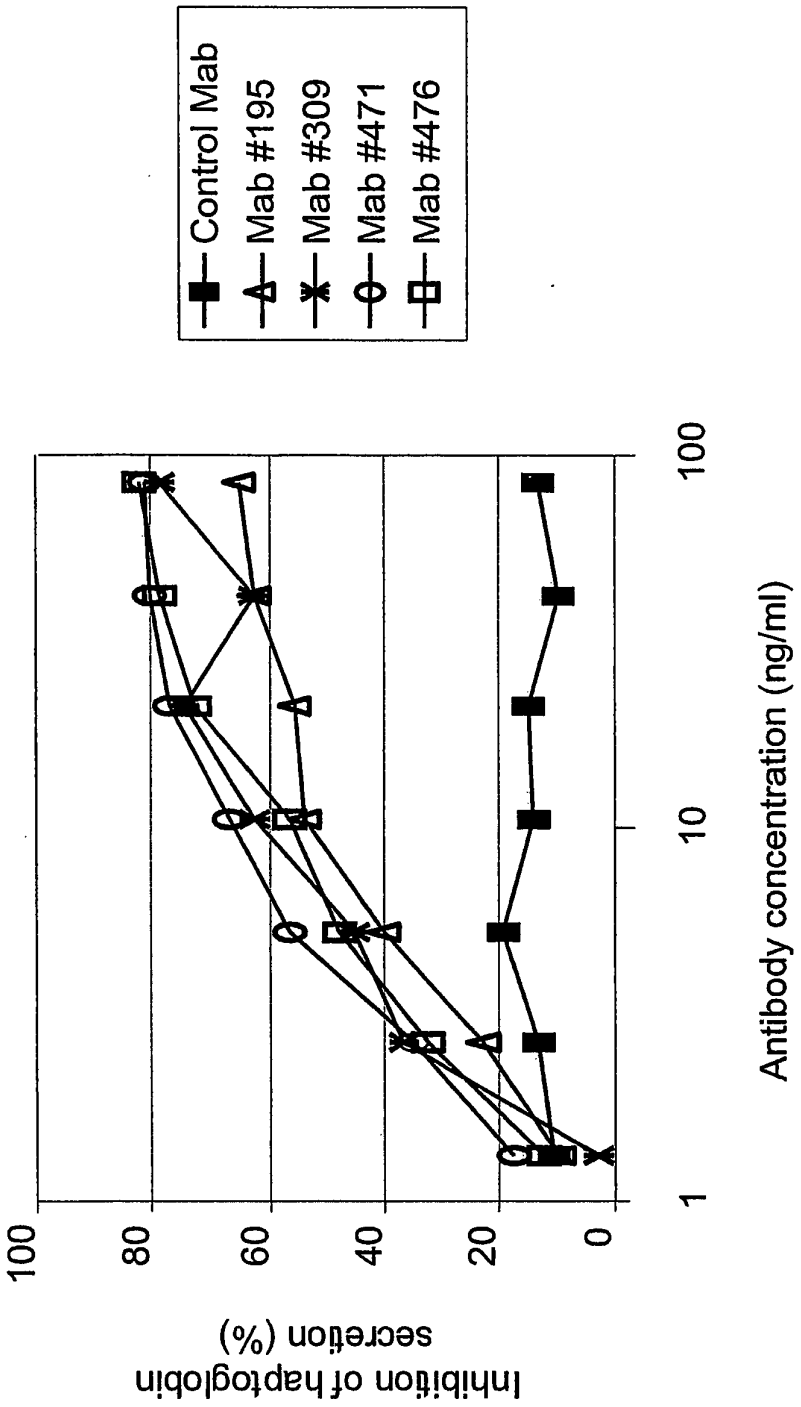

Control Mab
Mab #195
Mab #309
Mab #471
Mab #476
Inhibition of haptoglobin secretion (%)
0
20
40
60
80
100
1
10
100
Antibody concentration (ng/ml)

Figure 10A

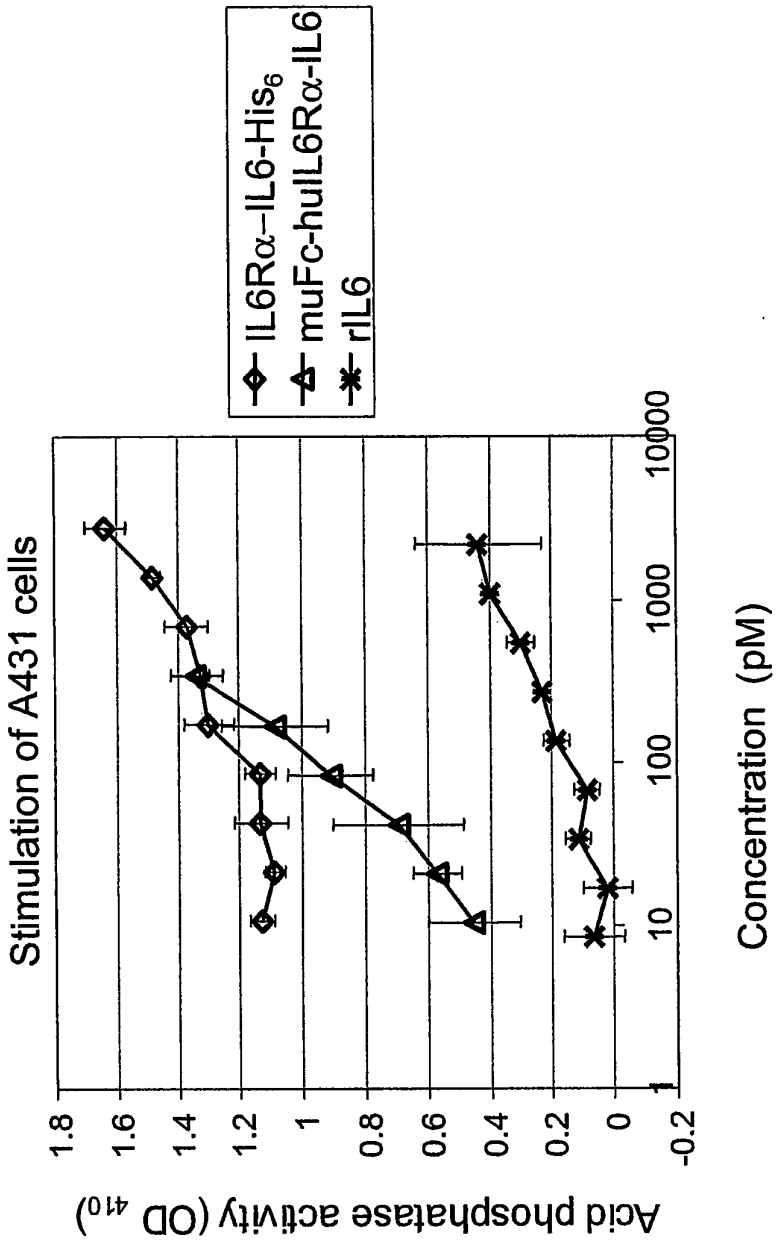

Stimulation of A431 cells
Acid phosphatase activity (OD 410)
Concentration (pM)
IL6Rα–IL6-His6
muFc-huIL6Rα-IL6
rIL6
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
1
10
100
1000
10000

Figure 10B-1

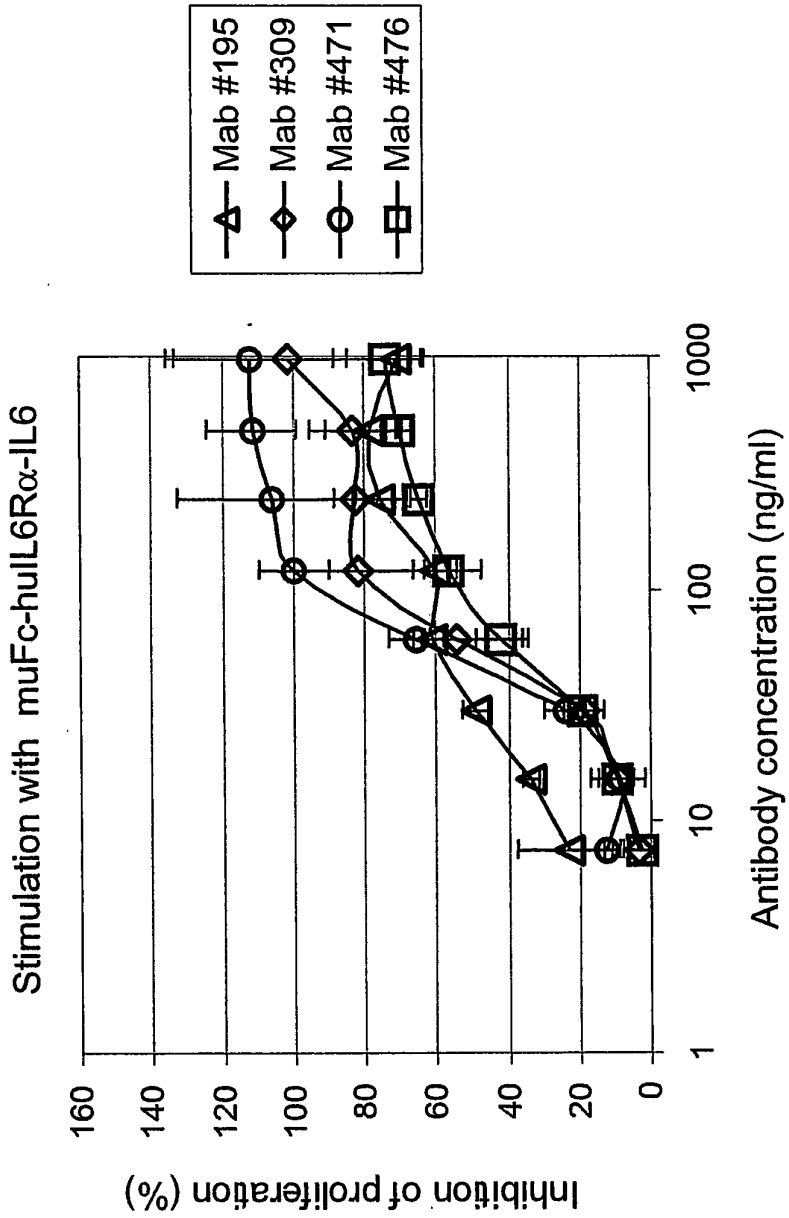
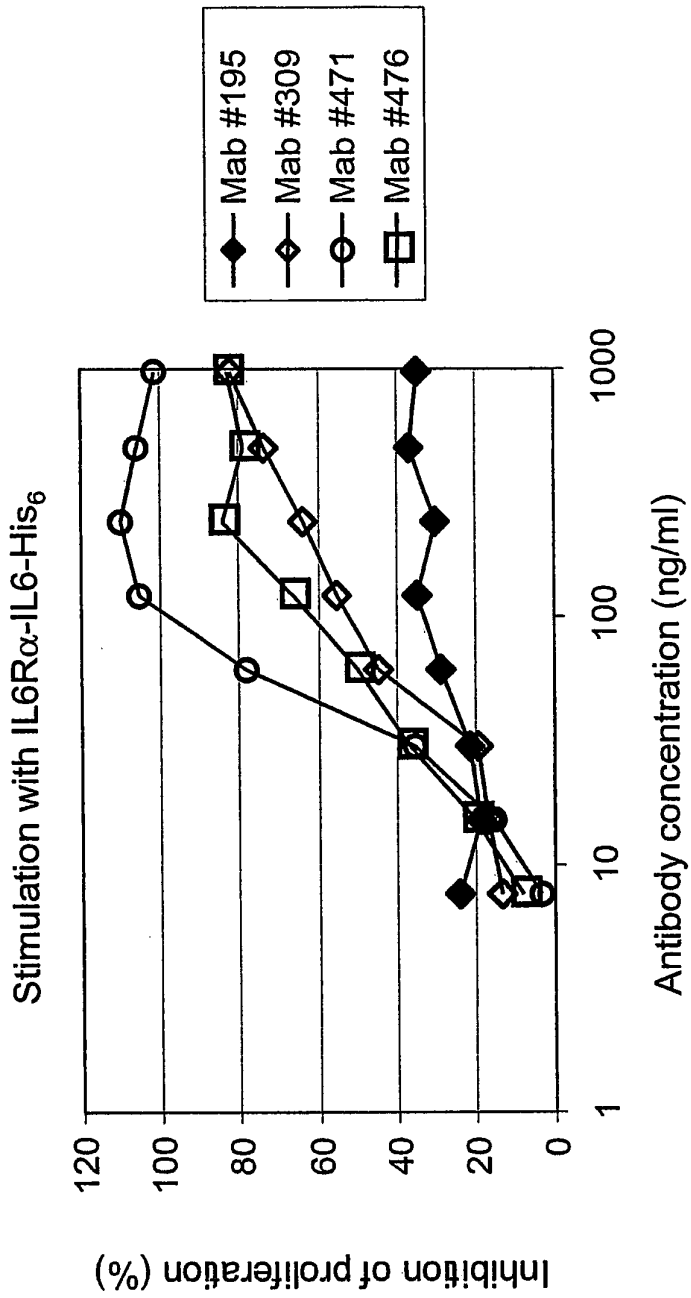

Mab #195
Mab #309
Mab #471
Mab #476
Stimulation with muFc-huIL6Rα-IL6
Inhibition of proliferation (%)
0
20
40
60
80
100
120
140
160
1
10
100
1000
Antibody concentration (ng/ml)

Figure 10B-2


Mab #195
Mab #309
Mab #471
Mab #476
Stimulation with IL6Rα-IL6-His6
Inhibition of proliferation (%)
120
100
80
60
40
20
0
1
10
100
1000
Antibody concentration (ng/ml)

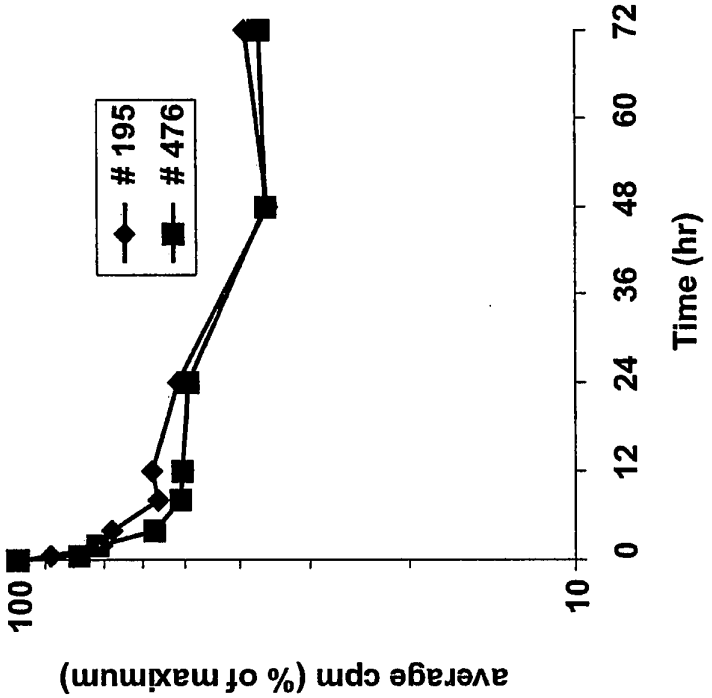
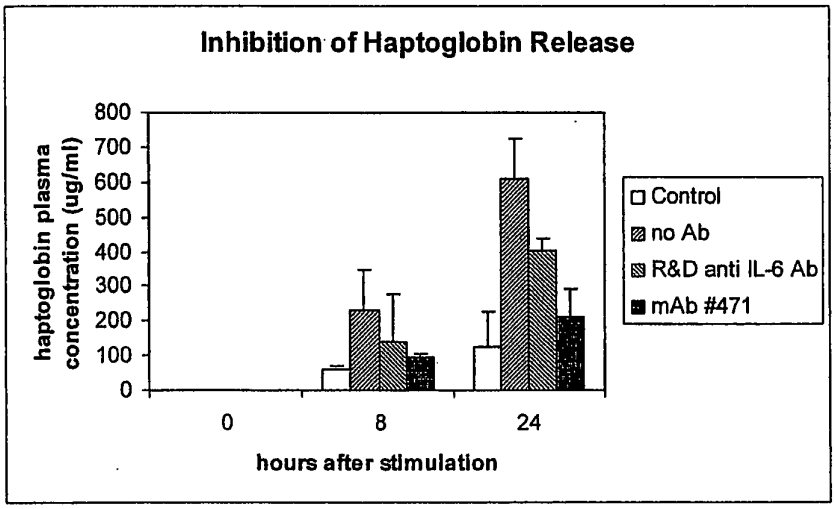


Figure 11

Figure 12. In vivo inhibition haptoglobin secretion.



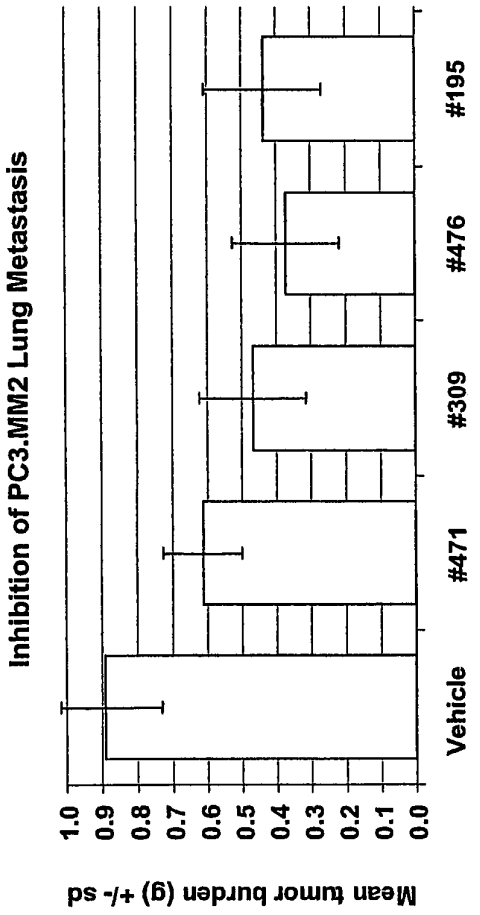

Inhibition of PC3.MM2 Lung Metastasis
Mean tumor burden (g) +/- sd
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Vehicle
#471
#309
#476
#195

Figure 13