ANTIBODY MOLECULES HAVING SPECICITY FOR HUMAN TUMOR NECROSIS FACTOR ALPHA, AND USE THEREOF

The present invention relates to an antibody molecule having specificity for antigenic determinants of human tumour necrosis factor alpha (TNFα). The present invention also relates to the therapeutic uses of the antibody molecule and methods for producing the antibody molecule.

This invention relates to antibody molecules. In an antibody molecule, there are two heavy chains and two light chains. Each heavy chain and each light chain has at its N-terminal end a variable domain. Each variable domain is composed of four framework regions (FRs) alternating with three complementarily determining regions (CDRs). The residues in the 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 (CDRH1), residues 50-65 (CDRH2) and residues 95-102 (CDRH3) according to the Kabat numbering.

The CDRs of the light chain variable domain are located at residues 24-34 (CDRL1), residues 50-56 (CDRL2) and residues 89-97 (CDRL3) according to the Kabat numbering.

Construction of CDR-grafted antibodies is described in European Patent Application EP-A-0239400, which discloses a process in which the CDRs of a mouse monoclonal antibody are grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. The

CDRs determine the antigen binding specificity of antibodies and are relatively short peptide sequences carried on the framework regions of the variable domains.

The earliest work on humanising monoclonal antibodies by CDR-grafting was carried out on monoclonal antibodies recognising synthetic antigens, such as NP. However, examples in which a mouse monoclonal antibody recognising lysozyme and a rat monoclonal antibody recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen *et al.* (Science, 239, 1534-1536, 1988) and Riechmann *et al.* (Nature, 332, 323-324, 1988), respectively.

Riechmann *et al.*, found that the transfer of the CDRs alone (as defined by Kabat (Kabat *et al.* (*supra*) and Wu *et al.*, J. Exp. Med., 132, 211-250, 1970)) was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. It was found that a number of framework residues have to be altered so that they correspond to those of the donor framework region. Proposed criteria for selecting which framework residues need to be altered are described in International Patent Application WO 90/07861.

A number of reviews discussing CDR-grafted antibodies have been published, including Vaughan *et al.* (Nature Biotechnology, 16, 535-539, 1998).

TNFα is a pro-inflammatory cytokine that is released by and interacts with cells of the immune system. Thus, TNFα is released by macrophages that have been activated by lipopolysaccharides (LPS) of gram negative bacteria. As such, TNFα appears to be an endogenous mediator of central importance involved in the development and pathogenesis of endotoxic shock associated with bacterial sepsis. TNFα has also been shown to be up-regulated in a number of human diseases, including chronic diseases such as rheumatoid arthritis, Crohn's disease, ulcerative colitis and multiple sclerosis. Mice transgenic for human TNFα produce high levels of TNFα constitutively and develop a spontaneous, destructive polyarthritis resembling rheumatoid arthritis (Kaffer *et al.*, EMBO J., 10, 4025-4031, 1991). TNFα is therefore referred to as a pro-inflammatory cytokine.

Monoclonal antibodies against TNFα have been described in the prior art. Meager *et al.*, (Hybridoma, 6, 305-311, 1987) describe murine monoclonal antibodies against recombinant TNFα. Fendly *et al.*, (Hybridoma, 6, 359-370, 1987) describe the use of murine monoclonal antibodies against recombinant TNFα in defining neutralising epitopes on TNF. Shimamoto *et al.*, (Immunology Letters, 17, 311-318, 1988) describe the use of murine monoclonal antibodies against TNFγ and their use in preventing endotoxic shock in mice. Furthermore, in International Patent Application WO 92/11383, recombinant

antibodies, including CDR-grafted antibodies, specific for TNFα are disclosed. Rankin *et al.*, (British J. Rheumatology, 34, 334-342, 1995) describe the use of such CDR-grafted antibodies in the treatment of rheumatoid arthritis. US-A-5 919 452 discloses anti-TNF chimeric antibodies and their use in treating pathologies associated with the presence of TNF.

Antibodies to TNFα have been proposed for the prophylaxis and treatment of endotoxic shock (Beutler *et al.*, Science, 234, 470-474, 1985). Bodmer *et al.*, (Critical Care Medicine, 21, S441-S446, 1993) and Wherry *et al.*, (Critical Care Medicine, 21, S436-S440, 1993) discuss the therapeutic potential of anti-TNFα antibodies in the treatment of septic shock. The use of anti-TNFα antibodies in the treatment of septic shock is also discussed by Kirschenbaum *et al.*, (Critical Care Medicine, 26, 1625-1626, 1998). Collagen-induced arthritis can be treated effectively using an anti-TNFα monoclonal antibody (Williams *et al.* (PNAS-USA, 89, 9784-9788, 1992)).

Increased levels of TNFα are found in both the synovial fluid and peripheral blood of patients suffering from rheumatoid arthritis. When TNFα blocking agents are administered to patients suffering from rheumatoid arthritis, they reduce inflammation, improve symptoms and retard joint damage (McKown *et al.* (Arthritis Rheum., 42, 1204-1208, 1999).

The use of anti-TNFα antibodies in the treatment of rheumatoid arthritis and Crohn's disease is discussed in Feldman *et al.*, (Transplantation Proceedings, 30, 4126-4127, 1998), Adorini *et al.*, (Trends in Immunology Today, 18, 209-211, 1997) and in Feldman *et al.*, (Advances in Immunology, 64, 283-350, 1997). The antibodies to TNFα used in such treatments are generally chimeric antibodies, such as those described in US-A-5 919 452.

Two TNFα blocking products are currently licensed for the treatment of rheumatoid arthritis. The first, called etanercept, is marketed by Immunex Corporation as Enbrel ™. It is a recombinant fusion protein comprising two p75 soluble TNF-receptor domains linked to the Fc portion of a human immunoglobulin. The second, called infliximab, is marketed by Centocor Corporation as Remicade™. It is a chimeric antibody having murine anti-TNFα variable domains and human IgG1 constant domains.

The prior art recombinant anti-TNFα antibody molecules generally have a reduced affinity for TNFα compared to the antibodies from which the variable regions or CDRs are

derived, generally have to be produced in mammalian cells and are expensive to manufacture. Prior art anti-TNFα antibodies are described in Stephens *et al.*, (Immunology, 85; 668-674, 1995), GB-A-2 246 570 and GB-A-2 297 145.

There is a need for an antibody molecule to treat chronic inflammatory diseases which can be used repeatedly and produced easily and efficiently. There is also a need for an antibody molecule which has high affinity for TNFα and low immunogenicity in humans.

In a first aspect, the present invention provides an antibody molecule having specificity for TNFα, comprising a heavy chain wherein the variable domain comprises a CDR (as defined by Kabat *et al.*, (*supra*)) having the sequence given as H1 in Figure 3 (SEQ ID NO:1) for CDRH1, as H2' in Figure 3 (SEQ ID NO:2) or as H2 in Figure 3 (SEQ ID NO:7) for CDRH2 or as H3 in Figure 3 (SEQ ID NO:3) for CDRH3.

The antibody molecule of the first aspect of the present invention comprises at least one CDR selected from H1, H2' or H2 and H3 (SEQ ID NO:1; SEQ ID NO:2 or SEQ ID NO:7 and SEQ ID NO:3) for the heavy chain variable domain. Preferably, the antibody molecule comprises at least two and more preferably all three CDRs in the heavy chain variable domain.

In a second aspect of the present invention, there is provided an antibody molecule having specificity for human TNFα, comprising a light chain wherein the variable domain comprises a CDR (as defined by Kabat *et al.*, (*supra*)) having the sequence given as L1 in Figure 3 (SEQ ID NO:4) for CDRL1, L2 in Figure 3 (SEQ ID NO:5) for CDRL2 or L3 in Figure 3 (SEQ ID NO:6) for CDRL3.

The antibody molecule of the second aspect of the present invention comprises at least one CDR selected from L1, L2 and L3 (SEQ ID NO:4 to SEQ ID NO:6) for the light chain variable domain. Preferably, the antibody molecule comprises at least two and more preferably all three CDRs in the light chain variable domain.

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

Preferably, the antibody molecule of the first or second aspect of the present invention comprises a heavy chain wherein the variable domain comprises a CDR (as defined by Kabat *et al.*, (*supra*)) having the sequence given as H1 in Figure 3 (SEQ ID NO:1) for CDRH1, as H2' or H2 in Figure 3 (SEQ ID NO:2 or SEQ ID NO:7) for CDRH2 or as H3 in Figure 3 (SEQ ID NO:3) for CDRH3 and a light chain wherein the variable

domain comprises a CDR (as defined by Kabat *et al.*, (*supra*)) having the sequence given as L1 in Figure 3 (SEQ ID NO:4) for CDRL1, as L2 in Figure 3 (SEQ ID NO:5) for CDRL2 or as L3 in Figure 3 (SEQ ID NO:6) for CDRL3.

The CDRs given in SEQ IDS NOS:1 and 3 to 7 and in Figure 3 referred to above are derived from a mouse monoclonal antibody hTNF40. However, SEQ ID NO:2 consists of a hybrid CDR. The hybrid CDR comprises part of heavy chain CDR2 from mouse monoclonal antibody hTNF40 (SEQ ID NO:7) and part of heavy chain CDR2 from a human group 3 germline V region sequence.

The complete sequences of the variable domains of the mouse hTNF40 antibody are shown in Figures 6 (light chain) (SEQ ID NO:99) and Figure 7 (heavy chain) (SEQ ID NO:100). This mouse antibody is referred to below as "the donor antibody".

A first alternatively preferred embodiment of the first or second aspect of the present invention is the mouse monoclonal antibody hTNF40 having the light and heavy chain variable domain sequences shown in Figure 6 (SEQ ID NO:99) and Figure 7 (SEQ ID NO:100), respectively. The light chain constant region of hTNF40 is kappa and the heavy chain constant region is IgG2a.

In a second alternatively preferred embodiment, the antibody according to either of the first and second aspects of the present invention is a chimeric mouse/human antibody molecule, referred to herein as the chimeric hTNF40 antibody molecule. The chimeric antibody molecule comprises the variable domains of the mouse monoclonal antibody hTNF40 (SEQ ID NOS:99 and 100) and human constant domains. Preferably, the chimeric hTNF40 antibody molecule comprises the human C kappa domain (Hieter *et al.*, Cell, 22, 197-207, 1980; Genebank accession number J00241) in the light chain and the human gamma 4 domains (Flanagan *et al.*, Nature, 300, 709-713, 1982) in the heavy chain.

In a third alternatively preferred embodiment, the antibody according to either of the first and second aspects of the present invention is a CDR-grafted antibody molecule. The term "a CDR-grafted antibody molecule" as used herein refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, a hybrid CDR) from the 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).

Preferably, such a CDR-grafted antibody has a variable domain comprising human acceptor framework regions as well as one or more of the donor CDRs referred to above.

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. 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. The preferred framework regions for the light chain are the human group 1 framework regions shown in Figure 1 (SEQ ID NOS:83, 85, 87 and 89). The preferred framework regions for the heavy chain are the human group 1 and group 3 framework regions shown in Figure 2 (SEQ ID NOS:91, 93, 95 and 97 and SEQ ID NOS:106, 107, 108 and 109), respectively.

In a CDR-grafted antibody of the present invention, it is preferred to use as the acceptor antibody one having chains which are homologous to the chains of the donor antibody. 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.

Also, in a CDR-grafted antibody of the present invention, the frameork 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. 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 human group 1 framework regions (shown in Figure 2) (SEQ ID NOS:91, 93, 95 and 97), then the acceptor framework regions of the heavy chain comprise, in addition to one or more donor CDRs, donor residues at positions 28, 69 and 71 (according to Kabat *et al. (supra)*).

Alternatively, if the acceptor heavy chain has group 1 framework regions, then the acceptor framework regions of the heavy chain comprise, in addition to one or more donor CDRs, donor residues at positions 28, 38, 46, 67, 69 and 71 (according to Kabat *et al. (supra)*.

Preferably, in a CDR-grafted antibody molecule of the present invention, if the acceptor heavy chain has human group 3 framework regions (shown in Figure 2) (SEQ ID NOS:106, 107, 108 and 109), then the acceptor framework regions of the heavy chain comprise, in addition to one or more donor CDRs, donor residues at positions 27, 28, 30, 48, 49, 69, 71, 73, 76 and 78 (according to Kabat *et al. (supra)*).

Preferably, in a CDR-grafted antibody molecule according to the present invention, if the acceptor light chain has human group 1 framework regions (shown in Figure 1) (SEQ ID NOS:83, 85, 87 and 89) then the acceptor framework regions of the light chain comprise donor residues at positions 46 and 60 (according to Kabat *et al.* (*supra*)).

Donor residues are residues from the donor antibody, i.e. the antibody from which the CDRs were originally derived.

The antibody molecule of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, modified Fab, Fab', $F(ab')_2$ or Fv fragment; a light chain or heavy chain monomer or dimer; 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.

Preferably the antibody molecule of the present invention is a Fab fragment. Preferably the Fab fragment has a heavy chain having the sequence given as SEQ ID NO:111 and a light chain having the sequence given as SEQ ID NO:113. The amino acid sequences given in SEQ ID NO:111 and SEQ ID NO:113 are preferably encoded by the nucleotide sequences given in SEQ ID NO:110 and SEQ ID NO:112, respectively.

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 residue to which the effector or reporter molecule may be attached. Such a modified Fab fragment preferably has a heavy chain having the sequence given as SEQ ID NO:115 and the light chain having the sequence given as SEQ ID NO:113. The amino acid sequence given in SEQ ID NO:115 is preferably encoded by the nucleotide sequence given in SEQ ID NO:114.

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 40 000Da 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 Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures.

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 EP-A-0392745.

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 13. As shown in Figure 13, the modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue is covalently linked to the maleimide group. To

each of the amine groups on the lysine residue is 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.

Preferably, in the compound shown in Figure 13, the heavy chain of the antibody part has the sequence given as SEQ ID NO:115 and the light chain has the sequence given in SEQ ID NO:113. This compound is referred to herein as CDP870.

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 TNFα activity.

Also, 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. 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 attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

The antibody molecule of the present invention preferably has a binding affinity of at least 0.85×10^{-10} M, more preferably at least 0.75×10^{-10} M and most preferably at least 0.5×10^{-10} M. (It is worth noting that the preferred humanised antibody molecule of the present invention, as described below, has an affinity of about 0.5×10^{-10} M, which is better than the affinity of the murine monoclonal antibody from which it is derived. The murine antibody has an affinity of about 0.85×10^{-10} M.)

Preferably, the antibody molecule of the present invention comprises the light chain variable domain hTNF40-gL1 (SEQ ID NO:8) and the heavy chain variable domain gh3hTNF40.4 (SEQ ID NO:11). The sequences of the variable domains of these light and heavy chains are shown in Figures 8 and 11, respectively.

The present invention also relates to variants of the antibody molecule of the present invention, which have an improved affinity for TNFα. 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 a 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.

In one preferred embodiment, the DNA sequence encodes a light chain and comprises the sequence shown in SEQ ID NO:8 (hTNF40-gL1) or SEQ ID NO:9 (h-TNF-40-gL2) or a degenerate equivalent thereof.

In an alternatively preferred embodiment, the DNA sequence encodes a heavy chain and comprises the sequence shown in SEQ ID NO:10 (gh1hTNF40.4) or SEQ ID NO:11 (gh3hTNF40.4) or a degenerate equivalent thereof.

The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences 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.

In a preferred embodiment, the present invention provides an *E. coli* expression vector comprising a DNA sequence of the present invention. Preferably the expression vector is pTTO(CDP870) as shown schematically in Figure 22.

The present invention also comprises vector pDNAbEng-G1 as shown in Figure 19.

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.

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.

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, in part, for expression of antibody fragments such as Fab and $F(ab')_2$ fragments, and especially Fv fragments and single chain antibody fragments, for example, single chain Fvs. Eukaryotic, e.g. mammalian, host cell expression systems may be used for production of larger antibody molecules, including complete antibody molecules. 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 comprising 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.

Preferably the process for the production of the antibody molecule of the present invention comprises culturing *E. coli* comprising an *E. coli* expression vector comprising the DNA sequence of the present invention under conditions suitable for leading to expression of protein from the DNA sequence and isolating the antibody molecule. The antibody molecule may be secreted from the cell or targeted to the periplasm by suitable signal sequences. Alternatively, the antibody molecules may accumulate within the cell's cytoplasm. Preferably the antibody molecule is targeted to the periplasm. Depending on

the antibody molecule being produced and the process used, it is desirable to allow the antibody molecules to refold and adopt a functional conformation. Procedures for allowing antibody molecules to refold are well known to those skilled in the art.

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.

The present invention also provides a therapeutic or diagnostic composition comprising an antibody molecule of the present invention in combination with a pharmaceutically acceptable excipient, diluent or carrier.

The present invention also provides a process for preparation of a therapeutic or diagnostic composition comprising admixing the antibody molecule of the present invention together with a pharmaceutically acceptable excipient, diluent or carrier.

The antibody molecule may be the sole active ingredient in the therapeutic 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 should 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 dose 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 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, an effective dose will be from 0.01 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 20 mg/kg, more preferably about 15 mg/kg. As shown in the Examples below, doses of 1, 5 and 20 mg/kg have been used to treat patients suffering from rheumatoid arthritis.

Compositions may be administered individually to a patient or may be administered in combination 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 degree to which the level of TNFα to be neutralised is, or is expected to be, raised above a desirable level, and on whether the antibody molecule is being used prophylactically or to treat an existing condition.

Thus, for example, where the product is for treatment or prophylaxis of a chronic inflammatory disease, such as rheumatoid arthritis, suitable doses of the antibody molecule of the present invention lie in the range of between 0.5 and 50 mg/kg, more preferably between 1 and 20 mg/kg and most preferably about 15 mg/kg. 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, per week or even once every 1 or 2 months.

A pharmaceutical composition may also contain a pharmaceutically acceptable carrier for administration of the antibody. The 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 WO98/20734), 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 the antibody molecule of the present invention for use in treating a disease mediated by TNFα.

The present invention further provides the use of the antibody molecule according to the present invention in the manufacture of a medicament for the treatment of a disease mediated by TNFα.

The antibody molecule of the present invention may be utilised in any therapy where it is desired to reduce the level of biologically active TNFα present in the human or animal body. The TNFα may be circulating in the body or present in an undesirably high level localised at a particular site in the body.

For example, elevated levels of TNFα are implicated in acute and chronic immune and immunoregulatory disorders, infections including septic, endotoxic and cardiovascular shock, inflammatory disorders, neurodegenerative diseases, malignant diseases and alcohol induced hepatitis. Details of the numerous disorders associated with elevated levels of TNFα are set out in US-A-5 919 452. The antibody molecule of the present invention may be utilised in the therapy of diseases mediated by TNFα. Particularly relevant diseases which may be treated by the antibody molecule of the present invention include sepsis, congestive heart failure, septic or endotoxic shock, cachexia, adult respiratory distress syndrome, AIDS, allergies, psoriasis, TB, inflammatory bone disorders, blood coagulation disorders, burns, rejection episodes following organ or tissue transplant, Crohn's disease and autoimmune diseases, such as thyroiditis and rheumatoid- and osteo-arthritis.

Additionally, the antibody molecule or composition may be used: to reduce side effects associated with TNFα generation during neoplastic therapy; to eliminate or reduce shock-related symptoms associated with the treatment or prevention of graft rejection by use of an anti-lymphocyte antibody; or for treating multi-organ failure.

The antibody molecule of the present invention is preferably used for treatment of rheumatoid- or osteo-arthritis.

The present invention also provides a method of treating human or animal subjects suffering from or at risk of a disorder mediated by TNFα, 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 elevated levels of TNFα.

The present invention also provides an antibody molecule comprising a hybrid CDR comprising a truncated donor CDR sequence wherein the missing portion of the truncated donor CDR is replaced by a different sequence and forms a functional CDR. The term "hybrid CDR" as used herein means a CDR comprising a donor CDR which has been truncated at one or more positions, for example at one or both of its ends. The missing portion of the truncated donor CDR is replaced by a different sequence to form a complete and functional CDR. The hybrid CDR has at least one amino acid change compared to the complete donor CDR. The sequence replacing the truncated portion of the CDR can be any sequence. Preferably the non-donor part of the CDR sequence is from the antibody from which the framework regions of the antibody molecule are derived, such as a germline antibody sequence.

It has been found that antibody molecules comprising a hybrid CDR retain substantially the same binding affinity as an antibody molecule comprising complete donor CDRs. The term "substantially the same binding affinity" as used herein means at least 70%, more preferably at least 85% and most preferably at least 95% of the binding affinity of the corresponding antibody molecule comprising complete donor CDRs. As noted above, in certain cases, the affinity of the antibody of the invention may be greater than that of the donor antibody. The use of a hybrid CDR provides the advantages of reducing the amount of foreign (i.e. donor) sequence present in the antibody molecule and may increase the binding affinity of the antibody molecule compared to the corresponding antibody molecule comprising complete donor CDRs.

Any of the CDRs of the antibody molecule can be hybrid. Preferably CDR2 of the heavy chain is hybrid in the antibody molecule.

Preferably the truncation of the donor CDR is from 1 to 8 amino acids, more preferably from 4 to 6 amino acids. It is further preferred that the truncation is made at the C-terminus of the CDR.

Depending on the sequence of the truncated portion of the CDR and the sequence of the different sequence replacing the missing portion, a number of amino acid changes may

be made. Preferably at least 2 amino acid changes are made, more preferably at least 3 amino acid changes are made and most preferably at least 4 amino acid changes are made.

A particular embodiment of this aspect of the invention is an antibody according to the first aspect of the invention wherein the second CDR in the heavy chain has the sequence given as SEQ ID NO:2. This has better affinity for its antigen than does the donor antibody from which part of the CDR is derived.

The present invention also provides a nucleic acid sequence which encodes the antibody molecule comprising a hybrid CDR of the present invention.

The present invention also provides an expression vector containing the nucleic acid sequence encoding the antibody molecule comprising a hybrid CDR of the present invention.

The present invention also provides a host cell transformed with the vector of the present invention.

The present invention also provides a process for the production of an antibody molecule comprising a hybrid CDR comprising culturing the host cell of the present invention and isolating the antibody molecule.

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

Figure 1 shows the framework regions of the human light chain subgroup 1 compared to the framework regions of the hTNF40 light chain (SEQ ID NOS:83 to 90);

Figure 2 shows the framework regions of the human heavy chain subgroup 1 and subgroup 3 compared to the framework regions of the hTNF40 heavy chain (SEQ ID NOS:91 to 98 and 106 to 109);

Figure 3 shows the amino acid sequence of the CDRs of hTNF40 (SEQ ID NOS:1 to 7), wherein CDR H2' is a hybrid CDR wherein the C-terminal six amino acids are from the H2 CDR sequence of a human subgroup 3 germline antibody and the amino acid changes to the sequence resulting from this hybridisation are underlined;

Figure 4 shows vector pMR15.1;

Figure 5 shows vector pMR14;

Figure 6 shows the nucleotide and predicted amino acid sequence of the murine hTNF40Vl (SEQ ID NO: 99);

Figure 7 shows the nucleotide and predicted amino acid sequence of the murine hTNF40Vh (SEQ ID NO:100);

Figure 8 shows the nucleotide and predicted amino acid sequence of hTNF40-gL1 (SEQ ID NO:8);

Figure 9 shows the nucleotide and predicted amino acid sequence of hTNF40-gL2 (SEQ ID NO:9);

Figure 10 shows the nucleotide and predicted amino acid sequence of gh1hTNF40.4 (SEQ ID NO:10);

Figure 11 shows the nucleotide and predicted amino acid sequence of gh3hTNF40.4 (SEQ ID NO:11);

Figure 12 shows vector CTIL5-gL6;

Figure 13 shows the structure of a compound called CDP870 comprising a modified Fab fragment derived from antibody hTNF40 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;

Figure 14 shows vector pTTQ9;

Figure 15 shows the sequence of the OmpA oligonucleotide adapter (SEQ ID NO:101);

Figure 16 shows vector pACYC184;

Figure 17 shows vector pTTO-1;

Figure 18 shows vector pTTO-2;

Figure 19 shows vector pDNAbEng-G1;

Figure 20 shows the oligonucleotide cassettes encoding different intergenic sequences for *E. coli* modified Fab expression (SEQ ID NOS:102 to 105);

Figure 21 shows periplasmic modified Fab accumulation of IGS variants;

Figure 22 shows vector pTTO(CDP870);

Figure 23 shows the disease activity score (DAS) in patients treated with different doses of CDP870 and placebo. Median and IQ ranges are presented for the per-protocol population with last observation carried forward. Small squares indicate placebo, diamonds indicate 1 mg/kg, triangles indicate 5 mg/kg and large squares indicate 20 mg/kg;

Figure 24 shows the tender joint count, swollen joint count, pain score, assessor's global assessment of disease activity, modified health assessment questionnaire (HAQ), C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in patients treated with different doses of CDP870 and placebo. Median and IQ range are presented for the per-

protocol population with last observation carried forward. Small squares indicate placebo, diamonds indicate 1 mg/kg, triangles indicate 5 mg/kg and large squares indicate 20 mg/kg.

EXAMPLES

Gene Cloning and Expression of a Chimeric hTNF40 Antibody Molecule

RNA Preparation from hTNF40 Hybridoma Cells

Total RNA was prepared from 3 x 10^7 hTNF40 hybridoma cells as described below. Cells were washed in physiological saline and dissolved in RNAzol (0.2 ml per 10^6 cells). Chloroform (0.2 ml per 2 ml homogenate) was added, the mixture shaken vigorously for 15 seconds and then left on ice for 15 minutes. The resulting aqueous and organic phases were separated by centrifugation for 15 minutes in an Eppendorf centrifuge and RNA was precipitated from the aqueous phase by the addition of an equal volume of isopropanol. After 15 minutes on ice, the RNA was pelleted by centrifugation, washed with 70% ethanol, dried and dissolved in sterile, RNAse free water. The yield of RNA was 400 µg.

PCR Cloning of hTNF40 Vh and Vl

cDNA sequences coding for the variable domains of hTNF40 heavy and light chains were synthesised using reverse transcriptase to produce single stranded cDNA copies of the mRNA present in the total RNA, followed by Polymerase Chain Reaction (PCR) on the cDNAs with specific oligonucleotide primers.

a) cDNA Synthesis

cDNA was synthesised in a 20 µl reaction volume containing the following reagents: 50mM Tris-HCl pH 8.3, 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$, 0.5 mM each deoxyribonucleoside triphosphate, 20 units RNAsin, 75 ng random hexanucleotide primer, 2 µg hTNF40 RNA and 200 units Moloney Murine Leukemia Virus reverse transcriptase. After incubation at 42°C for 60 minutes, the reaction was terminated by heating at 95°C for 5 minutes.

b) PCR

Aliquots of the cDNA were subjected to PCR using combinations of primers specific for the heavy and light chains. The nucleotide sequences of the 5' primers for the heavy and light chains are shown in Tables 1 and 2 respectively. These sequences all contain, in order, a

restriction site starting 7 nucleotides from their 5' ends, the sequence GCCGCCACC (SEQ ID NO:12), to allow optimal translation of the resulting mRNAs, an initiation codon and 20-30 nucleotides based on the leader peptide sequences of known mouse antibodies (Kabat *et al.*, Sequences of proteins of immunological interest, 5th Edition, 1991, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health).

The 3' primers are shown in Table 3. The light chain primer spans the J-C junction of the antibody and contains a restriction site for the enzyme Sp1I to facilitate cloning of the V1 PCR fragment. The heavy chain 3' primers are a mixture designed to span the J-C junction of the antibody. The 3' primer includes an ApaI restriction site to facilitate cloning. The 3' region of the primers contains a mixed sequence based on those found in known mouse antibodies (Kabat *et al.*, 1991, *supra*).

The combinations of primers described above enable the PCR products for Vh and V1 to be cloned directly into an appropriate expression vector (see below) to produce chimeric (mouse-human) heavy and light chains and for these genes to be expressed in mammalian cells to produce chimeric antibodies of the desired isotype.

Incubations (100 µl) for the PCR were set up as follows. Each reaction contained 10 mM Tris-HC1 pH 8.3, 1.5 mM $MgC1_2$, 50 mM KC1, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 10 pmoles 5' primer mix (Table 4), 10 pmoles 3' primer (CL12 (light chain) or R2155 (heavy chain) (Table 3)), 1 µl cDNA and 1 unit Taq polymerase. Reactions were incubated at 95°C for 5 minutes and then cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, aliquots of each reaction were analysed by electrophoresis on an agarose gel. Light chain reactions containing 5' primer mixes from light chain pools 1, 2 and 7 produced bands with sizes consistent with full length Vl fragments while the reaction from heavy chain reaction pool 3 produced a fragment with a size expected of a Vh gene. The band produced by the light chain pool 1 primers was not followed up as previous results had shown that this band corresponds to a light chain pseudogene produced by the hybridoma cell. The band produced by the light chain pool 7 primers was weaker than the band from the pool 2 primers and therefore was not followed up. Only the band from light chain reaction pool 2, which was the strongest band, was followed up.

c) Molecular Cloning of the PCR Fragments

The DNA fragments produced in the light chain reaction pool 2 were digested with the enzymes BstBI and Sp1I, concentrated by ethanol precipitation, electrophoresed on a

1.4% agarose gel and DNA bands in the range of 400 base pairs recovered. These were cloned by ligation into the vector pMR15.1 (Figure 4) that had been restricted with BstBI and SplI. After ligation, mixtures were transformed into *E. coli* LM 1035 and plasmids from the resulting bacterial colonies screened for inserts by digestion with BstBI and SplI. Representatives with inserts from each ligation were analysed further by nucleotide sequencing.

In a similar manner, the DNA fragments produced in heavy chain reaction pool 3 were digested with HindIII and ApaI and cloned into the vector pMR14 (Figure 5) that had been restricted with HindIII and ApaI. Again, representative plasmids containing inserts were analysed by nucleotide sequencing.

d) Nucleotide Sequence Analysis

Plasmid DNA from a number of isolates containing Vh inserts was sequenced using the primers R1053 (see Table 5) (which primes in the 3' region of the HCMV promoter in pMR14) and R720 (see Table 5) (which primes in the 5' region of human C – gamma 4 and allows sequencing through the DNA insert on pMR14). It was found that the nucleotide sequences of the Vh insert in a number of clones were identical, except for differences in the signal peptide and J regions. This indicated that the clones examined are independent isolates arising from the use of different primers from the mixture of oligonucleotides during the PCR stage. The determined nucleotide sequence and predicted amino acid sequence of the variable domain of the heavy chain of antibody hTNF40 (hTNF40Vh) are given in Figure 7 (SEQ ID NO:100).

To analyse the light chain clones, the sequence derived from priming with R1053 (see Table 5) and R684 (SEQ ID NO:62) (which primes in the 5' region of human C-kappa and allows sequencing through the DNA insert on pMR15.1) was examined. The nucleotide sequence and predicted amino acid sequence of the Vl genes arising from reactions in pool 2 were similarly analysed. Again it was found that the nucleotide sequences of the Vl insert in a number of clones were identical, except for differences in the signal peptide and J regions, indicating that the clones examined were independent isolates arising from the use of different primers from the mixture of oligonucleotides used during the PCR stage. The determined nucleotide sequence and predicted amino acid sequence of the variable domain of the light chain of antibody hTNF40 (hTNF40V1) are given in Figure 6 (SEQ ID NO:99).

TABLE 1

Oligonucleotide primers for the 5' region of mouse heavy chains.

CH1 : 5'ATGAAATGCAGCTGGGTCAT(G,C)TTCTT3' (SEQ ID NO:13)

CH2 : 5'ATGGGATGGAGCT(A,G)TATCAT(C,G)(C,T)TCTT3' (SEQ ID NO:14)

CH3 : 5'ATGAAG(A,T)TGTGGTTAAACTGGGTTTT3' (SEQ ID NO:15)

CH4 : 5'ATG(G,A)ACTTTGGG(T,C)TCAGCTTG(G,A)T3' (SEQ ID NO:16)

CH5 : 5'ATGGACTCCAGGCTCAATTTAGTTTT3' (SEQ ID NO:17)

CH6 : 5'ATGGCTGTC(C,T)T(G,A)G(G,C)GCT(G,A)CTCTTCTG3' (SEQ ID NO:18)

CH7 : 5'ATGG(G,A)ATGGAGC(G,T)GG(G,A)TCTTT(A,C)TCTT3' (SEQ ID NO:19)

CH8 : 5'ATGAGAGTGCTGATTCTTTTGTG3' (SEQ ID NO:20)

CH9 : 5'ATGG(C,A)TTGGGTGTGGA(A,C)CTTGCTATT3' (SEQ ID NO:21)

CH10 : 5'ATGGGCAGACTTACATTCTCATTCCT3'(SEQ ID NO:22)

CH11 : 5'ATGGATTTTGGGCTGATTTTTTTTATTG3' (SEQ ID NO:23)

CH12 : 5'ATGATGGTGTTAAGTCTTCTGTACCT3' (SEQ ID NO:24)

Each of the above primers has the sequence 5'GCGCGCAAGCTTGCCGCCACC3' (SEQ ID NO:25) added to its 5' end.

TABLE 2

Oligonucleotide primers for the 5' region of mouse light chains.

CL1 : 5'ATGAAGTTGCCTGTTAGGCTGTTGGTGCT3' (SEQ ID NO:26)

CL2 : 5'ATGGAG(T,A)CAGACACACTCCTG(T,C)TATGGGT3' (SEQ ID NO:27)

CL3 : 5'ATGAGTGTGCTCACTCAGGTCCT3' (SEQ ID NO:28)

CL4 : 5'ATGAGG(G,A)CCCCTGCTCAG(A,T)TT(C,T)TTGG3' (SEQ ID NO:29)

CL5 : 5'ATGGATTT(T,A)CAGGTGCAGATT(T,A)TCAGCTT3' (SEQ ID NO:30)

CL5A : 5'ATGGATTT(T,A)CA(A,G)GTGCAGATT(T,A)TCAGCTT3' (SEQ ID NO:31)

CL6 : 5'ATGAGGT(T,G)C(T,C)(T,C)TG(T,C)T(G,C)AG(T,C)T(T,C)CTG(A,G)G3'(SEQ ID NO:32)

CL7 : 5'ATGGGC(T,A)TCAAGATGGAGTCACA3' (SEQ ID NO:33)

CL8 : 5'ATGTGGGGA(T,C)CT(G,T)TTT(T,C)C(A,C)(A,C)TTTTTCAAT3'(SEQ ID NO:34)

CL9 : 5'ATGGT(G,A)TCC(T,A)CA(G,C)CTCAGTTCCTT3' (SEQ ID NO:35)

CL10 : 5'ATGTATATATGTTTGTTGTCTATTTC3' (SEQ ID NO:36)

CL11 : 5'ATGGAAGCCCCAGCTCAGCTTCTCTT3'(SEQ ID NO:37)

CL12A : 5'ATG(A,G)AGT(T,C)(A,T)CAGACCCAGGTCTT(T,C)(A,G)T3' (SEQ ID NO:38)

CL12B : 5'ATGGAGACACATTCTCAGGTCTTTGT3' (SEQ ID NO:39)

CL13 : 5'ATGGATTCACAGGCCCAGGTTCTTAT3' (SEQ ID NO:40)

CL14 : 5'ATGATGAGTCCTGCCCAGTTCCTGTT3' (SEQ ID NO:41)

CL15 : 5'ATGAATTTGCCTGTTCATCTCTTGGTGCT3' (SEQ ID NO:42)

CL16 : 5'ATGGATTTTCAATTGGTCCTCATCTCCTT3' (SEQ ID NO:43)

CL17A : 5'ATGAGGTGCCTA(A,G)CT(C,G)AGTTCCTG(A,G)G3' (SEQ ID NO:44)

CL17B : 5'ATGAAGTACTCTGCTCAGTTTCTAGG3' (SEQ ID NO:45)

CL17C : 5'ATGAGGCATTCTCTTCAATTCTTGGG3' (SEQ ID NO:46)

Each of the above primers has the sequence 5'GGACTGTTCGAAGCCGCCACC3' (SEQ ID NO:47) added to its 5' end.

TABLE 3

Oligonucleotide primers for the 3' ends of mouse Vh and Vl genes.

Light chain (CL12) :

5'GGATACAGTTGGTGCAGCATCCGTACGTTT3' (SEQ ID NO:48)

Heavy chain (R2155) :

5'GCAGATGGGCCCTTCGTTGAGGCTG(A,C)(A,G)GAGAC(G,T,A)GTGA3' (SEQ ID NO:49)

TABLE 4

a) 5' Primer mixtures for light chain PCR reactions

pool 1 : CL2.
pool 2 : CL7.
pool 3 : CL13.
pool 4 : CL6.
pool 5 : CL5A, CL9, CL17A.
pool 6 : CL8.
pool 7 : CL12A.
pool 8 : CL1, CL3, CL4, CL5, CL10, CL11, CL2B, CL14, CL15, CL16, CL17B, CL17C

b) 5' Primer mixtures for heavy chain PCR reactions

pool 1 : CH1, CH2, CH3, CH4.
pool 2 : CH5, CH6, CH7, CH8.
pool 3 : CH9, CH10, CH11, CH12.

Table 5

Primers used in nucleotide sequence analysis

R1053 : 5'GCTGACAGACTAACAGACTGTTCC3' (SEQ ID NO:50)
R720 : 5'GCTCTCGGAGGTGCTCCT3' (SEQ ID NO:51)

Evaluation of Activities of Chimeric Genes

The activities of the chimeric genes were evaluated by expressing them in mammalian cells and purifying and quantitating the newly synthesised antibodies. The methodology for this is described below, followed by a description of the biochemical and cell based assays used for the biological characterisation of the antibodies.

a) Production of Chimeric hTNF40 Antibody Molecule

Chimeric antibody for biological evaluation was produced by transient expression of the appropriate heavy and light chain pairs after co-transfection into Chinese Hamster Ovary (CHO) cells using calcium phosphate precipitation.

On the day prior to transfection, semi-confluent flasks of CHO-L761 cells were trypsinised, the cells counted and T75 flasks set up each with 10^7 cells.

On the next day, the culture medium was changed 3 hours before transfection. For transfection, the calcium phosphate precipitate was prepared by mixing 1.25 ml of 0.25 M $CaCl_2$ containing 50 µg of each of heavy and light chain expression vectors with 1.25 ml of 2 x HBS (16.36 g NaCl, 11.0 g HEPES and 0.4 g Na_2HPO_4 in 1 litre water with the pH adjusted to 7.1 with NaOH) and adding immediately into the medium of the cells. After 3 hours at 37°C in a CO_2 incubator, the medium and precipitate were removed and the cells shocked by the addition of 15 ml 15% glycerol in phosphate buffered saline (PBS) for 1 minute. The glycerol was removed, the cells washed once with PBS and incubated for 48-96 hours in 25 ml medium containing 10 mM sodium butyrate. Antibody could be purified from the culture medium by binding to and elution from protein A-Sepharose.

b) ELISA

For the ELISA, Nunc ELISA plates were coated overnight at 4°C with a $F(ab)_2$ fragment of a polyclonal goat anti-human Fc fragment specific antibody (Jackson Immunoresearch, code 109-006-098) at 5 µg/ml in coating buffer (15 mM sodium carbonate, 35 mM sodium hydrogen carbonate, pH 6.9). Uncoated antibody was removed by washing 5 times with distilled water. Samples and purified standards to be quantitated were diluted to approximately 1 µg/ml in conjugate buffer (0.1 M Tris-HCl, pH 7.0, 0.1 M NaCl, 0.2% v/v Tween 20, 0.2% w/v Hammersten casein). The samples were titrated in the microtitre wells in 2-fold dilutions to give a final volume of 0.1 ml in each well and the plates incubated at room temperature for 1 hour with shaking. After the first incubation step the plates were washed 10 times with distilled water and then incubated for 1 hour as before with 0.1 ml of a mouse monoclonal anti-human kappa (clone GD12) peroxidase conjugated antibody (The Binding Site, code MP135) at a dilution of 1 in 700 in conjugate buffer. The plate was washed again and substrate solution (0.1 ml) added to each well. Substrate solution contained 150 µl N,N,N,N-tetramethylbenzidine (10 mg/ml in DMSO), 150 µl hydrogen peroxide (30% solution) in 10 ml 0.1 M sodium acetate/sodium citrate, pH 6.0. The plate was developed for 5-10 minutes until the absorbance at 630 nm was approximately 1.0 for the top standard. Absorbance at 630 nm was measured using a plate reader and the concentration of the sample determined by comparing the titration curves with those of the standard.

c) **Determination of Affinity constants by BiaCore analysis.**

The binding interaction between hTNF40 and human TNF was investigated using BIA technology. An affinity purified goat polyclonal antibody, directed against the constant region of hTNF40, was immobilised on the dextran polymer sensor chip surface using standard NHS/EDC chemistry. Relatively low levels (200-500 RU) of hTNF40 were captured to ensure mass transport effects were minimised. Human TNF at different concentrations was passed over the captured hTNF40 to allow assessment of the association kinetics. Following the injection of ligand, buffer was passed over the surface so that the dissociation could be measured. The association and dissociation rate constants for the interaction between solid phase hTNF40 and human TNF were calculated, and a K_D value was derived.

EXAMPLE 1

CDR-Grafting of hTNF40

The molecular cloning of genes for the variable regions of the heavy and light chains of the hTNF40 antibody and their use to produce chimeric (mouse-human) hTNF40 antibodies has been described above. The nucleotide and amino acid sequences of the murine hTNF40 Vl and Vh are shown in Figures 6 and 7 (SEQ ID NOS:99 and 100), respectively. This example describes the CDR-grafting of the hTNF40 antibody.

CDR-Grafting of hTNF40 Light Chain

Alignment of the framework regions of hTNF40 light chain with those of the four human light chain subgroups (Kabat *et al.*, 1991, *supra*) revealed that hTNF40 was most homologous to antibodies in human light chain subgroup 1. Consequently, for constructing the CDR-grafted light chain, the framework regions chosen corresponded to those of the human group 1 consensus sequence.

A comparison of the amino acid sequences of the framework regions of murine hTNF40 and the consensus human group 1 light chains is given in Figure 1 and shows that there are 22 differences (underlined) between the two sequences. Analysis of the contribution that any of these framework differences might have on antigen binding identified 2 residues for investigation; these are at positions 46 and 60. Based on this analysis, two versions of the CDR-grafted light chain were constructed. In the first of these, hTNF40-gL1 (SEQ ID NO:8), residues 46 and 60 are derived from the hTNF40 light

chain while in the second, hTNF40-gL2 (SEQ ID NO:9), all residues are human consensus except residue number 60 which is from the hTNF40 light chain.

Construction of CDR-Grafted Light Chain hTNF40-gL1.

The construction of hTNF40-gL1 is given below in detail. The following overlapping oligonucleotides (P7982-P7986) were used in the Polymerase Chain Reactions (PCR) to assemble a truncated grafted light chain. The assembled fragment lacks the antibody leader sequence and the first 17 amino acids of framework 1.

oligo 1 P7982:
5' GAATTCAGGGTCACCATCACTTGTAAAGCCAGTCAGAACGTAGGTACTAAC GTAGCCTGGTATCAGCAAA3' (SEQ ID NO:52)

oligo 2 P7983:
5' ATAGAGGAAAGAGGCACTGTAGATGAGGGCTTTTGGGGCTTTACCTGGTTT TTGCTGATACCAGGCTACGT3' (SEQ ID NO:53)

oligo 3 P7984:
5' TACAGTGCCTCTTTCCTCTATAGTGGTGTACCATACAGGTTCAGCGGATCCG GTAGTGGTACTGATTTCAC3' (SEQ ID NO:54)

oligo 4 P7985
5'GACAGTAATAAGTGGCGAAATCTTCTGGCTGGAGGCTACTGATCGTGAGGGT GAAATCAGTACCACTACCG3' (SEQ ID NO:55)

oligo 5 P7986:
5'ATTTCGCCACTTATTACTGTCAACAGTATAACATCTACCCACTCACATTCGGT CAGGGTACTAAAGTAGAAATCAAACGTACGGAATTC3' (SEQ ID NO:56)

Fwd P7981:
5'GAATTCAGGGTCACCATCACTTGTAAAGCC3' (SEQ ID NO:57)

Bwd P7980

5'GAATTCCGTACGTTTGATTTCTACTTTAGT3' (SEQ ID NO:58),

A PCR reaction, 100 µl, was set up containing, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 2 pmoles of P7982, P7983, P7984, P7985, P7986, 10 pmoles of P7980, P7981 and 1 unit of Taq polymerase. Reactions were cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, each reaction was analysed by electrophoresis on an agarose gel and the PCR fragment excised from the gel and recovered using a Mermaid Kit. The recovered fragment was restricted with the enzymes BstEII and SplI in the appropriate buffer. The resulting product was finally electrophoresed on an agarose gel and the 270 base pair DNA fragment recovered from a gel slice and ligated into vector CTIL5-gL6 (Figure 12), that had previously been digested with the same enzymes. The above vector provides the missing antibody leader sequence and the first 17 amino acids of framework 1.

The ligation mixture was used to transform E. coli strain LM1035 and resulting colonies analysed by PCR, restriction enzyme digests and nucleotide sequencing. The nucleotide and amino acid sequence of the Vl region of hTNF40-gL1 is shown in Figure 8 (SEQ ID NO:8).

Construction of CDR-Grafted Light Chain hTNF40-gL2.

hTNF40-gL2 (SEQ ID NO:9) was constructed using PCR. The following oligonucleotides were used to introduce the amino acid changes:

R1053: 5'GCTGACAGACTAACAGACTGTTCC3' (SEQ ID NO:59)

R5350: 5'TCTAGATGGCACACCATCTGCTAAGTTTGATGCAGCATAGAT CAGGAGCTTAGGAGC3' (SEQ ID NO:60)

R5349: 5'GCAGATGGTGTGCCATCTAGATTCAGTGGCAGTGGATCA GGCACAGACTTTACCCTAAC3' (SEQ ID NO:61)

R684: 5'TTCAACTGCTCATCAGAT3' (SEQ ID NO:62)

Two reactions, each 20 µl, were set up each containing 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 0.1 µg hTNF40-gL1, 6 pmoles of R1053/R5350 or R5349/R684 and 0.25 units Taq polymerase. Reactions were cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, each reaction was analysed by electrophoresis on an agarose gel and the PCR fragments excised from the gel and recovered using a Mermaid Kit.

Aliquots of these were then subjected to a second round of PCR. The reaction, 100 µl, contained 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 1/5 of each of the PCR fragments from the first set of reactions, 30 pmoles of R1053 and R684 and 2.5 units Taq polymerase. Reaction temperatures were as above. After the PCR, the mixture was extracted with phenol/chloroform and then with chloroform and precipitated with ethanol. The ethanol precipitate was recovered by centrifugation, dissolved in the appropriate buffer and restricted with the enzymes BstEII and SplI. The resulting product was finally electrophoresed on an agarose gel and the 270 base pair DNA fragment recovered from a gel slice and ligated into the vector pMR15.1 (Figure 4) that had previously been digested with the same enzymes.

The ligation mixture was used to transform E. coli LM1035 and resulting colonies analysed by PCR, restriction enzyme digests and nucleotide sequencing. The nucleotide and amino acid sequence of the Vl region of hTNF40-glL2 is shown in Figure 9 (SEQ ID NO:9).

CDR-Grafting of hTNF40 Heavy Chain

CDR-grafting of hTNF40 heavy chain was accomplished using the same strategy as described for the light chain. hTNF40 heavy chain was found to be most homologous to human heavy chains belonging to subgroup 1 and therefore the consensus sequence of the human subgroup 1 frameworks was chosen to accept the hTNF40 heavy chain CDRs.

To investigate the requirement of a homologous human framework to act as an acceptor framework for CDR grafting, a second framework, human group 3, was selected to humanise hTNF40 heavy chain.

A comparison of hTNF40 with the two different frameworks region is shown in Figure 2 where it can be seen that hTNF40 differs from the human subgroup 1 consensus at 32 positions (underlined) and differs from the human subgroup 3 consensus at 40 positions

(underlined). After analysis of the contribution that any of these might make to antigen binding, residues 28, 38, 46, 67, 69 and 71 were retained as donor in the CDR-grafted heavy chain gh1hTNF40.1, using the group 1 framework. Residues 27, 28, 30, 48, 49, 69, 71, 73, 76 and 78 were retained as donor in the CDR-grafted heavy chain, gh3hTNF40.4 using the group 3 framework. Residues 28, 69 and 71 were retained as donor in the CDR-grafted heavy chain, gh1hTNF40.4 using the group 1 framework.

Construction of CDR-Grafted Heavy Chain gh1hTNF40.4

gh1hTNF40.4 (SEQ ID NO:10) was assembled by subjecting overlapping oligonucleotides to PCR in the presence of the appropriate primers. The following oligonucleotides were used in the PCR:

Group 1 graft

oligo 1 P7989:
5'GAAGCACCAGGCTTCTTAACCTCTGCTCCTGACTGGACCAGCTGCACCTGAG AGTGCACGAATTC3' (SEQ ID NO:63)

oligo 2 P7990:
5'GGTTAAGAAGCCTGGTGCTTCCGTCAAAGTTTCGTGTAAGGCCTCAGGCTAC GTGTTCACAGACTATGGTA3' (SEQ ID NO:64)

oligo 3 P7991:
5'CCAACCCATCCATTTCAGGCCTTGTCCCGGGGCCTGCTTGACCCAATTCATAC CATAGTCTGTGAACACGT3' (SEQ ID NO:65)

oligo 4 P7995:
5'GGCCTGAAATGGATGGGTTGGATTAATACTTACATTGGAGAGCCTATTTATGT TGACGACTTCAAGGGCAGATTCACGTTC3' (SEQ ID NO:66)

oligo 5 P7992:
5'CCATGTATGCAGTGCGTTGTGGAGGTGTCTAGAGTGAACGTGAATCTGCCCTT GAA3' (SEQ ID NO:67)

oligo 6 P7993:
5'CCACAAGCACTGCATACATGGAGCTGTCATCTCTGAGATCCGAGGACACCGC AGTGTACTAT3' (SEQ ID NO:68)

oligo 7 P7994:
5'GAATTCGGTACCCTGGCCCCAGTAGTCCATGGCATAAGATCTGTATCCTCTAG CACAATAGTACACTGCGGTGTCCTC3' (SEQ ID NO:69)

Fwd: P7988:
5'GAATTCGTGCACTCTCAGGTGCAGCTGGTC3' (SEQ ID NO:70)

Bwd P7987:
5'GAATTCGGTACCCTGGCCCCAGTAGTCCAT3' (SEQ ID NO:71)

The assembly reaction, 100 µl, contained 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 2 pmole of each of p7989, p7990, p7991, p7995, p7992, p7993 and p7994, 10 pmoles of each of p7988 and p7987 and 1 unit Taq polymerase. Reactions were cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, the reaction was extracted with phenol/chloroform (1/1), then with chloroform and precipitated with ethanol. After centrifugation, the DNA was dissolved in the appropriate restriction buffer and digested with ApaLI and KpnI. The resulting fragment was isolated from an agarose gel and ligated into pMR14 (Figure 5) that had previously been digested with the same enzymes. pMR14 contains the human gamma 4 heavy chain constant region when pMR14 is cleaved with ApaLI and KpnI, the cleaved vector is able to receive the digested DNA such that the 3' end of the digested DNA joins in reading frame to the 5' end of the sequence encoding the gamma 4 constant region. Therefore, the heavy chain expressed from this vector will be a gamma 4 isotype. The ligation mixture was used to transform *E. coli* LM1035 and resulting bacterial colonies screened by restriction digest and nucleotide sequence analysis. In this way, a plasmid was identified containing the correct sequence for gh1hTNF40.4 (Figure 10) (SEQ ID NO:10).

Construction of CDR-Grafted Heavy Chain gh3hTNF40.4

gh3hTNF40.4 (SEQ ID NO:11) was assembled by subjecting overlapping oligonucleotides to PCR in the presence of the appropriate primers. The following oligonucleotides were used in the PCR:

Group 3 graft

oligo 1 P7999:
5'GATCCGCCAGGCTGCACGAGACCGCCTCCTGACTCGACCAGCTGAACCTCAG AGTGCACGAATTC3' (SEQ ID NO:72)

oligo 2 P8000:
5'TCTCGTGCAGCCTGGCGGATCGCTGAGATTGTCCTGTGCTGCATCTGGTTACG TCTTCACAGACTATGGAA3' (SEQ ID NO:73)

oligo 3 P8001
5'CCAACCCATCCATTTCAGGCCCTTTCCCGGGGCCTGCTTAACCCAATTCATTC CATAGTCTGTGAAGACGT3' (SEQ ID NO:74)

oligo 4 P7995:
5'GGCCTGAAATGGATGGGTTGGATTAATACTTACATTGGAGAGCCTATTTATGT TGACGACTTCAAGGGCAGATTCACGTTC3' (SEQ ID NO:66)

oligo 5 P7997:
5'GGAGGTATGCTGTTGACTTGGATGTGTCTAGAGAGAACGTGAATCTGCCCTT GAA3' (SEQ ID NO:75)

oligo 6 P7998:
5'CCAAGTCAACAGCATACCTCCAAATGAATAGCCTGAGAGCAGAGGACACCGC AGTGTACTAT3' (SEQ ID NO:76)

oligo 7 P7993:

5'GAATTCGGTACCCTGGCCCCAGTAGTCCATGGCATAAGATCTGTATCCTCTAG CACAATAGTACACTGCGGTGTCCTC3' (SEQ ID NO:77)

Fwd P7996:

5'GAATTCGTGCACTCTGAGGTTCAGCTGGTC3' (SEQ ID NO:78)

Bwd P7987:

5'GAATTCGGTACCCTGGCCCCAGTAGTCCAT3' (SEQ ID NO:71)

The assembly reaction, 100 µl, contained 10 mM Tris-HC1 pH 8.3, 1.5 mM $MgCl_2$, 50 mM KC1, 0.01% w/v gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 2 pmole of each of p7999, p8000, p8001, p7995, p7997, p7998 and p7993, 10 pmoles of each of p7996 and p7987 and 1 unit Taq polymerase. Reactions were cycled through 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. After 30 cycles, the reaction was extracted with phenol/chloroform (1/1), then with chloroform and precipitated with ethanol. After centrifugation, the DNA was dissolved in the appropriate restriction buffer and digested with ApaLI and KpnI. The resulting fragment was isolated from an agarose gel and ligated into pMR14 (Figure 5) that had previously been digested with the same enzymes. pMR14 contained the human gamma 4 heavy chain constant region. When pMR14 is cleaved with ApaLI and KpnI, the cleaved vector is able to receive the digested DNA such that the 3' end of the digested DNA joins in reading frame to the 5' end of the sequence encoding the gamma 4 constant region. Therefore, the heavy chain expressed from this vector will be a gamma 4 isotype. The ligation mixture was used to transform *E. coli* LM1035 and resulting bacterial colonies screened by restriction digestion and nucleotide sequence analysis. In this way, a plasmid was identified containing the correct sequence for gh3hTNF40.4 (SEQ ID NO:11) (Figure 11).

Production of CDR-Grafted Modified Fab Fragment.

A CDR-grafted, modified Fab fragment, based on antibody hTNF40, was constructed using the *E. coli* vector pTTO-1. The variable regions of antibody hTNF40 are sub-cloned into this vector and the intergenic sequence optimised to create pTTO(CDP870). The pTTO expression vector is designed to give rise to soluble,

periplasmic accumulation of recombinant proteins in *E. coli*. The main features of this plasmid are:

(i) tetracycline resistance marker - antibiotic not inactivated by the product of resistance gene, hence selection for plasmid-containing cells is maintained;

(ii) low copy number - origin of replication derived from plasmid p15A, which is compatible with plasmids containing colE1 derived replicons;

(iii) strong, inducible tac promoter for trancription of cloned gene(s);

(iv) $lacI^q$ gene - gives constitutive expression of the lac repressor protein, maintaining the tac promoter in the repressed state until induction with IPTG / allolactose;

(v) OmpA signal sequence - gives periplasmic secretion of cloned gene(s); and

(vi) translational coupling of OmpA signal sequence to a short lacZ peptide, giving efficient initiation of translation.

The vector has been developed for expression of modified Fab fragments from a dicistronic message by the design of a method to select empirically the optimum intergenic sequence from a series of four purpose-built cassettes. The application of this in the construction of pTTO(CDP870) is described.

Materials and Methods

DNA techniques

Standard procedures were used for protocols including DNA restriction, agarose gel electrophoresis, ligation and transformation. Restriction enzymes and DNA modifying enzymes were obtained from New England Biolabs or Boehringer Mannheim, and were used according to the supplier's recommendations. DNA fragments were purified from agarose using the GeneClean protocol (BIO 101). Oligonucleotides were supplied by Oswel Oligonucleotide Service and were synthesized at the 40 nm scale. Plasmid DNA was isolated using Plasmid DNA Mini / Midi kits from Qiagen. PCR was performed using Perkin Elmer 'Amplitaq' as recommended. DNA sequencing was performed using the Applied Biosystems Taq cycle sequencing kit.

Shake flask induction

E. coli W3110 cultures were grown in L-broth supplemented with tetracycline (7.5 µg/ml). For inductions, fresh overnight cultures (grown at 30°C) were diluted to OD_{600} of

0.1 into 200 ml L-broth in a 2 L baffled flask and were grown at 30•C in an orbital incubator. At OD_{600} of 0.5, IPTG was added to 200 μM. Samples (normalised for OD) were taken at intervals.

Periplasmic Extraction

Culture samples were chilled on ice (5 minutes) then cells were harvested by centrifugation. Following resuspension in extraction buffer (100 mM Tris.HCl, 10 mM EDTA, pH 7.4) samples were incubated overnight at 30•C, then clarified by centrifugation.

Assembly Assay

Modified Fab concentrations were determined by ELISA. Plates were coated at 4•C overnight with anti-human Fd 6045 (2 μg/ml in coating buffer, physiological saline, 100 μl per well). After washing, 100 μl of sample was loaded per well; purified A5B7 gamma-1 Fab', initially at 2 μg/ml, was used as a standard. Samples were serially diluted 2-fold across the plate in sample conjugate buffer (per litre: 6.05 g trisaminomethane; 2.92 g NaCl; 0.1 ml Tween-20; 1 ml casein (0.2%)); plates were incubated for 1 hour at room temperature, with agitation. Plates were washed and dried, then 100 μl of anti-human C-kappa (GD12)-peroxidase was added (diluted in sample conjugate buffer). Incubation was carried out at room temperature for 1 hour with agitation. Plates were washed and dried, then 100 μl of substrate solution was added (10 ml sodium acetate/citrate solution (0.1 M pH 6); 100 μl H_2O_2 solution; 100 μl tetramethylbenzidine solution (10 mg/ml in dimethylsulphoxide)). Absorbance at 630 nm was read 4 - 6 minutes after substrate addition.

Construction of Plasmid pTTO-1

(a) Replacement of the pTTQ9 Polylinker

Plasmid pTTQ9 was obtained from Amersham and is shown in Figure 14. An aliquot (2 μg) was digested with restriction enzymes SalI and EcoRI, the digest was run on a 1% agarose gel and the large DNA fragment (4520 bp) was purified. Two oligonucleotides were synthesized which, when annealed together, encode the OmpA polylinker region shown in Figure 15. This sequence has cohesive ends which are compatible with the SalI and EcoRI ends generated by restriction of pTTQ9. By cloning this oligonucleotide 'cassette' into the pTTQ9 vector, the SalI site is not regenerated, but the

EcoRI site is maintained. The cassette encodes the first 13 amino acids of the signal sequence of the *E. coli* outer-membrane protein Omp-A, preceded by the Shine Dalgarno ribosome binding site of the OmpA gene. In addition restriction sites for enzymes XbaI, MunI, StyI and SplI are present. The MunI and StyI sites are within the coding region of the OmpA signal sequence and are intended as the 5' cloning sites for insertion of genes. The two oligonucleotides which make up this cassette were annealed together by mixing at a concentration of 5 pmoles/µl and heating in a waterbath to 95°C for 3 minutes, then slow cooling to room temperature. The annealed sequence was then ligated into the SalI / EcoRI cut pTTQ9. The resulting plasmid intermediate, termed pTQOmp, was verified by DNA sequencing.

(b) Fragment Preparation and Ligation

Plasmid pTTO-1 was constructed by ligating one DNA fragment from plasmid pACYC184 to two fragments generated from pTQOmp. Plasmid pACYC184 was obtained from New England Biolabs, and a restriction map is shown in Figure 16. An aliquot (2 µg) was digested to completion with restriction enzyme StyI, then treated with Mung Bean Nuclease; this treatment creates blunt ends by cutting back 5' base overhangs. Following phenol extraction and ethanol precipitation, the DNA was restricted with enzyme PvuII, generating fragments of 2348, 1081, 412 and 403 bp. The 2348 bp fragment was purified after agarose gel electrophoresis. This fragment encodes the tetracycline resistance marker and the p15A origin of replication. The fragment was then treated with calf intestinal alkaline phosphatase to remove 5' terminal phosphates, thereby preventing the self-ligation of this molecule.

An aliquot (2 µg) of plasmid pTQOmp was digested with enzymes SspI and EcoRI, and the 2350 bp fragment was purified from unwanted fragments of 2040 bp and 170 bp following agarose gel electrophoresis; this fragment encodes the transcriptional terminator region and the $lacI^q$ gene. Another aliquot (2 µg) of pTQOmp was digested with EcoRI and XmnI, generating fragments of 2289, 1670, 350 and 250 bp. The 350 bp fragment, encoding the tac promoter, OmpA signal sequence and multicloning site, was gel purified.

The three fragments were then ligated, using approximately equimolar amounts of each fragment, to generate the plasmid pTTO-1. All cloning junctions were verified by DNA sequencing. The restriction map of this plasmid is shown in Figure 17. Plasmid pTTO-2 was then created by insertion of DNA encoding the human Ig light chain kappa constant domain. This was obtained as a Spl I – EcoRI restriction fragment from plasmid

pHC132, and inserted into the corresponding sites in pTTO-1. Plasmid pTTO-2 is shown in Figure 18.

Insertion of humanized hTNF40 variable regions into pTTO-2

The variable light chain region hTNF40gL1 (SEQ ID NO:8) was obtained by PCR 'rescue' from the corresponding vector for mammalian cell expression pMR10.1. The OmpA leader sequence replaces the native Ig leader. The sequence of the PCR primers is shown below:

5' primer:

CGCGCGGCAATTGCAGTGGCCTTGGCTGGTTTCGCTACCGTAGCGCAAG CTGACATTCAAATGACCCAGAGCCC (SEQ ID NO:79)

3' primer: TTCAACTGCTCATCAGATGG (SEQ ID NO:80)

Following PCR under standard conditions, the product was purified, digested with enzymes MunI and SplI then gel purified. The purified fragment was then inserted into the MunI / SplI sites of pTTO-2 to create the light chain intermediate pTTO(hTNF40L).

The variable heavy chain region of gh3hTNF40.4 was obtained in the same way from the vector pGamma-4. The sequence of the PCR primers is shown below:

5' primer:

GCTATCGCAATTGCAGTGGCGCTAGCTGGTTTCGCCACCGTGGCGCAAG CTGAGGTTCAGCTGGTCGAGTCAGGAGGC (SEQ ID NO:81)

3' primer: GCCTGAGTTCCACGACAC (SEQ ID NO:82)

Following PCR the product was purified, digested with enzymes NheI and ApaI then sub-cloned into the vector pDNAbEng-G1 (Figure 19). After verification by DNA sequencing, the heavy chain was restricted with enzyme EcoRI and sub-cloned into the EcoRI site of pTTO(hTNF40L) to create the *E. coli* expression plasmid pTTO(hTNF40).

Optimisation of Intergenic Sequence for Modified Fab Expression

In the pTTO vector, modified Fab expression occurs from a dicistronic message encoding first light chain then heavy chain. The DNA sequence between the two genes (intergenic sequence, IGS) can influence the level of expression of the heavy chain by affecting the rate of translational initiation. For example, a short intergenic sequence may result in translational coupling between the light and heavy chains, in that the translating ribosome may not fully dissociate from the mRNA after completing light chain synthesis before initiating heavy chain synthesis. The 'strength' of any Shine Dalgarno (SD) ribosome binding site (homolgy to 16S rRNA) can also have an effect, as can the distance and sequence composition between the SD and the ATG start codon. The potential secondary structure of mRNA around the ATG is another important factor; the ATG should be in a 'loop' and not constrained within a 'stem', while the reverse applies to the SD. Thus by modifying the composition and length of the IGS it is possible to modify the strength of translational initiation and therefore the level of heavy chain production. It is likely that an optimum rate of translational initiation needs to be achieved to maximise expression of the heavy chain of a given modified Fab. For example, with one modified Fab, a high level of expression may be tolerated, but for a different modified Fab with different amino acid sequence, a high level of expression might prove toxic, perhaps because of different efficiencies of secretion or folding. For this reason, a series of four intergenic sequences were designed (Figure 20), permitting the empirical determination of the optimum IGS for the hTNF40-based modified Fab. IGS1 and IGS2 have very short intergenic sequences (-1 and +1 respectively) and might be expected to give closely coupled translation; the SD sequences (underlined) are subtly different. These two sequences will most likely confer a high level of translational initiation. IGS3 and IGS4 have a longer distance between start and stop codons (+13) and differ in their sequence composition; IGS3 has a 'stronger' SD sequence. All sequences were studied for secondary structure (using m/fold program) and 'optimised' as far as possible; however, with tight coupling of translation of the two chains the lack of ribosomal dissociation means that the mRNA may not be 'naked', preventing secondary structure formation.

Cloning of IGS variants

The IGS cassettes shown in Figure 20 have flanking SacI and MunI cloning sites. They were built by annealing complementary oligonucleotide pairs. A vector fragment was prepared by digesting pTTO(hTNF40) with SacI and NotI, and a heavy chain fragment was

prepared by digesting pDNAbEngG1(hTNF40H) with MunI and NotI. Three-way ligations were then performed, using equimolar amounts of the two restriction fragments and approximately 0.05 pmoles of each annealed oligo cassette. This created the four expression plasmids pTTO(hTNF40 IGS-1), pTTO(hTNF40 IGS-2), pTTO(hTNF40 IGS-3), pTTO(hTNF40 IGS-4).

Shake flask expression analysis

The four plasmids were transformed into *E. coli* strain W3110, along with the original expression construct, and then analysed for expression in shake flasks as described. The results of a typical experiment are shown in Figure 21. The different intergenic sequences confer different expression profiles. IGS1 and IGS2 accumulate periplasmic modified Fab rapidly with a peak at 1 hour post induction, after which the level recovered falls. The peak is greater and the fall sharper for IGS1. These results are consistent with a high level of synthesis, as expected for close translational coupling for these constructs. IGS1 apparently confers a higher level of heavy chain expression than does IGS2. In this instance, it appears that this high level of expression is poorly tolerated, since periplasmic expression levels fall after the 1 hour peak. This is seen on the growth profile of the IGS1 culture (not shown), which peaks at 1 hour post induction before falling, suggesting cell death and lysis. IGS3 accumulates modified Fab more slowly but peaks at 2 hours post induction with a higher peak value (325 ng/ml/OD), before levels fall. The growth of this culture continued to 3 hours post induction and reached a higher peak biomass (not shown). This is consistent with a lower level of heavy chain synthesis. IGS4 accumulates material at a slower rate still and fails to reach the high peak of productivity of the other 3 constructs. All IGS variants out-perform the original vector significantly. The hypothesis that the different IGS sequences confer different rates of translational initiation is supported by these experimental results. For the hTNF40-based modified Fab it appears that a high rate of heavy chain translational initiation is poorly tolerated and is therefore not optimal. A slower rate, as conferred by IGS3, results in better growth characteristics and consequently a better yield accumulates over time.

Following comparison of productivity in the fermenter the IGS3 construct was selected as the highest yielding and was termed pTTO(CDP870) – see Figure 22.

The heavy chain encoded by the plasmid pTTO(CDP870) has the sequence given in SEQ ID NO:115 and the light chain has the sequence given in SEQ ID NO:113.

PEGylation of CDR-Grafted, hTNF40-based Modified 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). The PEGylated molecule is shown in Figure 13 and is called compound CDP870.

Efficacy of PEGylated CDR-Grafted, hTNF40-based Modified Fab (CDP870) in Treating Rheumatoid Arthritis.

CDP870 has a long half life of approximately 11 days.

We evaluated the safety and efficacy of intravenous CDP870 in a randomised double-blind placebo-controlled dose escalating trial in patients with RA.

Methods

Patients:

Patients aged between 18 and 75 years old and who satisfied the 1987 revised American College of Rheumatology (ACR) diagnostic criteria for rheumatoid arthritis (RA) (Arnett *et al.*, Arthritis Rheum., 31, 315-324, 1988) were recruited from outpatient Rheumatology clinics at London, Cambridge, Norfolk and Norwich (United Kingdom). Patients were required to have clinically active disease as defined by having at least 3 of the following criteria: $\geq$ 6 painful or tender joints; $\geq$ 45 minutes of early morning stiffness; and erythrocyte sedimentation rate (ESR) $\geq$ 28 mm/hr. They must have failed to respond to at least one Disease Modifying Anti-Rheumatic Drug (DRARD) and have been off treatment for at least 4 weeks. Corticosteroids were permitted if the dose was $\geq$ 7.5 mg/day of prednisolone. Pregnant women, nursing women and women of childbearing potential not using an effective method of contraception were excluded. Patients were also excluded if they had a previous history of malignancy, concomitant severe uncontrolled medical conditions, previous failure of TNFα-neutralizing therapy or allergy to polyethylene glycol. Written informed consent was obtained from each patient before enrolment. The study was approved by the local research ethics committees.

- 2 DEC 2004

Treatment protocol:

36 RA patients were divided into 3 groups, each to receive an increasing dose of the trial drug (1, 5 or 20mg/kg). Each group of 12 was randomly divided into 8 to receive CDP870 and 4 to receive placebo. CDP870 was given as a single intravenous infusion (100 ml in total) over 60 minutes. Placebo (sodium acetate buffer) was given similarly as a single intravenous infusion of 100 ml over 60 minutes. Treatment was given on an outpatient basis. After 8 weeks, all patients had the opportunity to receive an infusion of either 5 or 20 mg/kg of CDP870 in open fashion.

Clinical assessment:

RA disease activity was assessed based on the World Health Organization and International League of Associations for Rheumatology (Boers *et al.*, J. Rheumatol – Supplement, 41, 86-89, 1994) and European League Against Rheumatism (EULAR) (Scott *et al.*, Clin. Exp. Rheumatol., 10, 521-525, 1992) core data sets with 28 joint counts. Changes in disease activity were assessed by Disease Activity Score (Prevoo *et al.*, Arthritis Rheum., 38, 44-48, 1995) and the ACR responses criteria (Felson *et al.*, Arthritis Rheum., 38, 727-735, 1995). Assessments were carried out before treatment and at 1, 2, 4, 6 and 8 weeks after therapy. Patients were also assessed for safety and tolerance of the study drug. Haematology, biochemistry, anti-CDP870 antibodies and adverse events were assessed at each visit.

CDP870 plasma concentration and anti-CDP870 antibodies:

CDP870 was measured by enzyme-linked immunosorbent assay (ELISA). Serial dilutions of patients' plasma were incubated in microtitre plates (Nunc) coated with recombinant human TNFα (Strathmann Biotech GmbH, Hannover). Captured CDP870 was revealed with horseradish peroxidase conjugated goat anti-human kappa light chain (Cappel, ICN) followed by tetramethylbenzidine (TMB) substrate.

Antibodies to CDP870 was screened (at 1/10 plasma dilution) using a double antigen sandwich ELISA with biotinylated CDP870 as the second layer. Bound antibodies were revealed using HRP-streptavidin and TMB substrate. The assay was calibrated using a hyperimmune rabbit IgG standard. A unit of activity is equivalent to 1 μg of the rabbit standard.

Statistical Analysis

The study was exploratory in nature and the sample size was based on previous experience with similar agents. Efficacy of CDP870 was analysed by calculating disease activity score (DAS) and ACR20/50 responses for intention to treat and per-protocol using a closed testing procedure. The disease activity score was calculated as follows: DAS = 0.555 x square root of (28 tender joints) + 0.284 x square root of (28 swollen joints) + 0.7 x ln(ESR) + 0.0142 x (patient's global assessment). First, the pooled active groups were compared to placebo. If this comparison was significant at the 5% level, each dosage group was compared to placebo. All comparisons were two tailed with a significance level of 5%. All P-values were derived from exploratory analysis and should not be used for inferential interpretation.

Results

Demography:

36 patients with RA were recruited. Their demographic details are given in Table 6. The mean age was 56 years and 30 patients were female. The mean duration of RA was 13 years and 21 patients were rheumatoid factor positive. Patients in the different groups have similar demographic characteristics. In the blinded dosing period, 6/12 placebo-treated patients withdrew from the study for deteriorating RA $\geq$ 4, weeks after dosing. 2/24 CDP870-treated patients withdrew, both in the 1 mg/kg group, for deteriorating RA/lost to follow up > 4 weeks after dosing. The difference was statistically significant (p=0.009, Fisher exact test).

Table 6: Demographic details (mean $\pm$ standard deviation)

	Number	Sex (M:F)	Age	Duration of Disease	Rheuma-toid Factor	Number of previous DMARDs
Placebo	12	1.11	51±8	12±8	8(67%)	5±1
1 mg/kg	8	1:7	59±7	12±7	4(50%)	4±1
5m g/kg	8	2:6	54±13	13±5	5(63%)	5±2
20 mg/kg	8	2.6	61±11	14±13	4(50%)	4±2

Clinical Efficacy:

The proportion of patients with ACR20 improvement for the per-protocol population with last observation carried forward was 16.7, 50, 87.5 and 62.5% after placebo, 1, 5 and 20 mg/kg CDP870 (combined treatment effect p=0.012) at 4 weeks and 16.7, 25, 75 and 75% (p=0.032) at 8 weeks. Reduction in DAS scores (median) for the per-protocol population with last observation carried forward was 0.15, 1.14, 1.91 and 1.95 after placebo, 1, 5 and 20 mg/kg CDP870 (combined treatment effect p=0.001) at 4 weeks and 0.31, 0.09, 2.09 and 1.76 (p=0.008) at 8 weeks (Figure 23). Changes in individual components of the World Health Organization and International League of Associations for Rheumatology core data set are shown in Figure 24.

Following the open label dose of CDP870, similar beneficial effects were achieved. Of the 36 patients recruited into the study, 32 received a second infusion of CDP870. The proportion of patients with ACR20 improvement from pre-first infusion was 72.2 and 55.6% after 5 and 20 mg/kg CDP870 at 4 weeks and 55.6 and 66.7% at 8 weeks.

Adverse Events

Treatment was well tolerated with no infusion-related reaction. No allergic reaction or skin rash was reported. In the double-blind phase, there were 19, 38, 8 and 14 adverse events in the placebo, 1, 5 and 20 mg/kg groups respectively. The commonest was headache with 9 episodes in 5 patients (1 placebo, 3 at 1 mg/kg, 1 at 20 mg/kg). One patient who received placebo and 3 patients who received CDP870 (1 at 5 mg/kg and 2 at 20 mg/kg) developed lower respiratory tract infections. These were reported as mild or moderate. They were treated with oral antibiotics and resolved over 1-2 week period. Three patients each in the 1 and 5 mg/kg groups and one in the 20 mg/kg group developed a urinary tract infection 1-2 months after CDP870 treatment. One adverse event was described as severe which was an episode of neck pain occurring 3 days after infusion with 1 mg/kg. Increase in anti-nuclear antibody was seen in 4 patients: 1 in the placebo group (negative to 1/40), 2 in the 1 mg/kg group (negative to 1/40, negative to 1/80) and 1 in the 20 mg/kg group (negative to 1/40). No change was found in anti-DNA or anti-cardiolipin antibodies.

CDP870 Plasma Concentration and Anti-CDP870 levels

As expected, for all dose levls of CDP870, the peak plasma concentration occurred at the end of infusion and was dose proportional with plasma concentration declining slowly thereafter. The plasma concentration profile of CDP870 appeared very similar to that previously observed in volunteers where the half-life was calculated to be approximately 14 days. On re-dosing, a similar profile to single dose infusion was observed.

Following a single intravenous infusion, anti-CDP870 levels were low or undetectable.

Discussion

Neutralizing TNFα is an effective treatment strategy in RA. Currently, this requires the use of biological agents, such as a chimeric mAb or a soluble receptor/human Fc fusion protein, which are expensive to manufacture. A therapeutic TNFα neutralizing agent needs to bind TNFα with high affinity and have a long plasma half-life, low antigenicity and high tolerability and safety. It also needs to be accessible to all patients with RA who would benefit from TNFα blockade. One technology that could achieve these objectives is the conjugation with polyethelene glycol of a TNFα binding antibody fragment made in *E. coli*. In this preliminary study, we find that CDP870, a PEGylated, anti-TNFα, modified Fab, is effective and well tolerated by patients with RA.

In vitro studies have shown that CDP870 has similar TNFα neutralizing activity to the murine anti-TNFα parent antibody. This study confirms that CDP870 reduced inflammation and improved symptoms in RA. Clinical improvement as measured by the ACR20 response criteria in the 5 and 20 mg/kg groups (75%, 75%) was comparable to etanercept (60%) (Moreland *et al.*, Annals Int. Med., 130, 478-486, 1999) and infliximab (50%) (Maini *et al.*, Lancet, 354, 1932-1939, 1999). At the middle and highest dosage levels tested, the therapeutic effect lasted 8 weeks which is comparable to previous other mAbs (Elliott *et al.*, Lancet, 344, 1105-1110, 1994 and Rankin *et al.*, Br. J. Rheumatol., 34, 334-342, 1995). Previous study has shown that the therapeutic effect of anti-TNFα antibody is related to its plasma half-life and the generation of circulating antibodies (Maini *et al.*, Arthritis Rheum.*38*, (Supplement) : S186 1995 (Abstract)). Our study showed that CDP870 has a plasma half-life of 14 days which is equivalent to that of a whole antibody

(Rankin *et al.*, (*supra*)) and much longer than the half-life of unconjugated Fab' fragments. Further, CDP870 generated only very low levels of antibody response.

One of the important objectives of this study is to examine the tolerability and safety of administering this PEGylated Fab'. In our study, CDP870 appears well tolerated. Although further study will be needed to assess long-term toxicity, especially the risk of demyelinating disease, infection and skin rashes that have been reported with etanercept and infliximab.

In summary, CDP870 is therapeutically effective in RA and was well tolerated in this short-term study.

It should be understood that the above-described examples are merely exemplary and do not limit the scope of the present invention as defined in the following claims.

CLAIMS

1. An antibody molecule having specificity for human TNFα, comprising a heavy chain wherein the variable domain comprises a CDR having the sequence given as H1 in Figure 3 (SEQ ID NO:1) for CDRH1, as H2' in Figure 3 (SEQ ID NO:2) or as H2' in Figure 3 (SEQ ID NO:7) for CDRH2 or as H3 in Figure 3 (SEQ ID NO:3) for CDRH3.

2. An antibody molecule having specificity for human TNFα, comprising a light chain wherein the variable domain comprises a CDR having the sequence given as L1 in Figure 3 (SEQ ID NO:4) for CDRL1, as L2 in Figure 3 (SEQ ID NO:5) for CDRL2 or as L3 in Figure 3 (SEQ ID NO:6) for CDRL3.

3. The antibody molecule of claim 1 or claim 2 comprising a heavy chain wherein the variable domain comprises a CDR having the sequence given in SEQ ID NO:1 for CDRH1, SEQ ID NO:2 or SEQ ID NO:7, for CDRH2 or SEQ ID NO:3 for CDRH3 and a light chain wherein the variable domain comprises a CDR having the sequence given in SEQ ID NO:4 for CDRL1, SEQ ID NO:5 for CDRL2 or SEQ ID NO:6 for CDRL3.

4. The antibody molecule of claim 3, which comprises SEQ ID NO:1 for CDRH1, SEQ ID NO: 2 or SEQ ID NO:7 for CDRH2, SEQ ID NO:3 for CDRH3, SEQ ID NO:4 for CDRL1, SEQ ID NO:5 for CDRL2 and SEQ ID NO:6 for CDRL3.

5. The antibody molecule of any one of claims 1 to 4, which comprises SEQ ID NO:2 for CDRH2.

6. The antibody molecule of any one of claims 1 to 5, which is a CDR-grafted antibody molecule.

7. The antibody molecule of claim 6, wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

8. The antibody molecule of claim 7, wherein the human acceptor framework regions of the variable domain of the heavy chain are based on a human group 1 consensus sequence and comprise non-human donor residues at positions 28, 69 and 71.

9. The antibody molecule of claim 7, wherein the human acceptor framework regions of the variable domain of the heavy chain are based on a human group 1 consensus sequence and comprise non-human donor residues at positions 28, 38, 46, 67, 69 and 71.

10. The antibody molecule of claim 7, wherein the human acceptor framework regions of the variable domain of the heavy chain are based on a human group 3 consensus sequence and comprise non-human donor residues at positions 27, 28, 30, 48 49, 69, 71, 73 76 and 78.

11. The antibody molecule of any one of claims 7 to 10, wherein the human acceptor framework regions of the variable domain of the light chain are based on human group 1 consensus sequence and comprise non-human donor residues at positions 46 and 60.

12. The antibody molecule of any one of claims 1 to 11, comprising the light chain variable region hTNF40-gL1 (SEQ ID NO:8) and the heavy chain variable region gh3hTNF40.4 (SEQ ID NO:11).

13. The antibody molecule of any one of claims 1 to 12 which is a Fab fragment.

14. The antibody molecule of claims 12 and 13, which is a Fab fragment comprising a heavy chain having the sequence given in SEQ ID NO:111 and a light chain having the sequence given in SEQ ID NO:113.

15. The antibody molecule of any one of claims 1 to 12, which is a modified Fab fragment having at the C-terminal end of its heavy chain one or more amino acids to allow attachment of an effector or reporter molecule.

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16. The antibody molecule of claim 15, 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.

17. The antibody molecule of claim 12, which is a modified Fab fragment comprising a heavy chain having the sequence given in SEQ ID NO:115 and a light chain having the sequence given in SEQ ID NO:113.

18. An antibody molecule having specificity for human TNFα, having a light chain comprising the sequence given in SEQ ID NO:113.

19. An antibody molecule having specificity for human TNFα, having a light chain consisting of the sequence given in SEQ ID NO:113.

20. An antibody molecule having specificity for human TNFα, having a heavy chain comprising the sequence given in SEQ ID NO:115.

21. An antibody molecule having specificity for human TNFα, having a heavy chain consisting of the sequence given in SEQ ID NO:115.

22. An antibody molecule having specificity for human TNFα, having a light chain comprising the sequence given in SEQ ID NO:113 and a heavy chain comprising the sequence given in SEQ ID NO:115.

23. An antibody molecule having specificity for human TNFα, having a light chain consisting of the sequence given in SEQ ID NO:113 and a heavy chain consisting of the sequence given in SEQ ID NO:115.

24. A variant of the antibody molecule of any one of claims 1 to 23, which has an improved affinity for TNFα.

25. The variant of claim 24 which is obtained by an affinity maturation protocol.

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26. The antibody of any one of claims 1 to 3 which is murine anti-TNFα monoclonal antibody hTNF40.

27. The antibody molecule of any one of claims 1 to 3, which is a chimeric antibody molecule comprising the light and heavy chain variable domains of the monoclonal antibody of claim 26.

28. A compound comprising the antibody molecule of any one of claims 15 to 23 having covalently attached to an amino acid at or towards the C-terminal end of its heavy chain an effector or reporter molecule.

29. The compound of claim 28, which comprises an effector molecule.

30. The compound of claim 29, wherein the effector molecule comprises one or more polymers.

31. The compound of claim 30, wherein the one or more polymers is/are an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide.

32. The compound of claim 31, wherein the one or more polymers is/are a methoxypoly(ethyleneglycol).

33. A compound comprising the antibody molecule of claim 17 having attached to one of the cysteine residues 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 compound comprising an antibody molecule having specificity for human TNFα, having a light chain comprising the sequence given in SEQ ID NO:113 and a heavy chain comprising the sequence given in SEQ ID NO:115, having attached to one of the cysteine residues at the C-terminal end of the heavy chain one or more synthetic or naturally-occurring polymers.

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35. A compound comprising an antibody molecule having specificity for human TNFα, having a light chain consisting of the sequence given in SEQ ID NO:113 and a heavy chain consisting of the sequence given in SEQ ID NO:115, having attached to one of the cysteine residues at the C-terminal end of the heavy chain one or more synthetic or naturally-occurring polymers.

36. A compound comprising an antibody molecule having specificity for human TNFα, having a light chain comprising the sequence given in SEQ ID NO:113, having attached to one of the cysteine residues 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..

37. A compound comprising an antibody molecule having specificity for human TNFα, having a light chain consisting of the sequence given in SEQ ID NO:113, having attached to one of the cysteine residues 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.

38. A compound comprising an antibody molecule having specificity for human TNFα, having a heavy chain comprising the sequence given in SEQ ID NO:115, having attached to one of the cysteine residues 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.

39. A compound comprising an antibody molecule having specificity for human TNFα, having a heavy chain consisting of the sequence given in SEQ ID NO:115, having attached to one of the cysteine residues 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 methoxy poly(ethyleneglycol) residue having a molecular weight of about 20,000 Da.

40. A compound comprising an antibody molecule having specificity for human TNFα, having a light chain comprising the sequence given in SEQ ID NO:113 and a heavy chain

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comprising the sequence given in SEQ ID NO:115, having attached to one of the cysteine residues 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.

41. A compound comprising an antibody molecule having specificity for human TNFα, having a light chain consisting of the sequence given in SEQ ID NO:113 and a heavy chain consisting of the sequence given in SEQ ID NO:115, having attached to one of the cysteine residues 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.

42. An antibody molecule according to any one of claims 1 to 27, comprising a hybrid CDR comprising a truncated donor CDR sequence wherein the missing portion of the donor CDR is replaced by a different sequence and forms a functional CDR.

43. The antibody molecule of claim 42, wherein the missing part of the CDR sequence is from the antibody from which the framework regions of the antibody molecule are derived.

44. The antibody molecule of claim 43, wherein the missing part of the CDR sequence is derived from a germline antibody having consensus framework regions.

45. The antibody molecule of any one of claims 42 to 44, wherein CDRH2 of the heavy chain is hybrid in the antibody molecule.

46. The antibody molecule of any one of claims 42 to 45, wherein the truncation of the donor CDR is from 1 to 8 amino acids.

47. The antibody molecule of claim 46, wherein the truncation is from 4 to 6 amino acids.

48. The antibody molecule of any one of claims 42 to 47, wherein the truncation is made at the C-terminus of the CDR.

49. A DNA sequence which encodes the heavy and/or light chain of the antibody molecule of any one of claims 1 to 27 and 42 to 48.

50. The DNA sequence of claim 49 comprising the sequence shown in SEQ ID NO:8 or 10.

51. The DNA sequence of claim 49 comprising the sequence shown in SEQ ID NO:10 or 11.

52. The DNA sequence of claim 49 comprising the sequence shown in SEQ ID NO:110, 112 or 114.

53. A cloning or expression vector containing the DNA sequence of any one of claims 49 to 52.

54. An *E. coli* expression vector comprising the DNA sequence of any one of claims 49 to 52.

55. The *E. coli* expression vector of claim 54 which is pTTO(CDP870).

56. A host cell transformed with the vector of any one of claims 53 to 55.

57. A process for the production of the antibody molecule of any one of claims 1 to 27 and 42 to 48, comprising culturing the host cell of claim 56 and isolating the antibody molecule.

58. A process for the production of the antibody molecule of any one of claims 1 to 27 and 42 to 48, comprising culturing *E. coli* comprising an *E. coli* expression vector comprising the DNA sequence of any one of claims 53 to 55 and isolating the antibody molecule.

59. The process of claim 58 wherein the antibody molecule is targeted to the periplasm.

60. A therapeutic or diagnostic composition comprising the antibody molecule of any one of claims 1 to 27 and 42 to 48 or the compound of any one of claims 28 to 41.

61. The antibody molecule of any one of claims 1 to 27 and 42 to 48, having specificity for human TNFα, or the compound of any one of claims 28 to 41, for use in treating a pathology mediated by TNFα.

62. The antibody molecule of or compound claim 61, for use in treating rheumatoid- or osteo- arthritis.

63. Use of the antibody molecule of any one of claims 1 to 27 and 42 to 48, having specificity for human TNFα, or the compound of any one of claims 28 to 41 in the manufacture of a medicament for the treatment of a pathology mediated by TNFα.

64. The use of claim 63, wherein the pathology is rheumatoid- or osteo- arthritis.

65. The vector pDNAbEng-G1 as shown in Figure 19.

66. The vector pTTO(CDP870) as shown in Figure 22.

67. A polypeptide having the amino acid sequence given in any one of SEQ ID NOS:1 to 7.

SEQUENCE LISTING

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gac cgg gtc acc atc act tgt aaa gcc agt cag aac gta ggt act aac   96
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
             20                  25                  30

gta gcc tgg tat cag caa aaa cca ggt aaa gcc cca aaa gcc ctc atc   144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile
         35                  40                  45

tac agt gcc tct ttc ctc tat agt ggt gta cca tac agg ttc agc gga   192
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Tyr Arg Phe Ser Gly
     50                  55                  60

tcc ggt agt ggt act gat ttc acc ctc acg atc agt agc ctc cag cca   240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65                  70                  75                  80

gaa gat ttc gcc act tat tac tgt caa cag tat aac atc tac cca ctc   288
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ile Tyr Pro Leu
                 85                  90                  95

aca ttc ggt cag ggt act aaa gta gaa atc aaa                      321
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            100                 105
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  1               5                  10                  15

gac cgg gtc acc atc act tgt aaa gcc agt cag aac gta ggt act aac   96
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
            20                  25                  30

gta gcc tgg tat cag caa aaa cca ggt aaa gcc cca aaa ctc ctc atc   144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

tac agt gcc tct ttc ctc tat agt ggt gta cca tac agg ttc agc gga   192
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Tyr Arg Phe Ser Gly
    50                  55                  60

tcc ggt agt ggt act gat ttc acc ctc acg atc agt agc ctc cag cca   240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

gaa gat ttc gcc act tat tac tgt caa cag tat aac atc tac cca ctc   288
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ile Tyr Pro Leu
                85                  90                  95

aca ttc ggt cag ggt act aaa gta gaa atc aaa                       321
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
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tcc gtc aaa gtt tcg tgt aag gcc tca ggc tac gtg ttc aca gac tat   96
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

ggt atg aat tgg gtc aga cag gcc ccg gga caa ggc ctg gaa tgg atg   144
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

ggt tgg att aat act tac att gga gag cct att tat gct caa aag ttc   192
Gly Trp Ile Asn Thr Tyr Ile Gly Glu Pro Ile Tyr Ala Gln Lys Phe
    50                  55                  60
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cag ggc aga gtc acg ttc act cta gac acc tcc aca agc act gca tac   240
Gln Gly Arg Val Thr Phe Thr Leu Asp Thr Ser Thr Ser Thr Ala Tyr
 65                  70                  75                  80

atg gag ctg tca tct ctg aga tcc gag gac acc gca gtg tac tat tgt   288
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gct aga gga tac aga tct tat gcc atg gac tac tgg ggc cag ggt acc   336
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tca ctg aga ttg tcc tgt gct gca tct ggt tac gtc ttc aca gac tat   96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

gga atg aat tgg gtt aga cag gcc ccg gga aag ggc ctg gaa tgg atg   144
Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

ggt tgg att aat act tac att gga gag cct att tat gct gac agc gtc   192
Gly Trp Ile Asn Thr Tyr Ile Gly Glu Pro Ile Tyr Ala Asp Ser Val
    50                  55                  60

aag ggc aga ttc acg ttc tct cta gac aca tcc aag tca aca gca tac   240
Lys Gly Arg Phe Thr Phe Ser Leu Asp Thr Ser Lys Ser Thr Ala Tyr
 65                  70                  75                  80

ctc caa atg aat agc ctg aga gca gag gac acc gca gtg tac tat tgt   288
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

gct aga gga tac aga tct tat gcc atg gac tac tgg ggc cag ggt acc   336
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<210> 36
<211> 26

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL10

<400> 36
atgtatatat gtttgttgtc tatttc 26

<210> 37
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL11

<400> 37
atggaagccc cagctcagct tctctt 26

<210> 38
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL12A

<400> 38
atgragtywc agacccaggt cttyrt 26

<210> 39
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL12B

<400> 39
atggagacac attctcaggt ctttgt 26

<210> 40
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL13

<400> 40
atggattcac aggcccaggt tcttat 26

<210> 41
<211> 26
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL14

<400> 41
atgatgagtc ctgcccagtt cctgtt 26

<210> 42
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL15

<400> 42
atgaatttgc ctgttcatct cttggtgct 29

<210> 43
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL16

<400> 43
atggattttc aattggtcct catctcctt 29

<210> 44
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL17A

<400> 44
atgaggtgcc tarctsagtt cctgrg 26

<210> 45
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL17B

<400> 45
atgaagtact ctgctcagtt tctagg 26

<210> 46
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL17C

<400> 46
atgaggcatt ctcttcaatt cttggg 26

<210> 47
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer 5' end

<400> 47
ggactgttcg aagccgccac c 21

<210> 48
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer CL12

<400> 48
ggatacagtt ggtgcagcat ccgtacgttt 30

<210> 49
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer R2155

<400> 49
gcagatgggc ccttcgttga ggctgmrgag acdgtga 37

<210> 50
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer R1053

<400> 50
gctgacagac taacagactg ttcc 24

<210> 51
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer R720

<400> 51
gctctcggag gtgctcct 18

<210> 52
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide P7982

<400> 52
gaattcaggg tcaccatcac ttgtaaagcc agtcagaacg taggtactaa cgtagcctgg 60
tatcagcaaa 70

<210> 53
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide P7983

<400> 53
atagaggaaa gaggcactgt agatgagggc ttttggggct ttacctggtt tttgctgata 60
ccaggctacg t 71

<210> 54
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide P7984

<400> 54
tacagtgcct ctttcctcta tagtggtgta ccatacaggt tcagcggatc cggtagtggt 60
actgatttca c 71

<210> 55
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide P7985

<400> 55
gacagtaata agtggcgaaa tcttctggct ggaggctact gatcgtgagg gtgaaatcag 60
taccactacc g 71

<210> 56
<211> 89
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide P7986

<400> 56
atttcgccac ttattactgt caacagtata acatctaccc actcacattc ggtcagggta 60
ctaaagtaga aatcaaacgt acggaattc 89

<210> 57
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide P7981

<400> 57
gaattcaggg tcaccatcac ttgtaaagcc 30

<210> 58
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide P7980

<400> 58
gaattccgta cgtttgattt ctactttagt 30

<210> 59
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide R1053

<400> 59
gctgacagac taacagactg ttcc 24

<210> 60
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide R5350

<400> 60
tctagatggc acaccatctg ctaagtttga tgcagcatag atcaggagct taggagc 57

<210> 61
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide R5349

<400> 61
gcagatggtg tgccatctag attcagtggc agtggatcag gcacagactt taccctaac 59

<210> 62
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:oligonucleotide R684

<400> 62
ttcaactgct catcagat 18

<210> 63
<211> 65
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7989

<400> 63
gaagcaccag gcttcttaac ctctgctcct gactggacca gctgcacctg agagtgcacg 60
aattc 65

<210> 64
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7990

<400> 64
ggttaagaag cctggtgctt ccgtcaaagt ttcgtgtaag gcctcaggct acgtgttcac 60
agactatggt a 71

<210> 65
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7991

<400> 65
ccaacccatc catttcaggc cttgtcccgg ggcctgcttg acccaattca taccatagtc 60
tgtgaacacg t 71

<210> 66
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7995

<400> 66
ggcctgaaat ggatgggttg gattaatact tacattggag agcctattta tgttgacgac 60
ttcaagggca gattcacgtt c 81

<210> 67
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7992

<400> 67
ccatgtatgc agtgcgttgt ggaggtgtct agagtgaacg tgaatctgcc cttgaa 56

<210> 68
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7993

<400> 68
ccacaagcac tgcatacatg gagctgtcat ctctgagatc cgaggacacc gcagtgtact 60
at 62

<210> 69
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7994

<400> 69
gaattcggta ccctggcccc agtagtccat ggcataagat ctgtatcctc tagcacaata 60
gtacactgcg gtgtcctc 78

<210> 70
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7988

<400> 70
gaattcgtgc actctcaggt gcagctggtc 30

<210> 71
<211> 30
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7987

<400> 71
gaattcggta ccctggcccc agtagtccat 30

<210> 72
<211> 65
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7999

<400> 72
gatccgccag gctgcacgag accgcctcct gactcgacca gctgaacctc agagtgcacg 60
aattc 65

<210> 73
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P8000

<400> 73
tctcgtgcag cctggcggat cgctgagatt gtcctgtgct gcatctggtt acgtcttcac 60
agactatgga a 71

<210> 74
<211> 71
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P8001

<400> 74
ccaacccatc catttcaggc cctttcccgg ggcctgctta acccaattca ttccatagtc 60
tgtgaagacg t 71

<210> 75
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7997

<400> 75
ggaggtatgc tgttgacttg gatgtgtcta gagagaacgt gaatctgccc ttgaa 55

<210> 76
<211> 62

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7998

<400> 76
ccaagtcaac agcatacctc caaatgaata gcctgagagc agaggacacc gcagtgtact 60
at 62

<210> 77
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7993

<400> 77
gaattcggta ccctggcccc agtagtccat ggcataagat ctgtatcctc tagcacaata 60
gtacactgcg gtgtcctc 78

<210> 78
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer P7996

<400> 78
gaattcgtgc actctgaggt tcagctggtc 30

<210> 79
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' primer

<400> 79
cgcgcggcaa ttgcagtggc cttggctggt ttcgctaccg tagcgcaagc tgacattcaa 60
atgacccaga gccc 74

<210> 80
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 3' primer

<400> 80
ttcaactgct catcagatgg 20

<210> 81
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' primer

<400> 81
gctatcgcaa ttgcagtggc gctagctggt ttcgccaccg tggcgcaagc tgaggttcag 60
ctggtcgagt caggaggc 78

<210> 82
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 3' primer

<400> 82
gcctgagttc cacgacac 18

<210> 83
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 1 consensus framework L1

<400> 83
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
20

<210> 84
<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:hTNF40 framework L1

<400> 84
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Val Thr Cys
20

<210> 85
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 1 consensus framework L2

<400> 85
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
1 5 10 15

<210> 86
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:hTNF40 framework L2

<400> 86
Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile Tyr
1 5 10 15

<210> 87
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 1 consensus framework L3

<400> 87
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
20 25 30

<210> 88
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:hTNF40 framework L3

<400> 88
Gly Val Pro Tyr Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr
1 5 10 15

Leu Thr Ile Ser Thr Val Gln Ser Glu Asp Leu Ala Glu Tyr Phe Cys
20 25 30

<210> 89
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 1 consensus framework L4

<400> 89
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
1 5 10

<210> 90
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:hTNF40 framework L4

<400> 90
Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
1 5 10

<210> 91
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 1 consensus framework H1

<400> 91
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
20 25 30

<210> 92
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:hTNF40 framework H1

<400> 92
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr
20 25 30

<210> 93
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 1 consensus framework H2

<400> 93
Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly
1 5 10

<210> 94
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:hTNF40 framework H2

<400> 94
Trp Val Lys Gln Ala Pro Gly Lys Ala Phe Lys Trp Met Gly
1 5 10

<210> 95
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 1 consensus framework H3

<400> 95
Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu
1 5 10 15

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 96

<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:hTNF40 framework H3

<400> 96
Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe Leu Gln
1 5 10 15

Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg
20 25 30

<210> 97
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 1 consensus framework H4

<400> 97
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 98
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:hTNF40 framework H4

<400> 98
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
1 5 10

<210> 99
<211> 324
<212> DNA
<213> murine

<220>
<221> CDS
<222> (1)..(324)
<223> mouse hTNF40 light chain variable domain

<400> 99

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gac att gtg atg acc cag tct caa aaa ttc atg tcc aca tca gta gga   48
Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
 1               5                  10                  15

gac agg gtc agc gtc acc tgc aag gcc agt cag aat gtg ggt act aat   96
Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly Thr Asn
            20                  25                  30

gta gcc tgg tat caa cag aaa cca gga caa tct cct aaa gca ctg att   144
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
        35                  40                  45

tac tcg gca tcc ttc cta tat agt gga gtc cct tat cgc ttc aca ggc   192
Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Tyr Arg Phe Thr Gly
    50                  55                  60

agt gga tct ggg aca gat ttc act ctc acc atc agc act gtg cag tct   240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Thr Val Gln Ser
65                  70                  75                  80

gaa gac ttg gca gag tat ttc tgt cag caa tat aac atc tat cct ctc   288
Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Tyr Asn Ile Tyr Pro Leu
                85                  90                  95

acg ttc ggt gct ggg acc aag ctg gag ctg aaa cgt                   324
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
            100                 105
```

<210> 100
<211> 354
<212> DNA
<213> murine

<220>
<221> CDS
<222> (1)..(354)
<223> mouse hTNF40 heavy chain variable domain

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<400> 100
cag atc cag ttg gtg cag tct gga cct gag ctg aag aag cct gga gag   48
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1               5                  10                  15

aca gtc aag atc tcc tgc aag gct tct gga tat gtt ttc aca gac tat   96
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Val Phe Thr Asp Tyr
            20                  25                  30

gga atg aat tgg gtg aag cag gct cca gga aag gct ttc aag tgg atg   144
Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Ala Phe Lys Trp Met
        35                  40                  45

ggc tgg ata aac acc tac att gga gag cca ata tat gtt gat gac ttc   192
Gly Trp Ile Asn Thr Tyr Ile Gly Glu Pro Ile Tyr Val Asp Asp Phe
    50                  55                  60
```

```
aag gga cga ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc ttt   240
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Phe
 65                  70                  75                  80

ttg cag atc aac aac ctc aaa aat gag gac acg gct aca tat ttc tgt   288
Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
                85                  90                  95

gca aga ggt tac cgg tcc tat gct atg gac tac tgg ggt caa gga acc   336
Ala Arg Gly Tyr Arg Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110

tca gtc acc gtc tct tca                                          354
Ser Val Thr Val Ser Ser
        115
```

<210> 101
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (29)..(67)

<223> Description of Artificial Sequence:OmpA oligonucleotide adaptor

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<400> 101
tcgagttcta gataacgagg cgtaaaaa atg aaa aag aca gct atc gca att    52
                               Met Lys Lys Thr Ala Ile Ala Ile
                                 1               5

gca gtg gcc ttg gct ctgacgtacg agtcagg                           84
Ala Val Ala Leu Ala
     10
```

<210> 102
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (2)..(40)

<220>
<221> CDS
<222> (43)..(66)

<220>
<223> Description of Artificial Sequence:IGS cassette-1

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<400> 102
g agc tca cca gta aca aaa agt ttt aat aga gga gag tgt ta atg aag   48
```

```
  Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Xaa    Xaa Lys
    1               5                  10                  15

aag act gct ata gca att g                                      67
Lys Thr Ala Ile Ala Ile
                  20
```

<210> 103
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (2)..(43)

<220>
<221> CDS
<222> (45)..(68)

<220>
<223> Description of Artificial Sequence:IGS cassette-2

<400> 103

```
g agc tca cca gta aca aaa agt ttt aat aga gga gag tgt taa a atg   47
  Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys       Met
    1               5                  10                  15

aag aag act gct ata gca att g                                  69
Lys Lys Thr Ala Ile Ala Ile
                  20
```

<210> 104
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (2)..(43)

<220>
<221> CDS
<222> (57)..(80)

<220>
<223> Description of Artificial Sequence:IGS cassette-3

<400> 104

```
g agc tca cca gta aca aaa agc ttt aat aga gga gag tgt tga       43
  Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    1               5                  10

ggaggaaaaa aaa atg aag aaa act gct ata gca att g                81
              Met Lys Lys Thr Ala Ile Ala Ile
```

15 20

<210> 105
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (2)..(43)

<220>
<221> CDS
<222> (57)..(80)

<220>
<223> Description of Artificial Sequence:IGS cassette-4

<400> 105
g agc tca cca gta aca aaa agt ttt aat aga gga gag tgt tga 43
Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
1 5 10

cgaggattat ata atg aag aaa act gct ata gca att g 81
Met Lys Lys Thr Ala Ile Ala Ile
15 20

<210> 106
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 3 consensus framework H1

<400> 106
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 Thr Phe Ser
20 25 30

<210> 107
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 3 consensus framework H2

<400> 107

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

<210> 108
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 3 consensus framework H3

<400> 108
Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
20 25 30

<210> 109
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:human group 3 consensus framework H4

<400> 109
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> 110
<211> 648
<212> DNA
<213> Artificial Sequence

<220>
<223> Grafted Heavy Chain for Fab

<400> 110
gaggttcagc tggtcgagtc aggaggcggt ctcgtgcagc ctggcggatc actgagattg 60
tcctgtgctg catctggtta cgtcttcaca gactatggaa tgaattgggt tagacaggcc 120
ccgggaaagg gcctggaatg gatgggttgg attaatactt acattggaga gcctatttat 180
gctgacagcg tcaagggcag attcacgttc tctctagaca catccaagtc aacagcatac 240
ctccaaatga atagcctgag agcagaggac accgcagtgt actattgtgc tagaggatac 300
agatcttatg ccatggacta ctggggccag ggtaccctag tcacagtctc ctcagcttcc 360
accaagggcc catcggtctt ccccctggca ccctcctcca agagcacctc tgggggcaca 420
gcggccctgg gctgcctggt caaggactac ttccccgaac cggtgacggt gtcgtggaac 480
tcaggcgccc tgaccagcgg cgtgcacacc ttcccggctg tcctacagtc ctcaggactc 540
tactccctca gcagcgtggt gaccgtgccc tccagcagct tgggcaccca gacctacatc 600

tgcaacgtga atcacaagcc cagcaacacc aaggtcgaca agaaagtt 648

<210> 111
<211> 216
<212> PRT
<213> Artificial Sequence

<220>
<223> Grafted Heavy Chain for Fab

<400> 111
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 Tyr Val Phe Thr Asp Tyr
20 25 30

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

Gly Trp Ile Asn Thr Tyr Ile Gly Glu Pro Ile Tyr Ala Asp Ser Val
50 55 60

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

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

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

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Lys Val
210 215

<210> 112
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<223> Grafted Light Chain for Fab and Modified Fab

<400> 112
gacattcaaa tgacccagag cccatccagc ctgagcgcat ctgtaggaga ccgggtcacc 60
atcacttgta aagccagtca gaacgtaggt actaacgtag cctggtatca gcaaaaacca 120
ggtaaagccc caaaagccct catctacagt gcctctttcc tctatagtgg tgtaccatac 180
aggttcagcg gatccggtag tggtactgat ttcaccctca cgatcagtag cctccagcca 240
gaagatttcg ccacttatta ctgtcaacag tataacatct acccactcac attcggtcag 300
ggtactaaag tagaaatcaa acgtacggta gcggccccat ctgtcttcat cttcccgcca 360
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420
cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag 480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600
ctgagctcac cagtaacaaa aagctttaat agaggagagt gt 642

<210> 113
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<223> Grafted Light Chain for Fab and Modified Fab

<400> 113
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 Lys Ala Ser Gln Asn Val Gly Thr Asn
20 25 30

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

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Tyr 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 Tyr Asn Ile Tyr Pro Leu
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

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

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser

165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 114
<211> 687
<212> DNA
<213> Artificial Sequence

<220>
<223> Grafted Heavy Chain for Modified Fab

<400> 114
gaggttcagc tggtcgagtc aggaggcggt ctcgtgcagc ctggcggatc actgagattg 60
tcctgtgctg catctggtta cgtcttcaca gactatggaa tgaattgggt tagacaggcc 120
ccgggaaagg gcctggaatg gatgggttgg attaatactt acattggaga gcctatttat 180
gctgacagcg tcaagggcag attcacgttc tctctagaca catccaagtc aacagcatac 240
ctccaaatga atagcctgag agcagaggac accgcagtgt actattgtgc tagaggatac 300
agatcttatg ccatggacta ctggggccag ggtaccctag tcacagtctc ctcagcttcc 360
accaagggcc catcggtctt ccccctggca ccctcctcca agagcacctc tgggggcaca 420
gcggccctgg gctgcctggt caaggactac ttccccgaac cggtgacggt gtcgtggaac 480
tcaggcgccc tgaccagcgg cgtgcacacc ttcccggctg tcctacagtc ctcaggactc 540
tactccctca gcagcgtggt gaccgtgccc tccagcagct tgggcaccca gacctacatc 600
tgcaacgtga atcacaagcc cagcaacacc aaggtcgaca agaaagttga gcccaaatct 660
tgtgacaaaa ctcacacatg cgccgcg 687

<210> 115
<211> 229
<212> PRT
<213> Artificial Sequence

<220>
<223> Grafted Heavy Chain for Modified Fab

<400> 115
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 Tyr Val Phe Thr Asp Tyr
20 25 30

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

Gly Trp Ile Asn Thr Tyr Ile Gly Glu Pro Ile Tyr Ala Asp Ser Val
50 55 60

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

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
115 120 125

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

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145 150 155 160

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
165 170 175

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180 185 190

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195 200 205

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210 215 220

His Thr Cys Ala Ala
225