Antibody molecules having specificity for human OX40

The present invention relates to antibody molecules having specificity for antigenic determinants of OX40 and compositions comprising the same. The present invention also relates to the therapeutic uses of the antibody molecules, compositions and methods for producing said antibody molecules.

OX40 (also known as CD134, TNFRSF4, ACT35 or TXGP1L) is a member of the TNF receptor superfamily, which includes 4-1BB, CD27, CD30 and CD40. The extracellular ligand binding domain of OX40 is composed of 3 full cysteine-rich domains (CRDs) and a partial, fourth C-terminal CRD (Bodmer *et al.*, 2002, Trends Biochem Sci, 27, 19-26).

The ligand for OX40 is OX40L and 3 copies of OX40 bind to the trimeric ligand to form the OX40-OX40L complex (Compaan and Hymowitz, 2006, Structure, 14, 1321-1330). OX40 is a membrane-bound receptor; however a soluble isoform has also been detected (Taylor and Schwarz, 2001, J.Immunol. Methods, 255, 67-72). The functional significance of the soluble form is presently unknown. OX40 is not expressed on resting T cells, but is transiently expressed on activated T cells after ligation of the T cell receptor (TCR). The ligand for OX40, OX40L, is a member of the TNF family and is expressed on activated antigen presenting cells (APC), including B cells, macrophages, endothelial cells and dendritic cells (DC).

OX40 is a major costimulatory receptor with sequential engagement of CD28 and OX40 being required for optimal T cell proliferation and survival. Ligation of OX40 on activated T cells leads to enhanced cytokine production and proliferation of both CD4+ and CD8+ T cells (Gramaglia *et al.*, 2000, J. Immunol, 165, 3043-3050, Bansal-Pakala *et al.*, 2004, J.Immunol, 172, 4821-425) and can contribute to both ongoing Th1 and Th2 responses (Gramaglia *et al.*, 1998, J. Immuno., 161, 6510-6517, Arestides *et al.*, 2002, Eur. J. Immunol. 32, 2874-2880). OX40 costimulation prolongs T cell survival beyond the initial effector phase of the immune response and increases the number of memory T cells through inhibition of effector T cell death.

When immune activation is excessive or uncontrolled, pathological allergy, asthma, inflammation, autoimmune and other related diseases may occur. Because OX40 functions to enhance immune responses, it may exacerbate autoimmune and inflammatory diseases.

The role of OX40/OX40L interactions in models of disease has been demonstrated in OX40 knockout mice. In experimental allergic encephalomyelitis (EAE), a model of multiple sclerosis, less severe clinical signs of disease and reduced inflammatory infiltrate within the CNS was noted in OX40 knockout mice (Carboni *et al.*, 2003, J.Neuroimmunology, 145, 1-11). Also OX40 knockout mice primed and challenged with ovalbumin exhibit diminished lung inflammation (80 – 90% reduction in eosinophilia), reduced mucus production, and significantly attenuated airway hyper-reactivity (Jember *et al.*, 2001, J. Exp.Med., 193, 387-392). Monoclonal antibodies to murine OX40 ligand have shown beneficial effects in the collagen-induced arthritis model of rheumatoid arthritis (Yoshioka *et al.*, 2000, Eur. J. Immunol., 30, 2815-2823), EAE (Nohara *et al.*, 2001, J. Immunol., 166, 2108-2115), non-obese diabetic (NOD) mice (Pakala *et al.*, 2004, Eur. J. Immunol., 34, 3039-3046), colitis in T cell

restored mice (Malmstrom *et al.*, 2001, J. Immunol., 166, 6972-6981, Totsuka *et al.*, 2003, Am. J. Physiol. Gastrointest. Liver Physiol., 284, G595-G603) and models of lung inflammation (Salek-Ardakani *et al.*, 2003, J. Exp. Med., 198, 315-324, Hoshino *et al.*, 2003, Eur.J.Immunol, 33, 861-869). An antibody to human OX40L has been profiled in a model of lung inflammation in rhesus monkeys and resulted in reduced levels of IL-5, IL-13 and effector memory T cells in bronchiolar lavage fluid after allergen challenge (Seshasayee *et al.*, 2007, J. Clin.Invest, 117, 3868-3878).

An increase in the expression of OX40 has been noted in several autoimmune and inflammatory diseases. This includes an increase in OX40 expression on T cells isolated from the synovial fluid of rheumatoid arthritis patients (Brugnoni D *et al.*, 1998, Br.J. Rheum., 37, 584-585; Yoshioka *et al.*, 2000, Eur. J. Immunol., 30, 2815-2823; Giacomelli R *et al.*, 2001, Clin. Exp. Rheumatol., 19, 317-320). Similarly an increase in OX40 expression has been noted in gastrointestinal tissue from patients with ulcerative colitis and Crohn's disease (Souza *et al.*, 1999, Gut, 45, 856-863; Stuber *et al.*, 2000, Eur.J.Clin.Invest., 30, 594-599) and in active lesions of patients with multiple sclerosis (Carboni *et al.*, 2003, J.Neuroimmunology, 145, 1-11). OX40L can also be detected on human airway smooth muscle (ASM) and asthma patients ASM cells show greater inflammatory responses to OX40L ligation than healthy donors, indicating a role for the OX40/OX40L pathway in asthma (Burgess *et al.*, 2004, J. Allergy Clin Immunol., 113, 683-689; Burgess *et al.*, 2005, J. Allergy Clin Immunol., 115, 302-308). It has also been reported that CD4+ T cells isolated from the peripheral blood of systemic lupus erythematosus (SLE) patients express elevated levels of OX40 which is associated with disease activity (Patschan *et al.*, 2006, Clin. Exp. Immunol., 145, 235-242).

Given the role of OX40 in allergy, asthma and diseases associated with autoimmunity and inflammation, one approach to therapy in these diseases is to block OX40-OX40L signalling through the use of anti-OX40L antibodies or antagonistic anti-OX40 antibodies

Anti-OX40L antibodies have been described, see for example WO2006/029879. Numerous agonistic anti-OX40 antibodies have been described but very few antagonistic anti-OX40 antibodies are known. A rabbit polyclonal anti-mouse OX40 antibody was produced by Stuber *et al.*, 1996, J.Exp.Med, 183, 979-989 which blocks the interaction between OX40 and OX40L. Mouse monoclonal antibodies, 131 and 315 which bind human OX40 were generated by Imura *et al.*, 1996, J.Exp.Med, 2185-2195.

Fully human antagonistic antibodies have been described in WO2007/062245, the highest affinity of these antibodies had an affinity for cell surface expressed OX40 (activated T cells) of 11nM.

Humanised antagonistic antibodies have been described in WO2008/106116 and the antibody with the best affinity for OX40 had an affinity of 0.94nM.

Other anti-OX40 antibodies have been described, including murine L106 (US Patent number 6,277,962) and murine ACT35, commercially available from eBioscience.

We have previously described high affinity antagonistic anti-OX40 antibodies in International Patent application number WO2010/096418.

We have also previously described in International Patent application number WO2010/035012, a novel multi-specific antibody fusion molecule, hereinafter referred to as a Fab-dsFv and illustrated herein in Figure 1. The same application provides useful anti-albumin binding variable regions which may be used to extend the half-life of the molecule.

In the present invention these albumin binding variable regions have been improved and combined in the Fab-dsFv format with the anti-OX40 antibodies described in WO2010/096418. The new bispecific molecule of the present invention has improved efficacy in a number of *in vitro* and *in vivo* assays described herein when compared to the Fab'-PEG molecule previously described in WO2010/096418. Accordingly, the present invention provides a bispecific antibody fusion protein which binds both human OX40 and human serum albumin which is suitable for use in the treatment or prophylaxis of pathological disorders mediated by OX40 or associated with an increased level of OX40.

Brief Description of the Drawings

Figure 1 shows a bispecific antibody fusion of the present invention (Fab-dsFv format)

Figures 2-8 shows certain amino acid or DNA sequences relating to an antibody according to the disclosure

Figure 9a shows binding of AlexaFluor 488 labelled A26 Fab-dsFv to activated human $CD4^{-}OX40^{+}$ T cells

Figure 9b shows binding for A26 Fab', A26 Fab-Fv and A26 Fab'-PEG in the presence of 5% HSA on activated human CD4+, OX40+ T cells.

Figure 10a shows the effect of A26 Fab-dsFv on cytokine production from PBMC exposed to *Dermatophagoides pteronyssinus* allergic extract

Figure 10b shows the ability of A26 Fab-dsFv to inhibit $CD4^{-}$ and $CD8^{+}$ T cell proliferation in a Hu-NSG mouse model

Figure 11a shows inhibition of OX40L binding to human activated $CD4^{+}$ $OX40^{+}$ T cells by A26 Fab-dsFv

Figure 11b shows inhibition of OX40L binding to human activated $CD4^{+}$ $OX40^{+}$ T cells by A26 Fab', A26 Fab-dsFv, A26 Fab'-PEG and two controls.

Figure 12a shows A26 Fab-Fv inhibits a human mixed lymphocyte reaction (MLR)

Figure 12b shows A26 Fab-Fv inhibits IFN-gamma production during a human MLR

Figure 13 shows A26 Fab-Fv reduces the percentage of activated (CD25+) CD4+ T cells after secondary antigen re-stimulation with *Dermatophagoides pteronyssinus* allergenic extract

Figure 14 shows Fab-Fv and Fab-PEG administered prior to cell transfer dose dependency inhibits CD4+ and CD8+ T cell proliferation in the Hu-NSG model

Humanised CA044_00026 anti-OX40 antibody, is referred to herein as A26.

The antibody fusion molecule of the present invention, referred to herein as a Fab-dsFv, is illustrated in Figure 1. In the present invention the Fab portion (comprising the first heavy and light chain variable regions and the constant domains) binds human OX40 and the dsFv

portion (comprising the second heavy and light chain variable regions, linked by a disulphide bond) binds human serum albumin. In particular, the Fab portion comprises the CDRs derived from an antagonistic anti-OX40 antibody and the Fv portion comprises the heavy and light chain variable regions of a humanised anti-albumin antibody, and these albumin binding variable regions are linked by a disulphide bond.

Accordingly, the present invention provides a bispecific antibody fusion protein which binds human OX40 and human serum albumin comprising:

a heavy chain comprising, in sequence from the N-terminal, a first heavy chain variable domain (V_H1), a CH1 domain and a second heavy chain variable domain (V_H2),

a light chain comprising, in sequence from the N-terminal, a first light chain variable domain (V_L1), a CL domain and a second light chain variable domain (V_L2),

wherein said heavy and light chains are aligned such that V_H1 and V_L1 form a first antigen binding site and V_H2 and V_L2 form a second antigen binding site,

wherein the antigen bound by the first antigen binding site is human OX40 and the antigen bound by the second antigen binding site is human serum albumin, in particular

wherein the first variable domain of the heavy chain (V_H1) comprises the sequence given in SEQ ID NO:1 for CDR-H1, the sequence given in SEQ ID NO:2 for CDR-H2 and the sequence given in SEQ ID NO:3 for CDR-H3 and the first variable domain of the light chain (V_L1) comprises the sequence given in SEQ ID NO:4 for CDR-L1, the sequence given in SEQ ID NO:5 for CDR-L2 and the sequence given in SEQ ID NO:6 for CDR-L3,

wherein the second heavy chain variable domain (V_H2) has the sequence given in SEQ ID NO:11 and the second light chain variable domain (V_L2) has the sequence given in SEQ ID NO: 12 and

the second heavy chain variable domain (V_H2) and second light chain variable domain (V_L2) are linked by a disulphide bond.

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

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

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (Chothia, C. and Lesk, A.M. J. Mol. Biol., 196, 901-

917 (1987)), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus unless indicated otherwise 'CDR-H1' as employed herein is intended to refer to residues 26 to 35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.

The bispecific fusion protein of the present invention comprises a Fab fragment of the anti-OX40 antagonistic antibody previously described in WO2010/096418. As used herein, the term 'antagonistic' describes an antibody fusion protein that is capable of inhibiting and/or neutralising the biological signalling activity of OX40, for example by blocking binding or substantially reducing binding of OX40 to OX40 ligand and thus inhibiting the activation of OX40.

Screening for antibodies to identify those that bind OX40 can be performed using assays to measure binding to human OX40 and/or assays to measure the ability to block the binding of OX40 to its ligand, OX40L. An example of a binding assay is an ELISA, in particular, using a fusion protein of human OX40 and human Fc, which is immobilized on plates, and employing a conjungated secondary antibody to detect anti-OX40 antibody bound to the fusion protein. An example of a blocking assay is a flow cytometry based assay measuring the blocking of OX40 ligand fusion protein binding to OX40 on human CD4 cells. A fluorescently labelled secondary antibody is used to detect the amount of OX40 ligand fusion protein binding to the cell. This assay is looking for a reduction in signal as the antibody in the supernatant blocks the binding of ligand fusion protein to OX40. A further example of a blocking assay is an assay where the blocking of costimulation of naive human T cells mediated by OX40 ligand fusion protein coated to a plate is measured by measuring tritiated thymidine incorporation.

In the present invention, the variable regions are humanised. Humanised antibodies (which include CDR-grafted antibodies) are antibody molecules having one or more complementarity determining regions (CDRs) from a non-human species and a framework region from a human immunoglobulin molecule (see, *e.g.* US 5,585,089; WO91/09967). It will be appreciated that it may only be necessary to transfer the specificity determining residues of the CDRs rather than the entire CDR (see for example, Kashmiri *et al.*, 2005, Methods, 36, 25-34). Humanised antibodies may optionally further comprise one or more framework residues derived from the non-human species from which the CDRs were derived.

In the present invention the CDRs of V_H1 and V_L1 are derived from the antibody known as A26, described in WO2010/096418. Accordingly, in the bispecific antibody fusion protein of the present invention, the first variable domain of the heavy chain (V_H1) comprises the sequence given in SEQ ID NO:1 for CDR-H1, the sequence given in SEQ ID NO:2 or SEQ ID NO:23 for CDR-H2 and the sequence given in SEQ ID NO:3 for CDR-H3 and the first variable domain of the light chain (V_L1) comprises the sequence given in SEQ ID NO:4 or SEQ ID NO:24 for CDR-L1, the sequence given in SEQ ID NO:5 for CDR-L2 and the sequence given in SEQ ID NO:6 for CDR-L3.

It will be appreciated that one or more amino acid substitutions, additions and/or deletions may be made to the CDRs provided by the present invention without significantly altering the ability of the antibody to bind to OX40 and to neutralise OX40 activity. The effect of any amino acid substitutions, additions and/or deletions can be readily tested by one skilled in the art, for example by using the methods described in WO2010/096418, to determine OX40 binding and inhibition of the OX40/OX40L interaction. Accordingly, the present invention provides a bispecific antibody having specificity for human OX40 comprising CDRH-1 (SEQ ID NO:1), CDRH-2 (SEQ ID NO:2), CDRH-3 (SEQ ID NO:3), CDRL-1 (SEQ ID NO:4), CDRL-2 (SEQ ID NO:5) and CDRL-3 (SEQ ID NO:6) as shown in Figure 2(c), for example in which one or more amino acids, for example 1 or 2 amino acids, in one or more of the CDRs has been substituted with another amino acid, such as a similar amino acid as defined herein below.

In one embodiment, a bispecific antibody fusion protein of the present invention comprises a heavy chain, wherein the first variable domain of the heavy chain comprises three CDRs wherein the sequence of CDRH-1 has at least 90% identity or similarity to the sequence given in SEQ ID NO:1, CDRH-2 has at least 90% identity or similarity to the sequence given in SEQ ID NO:2 and/or CDRH-3 has at least 90% identity or similarity to the sequence given in SEQ ID NO:3. In another embodiment, a bispecific antibody fusion protein of the present invention comprises a heavy chain, wherein the variable domain of the heavy chain comprises three CDRs wherein the sequence of CDRH-1 has at least 95% or 98% identity or similarity to the sequence given in SEQ ID NO:1, CDRH-2 has at least 95% or 98% identity or similarity to the sequence given in SEQ ID NO:2 and/or CDRH-3 has at least 95% or 98% identity or similarity to the sequence given in SEQ ID NO:3.

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

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains). Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987, Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991, the BLAST™ software available from NCBI (Altschul, S.F. *et al.,* 1990, J. Mol. Biol. 215:403-410; Gish, W.

& States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. *et al.,* 1996, Meth. Enzymol. 266:131-141; Altschul, S.F. *et al.,* 1997, Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656,).

In another embodiment, a bispecific antibody fusion protein of the present invention comprises a light chain, wherein the first variable domain of the light chain comprises three CDRs wherein the sequence of CDRL-1 has at least 90% identity or similarity to the sequence given in SEQ ID NO:4, CDRL-2 has at least 90% identity or similarity to the sequence given in SEQ ID NO:5 and/or CDRL-3 has at least 90% identity or similarity to the sequence given in SEQ ID NO:6. In another embodiment, a bispecific antibody fusion protein of the present invention comprises a light chain, wherein the first variable domain of the light chain comprises three CDRs wherein the sequence of CDRL-1 has at least 95% or 98% identity or similarity to the sequence given in SEQ ID NO:4, CDRL-2 has at least 95% or 98% identity or similarity to the sequence given in SEQ ID NO:5 and/or CDRL-3 has at least 95% or 98% identity or similarity to the sequence given in SEQ ID NO:6.

In one embodiment the Fab portion of the bispecific antibody fusion protein provided by the present invention is a humanised or CDR-grafted antibody molecule comprising one or more of the CDRs provided in SEQ ID NOs:1, 2, 3, 4, 5 and/or 6 (Figure 2 (c)) or variants thereof. As used herein, the term 'CDR-grafted antibody molecule' refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody). For a review, see Vaughan *et al*, Nature Biotechnology, 16, 535-539, 1998. In one embodiment rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs described herein above are transferred to the human antibody framework (see for example, Kashmiri *et al.*, 2005, Methods, 36, 25-34). In one embodiment only the specificity determining residues from one or more of the CDRs described herein above are transferred to the human antibody framework. In another embodiment only the specificity determining residues from each of the CDRs described herein above are transferred to the human antibody framework.

When the CDRs or specificity determining residues 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. Suitably, the CDR-grafted antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs or specificity determining residues described above. Thus, provided in one embodiment is a neutralising CDR-grafted antibody wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

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

POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at: http://vbase.mrc-cpe.cam.ac.uk/

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

A suitable framework region for the first heavy chain variable domain (VH1) of the present invention is derived from the human sub-group VH3 sequence 1-3 3-07 together with JH4. A suitable framework region for the light chain for the first light chain variable domain (VL1) is derived from the human germline sub-group VK1 sequence 2-1 1-02 together with JK4.

Also, in a CDR-grafted antibody variable region of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann *et al.*, 1998, Nature, 332, 323-324). 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.

Suitably, in the first heavy chain variable region (VH1) of the present invention, if the acceptor heavy chain has the human VH3 sequence 1-3 3-07 together with JH4, then the acceptor framework regions of the heavy chain comprise, in addition to one or more donor CDRs, a donor residue at at least one of positions 37, 73, 78 or 94 (according to Kabat et al., (supra)). Accordingly, provided is a bispecific antibody fusion protein, wherein at least the residues at positions 37, 73, 78 and 94 of the first variable domain of the heavy chain are donor residues.

Suitably, in the first light chain variable region (VL1) of the present invention, if the acceptor light chain has the human sub-group VK1 sequence 2-1 1-02 together with JK4, then the acceptor framework regions of the light chain comprise, in addition to one or more donor CDRs, a donor residue at at least one of positions 64 or 71. Accordingly, provided is a bispecific antibody fusion protein wherein at least the residues at positions 64 and 71 of the first variable domain of the light chain are donor residues.

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

In one embodiment, a bispecific antibody fusion protein of the present invention comprises a heavy chain, wherein the first variable domain of the heavy chain (V_H1) comprises the sequence given in Figure 2 (b) SEQ ID NO:8.

It will be appreciated that one or more amino acid, for example 1 or 2 amino acid, substitutions, additions and/or deletions may be made to the first heavy and light chain variable domains, provided by the present invention, without significantly altering the ability of the antibody fusion protein to bind to OX40 and to neutralise OX40 activity. The effect of any

amino acid substitutions, additions and/or deletions can be readily tested by one skilled in the art, for example by using the methods described in WO2010/096418, to determine OX40 binding and ligand blocking.

In one embodiment, a bispecific antibody fusion protein of the present invention comprises a heavy chain, wherein the first variable domain of the heavy chain comprises a sequence having at least 60% identity or similarity to the sequence given in Figure 2(b) SEQ ID NO:8. In one embodiment, an antibody fusion protein of the present invention comprises a heavy chain (VH1), wherein the first variable domain of the heavy chain comprises a sequence having at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:8.

In one embodiment, a bispecific antibody fusion protein of the present invention comprises a light chain, wherein the first variable domain of the light chain (VL1) comprises the sequence given in Figure 2 (a) SEQ ID NO:7.

In another embodiment, a bispecific antibody fusion protein of the present invention comprises a light chain, wherein the first variable domain of the light chain comprises a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:7. In one embodiment the antibody fusion protein of the present invention comprises a light chain, wherein the first variable domain of the light chain comprises a sequence having at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO: 7.

In one embodiment a bispecific antibody fusion protein of the present invention comprises a heavy chain, wherein the first variable domain of the heavy chain (VH1) comprises the sequence given in SEQ ID NO:8 and a light chain, wherein the first variable domain of the light chain (VL1) comprises the sequence given in SEQ ID NO:7.

In another embodiment of the invention, the antibody fusion protein comprises a heavy chain and a light chain, wherein the first variable domain of the heavy chain comprises a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:8 and the first variable domain of the light chain comprises a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:7. Suitably, the antibody fusion protein comprises a heavy chain, wherein the first variable domain of the heavy chain comprises a sequence having at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:8 and a light chain, wherein the first variable domain of the light chain comprises a sequence having at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:7.

In the bispecific antibody fusion protein of the present invention the heavy chain comprises a CH1 domain and light chain comprises a CL domain, either kappa or lambda.

In one embodiment a bispecific antibody fusion protein of the present invention comprises a heavy chain, wherein the heavy chain comprises the sequence given in SEQ ID NO:10 and a light chain, wherein the light chain comprises the sequence given in SEQ ID NO:9.

It will be appreciated that one or more amino acid, for example 1 or 2 amino acid, substitutions, additions and/or deletions may be made to the antibody variable and/or constant

domains provided by the present invention without significantly altering the ability of the antibody to bind to OX40 and to neutralise OX40 activity. The effect of any amino acid substitutions, additions and/or deletions can be readily tested by one skilled in the art, for example by using the methods described in WO2010096418, to determine OX40 binding and blocking of the OX40/OX40L interaction.

In one embodiment of the invention, the antibody fusion protein comprises a heavy chain, wherein the VH1 and CH1 domains of heavy chain comprise a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:10. Suitably, the antibody fusion comprises a heavy chain, wherein the VH1 and CH1 domains of the heavy chain comprise a sequence having at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:10.

In one embodiment a bispecific antibody fusion molecule according to the present invention comprises a light chain comprising the sequence given in Figure 2(d), SEQ ID NO:9.

In one embodiment of the invention, the antibody fusion protein comprises a light chain, wherein the V_L1 and the CH1 domains of the light chain comprise a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:9. For example, the antibody fusion protein comprises a light chain, wherein the VL1 and CL domains of the light chain comprise a sequence having at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:9.

The second antigen bound by the bispecific antibody fusion protein of the present invention is human serum albumin. This is bound by the Fv portion of the Fab-dsFv which is made up of the second heavy and light chain variable domains, V_H2 and V_L2. In the present invention, V_H2 and V_L2 are derived from one of the antibodies described in WO2010/035012 and represent an improved, more human graft of that antibody.

In one embodiment the second heavy chain variable domain (V_H2) has the sequence given in Figure 3(a) SEQ ID NO:11.

In one embodiment the second light chain variable domain (V_L2) has the sequence given in Figure 3(b) SEQ ID NO:12.

Accordingly, the present invention provides a bispecific antibody fusion protein which binds human OX40 and human serum albumin comprising:

a heavy chain comprising, in sequence from the N-terminal, a first heavy chain variable domain (V_H1), a CH1 domain and a second heavy chain variable domain (V_H2),

a light chain comprising, in sequence from the N-terminal, a first light chain variable domain (V_L1), a CL domain and a second light chain variable domain (V_L2),

wherein said heavy and light chains are aligned such that V_H1 and V_L1 form a first antigen binding site and V_H2 and V_L2 form a second antigen binding site,

wherein the antigen bound by the first antigen binding site is human OX40 and the antigen bound by the second antigen binding site is human serum albumin,

wherein the first variable domain of the heavy chain (V_H1) comprises the sequence given in SEQ ID NO:1 for CDR-H1, the sequence given in SEQ ID NO:2 for CDR-H2 and the sequence given in SEQ ID NO:3 for CDR-H3 and the first variable domain of the light chain

(V_L1) comprises the sequence given in SEQ ID NO:4 for CDR-L1, the sequence given in SEQ ID NO:5 for CDR-L2 and the sequence given in SEQ ID NO:6 for CDR-L3,

wherein the second heavy chain variable domain (V_H2) has the sequence given in SEQ ID NO:11 and the second light chain variable domain (V_L2) has the sequence given in SEQ ID NO: 12 and

the second heavy chain variable domain (V_H2) and second light chain variable domain (V_L2) are linked by a disulphide bond.

Preferably the CH1 domain and the second heavy chain variable domain (V_H2) are connected via a linker and the CL domain and the second light chain variable domain (V_L2) are connected via linker. Any suitable peptide linker sequence may be used and these may be the same in each chain or different. Suitable linkers have previously been described in WO2010/035012 and are incorporated herein by reference. Examples of suitable linkers are shown in Figure 3 (c) and (d). In one embodiment the linker between the CH1 domain and the second heavy chain variable domain (V_H2) comprises or consists of the sequence given in Figure 3 (c) SEQ ID NO:13. In one embodiment the linker between the CH1 domain and the second heavy chain variable domain (V_H2) comprises or consists of the sequence given in Figure 3 (c) SEQ ID NO:14. In one embodiment the linker between the CL domain and the second light chain variable domain (V_L2) comprises or consists of the sequence given in Figure 3(d) SEQ ID NO:14.

In one embodiment the linker in the light chain is a 15 amino acid sequence, in particular GGGGSGGGGSGGGGS (SEQ ID NO: 29).

In one embodiment the linker in the heavy chain is a 16 amino acid sequence, in particular SGGGGSGGGGTGGGGS (SEQ ID NO: 30).

In one embodiment the present invention provides a bispecific antibody fusion protein in which the heavy chain comprises or consists of the sequence given in Figure 3(e) (SEQ ID NO:15) and the light chain comprises or consists of the sequence given in Figure 3(f) (SEQ ID NO:16).

In one embodiment of the invention, the bispecific antibody fusion protein comprises a heavy chain and a light chain, wherein the heavy chain comprises a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:15 and the light chain comprises a sequence having at least 60% identity or similarity to the sequence given in SEQ ID NO:16. Generally, the antibody fusion comprises a heavy chain, wherein the heavy chain comprises a sequence having at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:15 and a light chain, wherein the light chain comprises a sequence having at least 70%, 80%, 90%, 95% or 98% identity or similarity to the sequence given in SEQ ID NO:16.

The antibody fusion molecules of the present invention suitably have a high binding affinity, in particular picomolar affinity for human OX40 and nanomolar affinity for human serum albumin. Affinity may be measured using any suitable method known in the art, including Surface Plasmon Resonance e.g. BIAcore™, as described for OX40 in

WO2010096418 and serum albumin in WO2010/035012, using isolated natural or recombinant OX40 or serum albumin or a suitable fusion protein/polypeptide.

In one example affinity is measured using recombinant human OX40 extracellular domain as described in WO2010/096418. In one example the recombinant human OX40 extracellular domain used is a dimer, for example an Fc fusion dimer. Suitably the antibody fusion molecules of the present invention have a binding affinity for isolated human OX40 of about 200pM or less. In one embodiment the antibody molecule of the present invention has a binding affinity of about 100 pM or less. In one embodiment the antibody molecule of the present invention has a binding affinity of about 50pM or less. In one embodiment the antibody fusion molecule of the present invention has a binding affinity of about 40pM or less.

The antibody fusion molecules of the present invention suitably have a high binding affinity for human OX40 expressed on the surface of activated T cells, for example nanomolar or picomolar affinity. Affinity may be measured using any suitable method known in the art, including the method as described in WO2010096418 using activated $CD4^+$ $OX40^-$ human T cells. In particular the antibody fusion molecules of the present invention have a binding affinity for cell surface expressed human OX40 of about 2nM or better. In one example the antibody molecules of the present invention have a binding affinity for cell surface expressed human OX40 of about 1nM or better. In another example the antibody molecules of the present invention have a binding affinity for cell surface expressed human OX40 of about 0.5 nM or better. In another example the antibody molecules of the present invention have a binding affinity for cell surface expressed human OX40 of about 0.2 nM or better.

Suitably the antibody fusion molecules of the present invention have a binding affinity for isolated human serum albumin about 50nM or less. Suitably the antibody fusion molecules of the present invention have a binding affinity for isolated human serum albumin of about 20nM or less. In one embodiment the antibody molecule of the present invention has a binding affinity of about 10nM or less. In one embodiment the antibody molecule of the present invention has a binding affinity of about 5nM or less. In one embodiment the antibody fusion molecule of the present invention has a binding affinity of about 2nM or less.

The antibody fusion molecules of the present invention can bind human serum albumin and cynomologous, mouse and rat serum albumin. In one embodiment the antibody fusion protein of the present invention bind cynomologus serum albumin with an affinity of 5nM or less. In one embodiment the antibody fusion protein of the present invention binds mouse serum albumin with an affinity of 5nM or less.

The antibody fusion molecules of the present invention are able to bind human OX40 and human serum albumin simultaneously.

Advantageously, the fusion molecules of the present invention have a high affinity for OX40 and also have a adequate half-life in vivo to be therapeutically useful, for example the half-life is in the range 5-15 days, such as 7-11 days.

It will be appreciated that the affinity of antibody fusion protein provided by the present invention for human OX40 and/or human serum albumin may be altered using any suitable method known in the art. The present invention therefore also relates to variants of the antibody

molecules of the present invention, which have an improved affinity for OX40 or human serum albumin. 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.

In one embodiment the bispecific antibody fusion molecules of the present invention block the interaction between OX40 and OX40L. Numerous assays suitable for determining the ability of an antibody to block this interaction are described in WO2010/096418. In one embodiment the present invention provides an antibody fusion protein having specificity for human OX40 which is capable of inhibiting the binding of human OX40L (tested at a final concentration of 2μg/ml) to activated human CD4+OX40+ T cells by 50% at a concentration of less than 0.5nM. In one embodiment the human OX40L used in the assay is natural human OX40. In one embodiment the human OX40 used in the assay is recombinant human OX40.

If desired an antibody for use in the present invention may be conjugated to one or more effector molecule(s). It will be appreciated that the effector molecule may comprise a single effector molecule or two or more such molecules so linked as to form a single moiety that can be attached to the antibodies of the present invention. Where it is desired to obtain an antibody fragment linked to an effector 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 molecule. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom *et al.*, Controlled Drug Delivery, 2nd Ed., Robinson *et al.*, eds., 1987, pp. 623-53; Thorpe *et al.*, 1982 , Immunol. Rev., 62:119-58 and Dubowchik *et al.*, 1999, Pharmacology and Therapeutics, 83, 67-123). Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 89/00195, WO 89/01476 and WO 03/031581. Alternatively, where the effector molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO 86/01533 and EP0392745.

The term effector molecule as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

Examples of effector molecules may include cytotoxins or cytotoxic agents including any agent that is detrimental to (*e.g.* kills) cells. Examples include combrestatins, dolastatins, epothilones, staurosporin, maytansinoids, spongistatins, rhizoxin, halichondrins, roridins, hemiasterlins, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy

anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Effector molecules also include, but are not limited to, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.* daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.* dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (*e.g.* vincristine and vinblastine).

Other effector molecules may include chelated radionuclides such as ^{111}In and ^{90}Y, Lu^{177}, Bismuth^{213}, Californium^{252}, Iridium^{192} and Tungsten^{188}/Rhenium^{188}; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as insulin, tumour necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor and immunoglobulins.

Other effector molecules may include detectable substances useful for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I, ^{131}I, ^{111}In and ^{99}Tc.

Where the effector molecule is a polymer it 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.

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

Specific 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.

Specific 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, for example from 5000 to 40000Da such as from 20000 to 40000Da.

In one example suitable effector molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fusion protein, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see for example US 5,219,996; US 5,667,425; WO98/25971).

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

Examples of suitable sequences are provided in Figure 5 (a) SEQ ID NO:21; Figure 5 (b) SEQ ID NO:22; Figure 6 (a) SEQ ID NO:23; Figure 6 (b) SEQ ID NO:24. Nucleotides 1-63 in SEQ ID NO 21 and 1-63 in SEQ ID NO:23 encode the signal peptide sequence OmpA which is cleaved to give an antagonistic antibody fusion molecule of the present invention.

The present invention also provides an isolated DNA sequence encoding the heavy chain of an antibody fusion protein of the present invention which comprises SEQ ID NO:21 or SEQ ID NO:22. The present invention also provides an isolated DNA sequence encoding the light chain of an antibody fusion molecule of the present invention which comprises SEQ ID NO:23 or SEQ ID NO:24.

Other examples of suitable sequences are provided in Figure 7 (a) SEQ ID NO:25; Figure 7 (b) SEQ ID NO:26; Figure 8 (a) SEQ ID NO:27; Figure 6 (b) SEQ ID NO:28. Nucleotides 1-57 in SEQ ID NO 25 and 1-60 in SEQ ID NO 27 encode the signal peptide sequence from mouse antibody B72.3 (Whittle *et al.*, 1987, Protein Eng. 1(6) 499-505.) which is cleaved to give an antagonistic antibody fusion molecule of the present invention. The present invention also provides an isolated DNA sequence encoding the heavy chain of an antibody fusion protein of the present invention which comprises SEQ ID NO:25 or SEQ ID NO:26. The present invention also provides an isolated DNA sequence encoding the light chain of an antibody fusion molecule of the present invention which comprises SEQ ID NO:27 or SEQ ID NO:28.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences of the present invention. Accordingly, provided is a cloning or expression vector comprising one or more DNA sequences encoding an antibody fusion protein of the present invention. Suitably, 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. Suitably, a vector according to the present invention comprises the sequences given in SEQ ID NO:21 and SEQ ID NO:23. Nucleotides 1-63 in SEQ ID NO 21 and 1-63 in SEQ ID NO 23 encode the signal peptide sequence from OmpA.

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

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

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

For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second

vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

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

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

The antibody fusion molecule may be the sole active ingredient in the pharmaceutical or diagnostic composition or may be accompanied by other active ingredients including other antibody ingredients, for example anti-TNF, anti- IL-1β, anti-T cell, anti-IFNγ or anti-LPS antibodies, or non-antibody ingredients such as xanthines. Other suitable active ingredients include antibodies capable of inducing tolerance, for example, anti-CD3 or anti-CD4 antibodies.

In a further embodiment the antibody fusion protein or composition according to the disclosure is employed in combination with a further pharmaceutically active agent, for example a corticosteroid (such as fluticasonoe propionate) and/or a beta-2-agonist (such as salbutamol, salmeterol or formoterol) or inhibitors of cell growth and proliferation (such as rapamycin, cyclophosphmide, methotrexate) or alternative a CD28 and /or CD40 inhibitor. In one embodiment the inhitor is a small molecule. In another embodiment the inhibitor is an antibody specific to the target.

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

The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation

and is within the judgement of the clinician. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 50 mg/kg, for example 0.1 mg/kg to 20 mg/kg. Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose.

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

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

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

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

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

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Suitable 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, in one or more embodiments the compositions are adapted for administration to human subjects.

Suitably in formulations according to the present disclosure, the pH of the final formulation is not similar to the value of the isoelectric point of the antibody or fragment, for

example if the pH of the formulation is 7 then a pI of from 8-9 or above may be appropriate. Whilst not wishing to be bound by theory it is thought that this may ultimately provide a final formulation with improved stability, for example the antibody or fragment remains in solution.

In one aspect advantageously the fusion molecule of the present disclosure does not have a pI which corresponds to an overall neutral molecule. This renders the molecule less susceptible to aggregation.

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).

In one embodiment the formulation is provided as a formulation for topical administrations including inhalation.

Suitable inhalable preparations include inhalable powders, metering aerosols containing propellant gases or inhalable solutions free from propellant gases. Inhalable powders according to the disclosure containing the active substance may consist solely of the abovementioned active substances or of a mixture of the abovementioned active substances with physiologically acceptable excipient.

These inhalable powders may include monosaccharides (e.g. glucose or arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and polysaccharides (e.g. dextranes), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g. sodium chloride, calcium carbonate) or mixtures of these with one another. Mono- or disaccharides are suitably used, the use of lactose or glucose, particularly but not exclusively in the form of their hydrates.

Particles for deposition in the lung require a particle size less than 10 microns, such as 1-9 microns for example from 0.1 to 5 μm, in particular from 1 to 5 μm. The particle size of the active ingredient (such as the antibody or fragment) is of primary importance.

The propellent gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellent gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as chlorinated and/or fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The abovementioned propellent gases may be used on their own or in mixtures thereof.

Particularly suitable propellent gases are halogenated alkane derivatives selected from among TG 11, TG 12, TG 134a and TG227. Of the abovementioned halogenated hydrocarbons, TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof are particularly suitable.

The propellent-gas-containing inhalable aerosols may also contain other ingredients such as cosolvents, stabilisers, surface-active agents (surfactants), antioxidants, lubricants and means for adjusting the pH. All these ingredients are known in the art.

The propellant-gas-containing inhalable aerosols according to the invention may contain up to 5 % by weight of active substance. Aerosols according to the invention contain, for example, 0.002 to 5 % by weight, 0.01 to 3 % by weight, 0.015 to 2 % by weight, 0.1 to 2 % by weight, 0.5 to 2 % by weight or 0.5 to 1 % by weight of active ingredient.

Alternatively topical administrations to the lung may also be by administration of a liquid solution or suspension formulation, for example employing a device such as a nebulizer, for example, a nebulizer connected to a compressor (e.g., the Pari LC-Jet Plus(R) nebulizer connected to a Pari Master(R) compressor manufactured by Pari Respiratory Equipment, Inc., Richmond, Va.).

The antibody fusion protein of the invention can be delivered dispersed in a solvent, e.g., in the form of a solution or a suspension. It can be suspended in an appropriate physiological solution, e.g., saline or other pharmacologically acceptable solvent or a buffered solution. Buffered solutions known in the art may contain 0.05 mg to 0.15 mg disodium edetate, 8.0 mg to 9.0 mg NaCl, 0.15 mg to 0.25 mg polysorbate, 0.25 mg to 0.30 mg anhydrous citric acid, and 0.45 mg to 0.55 mg sodium citrate per 1 ml of water so as to achieve a pH of about 4.0 to 5.0. A suspension can employ, for example, lyophilised antibody.

The therapeutic suspensions or solution formulations can also contain one or more excipients. Excipients are well known in the art and include buffers (e.g., citrate buffer, phosphate buffer, acetate buffer and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (e.g., serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. Solutions or suspensions can be encapsulated in liposomes or biodegradable microspheres. The formulation will generally be provided in a substantially sterile form employing sterile manufacture processes.

This may include production and sterilization by filtration of the buffered solvent/solution used for the formulation, aseptic suspension of the antibody in the sterile buffered solvent solution, and dispensing of the formulation into sterile receptacles by methods familiar to those of ordinary skill in the art.

Nebulizable formulation according to the present disclosure may be provided, for example, as single dose units (e.g., sealed plastic containers or vials) packed in foil envelopes. Each vial contains a unit dose in a volume, e.g., 2 mL, of solvent/solutionbuffer.

The antibody fusion proteins disclosed herein may be suitable for delivery via nebulisation.

It is also envisaged that the antibody of the present invention may be administered by use of gene therapy. In order to achieve this, DNA sequences encoding the heavy and light chains of the antibody molecule under the control of appropriate DNA components are introduced into a patient such that the antibody chains are expressed from the DNA sequences and assembled *in situ*.

The present invention also provides an antibody fusion molecule (or compositions comprising same) for use in the control of inflammatory diseases, for example acute or chronic inflammatory disease. Suitably, the antibody molecule (or compositions comprising same) can be used to reduce the inflammatory process or to prevent the inflammatory process. In one embodiment there is provided an *in vivo* reduction of activated T cells, in particular those involved in inappropriate inflammatory immune responses, for example recruited to the vicinity/location of such a response.

Reduction of activated T cells, as employed herein, may be a reduction, 10, 20, 30, 40, 50, 60, 70, 80, 90 or more percent in comparison to before treatment or without treatment. Advantageously, treatment with an antibody, fragment or composition according to the present invention, may allow the reduction in the level of activated T cells, without reducing the patients general level of T cells (unactivated T cells). This may result in fewer side effects, and possibly prevent T cell depletion in the patient.

The present invention also provides the antibody fusion molecule of the present invention for use in the treatment or prophylaxis of a pathological disorder that is mediated by OX40 or associated with an increased level of OX40. The pathological condition, may, for example be selected from the group consisting of infections (viral, bacterial, fungal and parasitic), endotoxic shock associtated with infection, arthritis, rheumatoid arthritis, asthma, COPD, pelvic inflammatory disease, Alzheimer's Disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, psoriasis, vasculitis, surgical adhesions, stroke, Type I Diabetes, lyme disease, arthritis, meningoencephalitis, autoimmune uveitis, immune mediated inflammatory disorders of the central and peripheral nervous system such as multiple sclerosis, lupus (such as systemic lupus erythematosus and lupus nephritis) and Guillain-Barr syndrome, Atopic dermatitis, autoimmune hepatitis, fibrosing alveolitis, Grave's disease, IgA nephropathy, idiopathic thrombocytopenic purpura, Meniere's disease, pemphigus, primary biliary cirrhosis, sarcoidosis, scleroderma, Wegener's granulomatosis, other autoimmune disorders, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, heart disease including ischaemic diseases such as myocardial infarction as well as atherosclerosis, intravascular coagulation, bone resorption, osteoporosis, osteoarthritis, periodontitis and hypochlorhydia.

In one embodiment the antibody fusion protein according to the invention is employed in the treatment of allergy, COPD, autoimmune disease, rheumatoid arthritis, asthma, graft versus host disease, Crohn's disease, ulcerative colitis, type- 1 diabetes, multiple sclerosis, Systemic lupus erythematosis, lupus nephritis, Myasthenia Gravis, Grave's disease, transplant rejection, Wegener's granulomatosis, Henoch-Schonlein purpura, systemic sclerosis or viral-induced lung inflammation.

In one embodiment the antibody fusion protein according to the invention is employed in the treatment of a disease selected from the group consisting of allergy, COPD, autoimmune disease, rheumatoid arthritis, asthma, graft versus host disease, Crohn's disease, ulcerative colitis, type- 1 diabetes, multiple sclerosis, Systemic lupus erythematosis, lupus nephritis, Myasthenia Gravis, Grave's disease, transplant rejection, Wegener's granulomatosis, Henoch-Schonlein purpura, systemic sclerosis and viral-induced lung inflammation.

The present invention also provides an antibody fusion molecule according to the present invention for use in the treatment or prophylaxis of pain, particularly pain associated with inflammation.

In one embodiment the mechanism through which the fusion molecules of the present disclosure work include one or more of inhibition of T cell proliferations or survival, enhancement of TReg generation, reduced differentiation of B cells and/or decreased cytokine production.

The present invention further provides the use of an antibody fusion molecule or composition according to the present invention in the manufacture of a medicament for the treatment or prophylaxis of a pathological disorder that is mediated by OX40 or associated with an increased level of OX40, in particular the pathological disorder is rheumatoid arthritis, asthma or COPD.

The present invention further provides the use of an antibody molecule, fragment or composition according to the present invention in the manufacture of a medicament for the treatment or prophylaxis of one or more medical indications described herein.

An antibody fusion molecule or composition of the present invention may be utilised in any therapy where it is desired to reduce the effects of OX40 in the human or animal body. OX40 may be circulating in the body or may be present in an undesirably high level localised at a particular site in the body, for example a site of inflammation.

In one embodiment the antibody fusion molecule of the present invention or a composition comprising the same is used for the control of inflammatory disease, e.g. as described herein.

The present invention also provides a method of treating human or animal subjects suffering from or at risk of a disorder mediated by OX40, the method comprising administering to the subject an effective amount of the antibody fusion molecule of the present invention, or a composition comprising the same.

In one embodiment there is provided a purified bispecific antibody fusion protein which binds human OX40 and human serum albumin, in substantially purified from, in particular free or substantially free of endotoxin and/or host cell protein or DNA.

Purified form as used *supra* is intended to refer to at least 90% purity, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% w/w or more pure.

Substantially free of endotoxin is generally intended to refer to an endotoxin content of 1 EU per mg antibody product or less such as 0.5 or 0.1 EU per mg product.

Substantially free of host cell protein or DNA is generally intended to refer to host cell protein and/or DNA content 400μg per mg of antibody product or less such as 100μg per mg or less, in particular 20μg per mg, as appropriate.

The antibody fusion molecule of the present invention may also be used in diagnosis, for example in the *in vivo* diagnosis and imaging of disease states involving OX40.

Advantageously, the present fusion molecules are thought to be safe for administration to humans at a proper therapeutic dose, in particular because they are not superagonists and are unlikely to cause cytokine storm.

Superagonist as employed herein refers to an antibody which expands T cells in the absence of TCR engagement.

In one embodiment A26 Fab-Fv reduces the Division Index indicating that fewer cells in the population are committed to division; this effect is presumably mediated by the NK cells that are expressing OX40. The Division Index represents the average number of cell divisions that a cell in the original population has undergone and includes the undivided cells.

The Proliferation Index reflects proliferation of the responding population only, and in one embodiment the inhibitory effect of A26 Fab-Fv using this measure is relatively reduced.

Comprising in the context of the present specification is intended to meaning including.

Where technically appropriate embodiments of the invention may be combined.

Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

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

EXAMPLES

Figures in detail:

Figure 1: A bispecific antibody fusion protein of the present invention, referred to as a Fab-dsFv.

Figure 2:

a) Light chain V region of antibody A26 (SEQ ID NO:7)

b) Heavy chain V region of antibody A26 (SEQ ID NO:8)

c) CDRH1 (SEQ ID NO:1), CDRH2 (SEQ ID NO:2), CDRH3 (SEQ ID NO:3), CDRL1 (SEQ ID NO:4), CDRL2 (SEQ ID NO:5) and CDRL3 (SEQ ID NO:6) of antibody A26.

d) Light chain of antibody A26 Fab component (SEQ ID NO:9)

e) Heavy chain of antibody A26 Fab component (SEQ ID NO:10)

Figure 3

a) Heavy chain of anti-albumin Fv component 645gH5 (SEQ ID NO:11)
b) Light chain of anti-albumin Fv component 645gL4 (SEQ ID NO:12)
c) Linker 1 (SEQ ID NO:13)
d) Linker 2 (SEQ ID NO:14)
e) Fab-dsFv heavy chain (SEQ ID NO:15)
f) Fab-dsFv light chain (SEQ ID NO:16)

Figure 4

a) 645g1 heavy chain variable domain (SEQ ID NO:17)
b) 645g1 light chain variable domain (SEQ ID NO:18)
c) A26 Fab-dsFv 645gH1 (SEQ ID NO:19)
d) A26 Fab-dsFv 645gL1 (SEQ ID NO:20)

Figure 5

a) DNA encoding heavy chain of the Fab-dsFv including OmpA leader (SEQ ID NO:21)
b) DNA encoding heavy chain of the Fab-dsFv without OmpA leader (SEQ ID NO:22)

Figure 6

a) DNA encoding light chain of the Fab-dsFv including OmpA leader (SEQ ID NO:23)
b) DNA encoding light chain of the Fab-dsFv without OmpA leader (SEQ ID NO:24)

Figure 7

a) DNA encoding heavy chain of the Fab-dsFv including B72.3 leader (SEQ ID NO:25)
b) DNA encoding heavy chain of the Fab-dsFv without B72.3 leader (SEQ ID NO:26)

Figure 8

a) DNA encoding light chain of the Fab-dsFv including B72.3 leader (SEQ ID NO:27)
b) DNA encoding light chain of the Fab-dsFv without B72.3 leader (SEQ ID NO:28)

Figure 9a shows binding of AlexaFluor 488 labelled A26 Fab-dsFv to activated human $CD4^+OX40^+$ T cells

Figure 9b shows binding for A26 Fab', A26 Fab-Fv and A26 Fab'-PEG in the presence of 5% HSA on activated human CD4+, OX40+ T cells

Figure 10a shows the effect of A26 Fab-dsFv on cytokine production from PBMC exposed to *Dermatophagoides pteronyssinus* allergic extract

Figure 10b shows the ability of A26 Fab-dsFv to inhibit $CD4^+$ and $CD8^+$ T cell proliferation in a Hu-NSG mouse model

Figure 11a shows inhibition of OX40L binding to human activated $CD4^+$ $OX40^+$ T cells by A26 Fab-dsFv

Figure 11b shows inhibition of OX40L binding to human activated $CD4^+$ $OX40^+$ T cells by A26 Fab', A26 Fab-dsFv, A26 Fab'-PEG and two controls.

Figure 12a shows A26 Fab-Fv inhibits a human missed lymphocyte reaction (MLR)

Figure 12b shows A26 Fab-Fv inhibits IFN-gamma production during a human MLR

Figure 13 shows A26 Fab-Fv reduces the percentage of activated (CD25+) CD4+ T cells after secondary antigen re-stimulation with *Dermatophagoides pteronyssinus* allergenic extract

Figure 14 shows Fab-Fv and Fab-PEG administered prior to cell transfer dose dependency inhibits CD4+ and CD8+ T cell proliferation in the Hu-NSG model

DNA manipulations and general methods

Competent *E. coli* strains were used for transformations and routine culture growth. DNA restriction and modification enzymes were obtained from Roche Diagnostics Ltd. and New England Biolabs. Plasmid preparations were performed using Maxi Plasmid purification kits (QIAGEN, catalogue No. 12165). DNA sequencing reactions were performed using the ABI Prism Big Dye terminator sequencing kit (catalogue No. 4304149) and run on an ABI 3100 automated sequencer (Applied Biosystems). Data was analysed using the program Sequencher (Genecodes). Oligonucleotides were obtained from Simga or Invitrogen. Genes encoding initial V-region sequences were constructed by an automated synthesis approach by DNA2.0, and modified to generate the grafted versions by oligonucleotide directed mutagenesis. The concentration of Fab-Fv was determined by a Protein-G based HPLC method.

EXAMPLE 1

Generation and analysis of different humanisation grafts of 645 in A26Fab-645dsFv

We have previously described the Fab-dsFv antibody format (Figure 1) (sometimes referred to herein simply as Fab-Fv) and a humanised anti-albumin antibody known as '645gH1gL1' in WO2010/035012. We have also previously described the generation of a humanised antagonistic anti-OX40 antibody known as 'A26' and a PEGylated Fab' fragment thereof in WO2010/096418. Here we describe the generation of a new improved humanised graft of antibody '645' known as 645dsgH5gL4 and the generation of a Fab-dsFv antibody molecule incorporating that graft in the Fv component and the 'A26' variable regions in the Fab component. The variable regions of A26 are given in Figure 2a and b (SEQ ID NOs 7 and 8). The variable and constant region sequences of A26 combined are given in Figure 2d and e (SEQ ID NOs 9 and 10).

The sequences of 645gH1 and gL1 are given in Figure 4(a) and (b), SEQ ID NOs 17 and 18.

Where the term Fab'-PEG or A26 Fab'-PEG is used this refers to the A26 Fab-40K PEG' described in WO2010/096418.

1.1. Construction of A26Fab-645dsFv(gH1gL1) and A26Fab-645dsFv(gH5gL4)G4S linker plasmids

The total coding region of A26Fab-645dsFv(gL1) light chain (SEQ ID NO:20) was cloned into a UCB mammalian expression vector under the control of the HCMV-MIE promoter and SV40E polyA sequence. The light chain variable region of 645dsFv(gL1) (SEQ ID NO:18) was mutated to 645dsFv(gL4) (SEQ ID NO:12) by an overlapping PCR method. The total

coding region of A26Fab-645dsFv(gH1) heavy chain (SEQ ID NO:19 was cloned into a UCB mammalian expression vector under the control of the HCMV-MIE promoter and SV40E polyA sequence. The heavy chain variable region of 645dsFv(gH1) (SEQ ID NO:17) was mutated to 645dsFv(gH5) (SEQ ID NO:11) by an overlapping PCR method. The constructs were verified by sequencing. Both constructs contained the 3xG4S linker given in SEQ ID NO:14, Figure 3(d).

1.2 Mammalian expression of A26Fab-645dsFv(gH1gL1) and A26Fab-645dsFv(gH5gL4)

HEK293 cells were transfected with the heavy and light chain plasmids using Invitrogen's 293fectin transfection reagent according to the manufacturer's instructions. Briefly, 25µg heavy chain plasmid and 25µg light chain plasmid were incubated with 100µl 293fectin and 1700µl Optipro media for 20mins at RT. The mixture was then added to $50x10^6$ HEK293 cells in 50ml suspension and incubated for 6 days with shaking at 37°C. After 6 days the supernatant was collected by centrifugation at 1500xg for 10 minutes to remove the cells and then 0.22µm sterile filtered.

1.3 Protein-G purification of A26Fab-645dsFv(gH1gL1) and A26Fab-645dsFv(gH5gL4)

The ~50ml of 0.22µm filtered supernatants were concentrated to ~2ml using Amicon Ultra-15 concentrators with a 10kDa molecular weight cut off membrane and centrifugation at 4000xg in a swing out rotor. 1.8ml of concentrated supernatant was applied at 1ml/min to a 1ml Gammabind Plus Sepharose (GE Healthcare) column equilibrated in 20mM phosphate, 40mM NaCl pH7.4. The column was washed with 20mM phosphate, 40mM NaCl pH7.4 and the bound material eluted with 0.1M glycine/HCl pH2.7. The elution peak was collected and pH adjusted to ~pH7 with 2M Tris/HCl pH8.5. The pH adjusted elution was concentrated and diafiltered into 20mM phosphate, 150mM NaCl pH7.4 using Amicon Ultra-15 concentrators with a 10kDa molecular weight cut off membrane and centrifugation at 4000xg in a swing out rotor, to a final volume of ~0.3ml.

1.4 Size exclusion analysis A26Fab-645dsFv(gH1gL1) and A26Fab-645dsFv(gH5gL4)

Protein-G purified samples were analysed by size exclusion HPLC. The samples were separated on a Superdex 200 10/300 GL Tricorn column (GE Healthcare) developed with an isocratic gradient of PBS pH7.4 at 1ml/min. Peak detection was at 280nm and apparent molecular weight was calculated by comparison to a standard curve of known molecular weight proteins verses elution volume. Changing the humanisation graft of the 645dsFv from gH1gL1

to gH5gL4 resulted in an increase in the percentage monomer of the expressed A26Fab-645dsFv from 59% to 71% an increase of 12%, without any change in the thermal stability of the dsFv (data not shown) or in the affinity of binding of the dsFv to HSA (data not shown).

Example 2

2.1 BIAcore kinetics for A26 Fab-dsFv (645gH5gL4) binding OX40

In this and all subsequent examples the A26 Fab-dsFv 645gH5gL4 had the heavy chain sequence given in SEQ ID NO:15 (Figure 3 (e)) and the light chain sequence given in SEQ ID NO:16 (Figure 3(f)) i.e. the heavy chain contained the G4S, G4T, G4S linker given in SEQ ID NO:13, figure 3 (c).

BIA (Biamolecular Interaction Analysis) was performed using a BIAcore T200 (GE Healthcare). Affinipure $F(ab')_2$ Fragment goat anti-human IgG, $F(ab')_2$ fragment specific (Jackson ImmunoResearch) was immobilised on a CM5 Sensor Chip via amine coupling chemistry to a capture level of ≈5000 response units (RUs). HBS-EP buffer (10mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05 % Surfactant P20, GE Healthcare) was used as the running buffer with a flow rate of 10 µL/min. A 10 µL injection of A26 Fab' at 0.5µg/mL or A26Fab-dsFv at 1µg/mL was used for capture by the immobilised anti-human IgG-$F(ab')_2$. Human OX40 was titrated over the captured A26 at various concentrations (25nM to 1.5625nM) at a flow rate of 30 µL/min. The surface was regenerated by 2 x 10 µL injection of 50 mM HCl, followed by a 5 µL injection of 5 mM NaOH at a flowrate of 10µL/min. Background subtraction binding curves were analysed using the T200evaluation software (version 1.0) following standard procedures. Kinetic parameters were determined from the fitting algorithm.

Sample	ka(1/Ms)	kd(1/s)	KD(M)	KD(pM)
Fab'	2.18 ± 0.38 E+05	1.00 E-05	4.68E-11	46.8
Fab-Fv	2.55 ± 0.35 E+05	1.04 E-05	4.12E-11	41.2
Fab' PEG	2.33 ± 0.46 E+05	1.12 E-05	4.84E-11	48.4

Average of 4 determinations

Table 1

2.2. BIAcore kinetics for A26 Fab-dsFv (645gH5gL4) binding albumin

BIA (Biamolecular Interaction Analysis) was performed using a BIAcore T200 (GE Healthcare). Affinipure $F(ab')_2$ Fragment goat anti-human IgG, $F(ab')_2$ fragment specific (Jackson ImmunoResearch) was immobilised on a CM5 Sensor Chip via amine coupling chemistry to a capture level of ≈5000 response units (RUs). HBS-EP buffer (10mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05 % Surfactant P20, GE Healthcare) was used as the running buffer with a flow rate of 10 µL/min. A 10 µL injection of Fab-Fv at 0.75µg/mL was used for capture by the immobilised anti-human IgG-$F(ab')_2$. Human Serum Albumin (HSA), Mouse Serum albumin (MSA) and Cynomolgus Serum Albumin (CSA) was titrated over the captured Fab-Fv at various concentrations (50nM to 6.25nM) at a flow rate of 30 µL/min. The surface was regenerated by 2 x 10 µL injection of 50 mM HCl, followed by a 5 µL injection of 5 mM NaOH at a flowrate of 10µL/min. Background subtraction binding curves were analysed using the T200evaluation software (version 1.0) following standard procedures. Kinetic parameters were determined from the fitting algorithm.

Table 2

Sample	ka(1/Ms)	kd(1/s)	KD(M)	KD(nM)
HSA	5.84 E+04	1.63 E-04	2.93E-09	2.93
MSA	8.86 E+04	3.68 E-04	4.16E-09	4.16
CSA	7.1 E+04	1.89 E-04	2.66E-09	2.66

Average of 3 determinations

2.3 Demonstration of A26 Fab-dsFv(645gH5gL4) binding OX40 and albumin simultaneously

The simultaneous binding of human OX40 and Human Serum Albumin to A26 Fab-dsFv was assessed. The A26 Fab-dsFv construct was captured to the sensor chip surface as stated in the method for Biacore kinetics for binding A26 Fab-dsFv albumin. 50nM HAS, 25nM OX40 or a mixed solution with final concentration of 50nM HSA and 25nM OX40 were titrated separately over the captured A26 Fab-dsFv. The binding response for the combined HSA/OX40 solution was equivalent to the sum of the responses of the independent injections. This confirms that the Fab-dsFv is capable of simultaneous binding to both human OX40 and HSA.

Table 3

Sample	Analyte	Binding (RU)	
A26 Fab-Fv	hOX40	25	
	HSA	9	
	hOX40 + HSA	**35**	**(34)**

2.4 Cell-based affinity of A26 Fab-dsFv (645gH5gL4)

Methods:

A26 Fab-Fv binding to human activated $CD4^+OX40^+$ T cells.

PBMC were isolated by separation on a Ficoll gradient and activated with 4µg/mL PHA-L for 3 days at 37°C, 5% CO_2, 100% humidity. $CD4^+$ T cells were isolated by negative selection using magnetic beads ($CD4^+$ T cell Isolation Kit II for Human; Miltenyi Biotec). Approximately 1 x 10^5 cells were incubated in the presence of antibody in either Facs buffer (PBS/0.2% BSA/0.09% NaN3) or Facs buffer supplemented with 5% HSA at 4°C. The final concentration of the antibody ranged from 48nM – 0.0005nM)) . The cells were washed in PBS prior to analysis by flow cytometry using a FACScalibur (Becton Dickinson).Two titration data sets were produced in both buffer conditions, one with A26 Fab-dsFv and the second with an irrelevant control Fab-Fv to determine non-specific binding. The number of moles of bound antibody were calculated by using interpolated values from a standard curve generated by use of beads comprised of differing but known amounts of fluorescent dye. Geometric mean fluorescence values were determined in the flow cytometric analyses of cells and beads. Non-specific binding was subtracted from the A26 Fab-dsFv values and the specific binding curve thus generated analysed by non-linear regression using a one-site binding equation (Graphpad Prism®) to determine the K_D.

To determine the affinity of A26 Fab-dsFv for cell surface expressed antigen, saturation binding experiments were performed using activated $CD4^+OX40^+$ T cells, and Alexa Fluor 488-labelled A26 Fab-dsFv. Specific binding of antibody to receptor at equilibrium across a range of antibody concentrations was used to determine K_D, assuming that only a very small fraction of antibody was bound to receptor at any point on the binding curve.

Equilibrium binding is described using the following equation:

$$\text{Receptor}_{free} + \text{Antibody}_{free} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{Receptor-Antibody}$$

The rate of association of antibody with receptor = k_{on} x [Receptor $_{free}$] x [Antibody $_{free}$]

The rate of dissociation of receptor-antibody complex = k_{off} x [Receptor-Antibody]

At equilibrium, the association and dissociation rates are equal and an equation can be derived which describes the binding isotherm; on a semi-log plot the binding is sigmoidal. The K_D is

defined by k_{off} / k_{on} and can be calculated from the binding curve as the concentration at which half-maximal binding occurs.

Binding of AlexaFluor488- labelled A26 Fab-Fv to activated human $CD4^+OX40^+$ T cells was measured by flow cytometry across a 5-log concentration range.

A representative binding curve for A26 Fab-Fv is shown in Figure 9A.

The mean K_D value obtained on activated cells from 5 different donors is 145pM.

A comparator binding curve for A26 Fab, A26 Fab-Fv and A26 Fab-PEG is shown in Figure 9B

The graphs represents the mean of 4 or 5 experiments where a different donor was used in each experiment.

PBMC were isolated by separation on a Ficoll gradient and activated with 4µg/mL PHA-L for 3 days at 37°C, 5% CO_2, 100% humidity. Following this, $CD4^+$ T cells were isolated by negative selection using magnetic beads ($CD4^+$ T cell Isolation Kit II for Human; Miltenyi Biotec). Approximately 1 x 10^5 cells were incubated in the presence of antibody in either Facs buffer (PBS/0.2% BSA/0.09% NaN3) or Facs buffer supplemented with 5% HSA, at 4°C. The final concentration of the antibody ranged from 48nM – 0.0005nM. The cells were washed in PBS prior to analysis by flow cytometry using a FACScalibur (Becton Dickinson). Titration data sets were also produced for isotype control antibodies for each A26 format to determine non specific binding. The number of moles of bound antibody was calculated by using interpolated values from a standard curve generated from beads comprised of differing but known amounts of fluorescent dye. Geometric mean fluorescence values were determined in the flow cytometric analyses of cells and beads. Non-specific binding was subtracted from the A26 Fab-Fv values and the specific binding curve thus generated analysed by non-linear regression using a one-site binding equation (Graphpad Prism®) to determine the K_D.

Table 4: Mean K_D values for A26 antibodies in human cell affinity assays

Antibody Format	Cellular Affinity **HSA** K_D (nM) ± S.E.M	Cellular Affinity **NO HSA** K_D (nM) ± S.E.M
A26 Fab-Fv (*n*=5)	**0.145 ± 0.019**	**0.096 ± 0.017**
A26 Fab'PEG (*n=4)*	0.230 ± 0.057	0.322 ± 0.089
A26 Fab' (*n=4*)	0.068 ± 0.011	0.085 ± 0.031

Example 3: A26 Fab-Fv modulates cytokine production from PBMC exposed to *Dermatophagoides pteronyssinus* allergenic extract

PBMC were isolated from allergic volunteers by separation on a Ficoll gradient. Purified PBMC were exposed to 25µg/mL *Dermatophagoides pteronyssinus* allergenic extract in the presence of test antibody (concentration range 50µg/mL to 0.0005µg/mL) in a final volume of 200µL per well in a 96-well round-bottomed plate. After 6 days incubation at 37°C, 5% CO_2, 100% humidity, supernatants were harvested and assayed for IL-13 content by MSD. The graph in Figure 10 (a) shows representative data of 1 representative donor from 4, where the mean EC50 for inhibition of IL-13 production was 0.87nM (range from 0.6nM to 1.07nM).

Table 5: Mean EC_{50} values for A26 Antibodies in human HDM in vitro assays

EC_{50} values were calculated from individual donor inhibition curves by non-linear regression using Graphpad Prism® software

Antibody Format	**Inhibition of IL-13 Production EC_{50} (nM) ± S.E.M**	**Inhibition of IL-5 Production EC_{50} (nM) ± S.E.M**
A26 Fab-Fv (*n*=4)	**0.865 ± 0.112**	**0.785 ± 0.216**
A26 Fab'PEG (*n*=4)	**0.928 ± 0.282**	**1.310 ± 0.425**
A26 Fab' (*n*=4)	**0.335 ± 0.040**	**0.680 ± 0.223**

A26 Fab- Fv reduces the percentage of activated ($CD25^+$) $CD4^+$ T cells after secondary antigen re-stimulation with *Dermatophagoides pteronyssinus* allergenic extract

$CD4^+$ T cells from allergic donors were stimulated in vitro for 7 days with 25µg/ml Dermatophagoides pteronyssinus allergenic extract (Greer) and autologous APC, in the presence of no antibody or 10µg/ml A26 Fab'PEG, A26 Fab-Fv or Ctrl Fab' (A33 Fab'). Cells were washed and rested for 3 days and then re-stimulated with Dermatophagoides pteronyssinus extract as previously (Figure 13). After 3 days, the cells were washed and fluorescently stained for surface CD3, CD4 and CD25. Cells were then analysed by flow cytometry on a FACS Canto flow cytometer (BD). Cells were gated on live lymphocytes and $CD3^+CD4^-$ expression prior to analysis. Data represents n = 3 donors including mean. n.s, A26 Fab-Fv compared to Ctrl Fab' (significance measured using paired, 2 tailed T test).

Example 4: A26 Fab-Fv inhibits CD4+ and CD8+ T cell proliferation in a Hu-NSG mouse model.

Mice were dosed s.c with 0.03, 0.3, 3 or 30 mg/kg A26 Fab-Fv one day prior to transfer of 1 x 10^7 human PBMCs into the peritoneal cavity. After 14 days mice were bled by cardiac puncture under terminal aesthetic and then killed by cervical dislocation. The number of human $CD4^+$ and $CD8^+$ cells in the blood was then determined by FACS analysis (Figure 10 (b)). Data (n=10) is expressed as means ± SEM and statistical analysis is by one way ANOVA with Bonferroni post test. Values represent % inhibition ± SEM.

Results are shown in Figure 14.

The Hu-NSG model has demonstrated that A26 Fab-Fv profoundly inhibits human T cell proliferation *in vivo* and supports A26 Fab-Fv as a viable therapeutic candidate for the inhibition of T cell mediated pathologies. In addition, the Fab-Fv format conferred greater efficacy at lower doses than the Fab' PEG format. The decrease in this xeno-proliferative response of donor T cells may provide supporting evidence that A26 Fab-Fv could be a viable therapeutic for GVHD.

Example 5: Ligand-blocking capacity

The capacity of A26 Fab-dsFv to block the interaction between cell-surface expressed OX40 and recombinant OX40L was measured using a flow cytometry-based ligand blocking assay. Briefly, activated human $CD4^+OX40^+$ T cells were pre-incubated with a titration of A26 Fab-Fv. Recombinant OX40L was subsequently added to the cells and allowed to bind in the presence of A26 Fab-dsFv. The proportion of OX40L bound was then detected by flow

cytometry using a labelled secondary reagent. Figure11 shows an inhibition curve representing combined data from 3 independent donors and demonstrates that A26 Fab-dsFv is capable of completely blocking OX40L binding. The mean IC_{50} for inhibition of recombinant OX40L binding was 0.44 nM (n = 3 donors).

Methods: Inhibition of OX40L binding to human activated $CD4^+OX40^+$ T cells by A26 Fab-Fv

PBMC were isolated by separation on a Ficoll gradient and activated with 4 μg/mL PHA-L (Sigma) for 3 days at 37°C, 5% CO_2, 100% humidity. $CD4^+$ T cells were then purified from the culture by negative selection using MACS columns (Miltenyi Biotech, $CD4^+$ T cell isolation kit II). 2 x 10^5 $CD4^+$ T cells were incubated in the presence of A26 Fab-dsFv (final concentration range 10 μg/mL – 0.000056 μg/mL (136.6 nM – 0.000765 nM)) for 30 minutes at 4°C. OX40L (biotinylated CD252 muCD8, Ancell) was added at a final concentration of 2 μg/mL and incubated for a further 30 minutes at 4°C. Cells were washed and OX40L binding detected by incubation with PE-labelled streptavadin (Jackson Immunoresearch) prior to analysis by flow cytometry using a FACS Canto (Becton Dickinson). A matched non-OX40 binding Fab-dsFv was used as a control. The inhibition curve was analysed by non-linear regression (Graphpad Prism®) to determine the IC_{50}. An inhibition curve representing combined data from 3 independent donors is shown in Figure 11, where data points represent the mean and error bars represent SEM.

The mean EC_{50} for inhibition of recombinant OX40L binding to OX40 by A26 Fab-Fv was 0.445 nM. In comparison, A26 Fab'PEG was slightly less potent at ligand blocking (EC_{50} = 0.739 nM) whereas A26 Fab' had marginally greater potency (EC_{50} = 0.242 nM) than the Fab-Fv as shown below.

Table 6: EC_{50} values for inhibition of OX40L binding to human activated $CD4^+OX40^+$ T cells by A26 antibodies

Antibody format	EC_{50} ligand blocking (nM) Mean ± S.E.M.
A26 Fab-Fv (n = 3)	**0.445 ± 0.110**
A26 Fab'PEG (n = 3)	0.739 ± 0.166
A26 Fab' (n = 3)	0.242 ± 0.069

EC_{50} values were calculated from individual donor inhibition curves by non-linear regression using Graphpad Prism® software.

Example 6 Effect of A26 Fab-Fv in functional human in vitro assays

The effect of A26 Fab-Fv on OX40-OX40L dependent cellular interactions was assessed in a range of antigen-driven human lymphocyte assays. These assays were performed in the presence of 5% human serum to ensure saturation of the albumin binding site of the Fv region, as would be predicted to occur *in vivo*.

A26 Fab-Fv inhibits a mixed lymphocyte reaction

The one-way allogeneic mixed lymphocyte reaction (MLR) is an *in vitro* model of alloreactive T cell activation and proliferation (Bach *et al.,* 1964, O'Flaherty *et al.,* 2000). Donor T cells are activated through recognition of allogeneic MHC antigens on unrelated donor stimulator PBMCs, resulting in cellular proliferation and cytokine production (Lukacs *et al.,* 1993). T lymphocyte alloreaction has been shown to be driven by both the allogeneic MHC antigen and bound peptide (Sherman *et al.,* 1993). The magnitude of an MLR response correlates with the degree of MHC mis-matching between the responder-stimulator pair (Forrester *et al.,* 2004). An MLR response results in the proliferation of cells from the responding donor and the production of both Th1 (IL-2, IFN-γ and TNF-α) and Th2 (IL-4, IL-5, IL-10 and IL-13) T cell derived cytokines. The exact cytokine profile in an MLR is thought to be specific to the responder-stimulator pairing (Jordan *et al.,* 2002). MLR assays have been used widely in research to study T cell activation pathways, screen immunosuppressive drugs and predict possible donor organ rejection in transplant recipients (Bromelow *et al.,* 2001).

The effect of A26 Fab-Fv on *in vitro* human alloreactive T cell activation and proliferation was investigated using an MLR assay essentially as described by O'Flaherty *et al.,* 2000. PBMCs from two unrelated donors were co-cultured in the presence or absence of A26 Fab-Fv, A26 Fab' or A26 Fab'PEG and cellular proliferation measured by ^{3}H-thymidine incorporation. As shown in Figure 12, A26 Fab-Fv inhibited cellular proliferation in a concentration-dependent manner with an EC_{50} value of 0.56 nM (40.9 ng/mL) and a maximal inhibition of 55% (n = 3 donor pairings). A26 Fab-Fv was slightly more potent than A26 Fab'PEG, which had an EC_{50} value of 0.88 nM, while A26 Fab' had an EC_{50} value of 0.25 nM as shown in Table 7:.

Human PBMCs from two unrelated donors were isolated from whole blood. Cells from one donor were inactivated by γ-irradiation to generate the stimulator population. Cells from the remaining donor formed the responder population. Stimulator and responder populations were mixed at a 1:1 ratio ($1x10^5$ cells/donor) and cultured in the presence A26 Fab', A26 Fab-Fv or

A26 Fab'PEG (0.4ng-25μg/mL) for 6 days. In-house reagent CA162-01297.1 Fab-Fv was utilized as an isotype-matched control. Cellular proliferation was measured at day 6 by ^{3}H-thymidine incorporation (0.5 μCi/well). Data is displayed as percentage inhibition relative to the responder plus stimulator response in the absence of biologic reagent, and is the combined data from three donor pairings. EC_{50} values were calculated using Graphpad Prism® software.

Table 7 EC_{50} values for inhibition of human MLR proliferative response by A26 antibodies

Antibody format	**EC_{50} (nM) Mean ± S.E.M.**
A26 Fab-Fv (n = 3)	**0.56 ± 0.12**
A26 Fab'PEG (*n* = 3)	0.88 ± 0.44
A26 Fab' (*n* = 3)	0.25 ± 0.06

Supernatants from the human MLR were also analysed to investigate the effect of A26 Fab-Fv on cytokine production. As shown in Figure 12B, A26 Fab-Fv significantly inhibited production of IFN-γ in the MLR by an average of 81% (*n* = 3 donor pairings).

Human PBMCs from two unrelated donors were isolated from whole blood. Cells from one donor were inactivated by γ-irradiation to generate the stimulator population. Cells from the remaining donor formed the responder population. Stimulator and responder populations were mixed at a 1:1 ratio ($1x10^5$ cells/donor) and cultured in the presence of 25 μg/ml A26 Fab', A26 Fab-Fv or A26 Fab'PEG or controls (A33 Fab' or CA162.01297.1) for 6 days. Supernatants were harvested and assayed for IFN-γ content using an MSD assay. The percent inhibition was calculated relative to cells cultured with no antibody. The graphs represent pooled data from three donors (mean ± S.E.M). ** = $p<0.01$; A26 Fab-Fv compared with Ctrl Fab-Fv (significance measured using paired, 2-tailed T-test).

Example 7 A26 Fab-Fv binding to NK cells in a human MLR

The effect of A26 Fab-Fv on NK cell division within an MLR was investigated. T lymphocyte alloreaction drives the mixed lymphocyte response and A26 Fab-Fv profoundly inhibits T cell division and IFNγ production in this system. Inhibition of NK cell division could also contribute to reduced IFNγ production. Using CFSE-labelled responder cells inhibition of NK cell division was demonstrated by FACS analysis of the dividing population (data not shown). Two different measures of cell division are shown. The Division Index represents the average

number of cell divisions that a cell in the original population has undergone and includes the undivided cells; A26 Fab-Fv reduces the Division Index indicating that fewer cells in the population are committed to division; this effect is presumably mediated by the NK cells that are expressing OX40. The Proliferation Index reflects proliferation of the responding population only, and the inhibitory effect of A26 Fab-Fv using this measure is relatively reduced.

Example 8 **Mean K_D / EC_{50} values for A26 Fab-Fv in human *in vitro* assays**

Binding / Functional Assay	**Mean K_D / EC_{50} (nM) ± S.E.M.**	**Mean K_D / EC_{50} (µg/mL)**
Affinity (n = 5)	0.145 ± 0.019	0.011
OX40L blocking (n = 3)	0.445 ± 0.110	0.033
Mixed Lymphocyte Reaction - Inhibition of Proliferation (n = 3)	0.558 ± 0.121	0.041
House Dust Mite - Inhibition of IL-13 production (n = 4)	0.865 ± 0.112	0.063

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

CLAIMS

1. An antibody fusion protein that binds human OX40 and human serum albumin comprising:

a heavy chain comprising, in sequence from the N-terminal, a first heavy chain variable domain (V_H1), a CH1 domain and a second heavy chain variable domain (V_H2),

a light chain comprising, in sequence from the N-terminal, a first light chain variable domain (V_L1), a CL domain and a second light chain variable domain (V_L2),

wherein said heavy and light chains are aligned such that VH1 and VL1 form a first antigen binding site and VH2 and VL2 form a second antigen binding site,

wherein the antigen bound by the first antigen binding site is human OX40 and the antigen bound by the second antigen binding site is human serum albumin,

wherein the first variable domain of the heavy chain (V_H1) comprises the sequence given in SEQ ID NO:1 for CDR-H1, the sequence given in SEQ ID NO:2 for CDR-H2 and the sequence given in SEQ ID NO: 3 for CDR-H3 and the first variable domain of the light chain (V_L1) comprises the sequence given in SEQ ID NO:4 for CDR-L1, the sequence given in SEQ ID NO:5 for CDR-L2 and the sequence given in SEQ ID NO:6 for CDR-L3,

wherein the second heavy chain variable domain (V_H2) has the sequence given in SEQ ID NO: 11 and the second light chain variable domain (V_L2) has the sequence given in SEQ ID NO: 12 and

the second heavy chain variable domain (V_H2) and second light chain variable domain (V_L2) are linked by a disulphide bond.

2. An antibody fusion protein according to claim 1, which antagonises the binding of OX40 to OX40L.

3. An antibody fusion protein according to claim 1 or claim 2, in which there is a peptide linker between the CH1 domain and the second heavy chain variable domain (V_H2).

4. An antibody fusion protein according to any one of claims 1 to 3 in which there is a peptide linker between the CL domain and the second light chain variable domain (V_L2).

5. An antibody fusion protein according to any one of claims 1 to 4, wherein the first heavy chain variable domain (V_H1) comprises the sequence given in SEQ ID NO:8.

6. An antibody fusion protein according to any one of claims 1 to 5, wherein the first light chain variable domain (V_L1) comprises the sequence given in SEQ ID NO:7.

7. An antibody fusion protein according to any one of claims 1 to 6, wherein the heavy chain comprises or consists of the sequence given in SEQ ID NO:15.

8. An antibody fusion protein according to any one of claims 1 to 7, wherein the light chain comprises or consists of the sequence given in SEQ ID NO:16.

9. An antibody fusion protein which binds human OX40 and human serum albumin, having a heavy chain comprising the sequence given in SEQ ID NO:15 and a light chain comprising the sequence given in SEQ ID NO:16.

10. An antibody fusion protein according to claim 9, wherein the heavy chain consists of the sequence given in SEQ ID NO:15 and the light chain consists of the sequence given in SEQ ID NO:16.

11. An isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody fusion protein according to any one of claims 1 to 10.

12. A cloning or expression vector comprising one or more DNA sequences according to claim 11.

13. A vector according to claim 12, wherein the vector comprises the sequences given in SEQ ID NO:22 and SEQ ID NO:24.

14. A host cell comprising one or more cloning or expression vectors according to claim 12 or claim 13.

15. A process for the production of an antibody fusion protein, comprising culturing the host cell of claim 13 and isolating the antibody fusion protein.

16. A pharmaceutical composition comprising an antibody fusion protein according to any one of claims 1 to 10, in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

17. A pharmaceutical composition according to claim 16, additionally comprising one or more other active ingredients.

18. An antibody fusion protein according to any one of claims 1 to 10 or a pharmaceutical composition according to claim 16 or claim 17, for use in therapy.

19. An antibody fusion protein according to any one of claims 1 to 10 or a pharmaceutical composition according to claim 16 or claim 17, for use in the treatment or prophylaxis of a pathological disorder that is mediated by OX40 or that is associated with an increased level of OX40, wherein the pathological disorder is selected from the group consisting of allergy, COPD, autoimmune disease, rheumatoid arthritis, asthma, graft versus host disease, Crohn's disease, ulcerative colitis, type- 1 diabetes, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, myasthenia gravis, Grave's disease, transplant rejection, Wegener's granulomatosis, Henoch-Schönlein purpura, systemic sclerosis and viral-induced lung inflammation.

20. The use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for the treatment or prophylaxis of a pathological disorder that is mediated by OX40 or that is associated with an increased level of OX40, wherein the pathological disorder is a disease selected

from the group consisting of allergy, COPD, autoimmune disease, rheumatoid arthritis, asthma, graft versus host disease, Crohn's disease, ulcerative colitis, type- 1 diabetes, multiple sclerosis, systemic lupus erythematosus, lupus nephritis, myasthenia gravis, Grave's disease, transplant rejection, Wegener's granulomatosis, Henoch-Schönlein purpura, systemic sclerosis and viral-induced lung inflammation.

21. An antibody fusion protein according to any one of claims 1 to 10 for use in treating allergy.

22. An antibody fusion protein according to any one of claims 1 to 10 for use in treating COPD.

23. An antibody fusion protein according to any one of claims 1 to 10 for use in treating autoimmune disease.

24. An antibody fusion protein according to any one of claims 1 to 10 for use in treating rheumatoid arthritis.

25. An antibody fusion protein according to any one of claims 1 to 10 for use in treating asthma.

26. An antibody fusion protein according to any one of claims 1 to 10 for use in treating graft versus host disease.

27. An antibody fusion protein according to any one of claims 1 to 10 for use in treating Crohn's disease.

28. An antibody fusion protein according to any one of claims 1 to 10 for use in treating ulcerative colitis.

29. An antibody fusion protein according to any one of claims 1 to 10 for use in treating type-1 diabetes.

30. An antibody fusion protein according to any one of claims 1 to 10 for use in treating multiple sclerosis.

31. An antibody fusion protein according to any one of claims 1 to 10 for use in treating systemic lupus erythematosus.

32. An antibody fusion protein according to any one of claims 1 to 10 for use in treating lupus nephritis.

33. An antibody fusion protein according to any one of claims 1 to 10 for use in treating myasthenia gravis.

34. An antibody fusion protein according to any one of claims 1 to 10 for use in treating Grave's disease.

35. An antibody fusion protein according to any one of claims 1 to 10 for use in treating transplant rejection.

36. An antibody fusion protein according to any one of claims 1 to 10 for use in treating Wegener's granulomatosis.

37. An antibody fusion protein according to any one of claims 1 to 10 for use in treating Henoch-Schönlein purpura.

38. An antibody fusion protein according to any one of claims 1 to 10 for use in treating systemic sclerosis.

39. An antibody fusion protein according to any one of claims 1 to 10 for use in treating viral-induced lung inflammation.

40. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating allergy.

41. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating COPD.

42. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating autoimmune disease.

43. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating rheumatoid arthritis.

44. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating asthma.

45. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating graft versus host disease.

46. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating Crohn's disease.

47. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating ulcerative colitis.

48. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating type-1 diabetes.

49. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating multiple sclerosis.

50. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating systemic lupus erythematosus.

51. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating lupus nephritis.

52. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating myasthenia gravis.

53. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating Grave's disease.

54. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating transplant rejection.

55. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating Wegener's granulomatosis.

56. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating Henoch-Schönlein purpura.

57. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating systemic sclerosis.

58. Use of an antibody fusion protein according to any one of claims 1 to 10 in the manufacture of a medicament for treating viral-induced lung inflammation.