Antibody molecules having specificity for human IL-1β

The present invention relates to an antibody molecule having specificity for antigenic determinants of IL-1β. The present invention also relates to the therapeutic uses of the antibody molecule and methods for producing the antibody molecule.

The pro-inflammatory cytokine interleukin-1β (IL-1β) is a member of the IL-1 gene family, which also includes IL-1α and the IL-1 receptor antagonist (IL-1RA) (reviewed by Dinarello, 1996, Blood, 87, 6, 2095-2147). IL-1β is primarily involved in inflammation and has direct effects on endothelial cells and macrophages as well as on both T and B cells. It stimulates bone marrow stromal cells to produce IL-6 as well as a number of colony-stimulating factors and also induces the production of TNFα.

IL-1β is implicated in many pathological conditions that are associated with inflammation. These include infections (viral, bacterial, fungal and parasitic), endotoxic shock, arthtitis, rheumatoid arthritis, pelvic inflammatory disease, multiple sclerosis, asthma, osteoarthritis, psoriasis, Alzheimer's Disease, Crohn's disease, Peyronies's disease, heart disease (such as atherosclerosis), colon cancer, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, meningoencephalitis, other autoimmune disorders, pancreatitis, trauma (surgery), graft-versus host disease and transplant rejection.

IL-1β is also implicated in cancer, osteoporosis and pain signalling.

The involvement of IL-1β in inflammation, pain and other pathological conditions suggests that IL-1β is a good target for drugs and other molecules for the prophylaxis and/or treatment of these conditions.

The mature 17 kDa form of IL-1β exerts its biological effects by binding to the IL-1 receptor IL-1R. Two types of IL-1R exist: the type I receptor IL-1RI and the type II receptor IL-1RII. Binding of IL-1β to IL-1RI leads to recruitment of the receptor accessory protein and signalling. IL-1RII on the other hand, has been termed a 'decoy' receptor, as binding of IL-1β does not transduce a signal. There may be expected to be at least three types of antibody which bind IL-1β:

(i) antibodies which bind IL-1β but which do not neutralise IL-1RI biological activity (a non-neutralising antibody);

(ii) antibodies which bind IL-1β and which neutralise IL-1RI biological activity by blocking binding to the IL-1RI; and

(iii) antibodies which bind IL-1β and which neutralise IL-1RI biological activity but do not block binding to the IL-1RI, such as those antibodies described in US2003/0026806.

Anti IL-1β antibodies have been identified and proposed for use in the treatment of IL-1β mediated diseases and disorders; see for example WO 95/01997.

We have now identified an improved IL-1β antibody that is particularly efficacious *in vivo,* for example in the *in vivo* inflammation models described herein. The antibody is a neutralising antibody as defined in alternative (ii), above.

Summary of the invention

In a first aspect, the present invention provides a neutralising antibody having specificity for human IL-1β, comprising a heavy chain, wherein the variable domain of the heavy chain comprises at least one of a CDR (complementarity determining region) having the sequence given in SEQ ID NO:5 for CDR-H1, a CDR having the sequence given in SEQ ID NO:6 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:7 for CDR-H3.

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat *et al.* This system is set forth in Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat *et al.* (*supra*)"). This numbering system is used in the present specification except where otherwise indicated.

The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (Chothia, C. and Lesk, A.M. J. Mol. Biol., 196, 901-917 (1987)), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus 'CDR-H1', as used herein, comprises residues 26 to 35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system. As used herein, the term 'neutralising antibody' describes an antibody that is capable of neutralising the biological signalling activity of IL-1β, in particular by blocking binding of IL-1β to the IL-1RI.

Preferably, an antibody of the first aspect of the present invention comprises a heavy chain wherein at least two of CDR-H1, CDR-H2 and CDR-H3 of the variable domain of the heavy chain are selected from the following: the sequence given in SEQ ID NO:5 for CDR-H1, the sequence given in SEQ ID NO:6 for CDR-H2 and the sequence given in SEQ ID NO:7 for CDR-H3. For example, the antibody may comprise a heavy chain wherein CDR-H1 has the sequence given in SEQ ID NO:5 and CDR-H2 has the sequence given in SEQ ID NO:6. Alternatively, the antibody may comprise a heavy chain wherein CDR-H1 has the sequence given in SEQ ID NO:5 and CDR-H3 has the sequence given in SEQ ID NO:7, or the antibody may comprise a heavy chain wherein CDR-H2 has the sequence given in SEQ ID NO:6 and CDR-H3 has the sequence given in SEQ ID NO:7. For the avoidance of doubt, it is understood that all permutations are included.

More preferably, the antibody of the first aspect of the present invention comprises a heavy chain, wherein the variable domain comprises the sequence given in SEQ ID NO:5 for CDR-H1, the sequence given in SEQ ID NO:6 for CDR-H2 and the sequence given in SEQ ID NO:7 for CDR-H3.

Even more preferably, the antibody of the first aspect of the present invention comprises a heavy chain, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:3.

Alternatively, the antibody of the first aspect of the present invention comprises a heavy chain, wherein the variable domain of the heavy chain comprises a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:3.

"Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains). Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New

York, 1991). Preferably, the antibody of the first aspect of the present invention comprises a heavy chain, wherein the variable domain of the heavy chain comprises a sequence having at least 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:3.

In a second aspect, the present invention provides a neutralising antibody having specificity for human IL-1β, comprising a light chain, wherein the variable domain of the light chain comprises at least one of a CDR (complementarity determining region) having the sequence given in SEQ ID NO:8 for CDR-L1, a CDR having the sequence given in SEQ ID NO:9 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:10 for CDR-L3.

Preferably, the antibody of the second aspect of the present invention comprises a light chain, wherein at least two of CDR-L1, CDR-L2 and CDR-L3 of the variable domain of the light chain are selected from the following: the sequence given in SEQ ID NO:8 for CDR-L1, the sequence given in SEQ ID NO:9 for CDR-L2 and the sequence given in SEQ ID NO:10 for CDR-L3. For example, the antibody may comprise a light chain wherein CDR-L1 has the sequence given in SEQ ID NO:8 and CDR-L2 has the sequence given in SEQ ID NO:9. Alternatively, the antibody may comprise a light chain wherein CDR-L1 has the sequence given in SEQ ID NO:8 and CDR-L3 has the sequence given in SEQ ID NO:10, or the antibody may comprise a light chain wherein CDR-L2 has the sequence given in SEQ ID NO:9 and CDR-L3 has the sequence given in SEQ ID NO:10. For the avoidance of doubt, it is understood that all permutations are included.

More preferably, the antibody of the second aspect of the present invention comprises a light chain, wherein the variable domain comprises the sequence given in SEQ ID NO:8 for CDR-L1, the sequence given in SEQ ID NO:9 for CDR-L2 and the sequence given in SEQ ID NO:10 for CDR-L3.

Even more preferably, the antibody of the second aspect of the present invention comprises a light chain, wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:4.

Alternatively, the antibody of the second aspect of the present invention comprises a light chain, wherein the variable domain of the light chain comprises a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:4. Preferably, the antibody of the second aspect of the present invention comprises a light chain, wherein the variable domain of the light chain comprises a sequence having at least 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:4.

The antibody molecules of the first and second aspects of the present invention preferably comprise a complementary light chain or a complementary heavy chain, respectively.

Preferably, the antibody according to either of the first and second aspects of the present invention comprises a heavy chain, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:5 for CDR-H1, the sequence given in SEQ ID NO:6 for CDR-H2 and the sequence given in SEQ ID NO:7 for CDR-H3 and a light chain wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:8 for CDR-L1, the sequence given in SEQ ID NO:9 for CDR-L2 and the sequence given in SEQ ID NO:10 for CDR-L3.

In a most preferred embodiment of the first and second aspects of the invention, the antibody comprises a heavy chain, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:3 and a light chain, wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:4.

In a third aspect of the present invention, there is provided an antibody according to either the first or the second aspect of the invention, wherein said antibody is a monoclonal antibody.

In a preferred embodiment of the third aspect of the invention, the monoclonal antibody comprises a heavy chain, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:3 and a light chain, wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:4.

In an alternatively preferred embodiment of the third aspect of the invention, the monoclonal antibody is a murine monoclonal antibody, wherein the monoclonal antibody comprises a heavy chain and a light chain, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:3, and wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:4. This murine monoclonal antibody is referred to herein as 'IC8' or as the "donor" antibody or as the "murine monoclonal antibody". The complete nucleotide and amino acid sequences of the variable domains of the heavy and light chains of mouse monoclonal antibody IC8 are shown in Figure 1 and are given in SEQ ID NOS: 1 to 4. The CDRs given in SEQ ID NOS: 5 to 10 are derived from murine monoclonal antibody IC8.

In a fourth aspect of the invention, there is provided a CDR-grafted antibody molecule, wherein one or more of the CDRs have been obtained from the murine monoclonal antibody IC8. As used herein, the term 'CDR-grafted antibody molecule' refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody). For a review, see Vaughan *et al*, Nature Biotechnology, 16, 535-539, 1998.

When the CDRs are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Preferably, the CDR-grafted antibody of the fourth aspect of the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs derived from the donor antibody as referred to above. Thus, provided is a neutralising CDR-grafted antibody wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al., supra*). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used.

In a CDR-grafted antibody of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

The preferred framework region for the heavy chain of the CDR-grafted antibody of the present invention is derived from the human sub-group VH3 sequence 3-11 (DP-35) shown in Figure 3 (SEQ ID NO:12) together with JH4. Accordingly, provided is a neutralising CDR-grafted antibody comprising at least one non-human donor CDR wherein the heavy chain framework region is derived from the human subgroup sequence 3-11 (DP-35) together with JH4. The sequence of human JH4 is as follows: .(YFDY)WGQGTLVTVSS (SEQ ID NO:74). The YFDY motif is part of CDR-H3 and is not part of framework 4 (Ravetch, JV. *et al.*, 1981, *Cell*, 27, 583-591). The donor sequence is the IC8 VH sequence (SEQ ID NO:11) shown in Figure 3a.

The preferred framework region for the light chain of the CDR-grafted antibody of the present invention is derived from the human germline sub-group VK1 sequence O12 (DPK9) shown in Figure 3 (SEQ ID NO:17) together with JK1. Accordingly, provided is a neutralising CDR-grafted antibody comprising at least one non-human donor CDR wherein the light chain framework region is derived from the human subgroup sequence O12 (DPK9) together with JK1. The JK1 sequence is as follows: (WT)FGQGTKVEIK (SEQ ID NO:75). The WT motif is part of CDR-L3 and is not part of framework 4 (Hieter, PA., *et al.*, 1982, J. Biol. Chem., 257, 1516-1522). The donor sequence is the IC8 VL sequence (SEQ ID NO:16) shown in Figure 3b.

Also, in a CDR-grafted antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual

residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann et al. Nature, 332, 323-324, 1988). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967.

Preferably, in a CDR-grafted antibody molecule of the present invention, if the acceptor heavy chain has the human DP-35+JH4 sequence, then the acceptor framework regions of the heavy chain comprise, in addition to one or more donor CDRs, a donor residue at position 44 (according to Kabat et al.,(supra). The surprising effect on affinity of changing residue 44 to a donor residue was not expected. Thus, in any antibody humanisation process, it will be worth additionally examining the effect of having residue 44 as a donor or acceptor residue. Accordingly, provided is a CDR-grafted antibody, wherein at least the residue at position 44 of the variable domain of the heavy chain is a donor residue.

Alternatively or additionally, if the acceptor heavy chain has the human DP-35+JH4 sequence, then the acceptor framework regions of the heavy chain preferably comprise, in addition to one or more donor CDRs, a donor residue at position 89 (according to Kabat et al., supra). Accordingly, provided is a CDR-grafted antibody, wherein at least the residue at position 44 and/or position 89 of the variable domain of the heavy chain is a donor residue.

Preferably, in a CDR-grafted antibody molecule according to the present invention, if the acceptor light chain has the human sub-group DPK9+JK1 sequence, then the acceptor framework regions of the light chain comprise donor residues at positions 45, 70 and 85 and may additionally comprise donor residues at positions 40 and 48 (according to Kabat et al., supra). Accordingly, provided is a CDR-grafted antibody wherein at least the residue at position 40, 45, 48, 70 and/or 85 is a donor residue. Also provided is a CDR-grafted antibody wherein the residues at positions 45, 70 and 85 are donor residues.

Donor residues are residues from the donor antibody, i.e. the antibody from which the CDRs were originally derived, which in the case of the present invention is the murine monoclonal antibody IC8.

In an alternative embodiment of the first or fourth aspects of the present invention, the heavy chain preferably comprises the sequence of gH1 (SEQ ID NO:13), gH2 (SEQ ID NO:14) or gH3 (SEQ ID NO:15). The sequences of the variable domains of these grafted heavy chains are shown in Figure 3a.

In an alternative embodiment of the second or fourth aspects of the present invention, the light chain preferably comprises the sequence of gL1 (SEQ ID NO:18), gL2 (SEQ ID

NO:19) or gL3 (SEQ ID NO:20). The sequences of the variable domains of these grafted light chains are shown in Figure 3b.

More preferably, an antibody molecule according to the alternative embodiment of the second or fourth aspects of the present invention comprises a heavy chain comprising the sequence of gH1 (SEQ ID NO:13), gH2 (SEQ ID NO:14) or gH3 (SEQ ID NO:15) and a light chain comprising the sequence of gL1 (SEQ ID NO:18), gL2 (SEQ ID NO:19) or gL3 (SEQ ID NO:20).

Even more preferably, the heavy chain of the antibody molecule according to the alternative embodiment of the second or fourth aspects of the present invention comprises variable domain gH3 (SEQ ID NO:15) and the light chain of the antibody molecule of the present invention comprises variable domain gL3 (SEQ ID NO:20).

In a fifth aspect of the invention, there is provided an antibody according to any one of the first to fourth aspects of the present invention, which binds to the same epitope as IC8. Alternatively, there is provided a neutralising antibody having specificity for human IL-1β, which binds to the same epitope as an antibody whose heavy chain comprises the sequence gH3 (SEQ ID NO:15) and whose light chain comprises the sequence gL3 (SEQ ID NO:20).

The antibody molecule of the present invention may comprise a complete antibody molecule having full length heavy and light chains or a fragment thereof, such as a Fab, modified Fab, Fab', F(ab')2, Fv or scFv fragment. Alternatively, it may comprise a light chain or heavy chain monomer or dimer or a single chain antibody, e.g. a single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker. Similarly, the heavy and light chain variable regions may be combined with other antibody domains as appropriate.

The antibody molecule of the present invention may have an effector or a reporter molecule attached to it. For instance, it may have a macrocycle for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure.

Preferably, the antibody of the present invention may be modified to enable an effector or reporter molecule to be attached to it. Most preferably, the antibody molecule of the present invention is a modified Fab fragment as described below.

Alternatively, procedures of recombinant DNA technology may be used to produce an antibody molecule in which the Fc fragment (CH2, CH3 and hinge domains), the CH2 and CH3 domains or the CH3 domain of a complete immunoglobulin molecule has (have) been replaced by, or has (have) attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule. Alternatively, it is preferred that the antibody molecule of the present invention is a modified Fab fragment wherein the

modification is the addition to the C-terminal end of its heavy chain one or more amino acids to allow the attachment of an effector or reporter molecule. Preferably, the additional amino acids form a modified hinge region containing one or two cysteine residues to which the effector or reporter molecule may be attached.

Also provided is a neutralising antibody molecule according to the present invention having an effector or a reporter molecule attached to it. Effector or reporter molecules include a molecule such as a cytotoxic agent, a radionuclide or drug moiety. Other molecules which may be attached include a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as tumour necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

A preferred effector group is a polymer molecule, which may be attached to the modified Fab fragment to increase its half-life *in vivo*.

The polymer molecule may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or hetero- polysaccharide.

Particular optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.

Particular examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

Particular naturally occurring polymers include lactose, amylose, dextran, glycogen or derivatives thereof.

"Derivatives" as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, preferably from 5000 to 40000Da and more

preferably from 25000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product. Thus, for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the product remains in the circulation, it may be advantageous to use a higher molecular weight polymer, for example having a molecular weight in the range from 25000Da to 40000Da.

Particularly preferred polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 25000Da to about 40000Da.

Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond.

Where desired, the antibody fragment may have one or more effector or reporter molecules attached to it. The effector or reporter molecules may be attached to the antibody fragment through any available amino acid side-chain or terminal amino acid functional group located in the fragment, for example any free amino, imino, hydroxyl or carboxyl group.

An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an α-halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

As regards attaching poly(ethyleneglycol) (PEG) moieties, reference is made to "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York; "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York.

Where it is desired to obtain an antibody fragment linked to an effector or reporter molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector or reporter molecule either before or after reaction with the activated polymer as appropriate.

Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 89/00195 and WO 89/01476. Alternatively, where the effector or reporter molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO 86/01533 and EP0392745.

Preferably, the modified Fab fragment of the present invention is PEGylated (i.e. has PEG (poly(ethyleneglycol)) covalently attached thereto) according to the method disclosed in EP-A-0948544. Preferably the antibody molecule of the present invention is a PEGylated modified Fab fragment as shown in Figure 12. The modified Fab fragment preferably has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue is preferably covalently linked to the maleimide group. To each of the amine groups on the lysine residue is preferably attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000 Da. The total molecular weight of the entire effector molecule is therefore approximately 40,000 Da. Accordingly, provided is a neutralising antibody having attached to one of the cysteine residues at the C-terminal end of the heavy chain a lysyl-maleimide or lysyl bis-maleimide group wherein each amino group of the lysyl residue has covalently linked to it a methoxypoly(ethyleneglycol) residue having a molecular weight of about 20,000 Da. For example, the molecular weight may be 15,000-25,000Da, or preferably 18,000-22,000Da, and even more preferably 19,000-21,000Da.

In a preferred embodiment, the present invention provides a neutralising antibody molecule having specificity for human IL-1β, which is a modified Fab fragment having a heavy chain comprising the sequence given in SEQ ID NO:15 and a light chain comprising the sequence given in SEQ ID NO:20 and having at the C-terminal end of its heavy chain a modified hinge region containing one cysteine residue to which an effector or reporter molecule may be attached.

In another preferred embodiment, provided is a neutralising antibody molecule having specificity for human IL-1β, which is a modified Fab fragment having a heavy chain comprising the sequence given in SEQ ID NO:15 and a light chain comprising the sequence given in SEQ ID NO:20 and having at the C-terminal end of its heavy chain a modified hinge region containing one cysteine residue to which an effector or reporter molecule is attached.

More preferably, provided is a neutralising antibody molecule having specificity for human IL-1β, which is a modified Fab fragment having a heavy chain comprising the sequence given in SEQ ID NO:15 and a light chain comprising the sequence given in SEQ ID NO:20 having attached to the cysteine residue at the C-terminal end of the heavy chain a lysyl-maleimide group, wherein each amino group of the lysyl residue has covalently linked to it a methoxypoly(ethyleneglycol) residue having a molecular weight of about 20,000 Da.

Even more preferably, provided is a neutralising antibody molecule, wherein its heavy chain comprises or consists of amino acid residue numbers 22 to 251 of the sequence given in SEQ ID NO:71, and wherein its light chain comprises or consists of amino acid residue numbers 22 to 235 of the sequence given in SEQ ID NO:70. Amino acid residue numbers 1 to 21 of the sequences given in SEQ ID NOS: 70 and 71 represent the *E. Coli* leader sequence which is most preferably cleaved to give a neutralising antibody molecule of the present invention.

Most preferably, provided is a neutralising antibody molecule, wherein its heavy chain comprises or consists of amino acid residue numbers 22 to 251 of the sequence given in SEQ ID NO:71, and wherein its light chain comprises or consists of amino acid residue numbers 22 to 235 of the sequence given in SEQ ID NO:70 having attached to the cysteine residue at the C-terminal end of the heavy chain a lysyl-maleimide group, wherein each amino group of the lysyl residue has covalently linked to it a methoxypoly(ethyleneglycol) residue having a molecular weight of about 20,000 Da.

Also provided is a neutralising antibody molecule having specificity for human IL-1β, which binds to the same epitope as a neutralising antibody which comprises a light chain comprising the sequence given in SEQ ID NO:20.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simply blocking IL-1β activity.

The antibody molecule of the present invention preferably has a binding affinity of at least 4.4×10^{-10}M, more preferably at least 3.2×10^{-10}M.

The present invention also relates to variants of the antibody molecules of the present invention, which have an improved affinity for IL-1β. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang *et al.*, J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks *et al.*, Bio/Technology, 10, 779-783, 1992), use of mutator strains of *E. coli* (Low *et al.*, J. Mol. Biol., 250, 359-368, 1996), DNA shuffling (Patten *et al.*, Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson *et al.*, J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Crameri *et al.*, Nature, 391, 288-291, 1998). Vaughan *et al.* (*supra*) discusses these methods of affinity maturation.

The present invention also provides an isolated DNA sequence encoding the heavy and/or light chain(s) of the antibody molecule of the present invention. Preferably, the DNA sequence encodes the heavy or the light chain of the antibody molecule of the present invention. The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

DNA sequences which encode the antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA sequences coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

DNA coding for acceptor framework sequences is widely available to those skilled in the art and can be readily synthesised on the basis of their known amino acid sequences.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody molecule of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences of the present invention. Accordingly, provided is a cloning or expression vector comprising one or more DNA sequences encoding an antibody of the present invention. Preferably, the cloning or expression vector comprises two DNA sequences, encoding the light chain and the heavy chain of the antibody molecule of the present invention, respectively. Preferably, a vector according to the present invention comprises the sequence given in SEQ ID NO:69.

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

Also provided is a host cell comprising one or more cloning or expression vectors comprising one or more DNA sequences encoding an antibody of the present invention. Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used or eukaryotic, for example mammalian, host cell expression systems may also be used. Suitable mammalian host cells include CHO, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell containing a vector of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

As the antibodies of the present invention are useful in the treatment and/or prophylaxis of a pathological condition, the present invention also provides a pharmaceutical or diagnostic composition comprising an antibody molecule of the present invention in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier. Accordingly, provided is the use of an antibody of the invention for the manufacture of a medicament. The composition will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically-acceptable adjuvant.

The present invention also provides a process for preparation of a pharmaceutical or diagnostic composition comprising adding and mixing the antibody molecule of the present invention together with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

The antibody molecule may be the sole active ingredient in the pharmaceutical or diagnostic composition or may be accompanied by other active ingredients including other antibody ingredients, for example anti-T cell, anti-IFNγ or anti-LPS antibodies, or non-antibody ingredients such as xanthines.

The pharmaceutical compositions preferably comprise a therapeutically effective amount of the antibody of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any antibody, the therapeutically effective amount can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of

administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 20 mg/kg. Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose.

Compositions may be administered individually to a patient or may be administered in combination (*e.g.* simultaneously, sequentially or separately) with other agents, drugs or hormones.

The dose at which the antibody molecule of the present invention is administered depends on the nature of the condition to be treated, the extent of the inflammation present and on whether the antibody molecule is being used prophylactically or to treat an existing condition.

The frequency of dose will depend on the half-life of the antibody molecule and the duration of its effect. If the antibody molecule has a short half-life (e.g. 2 to 10 hours) it may be necessary to give one or more doses per day. Alternatively, if the antibody molecule has a long half life (e.g. 2 to 15 days) it may only be necessary to give a dosage once per day, once per week or even once every 1 or 2 months.

The pharmaceutically acceptable carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as

tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Preferred forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, it is preferred that the compositions are adapted for administration to human subjects.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO 98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

It is also envisaged that the antibody of the present invention will be administered by use of gene therapy. In order to achieve this, DNA sequences encoding the heavy and light chains of the antibody molecule under the control of appropriate DNA components are

introduced into a patient such that the antibody chains are expressed from the DNA sequences and assembled *in situ*.

The present invention also provides an antibody molecule for use in the control of inflammation. Preferaby, the antibody molecule can be used to reduce the inflammatory process or to prevent the inflammatory process.

The present invention also provides the antibody molecule of the present invention for use in the treatment or prophylaxis of a pathological disorder that is mediated by IL-1β or associated with an increased level of IL-1β. Preferably, the pathological condition is selected from the group consisting of infections (viral, bacterial, fungal and parasitic), endotoxic shock associtated with infection, arthritis, rheumatoid arthritis, pelvic inflammatory disease, Alzheimer's Disease, Crohn's disease, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, meningoencephalitis, other autoimmune disorders, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, cancer (both solid tumours such as melanomas, hepatoblastomas, sarcomas, squamous cell carcinomas, transitional cell cancers, ovarian cancers and hematologic malignancies and in particular acute myelogenous leukaemia, chronic myelogenous leukemia, gastric cancer and colon cancer), heart disease including ischaemic diseases such as myocardial infarction as well as atherosclerosis, intravascular coagulation, bone resporption, osteoporosis, periodontitis and hypochlorhydia.

The present invention also provides an antibody molecule according to the present invention for use in the treatment or prophylaxis of pain.

The present invention further provides the use of an antibody molecule according to the present invention in the manufacture of a medicament for the treatment or prophylaxis of a pathological disorder that is mediated by IL-1β or associated with an increased level of IL-1β.

The present invention further provides the use of an antibody molecule according to the present invention in the manufacture of a medicament for the treatment or prophylaxis of pain.

An antibody molecule of the present invention may be utilised in any therapy where it is desired to reduce the effects of IL-1β in the human or animal body. IL-1β may be circulating in the body of may be present in an undesirably high level localised at a particular site in the body, for example a site of inflammation.

The antibody molecule of the present invention is preferably used for the control of inflammation.

The present invention also provides a method of treating human or animal subjects suffering from or at risk of a disorder mediated by IL-1β, the method comprising administering to the subject an effective amount of the antibody molecule of the present invention.

The antibody molecule of the present invention may also be used in diagnosis, for example in the *in vivo* diagnosis and imaging of disease states involving IL-1β.

The present invention is further described by way of illustration only in the following examples, which refer to the accompanying Figures, in which:

Figure 1a) shows the nucleotide and amino acid sequence (SEQ ID NOS:1 and 3, respectively) of the variable domains of the heavy chain, and Figure 1b) shows the nucleotide and amino acid sequence (SEQ ID NOS:2 and 4, respectively) of the variable domains of the light chain of murine monoclonal antibody IC8.

Figure 2 shows the vectors MRR14 and pMRR10.

Figure 3 shows the graft design for the IC8 heavy (Figure 3a; SEQ ID NOS:11-15) and light chain (Figure 3b; SEQ ID NOS:16-20) sequences. The symbol (|) highlights differences between donor:acceptor:grafted framework sequences. CDR's are single underlined for the IC8 sequences. These are as defined by Kabat, except for CDR-H1 which encompasses both Kabat and Chothia definitions. Double-underlined sequences are donor residues retained in the grafts. Starred (*) residues are common in human sub-group VH3 germline sequences, but not present in this particular germline – these are not considered mouse residues even though they are present in the original donor sequence.

Figure 4 shows the nucleotide and amino acid sequences of the designed genes gH1 (Figure 4a) and gL1 (Figure 4b).

Figure 5 shows the oligonucleotides that were used for gene construction.

Figure 6 shows the oligonucleotide cassettes that were used for further grafts. Shown are: the IC8gL2 sense strand sequence (SEQ ID NO:59), the reverse strand sequence (SEQ ID NO:72) and the corresponding amino acid sequence (SEQ ID NO:60); the IC8gL3 sense strand sequence (SEQ ID NO:61), the reverse strand sequence (SEQ ID NO:73) and the corresponding amino acid sequence (SEQ ID NO:62); the IC8gH2 sense strand sequence (SEQ ID NO:63), the reverse strand sequence (SEQ ID NO:64) and the corresponding amino acid sequence (SEQ ID NO:65), and the IC8gH3 sense strand sequence (SEQ ID NO:66), the reverse strand sequence (SEQ ID NO:67) and the corresponding amino acid sequence (SEQ ID NO:68). Underlined residues denote changed amino acids.

Figure 7 shows the results from the IL-1β neutralisation assay with IC8 grafts.

Figure 8 shows a map of the vector pTTOD(Fab').

Figure 9 shows steps 1-4 in the cloning of IC8 V-region genes into the intermediate vector pTTOD(Fab').

Figure 10 shows a map of vector pTTOD(gH3gL3 Fab' IGS-2).

Figure 11 shows the coding and flanking sequence of pTTOD(gH3gL3 Fab' IGS-2); SEQ ID NO:69.

Figure 12 shows the structure of a modified Fab fragment derived from an antibody to IL-1β covalently linked via a cysteine residue to a lysyl-maleimide linker wherein each amino group on the lysyl residue has covalently attached to it a methoxy PEG residue wherein n is about 420.

DNA manipulations and general methods

E. coli strain INVαF' (Invitrogen) was used for transformation and routine culture growth. DNA restriction and modification enzymes were obtained from Roche Diagnostics Ltd. and New England Biolabs. Plasmid preparations were performed using Maxi Plasmid purification kits (QIAGEN, catalogue No. 12165). DNA sequencing reactions were performed using the ABI Prism Big Dye terminator sequencing kit (catalogue No. 4304149) and run on an ABI 3100 automated sequencer (Applied Biosystems). Data was analysed using the program AutoAssembler (Applied Biosystems). Oligonucleotides were obtained from OSWEL. The concentration of Fab' was determined using Fab' assembly ELISA.

Example 1: Gene cloning and expression of the variable regions from murine monoclonal antibody IC8

Preparation of total RNA

The hybridoma expressing IC8 was generated by Cistron using conventional hybridoma technology following immunisation of mice with human IL-1β protein. The hyriboma was then obtained by Celltech R&D Limited. Total RNA was prepared from IC8 hybridoma cells using the QIAGEN RNeasy kit (QIAGEN Ltd, catalogue no. 74104). The RNA obtained was reverse transcribed to cDNA using the Clontech cDNA Advantage RT for PCR kit (catalogue no. K1402).

PCR cloning of IC8 VH and VL regions

The cDNA prepared from hybridoma cells was used as the template for PCR in a series of reactions designed to amplify the V-region sequences. The reactions used a set of

'forward' degenerate primers designed to anneal to DNA within the conserved signal sequence, and a reverse primer annealing to DNA encoding the framework 4/constant region junction. PCR was performed using Taq Plus Precision (Stratagene, catalogue No. 600211) and a 0.25 mM concentration of dNTP. The resultant PCR products were cloned into sequencing vectors (InVitrogen Zero Blunt TOPO PCR cloning kit for sequencing, catalogue No. K2875) and the DNA sequence was determined. N-terminal protein sequencing of the purified IC8 antibody (from the hybridoma) was used to confirm that the translated sequences corresponded to the observed protein sequence. The V-region sequences are shown in Figure 1 and in SEQ ID NOS:1 to 4.

The murine V-region genes were then sub-cloned into the expression vectors pMRR10 and pMRR14 (Figure 2). These are separate vectors for expression of the light and heavy chain respectively and contain genomic DNA encoding constant region genes for human kappa light chain and gamma-4 heavy chain respectively.

The double chimeric IC8 antibody molecule cHcL was expressed by transient co-transfection of the heavy and light chain expression vectors described above (pMRR10 and pMRR14 containing IC8 VL and VH respectively) into CHO-L761 cells. Transfections were performed using the lipofectamine procedure according to manufacturer's instructions (InVitrogen, catalogue No. 18324).

Example 2: CDR-grafting of IC8

The CDRs from IC8 were CDR-grafted into human frameworks in order to reduce potential immunogenicity and to facilitate *E. coli* expression. Human germline acceptor frameworks were chosen from sub-groups VH3 and VK1. Figure 3 shows an alignment between the donor mouse sequence and the acceptor human frameworks. The heavy chain acceptor framework is the human germline sequence VH3-11 (DP-35), with framework 4 coming from this portion of the human JH-region germline JH4. The light chain acceptor framework is the human germline sequence O12 (DPK9), with framework 4 coming from this portion of the human JK-region germline JK1.

The alignment in Figure 3 shows that there are 13 framework differences between the donor and acceptor heavy chains (excluding CDRs). At each of these positions an analysis was made of the potential of that residue to contribute to antigen binding, through direct contact with antigen, through a role in CDR packing, or through involvement at the VL/VH interface; if considered important, the murine donor residue was retained. The light chain alignment shows that there are 15 framework differences between the donor and acceptor sequences (excluding CDRs). The potential of the murine residue to contribute to antigen binding was again analysed. In this way, three VH grafts and three VL grafts were designed.

These are also shown in Figure 3 and correspond to SEQ ID NO:13 (gH1), SEQ ID NO:14 (gH2), SEQ ID NO:15 (gH3), SEQ ID NO:18 (gL1), SEQ ID NO:19 (gL2) and SEQ ID NO:20 (gL3).

Example 3: Design and construction of genes for grafted sequences

Genes were designed to encode the grafted sequences, using codons frequently used in *E. coli* and avoiding 'rare' *E. coli* codons (Wada *et al.* (1991), Nucl. Acids Res., 19, 1981-86). Restriction sites were introduced into the DNA sequence at intervals to facilitate further genetic manipulation. Figure 4 shows the design of genes for gH1 and gL1, the sequences of which are given in SEQ ID NOS:21 and 22. The corresponding amino acid sequences are given in SEQ ID NOS:23 and 24, respectively.

Completely overlapping oligonucleotides were used to construct the genes encoding gH1 and gL1 (Figure 5; SEQ ID NOS:27-58). The oligonucleotides encoding the designed genes were annealed together by mixing at a concentration of 100 pmoles/μl in buffer (12.5 mM TrisHCl pH 7.5, 2.5 mM $MgCl_2$, 25 mM NaCl, 0.25 mM dithioerythritol) and heating to 95°C for 3 minutes in a PCR block programmed to cool down to 30°C at a rate of -0.01°C every 10 seconds. T4 DNA ligase (5U) and its appropriate reaction buffer were added, and the oligonucleotides were ligated together by incubation at 25°C for 1 hour. The ligated heavy and light chain genes were then amplified by PCR following addition of a 10-fold excess of 'end' primers T1 (SEQ ID NO:25) and B1 (SEQ ID NO:26). A proof-reading DNA polymerase was used for this amplification (Taq Plus Precision, Stratagene). Amplification products were run on a 1.5% agarose gel. The 400-450 band was isolated and gel purified then cloned into the intermediate vector pCR4 blunt TOPO according to the manufacturer's instructions (InVitrogen). This created the intermediate plasmids pCR4(IC8gL1) and pCR4(IC8gH1). These plasmids were then used as the templates to create the further grafted forms gL2, gL3, gH2 and gH3.

An oligonucleotide cassette replacement method was used to create the humanised grafts gL2 and gL3. Figure 6 shows the design of the oligonucleotide cassettes. To construct each variant, the vector pCR4(IC8gL1) was cut with the restriction enzymes KpnI and NheI. The resulting large vector fragment was gel purified from agarose and was used in ligation with the oligonucleotide cassette. Oligonucleotide pairs were annealed together by mixing at a concentration of 0.5 pmoles/μl in a volume of 200 μl buffer (12.5 mM TrisHCl pH 7.5, 2.5 mM $MgCl_2$, 25 mM NaCl, 0.25 mM dithioerythritol), and heating to 95°C for 3 minutes in a waterbath (volume 500 ml) then allowed to slow-cool to room temperature. The annealed oligonucleotide cassette was then diluted ten-fold in water before ligation into the

appropriately cut vector. DNA sequencing was used to confirm the correct sequence, creating plasmids pCR4(IC8gL2) and pCR4(IC8gL3).

The variant gH2 was constructed from pCR4(IC8gH1) using a PCR strategy. The reverse strand of the cassette shown in Figure 6 (SEQ ID NO:64) was used as a reverse primer in PCR using a vector specific 5' forward primer to generate a product representing the partial gH2 sequence. This was digested with restriction enzymes HindIII and BspEI then cloned into HindIII-BspEI restricted pCR4(IC8gH1) to create pCR4(IC8gH2).

The variant gH3 was constructed using a different PCR strategy. The two gH3 oligos shown in Figure 6 were used in separate PCR reactions: the sense strand (SEQ ID NO:66) as a forward primer using a vector-specific 3'reverse primer, and the non-sense strand (SEQ ID NO:67) as a reverse primer using a vector-specific 5' forward primer. In both cases the template was pCR4(IC8gH1). The two resulting amplification products were isolated and purified, then added together with both 5' and 3' vector-specific primers and cycled through additional PCR amplifications to generate a full length gH3 product. This was digested with HindIII and ApaI and inserted into pCR4(IC8gH1) restricted with HindIII-ApaI, to create pCR4(IC8gH3). All variants were confirmed by DNA sequencing.

Each of the heavy chain grafts was then sub-cloned into the expression vector pMRR14 as HindIII-ApaI fragments. Each of the 3 light chain grafts was sub-cloned into the light chain expression vector pMRR10 as SfuI-BsiWI fragments.

Selection of optimum grafted variant

The grafted antibodies were expressed by transient co-transfection of the grafted heavy and light chain expression vectors described above (pMRR10 and pMRR14 containing IC8 gL1, gL2 and gL3 and gH1, gH2 and gH3 respectively) into CHO-L761 cells. Transfections were performed using the lipofectamine procedure according to manufacturer's instructions (InVitrogen, catalogue no. 18324).

All combinations of grafted chain and chimeric chain were expressed and compared against the double chimeric antibody cHcL. Binding was assessed in a BIAcore assay and in an IL-1β neutralisation assay.

BIAcore assay

The assay format used anti-IL-1β antibody captured by anti-hFc with a titration of recombinant human IL-1β in the solution phase. Mouse anti-human IgG, Fc fragment-specific (Celltech) was immobilised on flowcell 2 of a CM5 Sensor Chip via amine coupling chemistry to a level of 12757RU. A blocked reference surface was prepared on flowcell 1 by

activation with EDC/NHS and deactivation with ethanolamine using the same volumes as for flowcell 2. HBS-EP buffer (10 mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20, Biacore AB) was used as the running buffer and assays were performed at 25°C. Anti-IL-1β antibody was passed over flowcells 1 and 2 and captured on the immobilised anti-hFc surface using a flowrate of 10 µl/min. IL-1β from 400-0 nM was injected over the blocked and captured anti-IL-1β antibody surface using a flowrate of 30 µl/min for 3 min. The anti-hFc surface was regenerated with a 30 µl injection of 40 mM HCl. Kinetic parameters were calculated using BIAevaluation 3.1 software.

For gIC8 Fab', the assay format used anti-IL-1β antibody captured by anti-hF(ab')$_2$ (where 'h' indicates that it is a human F(ab')$_2$) then IL-1β titrated over. Affinipure goat anti-human IgG, F(ab')$_2$ fragment-specific (Jackson ImmunoResearch Code 109-005-097) was immobilised on flowcell 2 of a CM5 Sensor Chip via amine coupling chemistry to a level of 13025RU. A blocked reference surface was prepared on flowcell 1 by activation with EDC/NHS and deactivation with ethanolaomine using the same volumes as for flowcell 2. HBS-EP buffer (10 mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20, Biacore AB) was used as the running buffer and assays were performed at 25°C. Anti-IL-1β antibody was passed over flowcells 1 and 2 and captured on the immobilised anti-hF(ab')$_2$ surface using a flowrate of 10 µl/min. IL-1β (Strathmann) from 400-0 nM was injected over the blocked and captured anti-IL-1β antibody surface using a flowrate of 30 µl/min for 3 min. The anti-hF(ab')$_2$ surface was regenerated with a 30 µl injection of 40 mM HCl followed by a 15 µl injection of 5 mM NaOH. Kinetic parameters were calculated using BIAevalutation 3.1 software.

Table 1 shows a summary of Biacore antigen affinity data. It is clear that the gH1 graft, which has donor residues at positions 44 and 89, has a higher affinity than gH2, which only has a donor residue at position 89. The gH2 graft was, therefore, rejected. Surprisingly, high affinity was observed in the gH3 graft in which only position 44 is a donor framework residue.

Table 1: Affinity by BIAcore

Anti-IL-1β	K_D (nM)
mIC8	0.30
cHcL	0.38
gH1gL1	0.35
gH1gL2	0.34

gH1gL3	0.30
gH2gL1	1.27
gH2gL3	1.14
gH2gL3	1.07
gH3gL1	0.28
gH3gL2	0.32
gH3gL3	**0.28**
gH3gL3 Fab'	0.32

In vitro neutralisation assay

Fibroblasts were grown to 80% confluence in 96 well plates. Antibodies were titrated in half log dilutions from 1 μg/ml and IL-1β was added to give 20 pg/ml final concentration. The plates were incubated at room temperature for 30 min. Culture medium was removed from fibroblast cultures and 100 μl antibody/IL-1β mix added to the appropriate wells and cultured overnight at 37°C. The amount of IL-6 produced in response to IL-1β was then estimated using the R&D Systems Human IL-6 Duoset Kit DY206.

The results of the neutralisation assay are shown in Figure 7, in which the grafted forms of the antibody (excluding gH2) are compared with the parent mouse antibody. As with the Biacore affinity data there is very little difference between any of the remaining grafts. Hence gL3gH3 was selected as the variant with optimum activity and the fewest number of mouse framework residues. As shown in Figure 3, the gH3 sequence has 1 donor framework residue, whilst the gL3 sequence has 3 donor framework residues.

In vivo neutralisation assay

To determine the neutralisation efficacy of the gH3gL3Fab' *in vivo*, the gH3gL3Fab' was tested in two *in vivo* models of inflammation.

Intraperitoneal gH3gL3Fab'-PEG(40K) / intraperitoneal hIL-1β in mice

Male Balb/c mice (18-25g) were injected intraperitoneally (i.p.) with gH3gL3Fab'-PEG(40K) (100μl, in PBS vehicle) or control Fab'-PEG(40K) (100μl, in PBS vehicle) 5 minutes prior to i.p. injection with hIL-1β (150 ng/kg) or vehicle (100μl PBS). After 120 minutes, mice were killed by cervical dislocation and peritoneal lavage performed (3ml HBSS (Hanks' Balanced Salts) + 0.25% BSA, 12mM HEPES). A total leukocyte count was

performed using a Coulter Counter. For identification of neutrophils, 50µl peritoneal lavage fluid was stained with 1:300 dilution of anti-CD45-CyChrome mAb and 1:300 dilution of anti phycoerythrin labelled GR-1 mAb (anti-Ly6G/Ly6C) for 20 min (4°C, in dark). Leukocytes were washed once in HBSS (0.25% BSA, 12mM HEPES), resuspended in 300µl HBSS (0.25% BSA, 12mM HEPES) and analysed by flow cytometry. Neutrophils were identified as $CD45^{+}GR\text{-}1^{HIGH}$. Murine monocyte chemoattractant protein-1 (mMCP-1) concentration in the peritoneal lavage samples was measured by sandwich ELISA according to the manufacturer's instructions (BD Biosciences OPT-EIA mMCP-1).

Intravenous gH3gL3 Fab'-PEG(40K) / intravenous hIL-1β in mice

Male Balb/c mice (18-25g) were anaesthetised with halothane and injected intravenously (i.v.) with gH3gL3Fab'-PEG(40K) (100µl, in PBS vehicle) or control Fab-PEG (100µl, in PBS vehicle) 15 minutes prior to i.v. injection with hIL-1β (37.5 or 50µg/kg) or vehicle (100µl PBS). After 90 minutes, a blood sample was taken by cardiac puncture into heparin (20µl, 100U/ml), and plasma was prepared by centrifugation (14,000 x g, 2 min, RT). Plasma samples were stored at -20°C. Plasma samples were assayed for murine interleukin-6 (mIL-6) by sandwich ELISA according to the manufacturer's instructions (BD Biosciences OPT-EIA mIL-6).

The results demonstrate that the pre-treatment of mice with i.p. gH3gL3Fab'-PEG(40K) was effective in reducing i.p. hIL-1β-induced neutrophil accumulation (Table 2) and mMCP-1 generation (Table 3). The pre-treatment of mice with i.v. gH3gL3Fab'-PEG (40K) was also effective in reducing i.v. hIL-1β-induced mIL-6 generation. Data from 3 separate experiments are summarised in Table 4.

No ill effects were observed at any of the doses used in either model. The antibody gH3gL3Fab' can, therefore, be expected to be useful in the treatment of inflammation and other IL-1β mediated diseases.

Table 2: Neutrophil accumulation

Dose hIL-1β i.p. (ng/kg)	gH3gL3Fab'-PEG(40K) ED_{50} (mg/kg)	Max inhibition (%, dose, mg/kg)
150	0.02	96 (1)
150	0.02	97 (1)
150	0.03	95 (1)

150	0.06	86 (0.1)
150	0.03	89 (1)
150	0.03	98 (0.3)

Table 3: mMCP-1 generation

Dose hIL-1β i.p. (ng/kg)	gH3gL3Fab'-PEG(40K) ED_{50} (mg/kg)	Max inhibition (%, dose, mg/kg)
150	0.02	98 (1)
150	0.01	100 (1)
150	0.02	98 (1)
150	0.04	92 (1)
150	0.02	98 (1)
150	NA	99 (0.3)

5

Table 4

Dose hIL-1β i.v. (ng/kg)	gH3gL3Fab'-PEG(40K) ED_{50} (mg/kg)	Max inhibition (%, dose, mg/kg)
50	1.88	75 (10)
37.5	5.39	81 (10)
37.5	2.95	86 (10)

Example 4: Cloning and expression of Fab' fragments

Cloning of selected V-regions into E. coli Fab' expression plasmid pTTOD(Fab')

The expression vector containing an irrelevant Fab', termed pTTOD(Fab'), is shown in Figure 8. The DNA encoding both the light chain and heavy chain is preceded by DNA encoding the E. coli OmpA signal sequence (Movva NR, Nakamura K and Inouye M. Amino acid sequence of the signal peptide of ompA protein, a major outer membrane protein of Escherichia coli. J Biol Chem. 1980; 255(1): 27-9). The V-regions of IC8 were cloned into this vector so that this signal sequence is maintained, directing the translocation of both chains to the E. coli periplasm. The signal peptide is cleaved on translocation, so that it does not form part of the product Fab' sequence. (note; the sequence of the OmpA signal peptide is amino acid residues 1 to 21 of the sequence given in SEQ ID NO:70. Plasmid pTTOD(Fab') was digested with resriction enzymes EcoRV and BsiWI (BsiWI and SplI are isoschizomers) to remove the irrelevant VL sequence and the IC8 gL3 V-region was inserted, following its isolation from pMRR10(IC8gL3). This created the cloning intermediate pTTOD(IC8gL3). pTTOD(IC8gL3) was then cleaved with BsiWI and ApaI and 2 fragments were inserted in a 3-way ligation; a c-kappa/IGS-fragment (either IGS-2 or IGS-3) and the IC8gH3 fragment (see schematic representation in Figure 9). In this way, the 2 vector variants pTTOD(IC8gL3gH3 IGS-2) and pTTOD(IC8gL3gH3 IGS-3) were constructed. These vary only in the nucleotide sequence between the light and heavy chain genes: IGS-2 confers very tight translational coupling between the 2 genes giving a rapid rate of heavy chain translation, IGS-3 confers a slower rate of heavy chain translation (see UK patent application No. 0129105).

Fermenter expression of Fab' in E. coli

Plasmids pTTOD(IC8gL3gH3 IGS-2) and pTTOD(IC8gL3gH3 IGS-3) were transformed into the *E. coli* strain W3110 using standard protocols. The soluble *E.coli* periplasmic Fab' was extracted using tris-EDTA buffer at 50°C. After extraction & cooling, the pH of the extract was adjusted and the cells removed by centrifugation and filtration. The clarified feedstream was diluted with water (approx 2-fold) in order to reduce the conductivity. Fab' was captured using cation-exchange chromatography (SP sepharose FF resin) and bound Fab' eluted using a NaCl step. The eluted product stream was concentrated, diafiltered into tris buffer using ultrafiltration and subjected to anion-exchange chromatography (Q sepharose FF resin) where the Fab' was contained in the unbound fraction. The purified Fab' was concentrated & diafiltered into reduction buffer (by ultrafiltration) followed by reduction using 2-mercaptoethylamine to activate the hinge thiol. The reductant is then removed by ultrafiltration buffer-exchange. N-terminal sequencing

confirmed the correct amino acid sequence and cleavage of the OmpA leader. Expression of Fab' by these two plasmids was compared.

Table 5 shows a comparison of fermenter Fab' yields for the IGS-2 and the IGS-3 variants. The IGS-2 construct clearly out-performs IGS-3 for Fab' productivity. Therefore the IGS-2 variant was selected. The plasmid map is shown in Figure 10. Figure 11 shows the Fab' coding and flanking sequence of pTTOD(gH3gL3 Fab' IGS-2).

Table 5: Fermenter expression yields; a comparison of anti IL-1β gIC8 periplasmic Fab' yields

Fermentations	Date	Construct	Peri. Fab' ELISA (mg/l)	Protein G A280 (mg/l)
FM264	25/09/01	gIC8 IGS2	602	588
FM265	25/09/01	gIC8 IGS2	742	533
FM266	25/09/01	gIC8 IGS3	243	
FM267	25/09/01	gIC8 IGS3	224	

Activity of E.coli-produced Fab'

Some of the purified *E. coli*-produced Fab' was then analysed for affinity in the Biacore assay, as shown in Table 1. As can be seen, this material retains effectively full activity in this assay.

PEGylation of the Fab

The purified modified Fab is site-specifically conjugated with a branched molecule of PEG. This is achieved by activation of a single cysteine residue in a truncated hinge region of the modified Fab, followed by reaction with (PEG)-lysyl maleimide as previously described (A. P. Chapman *et al.*, Nature Biotechnology 17, 780-783, 1999). A PEGylated molecule of the invention is represented in Figure 12. The PEG used was 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater). The resulting Fab'-PEG was purified by cation-exchange chromatography (SP sepharose HP) using a linear NaCl gradient elution. Purified Fab'-PEG was concentrated and formulated by ultrafiltration into 50mM sodium acetate + 125mM NaCl, pH 5.5 to produce the therapeutic neutralising antibody of the invention.

It will of course be understood that the present invention has been described by way of example only, is in no way meant to be limiting, and that modifications of detail can be made within the scope of the claims hereinafter. Preferred features of each embodiment of the invention are as for each of the other embodiments *mutatis mutandis*. All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

SEQUENCE LISTING

<110> CELLTECH R&D LIMITED

<120> A neutralising antibody having specificity for human IL-1β

<160> 71

<170> SeqWin99, version 1.02

<210> 1
<211> 414
<212> DNA
<213> Artificial Sequence

<220>
<223>

<220>
<221> IC8 VH nucleotide sequence

<400> 1
atggactttg ggctcagctt gattttcctt gtccttactt taaaaggtgt gcagtgtgat 60
gtgcagttgg tggagtctgg gggaggctta gtgaagcctg gagggtccct gaaactctcc 120
tgtgcagcct ctggattcga tttcagtagg tatgacatgt cttgggttcg ccagactccg 180
gagaagaggc tggagtgggt cgcatatatt agtagtggtg gtggtagcac ctactttcca 240
gacactgtga agggccgatt caccatctcc agagacaatg ccaagaacac cctgtacctg 300
caaatgaaca atctgcagtc tgaggacaca gccatgtttt actgtgcaag acagaacaag 360
aaattaacct ggtttgatta ctggggccag gggactctgg tcactgtctc ttca 414

<210> 2
<211> 384
<212> DNA
<213>

<220>
<221> IC8 VL sequence

<400> 2
atgagtgtgc tcactcaggt cctggcgttg ctgctgctgt ggcttgcagg tgccagatgt 60
gacatccaga tgactcagtc tccagcctcc ctatctgcat ctgtgggaga aactgtcacc 120
atcacatgtc gaacaagtgg gaatattcac aattatttaa catggtatca acagaatttg 180
ggaaaatctc ctcagctcct ggtctataat gcaaaaacct tagcagatgg tgtgccatca 240
aggttcagtg gcagtggatc aggaacacaa ttttctctca cgatcaacag cctgcagcct 300
gaagattttg ggaattatta ctgtcaacat ttttggagtc ttccattcac gttcggctcg 360
gggacaaagt tggaaataaa acgt 384

<210> 3
<211> 138
<212> PRT
<213>

<220>
<221> IC8 VH protein sequence

<400> 3
Met Asp Phe Gly Leu Ser Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
1 5 10 15

Val Gln Cys Asp Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
20 25 30

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe
35 40 45

Ser Arg Tyr Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
50 55 60

Glu Trp Val Ala Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr Phe Pro
65 70 75 80

Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Thr Leu Tyr Leu Gln Met Asn Asn Leu Gln Ser Glu Asp Thr Ala Met
100 105 110

Phe Tyr Cys Ala Arg Gln Asn Lys Lys Leu Thr Trp Phe Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
130 135

<210> 4
<211> 128
<212> PRT
<213>

<220>
<221> IC8 VL protein sequence

<400> 4
Met Ser Val Leu Thr Gln Val Leu Ala Leu Leu Leu Leu Trp Leu Ala
1 5 10 15

Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser
20 25 30

Ala Ser Val Gly Glu Thr Val Thr Ile Thr Cys Arg Thr Ser Gly Asn
35 40 45

Ile His Asn Tyr Leu Thr Trp Tyr Gln Gln Asn Leu Gly Lys Ser Pro
50 55 60

Gln Leu Leu Val Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Ser Leu Thr Ile Asn
85 90 95

Ser Leu Gln Pro Glu Asp Phe Gly Asn Tyr Tyr Cys Gln His Phe Trp
100 105 110

Ser Leu Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
115 120 125

<210> 5
<211> 10
<212> PRT
<213>

<220>
<221> IC8 VH CDR1

<400> 5
Gly Phe Asp Phe Ser Arg Tyr Asp Met Ser
1 5 10

<210> 6
<211> 17
<212> PRT
<213>

<220>
<221> IC8 VH CDR2

<400> 6
Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr Phe Pro Asp Thr Val Lys
1 5 10 15

Gly

<210> 7
<211> 10
<212> PRT
<213>

<220>
<221> IC8 VH CDR3

<400> 7
Gln Asn Lys Lys Leu Thr Trp Phe Asp Tyr
1 5 10

<210> 8
<211> 11
<212> PRT
<213>

<220>
<221> IC8 VL CDR1

<400> 8
Arg Thr Ser Gly Asn Ile His Asn Tyr Leu Thr
1 5 10

<210> 9
<211> 7
<212> PRT
<213>

<220>
<221> IC8 VL CDR2

<400> 9
Asn Ala Lys Thr Leu Ala Asp
1 5

<210> 10
<211> 9
<212> PRT
<213>

<220>
<221> IC8 VL CDR3

<400> 10
Gln His Phe Trp Ser Leu Pro Phe Thr
1 5

<210> 11
<211> 119
<212> PRT
<213>

<220>
<221> IC8 graft design VH

<400> 11
Asp Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
20 25 30

Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
35 40 45

Ala Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr Phe Pro Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Asn Leu Gln Ser Glu Asp Thr Ala Met Phe Tyr Cys
85 90 95

Ala Arg Gln Asn Lys Lys Leu Thr Trp Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 12
<211> 109
<212> PRT
<213>

<220>
<221> IC8 graft design 3.11 (DP-35)

<400> 12
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly

1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105

<210> 13
<211> 119
<212> PRT
<213>

<220>
<221> IC8 graft design gH1

<400> 13
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
20 25 30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Arg Leu Glu Trp Val
35 40 45

Ala Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr Phe Pro Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Gln Asn Lys Lys Leu Thr Trp Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 14
<211> 119
<212> PRT
<213>

<220>

<221> IC8 graft design gH2

<400> 14

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
            20                  25                  30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr Phe Pro Asp Thr Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95

Ala Arg Gln Asn Lys Lys Leu Thr Trp Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser
        115
```

<210> 15
<211> 119
<212> PRT
<213>

<220>
<221> IC8 graft design gH3

<400> 15

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
            20                  25                  30

Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Arg Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr Phe Pro Asp Thr Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gln Asn Lys Lys Leu Thr Trp Phe Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser
        115
```

<210> 16
<211> 108
<212> PRT
<213>

<220>
<221> IC8 graft design VL

<400> 16
Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Glu Thr Val Thr Ile Thr Cys Arg Thr Ser Gly Asn Ile His Asn Tyr
20 25 30

Leu Thr Trp Tyr Gln Gln Asn Leu Gly Lys Ser Pro Gln Leu Leu Val
35 40 45

Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Ser Leu Thr Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Gly Asn Tyr Tyr Cys Gln His Phe Trp Ser Leu Pro Phe
85 90 95

Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
100 105

<210> 17
<211> 106
<212> PRT
<213>

<220>
<221> IC8 graft design 012 (DPK9)

<400> 17
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Phe
85 90 95

Gly Gln Gly Thr Lys Val Glu Ile Lys Arg

100 105

<210> 18
<211> 108
<212> PRT
<213>

<220>
<221> IC8 graft design gL1

<400> 18
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Gly Asn Ile His Asn Tyr
20 25 30

Leu Thr Trp Tyr Gln Gln Lys Leu Gly Lys Ala Pro Gln Leu Leu Val
35 40 45

Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Asn Tyr Tyr Cys Gln His Phe Trp Ser Leu Pro Phe
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 19
<211> 108
<212> PRT
<213>

<220>
<221> IC8 graft design gL2

<400> 19
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Gly Asn Ile His Asn Tyr
20 25 30

Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Gln Leu Leu Val
35 40 45

Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Asn Tyr Tyr Cys Gln His Phe Trp Ser Leu Pro Phe
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 20
<211> 108
<212> PRT
<213>

<220>
<221> IC8 graft design gL3

<400> 20
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Gly Asn Ile His Asn Tyr
20 25 30

Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Gln Leu Leu Ile
35 40 45

Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Asn Tyr Tyr Cys Gln His Phe Trp Ser Leu Pro Phe
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 21
<211> 458
<212> DNA
<213>

<220>
<221> IC8 gH1

<400> 21
gaataaaagc ttgccgccac catggacttt gggctcagct tgattttcct tgtccttact 60
ttaaaaggtg tgcagtgtga ggtgcagctg gtcgagtctg gaggcgggct tgtccagcct 120
ggagggagcc tgcgtctctc ttgtgcagca agcggcttcg acttttcccg ttacgatatg 180
tcctgggtgc ggcaggcacc tgggaagcgc ctggagtggg tggcatacat tagctccgga 240
ggcggctcta catacttccc ggacaccgtc aagggccgtt tcaccatttc ccgggacaat 300
gcaaagaata ccctttacct ccagatgaac tctctccgcg cagaggacac agcaatgtat 360
tactgtgcac ggcagaacaa gaaactgacc tggtttgact actggggaca ggggaccctt 420
gtgacagtct cctctgcttc tacaaagggc ccaagaaa 458

<210> 22
<211> 414
<212> DNA
<213>

<220>
<221> IC8 gL1 nucleotide sequence

<400> 22
ggatgattcg aagccgccac catgagtgtg ctcactcagg tcctggcgtt gctgctgctg 60
tggcttgcag gtgccagatg tgatatccag atgacccaga gtccaagcag tctctccgcc 120
agcgtaggcg atcgtgtgac tattacctgt cgtaccagtg gcaacatcca taattacctg 180
acgtggtacc agcaaaaact gggcaaagcc ccgcagctcc tggtctataa cgcgaaaacg 240
ctagcagacg gtgtgccaag ccgtttcagt ggcagtggca gcggtactca gtttaccctc 300
acaatttcgt ctctccagcc ggaagatttc gccaattact attgtcagca cttttggagc 360
ctgcctttca ccttcggtca gggcactaaa gtagaaatca aacgtacggc gtgc 414

<210> 23
<211> 144
<212> PRT
<213>

<220>
<221> IC8 gH1 amino acid sequence

<400> 23
Met Asp Phe Gly Leu Ser Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
1 5 10 15

Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln
20 25 30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe
35 40 45

Ser Arg Tyr Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Arg Leu
50 55 60

Glu Trp Val Ala Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr Phe Pro
65 70 75 80

Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
85 90 95

Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Met
100 105 110

Tyr Tyr Cys Ala Arg Gln Asn Lys Lys Leu Thr Trp Phe Asp Tyr Trp
115 120 125

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130 135 140

<210> 24
<211> 129
<212> PRT
<213>

<220>
<221> IC8 gL1 amino acid sequence

<400> 24
Met Ser Val Leu Thr Gln Val Leu Ala Leu Leu Leu Leu Trp Leu Ala
1 5 10 15

Gly Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser

20 25 30

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Gly Asn
35 40 45

Ile His Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Leu Gly Lys Ala Pro
50 55 60

Gln Leu Leu Val Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Thr Leu Thr Ile Ser
85 90 95

Ser Leu Gln Pro Glu Asp Phe Ala Asn Tyr Tyr Cys Gln His Phe Trp
100 105 110

Ser Leu Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
115 120 125

Thr

<210> 25
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide primer

<220>
<221> gH1 End primer T1

<400> 25
gaataaaagc ttgccgccac c 21

<210> 26
<211> 22
<212> DNA
<213> oligonucleotide primer

<220>
<221> gH1 End primer B1

<400> 26
tttcttgggc cctttgtaga ag 22

<210> 27
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gH1 F1

<400> 27
atggactttg ggctcagctt gattttcctt gtccttactt taaaaggtgt gcag 54

<210> 28
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH1 F2

<400> 28
tgtgaggtgc agctggtcga gtctggaggc gggcttgtcc agcctggagg gagc 54

<210> 29
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH1 F3

<400> 29
ctgcgtctct cttgtgcagc aagcggcttc gacttttccc gttacgatat gtcc 54

<210> 30
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH1 F4

<400> 30
tgggtgcggc aggcacctgg gaagcgcctg gagtgggtgg catacattag ctcc 54

<210> 31
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH1 F5

<400> 31
ggaggcggct ctacatactt cccggacacc gtcaagggcc gtttcaccat ttcc 54

<210> 32
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gH1 F6

<400> 32
cgggacaatg caaagaatac cctttacctc cagatgaact ctctccgcgc agag 54

<210> 33
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gH1 F7

<400> 33
gacacagcaa tgtattactg tgcacggcag aacaagaaac tgacctggtt tgac 54

<210> 34
<211> 59
<212> DNA
<213>

<220>
<221> IC8gH1 F8

<400> 34
tactggggac aggggaccct tgtgacagtc tcctctgctt ctacaaaggg cccaagaaa 59

<210> 35
<211> 58
<212> DNA
<213>

<220>
<221> IC8gH1 R1

<400> 35
cagaggagac tgtcacaagg gtcccctgtc cccagtagtc aaaccaggtc agtttctt 58

<210> 36
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH1 R2

<400> 36
gttctgccgt gcacagtaat acattgctgt gtcctctgcg cggagagagt tcat 54

<210> 37
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gH1 R3

<400> 37
ctggaggtaa agggtattct ttgcattgtc ccgggaaatg gtgaaacggc cctt 54

<210> 38
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH1 R4

<400> 38
gacggtgtcc gggaagtatg tagagccgcc tccggagcta atgtatgcca ccca 54

<210> 39
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH1 R5

<400> 39
ctccaggcgc ttcccaggtg cctgccgcac ccaggacata tcgtaacggg aaaa 54

<210> 40
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gH1 R6

<400> 40
gtcgaagccg cttgctgcac aagagagacg caggctccct ccaggctgga caag 54

<210> 41
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gH1 R7

<400> 41
cccgcctcca gactcgacca gctgcacctc acactgcaca ccttttaaag taag 54

<210> 42
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gH1 R8

<400> 42
gacaaggaaa atcaagctga gcccaaagtc catggtggcg gcaagctttt attc 54

<210> 43
<211> 20
<212> DNA
<213>

<220>
<221> gL1 End primer T1

<400> 43
ggatgattcg aagccgccac 20

<210> 44

<211> 21
<212> DNA
<213>

<220>
<221> gL1 End primer B1

<400> 44
gcacgccgta cgtttgattt c 21

<210> 45
<211> 55
<212> DNA
<213>

<220>
<221> IC8 gL1 F1

<400> 45
catgagtgtg ctcactcagg tcctggcgtt gctgctgctg tggcttgcag gtgcc 55

<210> 46
<211> 57
<212> DNA
<213>

<220>
<221> IC8 gL1 F2

<400> 46
agatgtgata tccagatgac ccagagtcca agcagtctct ccgccagcgt aggcgat 57

<210> 47
<211> 57
<212> DNA
<213>

<220>
<221> IC8 gL1 F3

<400> 47
cgtgtgacta ttacctgtcg taccagtggc aacatccata attacctgac gtggtac 57

<210> 48
<211> 57
<212> DNA
<213>

<220>
<221> IC8 gL1 F4

<400> 48
cagcaaaaac tgggcaaagc cccgcagctc ctggtctata acgcgaaaac gctagca 57

<210> 49
<211> 57
<212> DNA
<213>

<220>
<221> IC8 gL1 F5

<400> 49
gacggtgtgc caagccgttt cagtggcagt ggcagcggta ctcagtttac cctcaca 57

<210> 50
<211> 57
<212> DNA
<213>

<220>
<221> IC8gL1 F6

<400> 50
atttcgtctc tccagccgga agatttcgcc aattactatt gtcagcactt ttggagc 57

<210> 51
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gL1 F7

<400> 51
ctgcctttca ccttcggtca gggcactaaa gtagaaatca aacgtacggc gtgc 54

<210> 52
<211> 54
<212> DNA
<213>

<220>
<221> IC8gL1 R1

<400> 52
tactttagtg ccctgaccga aggtgaaagg caggctccaa aagtgctgac aata 54

<210> 53
<211> 57
<212> DNA
<213>

<220>
<221> IC8 gL1 R2

<400> 53
gtaattggcg aaatcttccg gctggagaga cgaaattgtg agggtaaact gagtacc 57

<210> 54
<211> 57
<212> DNA
<213>

<220>
<221> IC8 gL1 R3

<400> 54

gctgccactg ccactgaaac ggcttggcac accgtctgct agcgttttcg cgttata 57

<210> 55
<211> 57
<212> DNA
<213>

<220>
<221> IC8 gL1 R4

<400> 55
gaccaggagc tgcggggctt tgcccagttt ttgctggtac cacgtcaggt aattatg 57

<210> 56
<211> 57
<212> DNA
<213>

<220>
<221> IC8gL1 R5

<400> 56
gatgttgcca ctggtacgac aggtaatagt cacacgatcg cctacgctgg cggagag 57

<210> 57
<211> 57
<212> DNA
<213>

<220>
<221> IC8gL1 R6

<400> 57
actgcttgga ctctgggtca tctggatatc acatctggca cctgcaagcc acagcag 57

<210> 58
<211> 54
<212> DNA
<213>

<220>
<221> IC8 gL1 R7

<400> 58
cagcaacgcc aggacctgag tgagcacact catggtggcg gcttcgaatc atcc 54

<210> 59
<211> 51
<212> DNA
<213>

<220>
<221> IC8gL2 Sequence

<400> 59
cagcaaaaac cgggcaaagc cccgcagctc ctggtctata acgcgaaaac g 51

<210> 60
<211> 21

<212> PRT
<213>

<220>
<221> IC8gL2 Sequence

<400> 60
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Gln Leu Leu Val Tyr Asn
1 5 10 15

Ala Lys Thr Leu Ala
20

<210> 61
<211> 51
<212> DNA
<213>

<220>
<221> IC8 gL3 sequence

<400> 61
cagcaaaaac cgggcaaagc cccgcagctc ctgatctata acgcgaaaac g 51

<210> 62
<211> 21
<212> PRT
<213>

<220>
<221> IC8 gL3 sequence

<400> 62
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Gln Leu Leu Ile Tyr Asn
1 5 10 15

Ala Lys Thr Leu Ala
20

<210> 63
<211> 48
<212> DNA
<213>

<220>
<221> IC8gH2 Sequence

<400> 63
cctgggaagg gcctggagtg ggtggcatac attagctccg gaggcggc 48

<210> 64
<211> 48
<212> DNA
<213>

<220>
<221> IC8gH2 Reverse Sequence

<400> 64

ggacccttcc cggacctcac ccaccgtatg taatcgaggc ctccgccg 48

<210> 65
<211> 16
<212> PRT
<213>

<220>
<221> IC8gH2 Sequence

<400> 65
Pro Gly Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser Gly Gly Gly
1 5 10 15

<210> 66
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH3 Sequence

<400> 66
gacacagcag tgtattactg tgcacggcag aacaagaaac tgacctggtt tgac 54

<210> 67
<211> 54
<212> DNA
<213>

<220>
<221> IC8gH3 Reverse Sequence

<400> 67
ctgtgtcgtc acataatgac acgtgccgtc ttgttctttg actggaccaa actg 54

<210> 68
<211> 18
<212> PRT
<213>

<220>
<221> IC8gH3 Sequence

<400> 68
Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gln Asn Lys Lys Leu Thr Trp
1 5 10 15

Phe Asp

<210> 69
<211> 2050
<212> DNA
<213>

<220>
<221> Coding and flanking sequence of pTTOD(gH3gL3 Fab' IGS-2)

<400> 69
aattctcatg tttgacagct tatcatcgac tgcacggtgc accaatgctt ctggcgtcag 60
gcagccatcg gaagctgtgg tatggctgtg caggtcgtaa atcactgcat aattcgtgtc 120
gctcaaggcg cactcccgtt ctggataatg ttttttgcgc cgacatcata acggttctgg 180
caaatattct gaaatgagct gttgacaatt aatcatcggc tcgtataatg tgtggaattg 240
tgagcggata acaatttcac acaggaaaca gcgatgagct tggctgcagg tcgagttcta 300
gataacgagg cgtaaaaaat gaaaaagaca gctatcgcaa ttgcagtggc cttggctggt 360
ttcgctaccg tagcgcaagc tgatatccag atgacccaga gtccaagcag tctctccgcc 420
agcgtaggcg atcgtgtgac tattacctgt cgtaccagtg gcaacatcca taattacctg 480
acgtggtacc agcaaaaacc gggcaaagcc ccgcagctcc tgatctataa cgcgaaaacg 540
ctagcagacg gtgtgccaag ccgtttcagt ggcagtggca gcggtactca gtttaccctc 600
acaatttcgt ctctccagcc ggaagatttc gccaattact attgtcagca cttttggagc 660
ctgcctttca ccttcggtca gggcactaaa gtagaaatca aacgtacggt agcggcccca 720
tctgtcttca tcttcccgcc atctgatgag cagttgaaat ctggaactgc ctctgttgtg 780
tgcctgctga ataacttcta tcccagagag gccaaagtac agtggaaggt ggataacgcc 840
ctccaatcgg gtaactccca ggagagtgtc acagagcagg acagcaagga cagcacctac 900
agcctcagca gcaccctgac gctgagcaaa gcagactacg agaaacacaa agtctacgcc 960
tgcgaagtca cccatcaggg cctgagctca ccagtaacaa aaagttttaa tagaggggag 1020
tgttaaaatg aagaagactg ctatagcaat tgcagtggcg ctagctggtt tcgccaccgt 1080
ggcgcaagct gaggttcagc tggtcgagtc tggaggcggg cttgtccagc ctggagggag 1140
cctgcgtctc tcttgtgcag caagcggctt cgacttttcc cgttacgata tgtcctgggt 1200
gcggcaggca cctgggaagc gcctggagtg ggtggcatac attagctccg gaggcggctc 1260
tacatacttc ccggacaccg tcaagggccg tttcaccatt tcccgggaca atgcaaagaa 1320
taccctttac ctccagatga actctctccg cgcagaggac acagcagtgt attactgtgc 1380
acggcagaac aagaaactga cctggtttga ctactgggga caggggaccc ttgtgacagt 1440
ctcctctgct tctacaaagg gcccatcggt cttccccctg gcaccctcct ccaagagcac 1500
ctctgggggc acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccggtgac 1560
ggtgtcgtgg aactcaggcg ccctgaccag cggcgtgcac accttcccgg ctgtcctaca 1620
gtcctcagga ctctactccc tcagcagcgt ggtgaccgtg ccctccagca gcttgggcac 1680
ccagacctac atctgcaacg tgaatcacaa gcccagcaac accaaggtcg acaagaaagt 1740
tgagcccaaa tcttgtgaca aaactcacac atgcgccgcg tgatgaggat ccaagcttgc 1800
ggccgcgaat tcactggccg tcgttttaca acgtcgtgac tgggaaaacc ctggcgttac 1860
ccaacttaat cgccttgcag cacatccccc tttcgccagc tcgcgtaata gcgaagaggc 1920
ccgcaccgat cgcccttccc aacagttgcg cagcctgaat ggcgaatggc gcctgatgcg 1980
gtattttctc cttacgcatc tgtgcggtat ttcacaccgc ataaattccc tgttttggcg 2040
gatgagagaa 2050

<210> 70
<211> 235
<212> PRT
<213>

<400> 70
Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala
1 5 10 15

Thr Val Ala Gln Ala Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
20 25 30

Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Thr Ser Gly
35 40 45

Asn Ile His Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Lys Ala
50 55 60

Pro Gln Leu Leu Ile Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro
65 70 75 80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Thr Leu Thr Ile
85 90 95

Ser Ser Leu Gln Pro Glu Asp Phe Ala Asn Tyr Tyr Cys Gln His Phe
100 105 110

Trp Ser Leu Pro Phe Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
115 120 125

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
130 135 140

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
145 150 155 160

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
165 170 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
180 185 190

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
195 200 205

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 71
<211> 251
<212> PRT
<213>

<400> 71
Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala
1 5 10 15

Thr Val Ala Gln Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu
20 25 30

Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe
35 40 45

Asp Phe Ser Arg Tyr Asp Met Ser Trp Val Arg Gln Ala Pro Gly Lys
50 55 60

Arg Leu Glu Trp Val Ala Tyr Ile Ser Ser Gly Gly Gly Ser Thr Tyr
65 70 75 80

Phe Pro Asp Thr Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
85 90 95

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
100 105 110

Ala Val Tyr Tyr Cys Ala Arg Gln Asn Lys Lys Leu Thr Trp Phe Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145 150 155 160

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
210 215 220

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
225 230 235 240

Lys Ser Cys Asp Lys Thr His Thr Cys Ala Ala
245 250

<210> 72
<211> 59
<212> DNA
<213>

<220>
<221> IC8gL2 Complementary Sequence

<400> 72

ctagcgtttt cgcgttatag accaggagct gcggggcttt gcccggtttt tgctggtac 59

<210> 73
<211> 59
<212> DNA
<213>

<220>
<221> IC8gL3 Complementary Sequence

<400> 73

ctagcgtttt cgcgttatag atcaggagct gcggggcttt gcccggtttt tgctggtac 59

<210> 74
<211> 15
<212> PRT
<213>

<400> 74

Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10 15

<210> 75
<211> 12
<212> PRT
<213>

<400> 75

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Iso Lys
1 5 10

CLAIMS:

1. A neutralising antibody having specificity for human IL-1β comprising a heavy chain, wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:5 for CDR-H1, a CDR having the sequence given in SEQ ID NO:6 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:7 for CDR-H3.

2. A neutralising antibody according to claim 1, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:5 for CDR-H1, the sequence given in SEQ ID NO:6 for CDR-H2 and the sequence given in SEQ ID NO:7 for CDR-H3.

3. A neutralising antibody having specificity for human IL-1β, comprising a light chain, wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:8 for CDR-L1, a CDR having the sequence given in SEQ ID NO:9 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:10 for CDR-L3.

4. A neutralising antibody according to claim 3, wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:8 for CDR-L1, the sequence given in SEQ ID NO:9 for CDR-L2 and the sequence given in SEQ ID NO:10 for CDR-L3.

5. A neutralising antibody according to claim 1 or 2, additionally comprising a light chain, wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:8 for CDR-L1, a CDR having the sequence given in SEQ ID NO:9 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:10 for CDR-L3.

6. A neutralising antibody according to claim 5, wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:8 for CDR-L1, the sequence given in SEQ ID NO:9 for CDR-L2 and the sequence given in SEQ ID NO:10 for CDR-L3.

7. A neutralising antibody according to any one of claims 1 to 6, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:5 for CDR-H1, the sequence given in SEQ ID NO:6 for CDR-H2 and the sequence given in SEQ ID NO:7 for CDR-H3 and wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:8 for CDR-L1, the sequence given in SEQ ID NO:9 for CDR-L2 and the sequence given in SEQ ID NO:10 for CDR-L3.

8. A neutralising antibody according to any one of claims 1 to 7, wherein said neutralising antibody is a CDR-grafted antibody molecule.

9. The antibody molecule of claim 8, wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

10. A CDR-grafted antibody molecule according to claim 9, wherein the heavy chain framework region is derived from human sub-group sequence 3-11 (DP-35) together with JH4.

11. A CDR-grafted antibody molecule according to claim 10, wherein the residue at at leastone of positions 44 and 89 of the variable domain of the heavy chain is a donor residue.

12. A CDR-grafted antibody molecule according to claim 10, wherein the residue at at least position 44 of the variable domain of the heavy chain is a donor residue.

13. A CDR-grafted antibody molecule according to any one of claims 8 to 12, wherein the light chain framework region is derived from human sub-group sequence O12 (DPK9) together with JK1.

14. A CDR-grafted antibody molecule according to claim 13, wherein the residue at at least one of positions 40, 45, 48, 70 and 85 is a donor residue.

15. A CDR-grafted antibody molecule according to claim 14, wherein the residues at positions 45, 70 and 85 are donor residues.

16. A CDR-grafted antibody according to any one of claims 8 to 15, wherein the heavy chain comprises the sequence gH3 (SEQ ID NO:15).

17. A CDR-grafted antibody according to any one of claims 8 to 15, wherein the light chain comprises the sequence gL3 (SEQ ID NO:20).

18. A CDR-grafted antibody according to any one of claims 8 to 15, wherein the heavy chain comprises the sequence gH3 (SEQ ID NO:15) and the light chain comprises the sequence gL3 (SEQ ID NO:20).

19. A neutralising antibody having specificity for human IL-1β, having a heavy chain comprising the sequence given in SEQ ID NO:3 and a light chain comprising the sequence given in SEQ ID NO:4.

20. A neutralising antibody having specificity for human IL-1β, having a variable domain at least 90% identical or similar to the antibody of any one of claims 2, 4, 18 or 19.

21. A neutralising antibody molecule according to any one of claims 1 to 20, wherein the antibody molecule is selected from the group consisting of: a complete antibody molecule having full length heavy and light chains or a fragment thereof, such as a Fab, modified Fab, Fab', $F(ab')_2$, Fv or scFv fragment.

22. A neutralising antibody molecule according to claim 21 that has been modified to enable an effector or reporter molecule to be attached to it.

23. A neutralising antibody molecule according to claim 22, wherein the modification is the addition to the C-terminal end of its heavy chain of one or more amino acids to allow the attachment of an effector or reporter molecule.

24. The antibody molecule of claim 23, wherein the additional amino acids form a modified hinge region containing one or two cysteine residues to which the effector or reporter molecule may be attached.

25. A neutralising antibody molecule according to any one of claims 22 to 24, having an effector or a reporter molecule attached to it.

26. A neutralising antibody molecule according to claim 25, wherein the effector molecule comprises one or more polymers.

27. A neutralising antibody molecule according to claim 26, wherein the one or more polymers is/are optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide.

28. A neutralising antibody molecule according to claim 26, wherein the one or more polymers is/are a methoxypoly(ethyleneglycol) or poly(ethyleneglycol).

29. A neutralising antibody molecule according to claim 25, having attached to one of the cysteine residues at the C-terminal end of the heavy chain a lysyl-maleimide or lysyl bis-maleimide group wherein each amino group of the lysyl residue has covalently linked to it a methoxypoly(ethyleneglycol) residue having a molecular weight of about 20,000 Da.

30. A neutralising antibody molecule having specificity for human IL-1β, which is a modified Fab fragment having a heavy chain comprising the sequence given in SEQ ID NO:15 and a light chain comprising the sequence given in SEQ ID NO:20 and having at the C-terminal end of its heavy chain a modified hinge region containing one cysteine residue to which an effector or reporter molecule may be attached.

31. A neutralising antibody molecule having specificity for human IL-1β, which is a modified Fab fragment having a heavy chain comprising the sequence given in SEQ ID NO:15 and a light chain comprising the sequence given in SEQ ID NO:20 and having at the C-terminal end of its heavy chain a modified hinge region containing one cysteine residue to which an effector or reporter molecule is attached.

32. A neutralising antibody molecule according to claim 31, wherein its heavy chain comprises or consists of amino acid residue numbers 22 to 251 of the sequence given in

SEQ ID NO:71, and wherein its light chain comprises or consists of amino acid residue numbers 22 to 235 of the sequence given in SEQ ID NO:70.

33. A neutralising antibody molecule according to claim 31 or claim 32, having attached to the cysteine residue at the C-terminal end of the heavy chain a lysyl-maleimide group, wherein each amino group of the lysyl residue has covalently linked to it a methoxypoly(ethyleneglycol) residue having a molecular weight of about 20,000 Da.

34. A neutralising antibody molecule having specificity for human IL-1β, which binds to the same epitope as the antibody of claim 18.

35. An isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody molecule according to any one of claims 1 to 34.

36. A cloning or expression vector comprising one or more DNA sequences according to claim 35.

37. The vector according to claim 36, wherein the vector comprises the sequence given in SEQ ID NO:69.

38. A host cell comprising one or more cloning or expression vectors according to claims 36 or 37.

39. A process for the production of the antibody molecule of any one of claims 1 to 34, comprising culturing the host cell of claim 38 and isolating the antibody molecule.

40. A pharmaceutical composition comprising an antibody molecule according to any one of claims 1 to 34, in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

41. A pharmaceutical composition according to claim 40, additionally comprising other active ingredients.

42. An antibody molecule according to any one of claims 1 to 34 or a pharmaceutical composition according to claim 40 or claim 41, for use in the treatment or prophylaxis of a pathological disorder that is mediated by IL-1β, or that is associated with an increased level of IL-1β.