

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 January 2007 (18.01.2007)

PCT

(10) International Publication Number
WO 2007/007173 A2

(51) International Patent Classification: **Not classified**

(21) International Application Number:
PCT/IB2006/001923

(22) International Filing Date: 5 July 2006 (05.07.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/697,453 8 July 2005 (08.07.2005) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NOVEL MADCAM ANTIBODIES

(57) Abstract: The invention provides new, improved anti-MAdCAM antibodies. Uses of these antibodies in medicine are also included, in particular for the treatment of inflammatory conditions such as inflammatory bowel disease.



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Novel MAdCAM Antibodies

The invention relates to an improved human antibody specific for Mucosal addressin cell adhesion molecule (MAdCAM).

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Mucosal addressin cell adhesion molecule (MAdCAM) is a member of the immunoglobulin superfamily of cell adhesion receptors. It is one of the adhesion molecules involved in the recruitment of lymphocytes to tissues when required, by means of interacting with an integrin molecule on the surface of the lymphocytes.

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It has been shown that antibodies that inhibit binding of MAdCAM to its integrin binding partner, $\alpha_4\beta_7$, for example anti-MAdCAM antibodies (e.g. MECA-367; US 5,403,919, US 5,538,724) or anti- $\alpha_4\beta_7$ antibodies (e.g. Act-1; US 6,551,593 or humanised Act-1, also called MLN02, described in WO 01/78779), can inhibit leukocyte extravasation into inflamed intestine, and can therefore be beneficial in the treatment of inflammatory bowel disease.

15

Anti-MAdCAM antibodies such as MECA-367, however, are not therapeutically useful in human patients; MECA-367 binds mouse MAdCAM, and does not show much affinity for the human MAdCAM molecule. In addition, being a rat antibody, it will lead to an immune response in human patients and therefore not be suitable for therapeutic use. Mouse monoclonal antibodies, directed against human MAdCAM have been described (WO 96/24673), but these are also likely to be immunogenic in humans. Recently, therapeutically useful, fully human anti-human MAdCAM antibodies with exquisite specificity and affinity to human and primate MAdCAM have been developed and disclosed in WO2005/067620.

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It is known that even fully human antibodies can still lead to an immune response in some patients, which is likely to be due to sequences in the variable region of the antibody. The antibodies of the present invention are modifications of the human anti-MAdCAM antibodies disclosed in WO2005/067620, which are likely to be even less prone to any immunogenicity problems in human patients.

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Aspects of the invention

One aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the heavy chain of 6.22.2-mod_V (SEQ ID NO: 2).

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Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the light chain of 6.34.2-mod_SSQ, 6.34.2-mod_QT, or 6.34.2-mod_SSQ,QT (SEQ ID NOs: 8, 10, 12).

40

Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the light chain of 6.67.1-mod_Y, 6.67.1-mod_TAP, or 6.67.1-mod_Y,TAP (SEQ ID NOs: 16, 18, 20).

Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the heavy chain of 6.77.1-mod_ΔS (SEQ ID NO: 22).

- 5 Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the light chain of 7.16.6_V, 7.16.6_S, or 7.16.6_VS (SEQ ID NOs: 30, 32, 34).

Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the heavy chain of 7.16.6_L (SEQ ID NO: 28).

10

Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the light chain of 7.16.6_V, 7.16.6_S, or 7.16.6_VS (SEQ ID NOs: 30, 32, 34) and the variable region of the heavy chain of 7.16.6_L (SEQ ID NO: 28).

- 15 Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the heavy chain of 9.8.2_ΔRGAYH,D (SEQ ID NO: 36).

Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, comprising the variable region of the light chain of 9.8.2-mod (SEQ ID NO: 38).

20

Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, wherein the antibody is selected from the group consisting of:

- (a) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 3;
- 25 (b) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NO: 5 and SEQ ID NOs: 7, 9 or 11;
- (c) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NO: 13 and SEQ ID NOs: 15, 17, or 19;
- (d) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in
- 30 SEQ ID NO: 21 and SEQ ID NO: 23;
- (e) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NOs: 25 or 27 and SEQ ID NOs: 29, 31, or 33; or
- (f) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NO: 35 and SEQ ID NO: 37.

35

Another aspect of the invention is a monoclonal antibody that specifically binds MAdCAM, wherein the antibody is selected from the group consisting of:

- (a) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 2 and SEQ ID NO: 4, without the signal sequences;
- 40 (b) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 6 and SEQ ID NOs: 8, 10, or 12, without the signal sequences;

- (c) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 14 and SEQ ID NOs: 16, 18, or 20, without the signal sequences;
- (d) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 22 and SEQ ID NO: 24, without the signal sequences;
- 5 (e) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NOs: 26 or 28 and SEQ ID NOs: 30, 32 or 34, without the signal sequences; or
- (f) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 36 and SEQ ID NO: 38, without the signal sequences.
- 10 Another aspect of the invention is a monoclonal antibody or antigen-binding portion thereof, wherein said antibody comprises:
- (a) a heavy chain of amino acid sequence of SEQ ID NO: 2 and the light chain amino acid sequence of SEQ ID NO: 4, without the signal sequences.
- (b) a heavy chain of amino acid sequence of SEQ ID NO: 6 and the light chain amino acid sequence
- 15 of SEQ ID NOs: 8, 10, or 12, without the signal sequences.
- (c) a heavy chain of amino acid sequence of SEQ ID NO: 14 and the light chain amino acid sequence of SEQ ID NOs: 16, 18, or 20, without the signal sequences.
- (d) a heavy chain of amino acid sequence of SEQ ID NO: 22 and the light chain amino acid sequence of SEQ ID NO: 24, without the signal sequences.
- 20 (e) a heavy chain of amino acid sequence of SEQ ID NOs: 26 or 28 and the light chain amino acid sequence of SEQ ID NOs: 30, 32 or 34, without the signal sequences.
- (f) a heavy chain of amino acid sequence of SEQ ID NO: 36 and the light chain amino acid sequence of SEQ ID NO: 38, without the signal sequences.
- 25 Another aspect of the invention is a human monoclonal antibody of the invention, wherein the heavy chain C-terminal lysine is cleaved.

Another aspect of the invention is a human monoclonal antibody of the invention, which is an IgG₂ isotype. A further aspect of the invention is a human monoclonal antibody of the invention, which is an

30 IgG₄ isotype. A further aspect of the invention is a human monoclonal antibody of the invention which comprises a κ light chain. A further aspect of the invention is a human monoclonal antibody of the invention which comprises a λ light chain. A preferred aspect of the invention is a human monoclonal antibody of the invention which is an IgG₂ isotype with a κ light chain.

- 35 Another aspect of the invention is a vector comprising any of the sequences of the variable regions of SEQ ID NOs: 1, 7, 9, 11, 15, 17, 19, 21, 27, 29, 31, 33, 35, or 37 or any of the sequences encoding the variable regions of SEQ ID NOs: 2, 8, 10, 12, 16, 18, 20, 22, 28, 30, 32, 34, 36 or 38. A further aspect of the invention is a host cell comprising any of the sequences of the variable regions of SEQ ID NOs: 1, 7, 9, 11, 15, 17, 19, 21, 27, 29, 31, 33, 35 or 37, or any of the sequences encoding the variable regions of
- 40 SEQ ID NOs: 2, 8, 10, 12, 16, 18, 20, 22, 28, 30, 32, 34, 36 or 38. The host cell can be a bacterium such as *E. coli*, a yeast such as *Saccharomyces cerevisiae* or *Pichia pastoris*, a plant cell, an insect cell, or a

mammalian cell. More preferably, the host cell is a mammalian cell. Most preferably, the host cell is a CHO cell or NS0 cell.

Another aspect of the invention is a method of producing the antibody of the invention, comprising culturing a host cell comprising any of the sequences of the variable regions of SEQ ID NOs: 1, 7, 9, 11, 15, 17, 19, 21, 27, 29, 31, 33, 35 or 37, or any of the sequences encoding the variable regions of SEQ ID NOs: 2, 8, 10, 12, 16, 18, 20, 22, 28, 30, 32, 34, 36 or 38, under conditions in which the antibody is expressed, and recovering the antibody from the host cell or its culture supernatant.

Another aspect of the invention is a transgenic animal or a transgenic plant, comprising any of the sequences of the variable regions of SEQ ID NOs: 1, 7, 9, 11, 15, 17, 19, 21, 27, 29, 31, 33, 35 or 37, or any of the sequences encoding the variable regions of SEQ ID NOs: 2, 8, 10, 12, 16, 18, 20, 22, 28, 30, 32, 34, 36 or 38.

One aspect of the invention is the use of the antibodies of the invention or an antigen-binding portion thereof for use as a medicament. Another aspect of the invention is the use of the antibodies of the invention or an antigen-binding portion thereof for the manufacture of a medicament for the treatment of conditions involving MAdCAM-mediated adhesion of leukocytes. Another aspect of the invention is the use of the antibodies of the invention or an antigen-binding portion thereof for the treatment of inflammatory conditions, such as but not limited to inflammatory diseases of the gastrointestinal tract including inflammatory bowel disease such as Crohn's disease, ulcerative colitis; diverticula disease, gastritis, liver disease, primary sclerosis, sclerosing cholangitis. Inflammatory diseases also include but are not limited to abdominal disease (including peritonitis, appendicitis, biliary tract disease), acute transverse myelitis, allergic dermatitis (including allergic skin, allergic eczema, skin atopy, atopic eczema, atopic dermatitis, cutaneous inflammation, inflammatory eczema, inflammatory dermatitis, flea skin, milial dermatitis, milial eczema, house dust mite skin), ankylosing spondylitis (Reiters syndrome), asthma, airway inflammation, atherosclerosis, arteriosclerosis, biliary atresia, bladder inflammation, breast cancer, cardiovascular inflammation (including vasculitis, rheumatoid nail-fold infarcts, leg ulcers, polymyositis, chronic vascular inflammation, pericarditis, chronic obstructive pulmonary disease), pancreatitis, including chronic pancreatitis, perineural inflammation, coeliac disease, colitis (including amoebic colitis, infective colitis, bacterial colitis, Crohn's colitis, ischemic colitis, ulcerative colitis, idiopathic proctocolitis, inflammatory bowel disease, pseudomembranous colitis), chloroma, collagen vascular disorders (rheumatoid arthritis, SLE, progressive systemic sclerosis, mixed connective tissue disease, diabetes mellitus), Crohn's disease (regional enteritis, granulomatous ileitis, ileocolitis, digestive system inflammation), demyelinating disease (including myelitis, multiple sclerosis, disseminated sclerosis, acute disseminated encephalomyelitis, perivenous demyelination, vitamin B12 deficiency, Guillain-Barre syndrome, MS-associated retrovirus), dermatomyositis, diverticulitis, emphysema, exudative diarrhea, fever in immunocompromised patients, gastritis, granulomatous hepatitis, granulomatous inflammation, cholecystitis, insulin-dependent diabetes mellitus, liver inflammatory diseases (liver fibrosis including but not limited to Hepatitis C-induced liver damage, alcoholic liver disease, non-alcoholic steatohepatitis (NASH); primary biliary cirrhosis, hepatitis, sclerosing cholangitis),

lung inflammation (idiopathic pulmonary fibrosis, eosinophilic granuloma of the lung, pulmonary histiocytosis X, peribronchiolar inflammation, acute bronchitis), lymphogranuloma venereum, malignant melanoma, mouth/tooth disease (including gingivitis, periodontal disease), metastatic cancers, mucositis, musculoskeletal system inflammation (myositis), nonalcoholic steatohepatitis (nonalcoholic fatty liver disease), ocular & orbital inflammation (including uveitis, optic neuritis, peripheral rheumatoid ulceration, peripheral corneal inflammation,), osteoarthritis, osteomyelitis, pharyngeal inflammation, polyarthritis, proctitis, psoriasis, radiation injury, sarcoidosis, sickle cell necropathy, superficial thrombophlebitis, systemic inflammatory response syndrome, thyroiditis, systemic lupus erythematosus, tropical sprue, graft versus host disease, acute burn injury, Behçet's syndrome, Sjögren's syndrome, uterine disorders such as endometriosis, dysmenorrhea or pelvic inflammatory disease. Preferably, the antibodies of the invention are used for the treatment of inflammatory bowel disease such as Crohn's disease and ulcerative colitis, graft versus host disease, liver fibrosis, uveitis. Even more preferably, the antibodies of the invention are used for the treatment of inflammatory bowel disease.

Another aspect of the invention is a method of treatment of a condition mentioned above, comprising administering to a patient in need of such treatment a therapeutically effective amount of an antibody of the invention.

Another aspect of the invention is a pharmaceutical composition comprising an antibody of the invention or an antigen-binding portion thereof and a pharmaceutically acceptable carrier or excipient.

Detailed description of the invention

A nucleic acid molecule encoding the entire heavy chain of an anti-MAdCAM antibody of the invention may be constructed by fusing a nucleic acid molecule encoding the entire variable domain of a heavy chain or an antigen-binding domain thereof with a constant domain of a heavy chain. Similarly, a nucleic acid molecule encoding the light chain of an anti-MAdCAM antibody may be constructed by fusing a nucleic acid molecule encoding the variable domain of a light chain or an antigen-binding domain thereof with a constant domain of a light chain. Nucleic acid molecules encoding the VH and VL regions may be converted to full-length antibody genes by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the heavy chain constant region (CH) segment(s) within the vector and the VL segment is operatively linked to the light chain constant region (CL) segment within the vector. Alternatively, the nucleic acid molecules encoding the VH or VL chains are converted into full-length antibody genes by linking, e.g., ligating, the nucleic acid molecule encoding a VH chain to a nucleic acid molecule encoding a CH chain using standard molecular biological techniques. The same may be achieved using nucleic acid molecules encoding VL and CL chains. The sequences of human heavy and light chain constant region genes are known in the art. See, e.g., Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed., NIH Publ. No. 91-3242 (1991). Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed from a cell into which they have been introduced and the anti-MAdCAM antibody isolated.

A nucleic acid molecule encoding either the heavy chain of an anti-MAdCAM antibody of the invention or an antigen-binding portion thereof, or the light chain of an anti-MAdCAM antibody of the invention or an antigen-binding portion thereof may be prepared, for example, by synthesizing appropriate nucleic acids using the sequence information provided herein, and splicing them together with the desired constant regions of immunoglobulin genes. The skilled person will be well aware of the appropriate methods.

The nucleic acid molecules may be used to recombinantly express large quantities of anti-MAdCAM antibodies, as described below. The nucleic acid molecules may also be used to produce chimeric antibodies, single chain antibodies, immunoadhesins, diabodies, mutated antibodies and antibody derivatives, as described further below. If the nucleic acid molecules are derived from a non-human, non-transgenic animal, the nucleic acid molecules may be used for antibody humanization, also as described below.

The invention provides vectors comprising the nucleic acid molecules of the invention that encode the heavy chain or the antigen-binding portion thereof. The invention also provides vectors comprising the nucleic acid molecules of the invention that encode the light chain or antigen-binding portion thereof. The invention also provides vectors comprising nucleic acid molecules encoding fusion proteins, modified antibodies, antibody fragments, and probes thereof.

To express the antibodies, or antibody portions used in the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. Expression vectors include plasmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, EBV derived episomes, and the like. The antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector. In a preferred embodiment, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed, as described above. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector can also encode a signal peptide that facilitates secretion of the antibody chain from a host cell.

The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

5 In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell
10 expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, see e.g., U.S. Pat. Nos. 5,168,062, 4,510,245, and 4,968,615, each of which is hereby incorporated by reference. Methods for expressing antibodies in plants, including a description of promoters and vectors, as well as transformation of plants are known in the art. See, e.g., United States Patent 6,517,529. Methods of expressing polypeptides in bacterial cells or fungal cells, e.g., yeast cells,
15 are also well known in the art.

20 In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors used in the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Pat. Nos.
25 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in *dhfr*⁻ host cells with methotrexate selection/amplification) and the neo gene (for G418 selection), and the glutamate synthetase gene.

30 Nucleic acid molecules encoding the heavy chain or an antigen-binding portion thereof and/or the light chain or an antigen-binding portion thereof of an anti-MAdCAM antibody, and vectors comprising these nucleic acid molecules, can be used for transformation of a suitable mammalian plant, bacterial or yeast host cell. Transformation can be by any known method for introducing polynucleotides into a host cell.
35 Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, biolistic injection and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming cells are well known in the
40 art. See, e.g., U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). Methods of transforming plant cells are well known in the art,

including, e.g., Agrobacterium-mediated transformation, biolistic transformation, direct injection, electroporation and viral transformation. Methods of transforming bacterial and yeast cells are also well known in the art.

5 Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NS0, SP2 cells, HEK-293T cells, NIH-3T3 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, 3T3 cells, and a number of other cell lines. Mammalian host cells include
10 human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. When recombinant expression vectors encoding the heavy chain or antigen-binding portion thereof, the light chain and/or antigen-binding portion thereof are introduced into mammalian host cells,
15 the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Plant host cells include, e.g., Nicotiana, Arabidopsis, duckweed, corn, wheat, potato, etc. Bacterial host cells include *E. coli* and *Streptomyces* species. Yeast host cells
20 include *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Pichia pastoris*.

Further, expression of antibodies used in the invention (or other moieties therefrom) from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain
25 conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, 0 338 841 and 0 323 997.

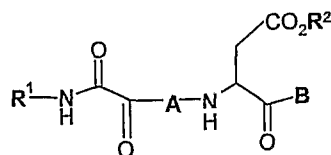
It is likely that antibodies expressed by different cell lines or in transgenic animals will have different glycosylation from each other. However, all antibodies encoded by the nucleic acid molecules provided
30 herein, or comprising the amino acid sequences provided herein are part of the instant invention, regardless of the glycosylation of the antibodies.

Transgenic non-human animals and transgenic plants comprising one or more nucleic acid molecules described above may be used to produce antibodies used in the invention. Antibodies can be produced
35 in and recovered from tissue or bodily fluids, such as milk, blood or urine, of goats, cows, horses, pigs, rats, mice, rabbits, hamsters or other mammals. See, e.g., U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957. As described above, non-human transgenic animals that comprise human immunoglobulin loci can be immunized with MAdCAM or a portion thereof. Methods for making antibodies in plants are described, e.g., in U.S. Patents 6,046,037 and 5,959,177, incorporated herein by
40 reference.

Non-human transgenic animals and transgenic plants are produced by introducing one or more nucleic acid molecules used in the invention into the animal or plant by standard transgenic techniques. See Hogan, *supra*. The transgenic cells used for making the transgenic animal can be embryonic stem cells, somatic cells or fertilized egg cells. The transgenic non-human organisms can be chimeric, nonchimeric heterozygotes, and nonchimeric homozygotes. See, e.g., Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual 2ed.*, Cold Spring Harbor Press (1999); Jackson et al., *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press (2000); and Pinkert, *Transgenic Animal Technology: A Laboratory Handbook*, Academic Press (1999). The transgenic non-human organisms may have a targeted disruption and replacement that encodes a heavy chain and/or a light chain of interest. The transgenic animals or plants may comprise and express nucleic acid molecules encoding heavy and light chains that combine to bind specifically to MAdCAM, preferably human MAdCAM. The transgenic animals or plants may comprise nucleic acid molecules encoding a modified antibody such as a single-chain antibody, a chimeric antibody or a humanized antibody. The anti-MAdCAM antibodies may be made in any transgenic animal. The non-human animals include mice, rats, sheep, pigs, goats, cattle or horses. The non-human transgenic animal expresses said encoded polypeptides in blood, milk, urine, saliva, tears, mucus and other bodily fluids.

Treatment may involve administration of one or more inhibitory anti-MAdCAM monoclonal antibodies of the invention, or antigen-binding fragments thereof, alone or with a pharmaceutically acceptable carrier. Inhibitory anti-MAdCAM antibodies and compositions comprising them, can be administered in combination with one or more other therapeutic, diagnostic or prophylactic agents. Such additional agents may be included in the same composition or administered separately. Additional therapeutic agents include anti-inflammatory or immunomodulatory agents. These agents include, but are not limited to, the topical and oral corticosteroids such as prednisolone, methylprednisolone, NCX-1015 or budesonide; the aminosalicylates such as mesalazine, olsalazine, balsalazide or NCX-456; the class of immunomodulators such as azathioprine, 6-mercaptopurine, methotrexate, cyclosporin, FK506, IL-10 (Ilodecakin), IL-11 (Oporelevkin), IL-12, MIF/CD74 antagonists, CD40 antagonists, such as TNX-100/5-D12, OX40L antagonists, GM-CSF, pimecrolimus or rapamycin; the class of anti-TNF α agents such as infliximab, adalimumab, CDP-870, onercept, etanercept; the class of anti-inflammatory agents, such as PDE-4 inhibitors (roflumilast, etc), TACE inhibitors (DPC-333, RDP-58, etc) and ICE inhibitors (VX-740, etc) as well as IL-2 receptor antagonists, such as daclizumab, the class of selective adhesion molecule antagonists, such as natalizumab, MLN-02, or alicaforsen, classes of analgesic agents such as, but not limited to, COX-2 inhibitors, such as rofecoxib, valdecoxib, celecoxib, P/Q-type voltage sensitive channel ($\alpha 2\delta$) modulators, such as gabapentin and pregabalin, NK-1 receptor antagonists, cannabinoid receptor modulators, and delta opioid receptor agonists, as well as anti-neoplastic, anti-tumor, anti-angiogenic chemotherapeutic agents, or anti-fibrotic agent including but not limited to protease inhibitors, preferably caspase inhibitors, more preferably the caspase inhibitor is a compound of formula I.

The compound of formula I



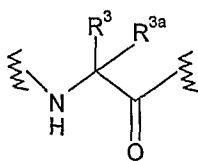
Formula I

wherein A, B, R1 and R2 are as defined below.

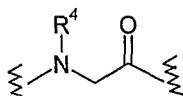
wherein

5

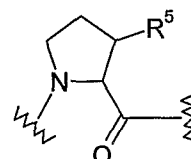
A is a natural or unnatural amino acid of Formula IIa-i:



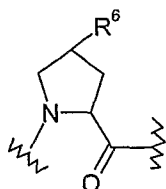
IIa



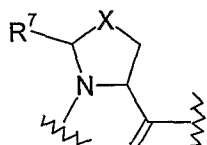
IIb



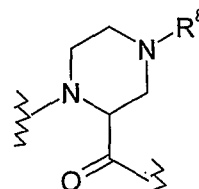
IIc



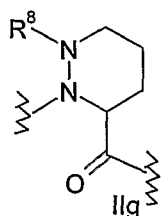
IIId



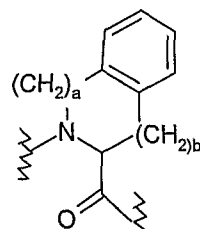
IIe



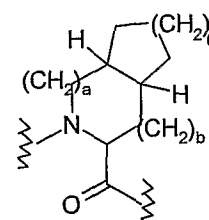
IIIf



IIg



IIh



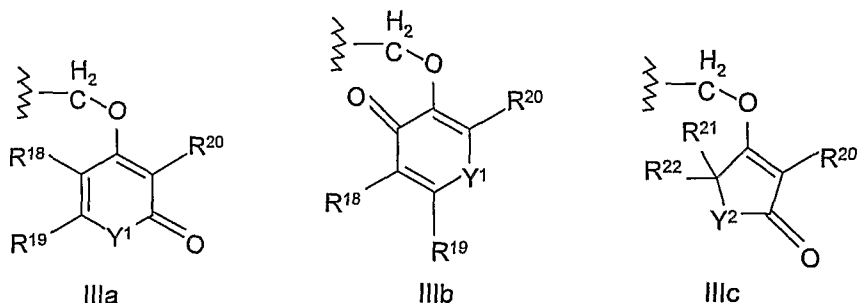
IIi

10

15

B is a hydrogen atom, a deuterium atom, alkyl, cycloalkyl, phenyl, substituted phenyl, naphthyl, substituted naphthyl, 2-benzoxazolyl, substituted 2-oxazolyl, (CH₂)_n-cycloalkyl, (CH₂)_n-phenyl, (CH₂)_n-(substituted phenyl), (CH₂)_n-(1 or 2-naphthyl), (CH₂)_n-(substituted 1 or 2-naphthyl), (CH₂)_n-(heteroaryl), (CH₂)_n-(substituted heteroaryl), halomethyl, CO₂R¹², CONR¹³R¹⁴, CH₂ZR¹⁵, CH₂OCO(aryl), CH₂OCO(heteroaryl), or CH₂OPO(R¹⁶)R¹⁷, where Z is an oxygen or a sulfur atom, or B is a group of the Formula IIIa-c:

20



R^1 is alkyl, cycloalkyl, (cycloalkyl)alkyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, naphthyl, substituted naphthyl, (1 or 2 naphthyl)alkyl, substituted (1 or 2 naphthyl)alkyl, heteroaryl, substituted heteroaryl, (heteroaryl)alkyl, substituted (heteroaryl)alkyl, $R^{1a}(R^{1b})N$, or $R^{1c}O$; and R^2 is hydrogen, lower alkyl, cycloalkyl, (cycloalkyl)alkyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, naphthyl, substituted naphthyl, (1 or 2 naphthyl)alkyl, or substituted (1 or 2 naphthyl)alkyl;

And wherein:

R^{1a} and R^{1b} are independently hydrogen, alkyl, cycloalkyl, (cycloalkyl)alkyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, naphthyl, substituted naphthyl, (1 or 2 naphthyl)alkyl, substituted (1 or 2 naphthyl)alkyl, heteroaryl, substituted heteroaryl, (heteroaryl)alkyl, or substituted (heteroaryl)alkyl, with the proviso that R^{1a} and R^{1b} cannot both be hydrogen;

R^{1c} is alkyl, cycloalkyl, (cycloalkyl)alkyl, phenyl, substituted phenyl, phenylalkyl, substituted phenylalkyl, naphthyl, substituted naphthyl, (1 or 2 naphthyl)alkyl, substituted (1 or 2 naphthyl)alkyl, heteroaryl, substituted heteroaryl, (heteroaryl)alkyl, or substituted (heteroaryl)alkyl;

R^3 is C_{1-6} alkyl, cycloalkyl, phenyl, substituted phenyl, $(CH_2)_nNH_2$, $(CH_2)_nNHCOR^9$, $(CH_2)_nN(C=NH)NH_2$, $(CH_2)_mCO_2R^2$, $(CH_2)_mOR^{10}$, $(CH_2)_mSR^{11}$, $(CH_2)_ncycloalkyl$, $(CH_2)_nphenyl$, $(CH_2)_n(substituted\ phenyl)$, $(CH_2)_n(1\ or\ 2-naphthyl)$ or $(CH_2)_n(heteroaryl)$, wherein heteroaryl includes pyridyl, thienyl, furyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, pyrazinyl, pyrimidyl, triazinyl, tetrazolyl, and indolyl;

R^{3a} is hydrogen or methyl, or R^3 and R^{3a} taken together are $-(CH_2)_d-$ where d is an integer from 2 to 6;

R^4 is phenyl, substituted phenyl, $(CH_2)_mphenyl$, $(CH_2)_m(substituted\ phenyl)$, cycloalkyl, or benzofused cycloalkyl;

R^5 is hydrogen, lower alkyl, cycloalkyl, phenyl, substituted phenyl, $(CH_2)_ncycloalkyl$, $(CH_2)_nphenyl$, $(CH_2)_n(substituted\ phenyl)$, or $(CH_2)_n(1\ or\ 2-naphthyl)$;

R^6 is hydrogen, fluorine, oxo, lower alkyl, cycloalkyl, phenyl, substituted phenyl, naphthyl, $(CH_2)_ncycloalkyl$, $(CH_2)_nphenyl$, $(CH_2)_n(substituted\ phenyl)$, $(CH_2)_n(1\ or\ 2-naphthyl)$, OR^{10} , SR^{11} , or $NHCOR^9$;

R^7 is hydrogen, oxo (i.e. =O), lower alkyl, cycloalkyl, phenyl, substituted phenyl, naphthyl, $(CH_2)_ncycloalkyl$, $(CH_2)_nphenyl$, $(CH_2)_n(substituted\ phenyl)$, or $(CH_2)_n(1\ or\ 2-naphthyl)$;

R^8 is lower alkyl, cycloalkyl, $(CH_2)_ncycloalkyl$, $(CH_2)_nphenyl$, $(CH_2)_n(substituted\ phenyl)$, $(CH_2)_n(1\ or\ 2-naphthyl)$, or COR^9 ;

R^9 is hydrogen, lower alkyl, cycloalkyl, phenyl, substituted phenyl, naphthyl, $(CH_2)_ncycloalkyl$, $(CH_2)_nphenyl$, $(CH_2)_n(substituted\ phenyl)$, $(CH_2)_n(1\ or\ 2-naphthyl)$, OR^{12} , or $NR^{13}R^{14}$;

R¹⁰ is hydrogen, lower alkyl, cycloalkyl, phenyl, substituted phenyl, naphthyl, (CH₂)_ncycloalkyl, (CH₂)_nphenyl, (CH₂)_n(substituted phenyl), or (CH₂)_n(1 or 2-naphthyl);

R¹¹ is lower alkyl, cycloalkyl, phenyl, substituted phenyl, naphthyl, (CH₂)_ncycloalkyl, (CH₂)_nphenyl, (CH₂)_n(substituted phenyl), or (CH₂)_n(1 or 2-naphthyl);

5 R¹² is lower alkyl, cycloalkyl, (CH₂)_ncycloalkyl, (CH₂)_nphenyl, (CH₂)_n(substituted phenyl), or (CH₂)_n(1 or 2-naphthyl);

R¹³ is hydrogen, lower alkyl, cycloalkyl, phenyl, substituted phenyl, naphthyl, substituted naphthyl, (CH₂)_ncycloalkyl, (CH₂)_nphenyl, (CH₂)_n(substituted phenyl), or (CH₂)_n(1 or 2-naphthyl);

R¹⁴ is hydrogen or lower alkyl; or

10 R¹³ and R¹⁴ taken together form a five to seven membered carbocyclic or heterocyclic ring, such as morpholine, or N-substituted piperazine;

R¹⁵ is phenyl, substituted phenyl, naphthyl, substituted naphthyl, heteroaryl, (CH₂)_nphenyl, (CH₂)_n(substituted phenyl), (CH₂)_n(1 or 2-naphthyl), or (CH₂)_n(heteroaryl);

R¹⁶ or R¹⁷ are independently lower alkyl, cycloalkyl, phenyl, substituted phenyl, naphthyl, phenylalkyl,

15 substituted phenylalkyl, or (cycloalkyl)alkyl;

R¹⁸ and R¹⁹ are independently hydrogen, alkyl, phenyl, substituted phenyl, (CH₂)_nphenyl, (CH₂)_n(substituted phenyl), or R¹⁸ and R¹⁹ taken together are -(CH=CH)₂-;

R²⁰ is hydrogen, alkyl, phenyl, substituted phenyl, (CH₂)_nphenyl, (CH₂)_n(substituted phenyl);

R²¹, R²² and R²³ are independently hydrogen, or alkyl;

20 X is CH₂, (CH₂)₂, (CH₂)₃, or S;

Y¹ is O or NR²³;

Y² is CH₂, O, or NR²³;

a is 0 or 1 and b is 1 or 2, provided that when a is 1 then b is 1;

c is 1 or 2, provided that when c is 1 then a is 0 and b is 1;

25 m is 1 or 2; and

n is 1, 2, 3, or 4;

or a pharmaceutically acceptable salt thereof.

As used herein, the term "alkyl" means a straight or branched C₁ to C₁₀ carbon chain, such as methyl, ethyl, tert-butyl, iso-propyl, n-octyl, and the like. The term "lower alkyl" means a straight chain or

30 branched C₁ to C₆ carbon chain, such as methyl, ethyl, iso-propyl, and the like.

Depending on the choice of solvent and other conditions known to the skilled person, these compounds may also take the ketal or acetal form, and the use of these forms in the combination of the invention is included in the invention.

35

These compounds can be prepared as described in WO 00/01666 or in US 6,544,951, hereby incorporated by reference in their entirety. Preferred subgroups are those listed in US 6,544,951.

Preferred are compounds selected from:

40 (3S)-3-[N-(N'-(2-Fluoro-4-Iodophenyl)Oxamyl)Valinyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

(3S)-3-[N-(N'-(2-Chlorophenyl)Oxamyl)Valinyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

(3S)-3-[N-(N'-(2-Bromophenyl)Oxamyl)Valinyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

5 (3S)-3-[N-(N'-(2-Fluorophenyl)Oxamyl)Valinyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

(3S)-3-[N-(N'-(2-Trifluoromethylphenyl)Oxamyl)Valinyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

(3S)-3-[N-(N'-(1-Anthryl)Oxamyl)Valinyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

10 (3S)-3-[N-(N'-(2-Tert-Butylphenyl)Oxamyl)Alaninyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

(3S)-3-[N-(N'-(2-Trifluoromethylphenyl)Oxamyl)Alaninyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

(3S)-3-[N-(N'-(2,6-Difluorophenyl)Oxamyl)Alaninyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-

15 Oxopentanoic acid;

(3S)-3-[N-(N'-(1-Naphthyl)Oxamyl)Alaninyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

(3S)-3-[N-(N'-(4-Methoxyphenyl)Oxamyl)Alaninyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid;

(3S)-3-[N-(N'-(2-Trifluoromethylphenyl)Oxamyl)Valinyl]Amino-4-Oxobutanoic acid;

20 (3S)-3-[N-(N'-(2-tert-Butylmethylphenyl)Oxamyl)Valinyl]Amino-4-Oxobutanoic acid;

(3S)-3-[N-(N'-(2-Benzylphenyl)Oxamyl)Valinyl]Amino-4-Oxobutanoic acid;

(3S)-3-[N-(N'-(2-Phenylphenyl)Oxamyl)Valinyl]Amino-4-Oxobutanoic acid.

25 Most preferably, the compound is (3S)-3-[N-(N'-(2-Tert-Butylphenyl)Oxamyl)Alaninyl]Amino-5-(2',3',5',6'-Tetrafluorophenoxy)-4-Oxopentanoic acid.

As used herein, "pharmaceutically acceptable carrier" means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption enhancing or delaying agents, and the like that are physiologically compatible. Some examples of pharmaceutically acceptable carriers are water, saline, phosphate buffered saline, acetate buffer with sodium chloride, dextrose, glycerol, Polyethylene glycol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are surfactants, wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody.

The compositions used in this invention may be in a variety of forms, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, lyophilized cake, dry powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those

used for passive immunization of humans. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular, intradermal). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular, intradermal or subcutaneous injection. If desired, the antibody may be administered by using a pump, enema, suppository, or indwelling reservoir or such like.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, lyophilized cake, dry powder, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the anti-MAdCAM antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile solution thereof. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. The desired characteristics of a solution can be maintained, for example, by the use of surfactants and the required particle size in the case of dispersion by the use of surfactants, phospholipids and polymers. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts, polymeric materials, oils and gelatin.

The antibodies of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous, intramuscular, intradermal or intravenous infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

In certain embodiments, the antibody compositions may be prepared with a carrier that will protect the antibody against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., *Sustained and Controlled Release Drug Delivery Systems* (J. R. Robinson, ed., Marcel Dekker, Inc., New York (1978)).

In certain embodiments, an anti-MAdCAM antibody of the invention can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) can also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the anti-MAdCAM antibodies can be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the

invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

The compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antigen-binding portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount may be less than the therapeutically effective amount.

Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a pre-determined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the anti-MAdCAM antibody or portion thereof and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an antibody for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.025 to 50 mg/kg, more preferably 0.1 to 50 mg/kg, more preferably 0.1-25, 0.1 to 10 or 0.1 to 3 mg/kg. In some embodiments, a formulation contains 5 mg/mL of antibody in a buffer of 20 mM sodium acetate, pH 5.5, 140 mM NaCl, and 0.2 mg/mL polysorbate 80. In other embodiments, a formulation contains 10 mg/ml of antibody in 2.73 mg/ml of sodium acetate trihydrate, 45 mg/ml of mannitol, 0.02 mg/ml of disodium EDTA dihydrate, 0.2 mg/ml of polysorbate 80, adjusted to pH 5.5 with glacial acetic acid, e.g. for intravenous use. In other embodiments, a formulation contains 50 mg/ml of antibody, 2.73 mg/ml of sodium acetate trihydrate, 45 mg/ml of mannitol, 0.02 mg/ml of disodium EDTA dihydrate, 0.4 mg/ml of polysorbate 80, adjusted to pH 5.5 with glacial acetic acid, e.g. for subcutaneous or intradermal use. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions,

and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

In a preferred embodiment, the antibody is administered in a formulation as a sterile aqueous solution having a pH that ranges from about 5.0 to about 6.5 and comprising from about 1 mg/ml to about 200 mg/ml of antibody, from about 1 millimolar to about 100 millimolar of histidine buffer, from about 0.01 mg/ml to about 10 mg/ml of polysorbate 80, from about 100 millimolar to about 400 millimolar of trehalose, and from about 0.01 millimolar to about 1.0 millimolar of disodium EDTA dihydrate.

Another aspect of the present invention provides kits comprising an anti-MAdCAM antibody or antibody portion of the invention or a composition comprising such an antibody. A kit may include, in addition to the antibody or composition, diagnostic or therapeutic agents. A kit can also include instructions for use in a diagnostic or therapeutic method. In a preferred embodiment, the kit includes the antibody or a composition comprising it and a diagnostic agent that can be used in a method described below. In another preferred embodiment, the kit includes the antibody or a composition comprising it and one or more therapeutic agents that can be used in a method described below.

The nucleic acid molecules of the instant invention can be administered to a patient in need thereof via gene therapy. The therapy may be either in vivo or ex vivo. In a preferred embodiment, nucleic acid molecules encoding both a heavy chain and a light chain are administered to a patient. In a more preferred embodiment, the nucleic acid molecules are administered such that they are stably integrated into chromosomes of B cells because these cells are specialized for producing antibodies. In a preferred embodiment, precursor B cells are transfected or infected ex vivo and re-transplanted into a patient in need thereof. In another embodiment, precursor B cells or other cells are infected in vivo using a recombinant virus known to infect the cell type of interest. Typical vectors used for gene therapy include liposomes, plasmids and viral vectors. Exemplary viral vectors are retroviruses, adenoviruses and adeno-associated viruses. After infection either in vivo or ex vivo, levels of antibody expression can be monitored by taking a sample from the treated patient and using any immunoassay known in the art or discussed herein.

In a preferred embodiment, the gene therapy method comprises the steps of administering an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof of an anti-MAdCAM antibody and expressing the nucleic acid molecule. In another embodiment, the gene therapy method comprises the steps of administering an isolated nucleic acid molecule encoding the light chain or an antigen-binding portion thereof of an anti-MAdCAM antibody and expressing the nucleic acid molecule. In a more preferred method, the gene therapy method comprises the steps of administering of an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof and an isolated nucleic acid molecule encoding the light chain or the antigen-binding portion thereof of an anti-MAdCAM antibody of the invention and expressing the nucleic acid molecules. The gene therapy method may also comprise the step of administering another anti-inflammatory or immunomodulatory agent.

Another aspect of the invention is a method of diagnosis of a condition where the antibody of the invention will be useful as a medicament, by testing whether abnormal binding of an antibody of the invention occurs in the patient. This can be done using various imaging techniques well known to the skilled person, such as x-ray analysis, gamma scintigraphy, magnetic resonance imaging (MRI), positron emission tomography or computed tomography (CT) and others.

One or more inhibitory anti-MAdCAM antibodies of the invention can be used as a vaccine or as adjuvants to a vaccine, and this is another aspect of the invention. In particular, because MAdCAM is expressed in lymphoid tissue, vaccine antigens can be advantageously targeted to lymphoid tissue by conjugating the antigen to an anti-MAdCAM antibody of the invention.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992), and Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is free of other proteins from the same species (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

A protein or polypeptide is "substantially pure," "substantially homogeneous" or "substantially purified" when at least about 60 to 75% of a sample exhibits a single species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence. In some embodiments, fragments are at least 5, 6, 8 or 10 amino acids long. In other embodiments, the fragments are at least 14 amino acids long, more preferably at least 20 amino acids long, usually at least 50 amino acids long, even more preferably at least 70, 80, 90, 100, 150 or 200 amino acids long.

The term "polypeptide analog" as used herein refers to a polypeptide that comprises a segment of at least 25 amino acids that has substantial identity to a portion of an amino acid sequence and that has at least one of the following properties: (1) specific binding to MAdCAM under suitable binding conditions, (2) ability to inhibit $\alpha_4\beta_7$ integrin and/or L-selectin binding to MAdCAM, or (3) ability to reduce MAdCAM cell surface expression *in vitro* or *in vivo*. Typically, polypeptide analogs comprise a conservative amino acid substitution (or insertion or deletion) with respect to the naturally-occurring sequence. Analogs typically are at least 20 amino acids long, preferably at least 50, 60, 70, 80, 90, 100, 150 or 200 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

An "immunoglobulin" is a tetrameric molecule. In a naturally-occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as κ and λ light chains. Heavy chains are classified as μ , δ , γ , α , or ϵ , and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 or more amino acids. See generally, *Fundamental Immunology*, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites.

Immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The

CDRs from the two chains of each pair are aligned by the framework regions to form an epitope-specific binding site. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk, *J. Mol. Biol.*, 196:901-917(1987); Chothia et al., *Nature*, 342:878-883(1989), each of which is incorporated herein by reference in their entirety.

The term "antibody" refers to an intact immunoglobulin or to an antigen-binding portion thereof that competes with the intact antibody for specific binding. In some embodiments, an antibody is an antigen-binding portion thereof. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen-binding portions include, *inter alia*, Fab, Fab', F(ab')₂, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. A Fab fragment is a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)₂ fragment is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consists of the VH and CH1 domains; an Fv fragment consists of the VL and VH domains of a single arm of an antibody; and a dAb fragment (Ward et al., *Nature*, 341:544-546(1989)) consists of a VH domain. The term is also intended to include modified versions of the antibody such as pegylated antibodies or antibodies conjugated to detectable moieties such as enzymes (e.g. horseradish peroxidase, alkaline phosphatase), radioisotopes, biotin, fluorescent labels, and others.

As used herein, an antibody that is referred to as, e.g., 1.7.2, 1.8.2, 6.14.2, 6.34.2, 6.67.1, 6.77.2, 7.16.6, 7.20.5, 7.26.4 or 9.8.2, is a monoclonal antibody that is produced by the hybridoma of the same name. For example, antibody 1.7.2 is produced by hybridoma 1.7.2. An antibody that is referred to as 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod is a monoclonal antibody whose sequence has been modified from its corresponding parent by site-directed mutagenesis.

A single-chain antibody (scFv) is an antibody in which VL and VH regions are paired to form a monovalent molecule via a synthetic linker that enables them to be made as a single protein chain (Bird et al., *Science*, 242:423-426 (1988) and Huston et al., *Proc. Natl. Acad. Sci. USA*, 85:5879-5883 (1988)). Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, e.g., Holliger, P., et al., *Proc. Natl. Acad. Sci. USA*, 90: 6444-6448 (1993) and Poljak, R. J., et al., *Structure*, 2:1121-1123 (1994)). One or more CDRs from an antibody of the invention may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to MAdCAM. An immunoadhesin may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may

incorporate the CDR(s) noncovalently. The CDRs permit the immunoadhesin to specifically bind to a particular antigen of interest.

An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a "bispecific" or "bifunctional" antibody (diabody) has two different binding sites.

An "isolated antibody" is an antibody that (1) is not associated with naturally-associated components, including other naturally-associated antibodies, that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature. Examples of isolated antibodies include an anti-MAdCAM antibody that has been affinity purified using MAdCAM, an anti-MAdCAM antibody that has been produced by a hybridoma or other cell line *in vitro*, and a human anti-MAdCAM antibody derived from a transgenic mammal or plant.

As used herein, the term "human antibody" means an antibody in which the variable and constant region sequences are human sequences. The term encompasses antibodies with sequences derived from human genes, but which have been changed, *e.g.*, to decrease possible immunogenicity, increase affinity, eliminate cysteines or glycosylation sites that might cause undesirable folding, etc. The term encompasses such antibodies produced recombinantly in non-human cells which might impart glycosylation not typical of human cells. The term also encompasses antibodies which have been raised in a transgenic mouse which comprises some or all of the human immunoglobulin heavy and light chain loci.

In one aspect, the invention provides a humanized antibody. In some embodiments, the humanized antibody is an antibody that is derived from a non-human species, in which certain amino acids in the framework and constant domains of the heavy and light chains have been mutated so as to avoid or abrogate an immune response in humans. In some embodiments, a humanized antibody may be produced by fusing the constant domains from a human antibody to the variable domains of a non-human species. Examples of how to make humanized antibodies may be found in United States Patent Nos. 6,054,297, 5,886,152 and 5,877,293. In some embodiments, a humanized anti-MAdCAM antibody of the invention comprises the amino acid sequence of one or more framework regions of one or more human anti-MAdCAM antibodies of the invention.

In another aspect, the invention includes the use of a "chimeric antibody". In some embodiments the chimeric antibody refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. In a preferred embodiment, one or more of the CDRs are derived from a human anti-MAdCAM antibody of the invention. In a more preferred embodiment, all of the CDRs are derived from a human anti-MAdCAM antibody of the invention. In another preferred embodiment, the CDRs from more than one human anti-MAdCAM antibody of the invention are mixed and matched in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the

light chain of a first human anti-MAdCAM antibody may be combined with CDR2 and CDR3 from the light chain of a second human anti-MAdCAM antibody, and the CDRs from the heavy chain may be derived from a third anti-MAdCAM antibody. Further, the framework regions may be derived from one of the same anti-MAdCAM antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody.

A "neutralizing antibody," "an inhibitory antibody" or antagonist antibody is an antibody that inhibits the binding of $\alpha_4\beta_7$ or $\alpha_4\beta_7$ -expressing cells, or any other cognate ligand or cognate ligand-expressing cells, to MAdCAM by at least about 20%. In a preferred embodiment, the antibody reduces inhibits the binding of $\alpha_4\beta_7$ integrin or $\alpha_4\beta_7$ -expressing cells to MAdCAM by at least 40%, more preferably by 60%, even more preferably by 80%, 85%, 90%, 95% or 100%. The binding reduction may be measured by any means known to one of ordinary skill in the art, for example, as measured in an *in vitro* competitive binding assay. An example of measuring the reduction in binding of $\alpha_4\beta_7$ -expressing cells to MAdCAM is presented in Example I.

Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the art following the teachings of this specification. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known (Bowie et al., *Science*, 253:164 (1991)).

The term " k_{off} " refers to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

The term " K_d " refers to the dissociation constant of a particular antibody-antigen interaction. An antibody is said to bind an antigen when the dissociation constant is $\leq 1 \mu\text{M}$, preferably $\leq 100 \text{ nM}$ and most preferably $\leq 10 \text{ nM}$.

The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or carbohydrate side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, s-N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the lefthand direction is the amino terminal direction and the righthand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g., for probes; although oligonucleotides may be double stranded, e.g., for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See, e.g., LaPlanche et al., *Nucl. Acids Res.* 14:9081 (1986); Stec et al., *J. Am. Chem. Soc.* 106:6077(1984); Stein et al., *Nucl. Acids Res.*, 16:3209(1988); Zon et al., *Anti-Cancer Drug Design* 6:539(1991); Zon et al., *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England(1991)); Stec et al., U.S. Patent No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews*, 90:543(1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

"Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. "High stringency" or "highly stringent" conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. An example of "high stringency" or "highly stringent" conditions is a method of incubating a polynucleotide with another polynucleotide, wherein one polynucleotide may be affixed to a solid surface such as a membrane, in a hybridization buffer of 6X SSPE or SSC, 50% formamide, 5X Denhardt's reagent, 0.5% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA at a hybridization temperature of 42°C for 12-16 hours, followed by twice washing at 55°C using a wash buffer of 1X SSC, 0.5% SDS. See also Sambrook et al., *supra*, pp. 9.50-9.55.

The term "percent sequence identity" in the context of nucleotide sequences refers to the residues in two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 18 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36, 48 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.3, Accelrys, San Diego, CA. FASTA, which includes, e.g., the programs FASTA2 and FASTA3, provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.*, 183: 63-98 (1990); Pearson, *Methods Mol. Biol.*, 132: 185-219 (2000); Pearson, *Methods Enzymol.*, 266: 227-258 (1996); Pearson, *J. Mol. Biol.*, 276: 71-84 (1998); herein incorporated by reference). Unless otherwise specified, default parameters for a particular program or algorithm are used. For instance, percent sequence identity between nucleotide sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in Wisconsin Package Version 10.3, herein incorporated by reference.

A reference to a nucleotide sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid molecule having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence.

In the molecular biology art, researchers use the terms "percent sequence identity", "percent sequence similarity" and "percent sequence homology" interchangeably. In this application, these terms shall have the same meaning with respect to nucleotide sequences only.

The term "substantial similarity" or "substantial sequence similarity," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or

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

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 75% or 80% sequence identity, preferably at least 90% or 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, *Methods Mol. Biol.*, 24: 307-31 (1994), herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; and 6) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al., *Science*, 256: 1443-45 (1992), herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., Wisconsin package Version 10.3. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in Wisconsin package Version 10.3. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (1990); Pearson (2000)). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. See, e.g., Altschul et al., *J. Mol. Biol.* 215: 403-410 (1990); Altschul et al., *Nucleic Acids Res.* 25:3389-402 (1997); herein incorporated by reference.

The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences.

As used herein, the terms "label" or "labeled" refers to incorporation of another molecule in the antibody. In one embodiment, the label is a detectable marker, *e.g.*, incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In another embodiment, the label or marker can be therapeutic, *e.g.*, a drug conjugate or toxin. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), magnetic agents, such as gadolinium chelates, toxins such as pertussis toxin, 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. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The following examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

ExamplesExample 1: Identification of potential immunogenic epitopes

5 CD4⁺ T (helper) cell epitopes are critical in driving T cell dependent immune responses to protein antigens. During the initiation of a T-cell dependent immune response dendritic cells capture, process and present protein antigen in the form of linear peptides bound in the groove of MHC class II. Binding of the T-cell receptor to the peptide/MHC-II complex by naïve CD4⁺ T cells can trigger a cascade in which T cells proliferate, differentiate and provide help by producing co-stimulatory cytokines (e.g. IL-4, IL-6) and
 10 by direct cell-cell contact (e.g. CD40) to B cells. The continued help from T cells is required during secondary B cell lymphopoiesis in which B cells rearrange their immunoglobulin chains to produce high affinity isotype switched immunoglobulin against the same antigen recognised by the T cells. It is the binding of the B cell produced immunoglobulins that can neutralise the effects of protein-based therapies. The identification of T cell epitopes contained within therapeutic antibodies is important in predicting
 15 immunogenicity in man. With their identification it may be desirable to engineer the MHC-II anchors out by site directed mutagenesis, generating non-immunogenic adducts that retain the therapeutic activity of the parent.

Six fully human anti-MAdCAM mAbs, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod, 7.16.6 and 9.8.2
 20 (previously described in WO2005/067620; hybridomas secreting 7.16.6 and 9.8.2 were deposited in the European Collection of Cell Cultures (ECACC), H.P.A at CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG on 9th September 2003 with the following deposit numbers: 7.16.6: Deposit No: 03090909; 9.8.2: Deposit No: 03090912), were subjected to an *ex vivo* assessment to identify potential T cell epitopes and determine their immunogenicity. Sequence overlapping peptides (15 mers of purity >90%) covering the
 25 framework (FR) and complementary determining regions (CDRs) of the heavy and kappa light chain counterparts of 6.22.2, 6.34.2, 6.67.1, 6.77.1, 7.16.6 and 9.8.2 were synthesised using standard methods. The peptides were screened against a panel of 40 human donors in multiple cultures of CD8⁺ T-cell depleted PMSC, providing a source of CD4⁺ cells and antigen presenting cells at physiological ratios. The 40 healthy donors were selected for screening on the basis of HLA-DR typing and represented >80%
 30 of the DR alleles expressed in the world's population. Individual peptides (1 and 5 µM) were screened in sextuplicate, in conjunction with two positive control peptides. Cells were treated with peptides for 7 days, then proliferation was measured by incorporation of ³H-thymidine (0.5 µCi/well; 18 hrs). The incorporation of radiolabel was determined by scintillation counting and expressed as a stimulation index (SI) as follows:

35

$$\text{SI} = \frac{\text{cpm of test peptide}}{\text{Cpm of untreated control}}$$

A T-cell epitope is defined as a peptide giving a SI >2 in two or more independent donors.

40

In addition to the peptide-induced proliferation response, peptides were tested on PBMC donors in an ELISPOT assay. This method captures released cytokines and enables quantification of activated cells within the whole population. The release of IL-2, the principle cytokine secreted by activated CD4⁺ T cells, was selected to identify T-cell stimulated by the peptide epitope. As with the proliferation assay, donor samples were incubated with peptide for 7 days prior to assessment. On analysis each stimulated T cell was represented as a spot of IL-2 release. For each donor and peptide (1 and 5 μ M) combination the number of spots/well were determined and a stimulation index calculated as a ratio:

$$SI = \frac{\text{spots/well of test peptide}}{\text{spots/well of untreated control}}$$

In general there should be good overlap in the responses observed between the proliferation and ELISPOT assays, though differences in the kinetics and magnitude of responses between the two assays can lead to some lack of correlation.

Table 1 describes the data from the T-cell proliferation and ELISPOT assays. Based on these analyses, T cell epitopes were identified in FR2/CDR2 region of the 6.22.2-mod heavy chain; CDR2, FR3/CDR3 and CDR3 of the kappa light chain of 6.34.2-mod light chain; FR3/CDR3 of the 6.67.1-mod kappa light chain; CDR3 of the 6.77.1-mod heavy chain; CDR2 of the 7.16.6 heavy chain and FR3/CDR3 of the 7.16.6 kappa light chain; and FR3/CDR3 of the 9.8.2 heavy chain.

Table 1. The induction of T cell proliferation and IL-2 production in PBMCs from 40 healthy volunteers incubated with different peptides derived from the anti-MAdCAM mAbs 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod, 7.16.6 and 9.8.2. The sequences of each peptide are indicated, residues which differ from the germline sequence are in bold.

Table 1:

Antibody	Chain	Sequence	Proliferation (SI >2)	ELISPOT (SI>2)
6.22.2	Heavy	SGFTFSSDGMHWVRQ		1
		EWVAIIWYDGSNKYY	2	
	Light	RASQRIGSSLHWYQQ		1
6.34.2	Heavy	AVISNDGNNKYYADS	1	
	Light	ISSYLNWFQKPGKA	1	
		YLNWFQKPGKAPKL	1	1
		APKLLIYAASGLKRG	3	1
		LLIYAASGLKRGVPS		1
		SGLKRGVPSRFGSG	1	
		QPEDFATYYCHQSYS	1	
		DFATYYCHQSYSLPF	2	
		CHQSYSLPFTFGPGT	1	3
		SYSLEPFTFGPGTKVD		1
6.67.1	Heavy	GRIYTSGGTNSNPSL	1	
		RDRTIIRGLIPSFF	1	
		ITIIRGLIPSFFDYW	1	
		LIPSFFDYWGQTLV	1	
	Light	PPKLLIYWASIREYG	1	
		AEDVAVYFCQQYYSI		1
		VAVYFCQQYYSIPPL		3
		YFCQQYYSIPPLTFG		1
		YSIPPLTFGGGKVE	1	
6.77.1	Heavy	AVYYCARDGYSSQWS	1	
		RDGYSSGWSYYYYYG	1	
		YSSGWSYYYYYGMDV	3	
		GWSYYYYYGMDVWGQ	4	
	Light	ISCKSSQSLLSDGK	1	
		EAEDVGVIYSCMQSIQ	1	
		VYSCMQSIQLMSSFG	1	
		SIQLMSSFGQGTKLE	1	1
7.16.6	Heavy	SVYSGNTNYAQKVQG	2	
		AVYYCAREGSSSSGD	1	
		SGDYYYGMDVWGQGT	1	
	Light	VEAEDVGIIYCMQNI	1	
		EDVGIIYCMQNIQLP	4	4
		GIYYCMQNIQLPWTF	1	1
9.8.2	heavy	VAVIWDGSNEYAD		1
		IWDGSNEYADSVK	1	1
		AEDTAVYYCARGAYH	1	1
		TAVYYCARGAYHFAY	3	
		ARGAYHFAYWGQGT	1	
	Light	SLQPEDIATYSCQHS	1	
		PEDIATYSCQHSNLT	1	
		IATYSCQHSNLTFTG	1	
		YSCQHSNLTFTGQGT	1	

Example 2: Characterisation of modified variants of 7.16.6

The T-cell proliferation and ELISPOT analysis identified a number of potential T-cell epitopes in the MAdCAM mAbs 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod, 7.16.6 and 9.8.2. The major T-cell epitope in the kappa light chain of 7.16.6 was reverted to germline by site directed mutagenesis (QuikChange, Stratagene) according to manufacturer's instructions. The sequences of the oligonucleotides used to generate the modified forms are indicated below: primer pairs 7.16.6_V5 and 7.16.6_V3 were used to generate the 7.16.6_V adduct (VH=wild type; VL=I90V); 7.16.6_S5 and 7.16.6_S3 were used to generate the 7.16.6_S adduct (VH= wild type; VL=N96S); 7.16.6_VS5 and 7.16.6_VS3 were used to generate the 7.16.6_VS adduct (VH=wild type; VL=I90V, N96S). The pEE12.1 vector containing the 7.16.6 heavy chain cDNA (described in WO2005/067620). Briefly, a functional eukaryotic expression vector containing the respective heavy and light chain sequences were assembled as follows: The heavy chain cDNA inserts were excised from the required pEE6.1 with NotI/Sall, and the purified fragments were cloned into identical sites into the corresponding pEE12.1 vector containing the required versions of the kappa light chain sequences.) was used as a template for the QuikChange PCR.

7.16.6_V5 GGCTGAGGATGTTGGGGTTTATTACTGCATGC

7.16.6_V3 GCATGCAGTAATAAACCCCAACATCCTCAGCC

7.16.6_S5 CTGCATGCAAAGTATACAGCTTCCGTGGAC

7.16.6_S3 GTCCACGGAAGCTGTATACTTTGCATGCAG

7.16.6_VS5 GGATGTTGGGGTTTATTACTGCATGCAAAGTATACAGCTTCCG

7.16.6_VS3 CGGAAGCTGTATACTTTGCATGCAGTAATAAACCCCAACATCC

pEE12.1 constructs containing the modified kappa light chains of 7.16.6_V, 7.16.6_S and 7.16.6_VS along with the parent 7.16.6 heavy chain were generated from the QuikChange PCR products and fully sequence verified. A similar PCR approach was used to generate the 7.16.6_L variant (VH=V64L, VL=wild type), using the following oligonucleotides and QuikChange PCR:

7.16.6_L5 CTATGCACAGAAGCTTCAGGGCAGAGTCAC

7.16.6_L3 GTGACTCTGCCCTGAAGCTTCTGTGCATAG

Parent kappa light chain and 7.16.6_VS variant were used to generate the 7.16.6_L and 7.16.6_LVS (VH=V64L; VL=I90V, N96S) variant as described in WO2005/067620. Recombinant material was purified by affinity chromatography from FreeStyle HEK293 cells (Invitrogen) transiently transfected with the appropriate construct.

The purified antibodies were assessed for activity in an adhesion assay using MAdCAM-IgG₁ Fc fusion protein:

An EcoRI/BglII cDNA fragment encoding the mature extracellular, immunoglobulin-like domain of MAdCAM was excised from a pINCY Incyte clone (3279276) and cloned into EcoRI/BamHI sites of the pIG1 vector (Simmons, D. L. (1993) in *Cellular Interactions in Development: A Practical Approach*, ed. Hartley, D. A. (Oxford Univ. Press, Oxford), pp. 93-127.) to generate an in frame IgG₁ Fc fusion. The resulting insert was excised with EcoRI/NotI and cloned into pCDNA3.1+ (Invitrogen). The MAdCAM-IgG₁ Fc cDNA in the vector was sequence confirmed. The amino acid sequence of the MAdCAM-IgG₁ Fc fusion protein is shown below:

MDFGLALLLAGLLGLLLGQSLQVKPLQVEPPEPVVAVALGASRQLTCRLACADRGASVQWRGLDTSLGAVQSDTGRS
VLTVRNASLSAAGTRVCVSGCGGRTFQHTVQLLVYAFPDQLTVSPAALVPGDPEVACTAHKVTPVDPNALSFSLLVG
GQELEGAQALGPVEVQEEEEEPQGDVLFVRVTERWRLPPLGTFVPPALYCQATMRPLGLELSHRQAIPVLHSPTSPE
PPDTTSPESPDTTSPESPDTTSPQEPDPTTSQEPDPTTSQEPDPTTSPEPPDKTSPEPAPQQGSTHTPRSPGSTRTRR
PEIQPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVHLQDNLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFPYSPDIAVEWESNGQPENNYKATPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL
SPGK

Underlined: signal peptide.

Bold: MAdCAM extracellular domain

CHO-DHFR cells were transfected with pCDNA3.1+ vector containing MAdCAM-IgG₁ Fc fusion protein cDNA and stable clones expressing MAdCAM-IgG₁ Fc fusion protein selected in Iscove's media containing 600 µg/mL G418 and 100 ng/mL methotrexate. For protein expression, a hollow fibre bioreactor was seeded with stably expressing MAdCAM-IgG₁ Fc CHO cells in Iscove's media containing 10% low IgG fetal bovine serum (Gibco), non essential amino acids (Gibco), 2 mM glutamine (Gibco), sodium pyruvate (Gibco), 100 µg/mL G418 and 100 ng/mL methotrexate, and used to generate concentrated media supernatant. The MAdCAM-IgG₁ Fc fusion protein was purified from the harvested supernatant by affinity chromatography. Briefly, supernatant was applied to a HiTrap Protein G Sepharose (5 mL, Pharmacia) column (2 mL/min), washed with 25 mM Tris pH 8, 150 mM NaCl (5 column volumes) and eluted with 100 mM glycine pH 2.5 (1 mL/min), immediately neutralising fractions to pH 7.5 with 1M Tris pH 8. Fractions containing MAdCAM-IgG₁ Fc fusion protein were identified by SDS-PAGE, pooled together and applied to a Sephacryl S100 column (Pharmacia), pre-equilibrated with 35 mM BisTris pH 6.5, 150 mM NaCl. The gel filtration was performed at 0.35 mL/min, collecting a peak of MAdCAM-IgG₁ Fc fusion protein in ca. 3 x 5 mL fractions. These samples were pooled and applied to a Resource Q (6 mL, Pharmacia) column, pre-equilibrated in 35 mM BisTris pH6.5. The column was washed with 5 column volumes of 35 mM Bis Tris pH 6.5, 150 mM NaCl (6 mL/min) and MAdCAM-IgG₁ Fc fusion protein eluted into a 4-6 mL fraction with 35 mM Bis Tris pH 6.5, 400 mM NaCl. At this stage the protein was 90% pure and migrating as a single band at approximately 68 kD by SDS-PAGE. For use as an immunogen and all subsequent assays, the material was buffer exchanged into 25 mM HEPES pH 7.5, 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 50% glycerol and stored as aliquots at -80°C.

100µL of a 4.5µg/mL solution of purified MAdCAM-IgG₁ Fc fusion protein in Dulbecco's PBS was adsorbed to 96 well Black Microfluor "B" u-bottom (Dynex #7805) plates overnight at 4°C. The MAdCAM coated plates were then inverted and excess liquid blotted off, prior to blocking at 37°C for at least 1 hour in 10% BSA/ PBS. During this time cultured JY cells were counted using tryptan blue exclusion (should

be approximately 8×10^5 cells/mL and 20×10^6 cells/assay plate pipetted into a 50 mL centrifuge tube. JY cells were cultured in RPMI1640 media (Gibco), containing 2 mM L-glutamine and 10% heat-inactivated fetal bovine serum (Life Technologies #10108-165) and seeded at $1-2 \times 10^5$ /mL every 2-3 days to prevent the culture from differentiating. The cells were washed twice with RPMI 1640 media (Gibco) containing 2 mM L-glutamine (Gibco) by centrifugation (240g), resuspending the final cell pellet at 2×10^6 cells/mL in RPMI 1640 for Calcein AM loading. Calcein AM (Molecular Probes #C-3099) was added to the cells as a 1:200 dilution in DMSO (ca. final concentration 5 μ M) and the cells protected from light during the course of the incubation (37°C for 30 min). During this cell incubation step the antibodies to be tested, were diluted as follows: for single dose testing, the antibodies were made up to 3 μ g/mL (1 μ g/mL final) in 0.1 mg/mL BSA (Sigma#A3059) in PBS; for full IC₅₀ curves, the antibodies were diluted in 0.1 mg/mL BSA/PBS, with 3 μ g/mL (1 μ g/mL final) being the top concentration, then doubling dilutions (1:2 ratio) across the plate. The final well of the row was used for determining total binding, so 0.1mg/ml BSA in PBS was used.

After blocking, the plate contents were flicked out and 50 μ L of antibodies/controls were added to each well and the plate incubated at 37°C for 20 min. During this time, Calcein-loaded JY cells were washed once with RPMI 1640 media containing 10% fetal bovine serum and once with 1 mg/mL BSA/PBS by centrifugation, resuspending the final cell pellet to 1×10^6 /mL in 1 mg/mL BSA/PBS. 100 μ L of cells were added to each well of the U bottomed plate, the plate sealed, briefly centrifuged (1000 rpm for 2 min) and the plate then incubated at 37°C for 45 min. At the end of this time, the plates were washed with a Skatron plate washer and fluorescence measured using a Wallac Victor² 1420 Multilabel Reader (excitation λ 485nm, emission λ 535nm count from top, 8 mm from bottom of plate, for 0.1 sec with normal emission aperture). For each antibody concentration, percent adhesion was expressed as a percentage of maximal fluorescence response in the absence of any antibody minus fluorescence associated with non-specific binding. The IC₅₀ value is defined as the anti-MAdCAM antibody concentration at which the adhesion response is decreased to 50% of the response in the absence of anti-MAdCAM antibody. Antibodies that were able to inhibit the binding of JY cells to MAdCAM-IgG₁ Fc fusion with an IC₅₀ value <0.1 μ g/mL, were considered to have potent antagonist activity and were progressed to the MAdCAM-CHO adhesion assay.

The IC₅₀ values are summarised in Table 2 (all datapoints given in μ g/mL):

Table 2. K_d, k_a, and k_d values for the interaction of MAdCAM with the anti-MAdCAM antibody 7.16.6 and 5 clones derived from it.

* Indicates that these off rate values are just below the lower limit ($5 \times 10^{-6} \text{ s}^{-1}$) for BIAcore. The affinity (K_D) values should therefore more accurately be quoted as:

- <15pM ($=5 \times 10^{-6}/3.3 \times 10^5$) for 7.16.6 and
- <27.3pM ($=5 \times 10^{-6}/1.83 \times 10^5$) for 7.16.6_V

The off rates for the other four antibodies are within the limits of the instrument.

	K _d (pM)	k _a (M ⁻¹ s ⁻¹)	k _d (s ⁻¹)	IC ₅₀ (ng/mL)	s.d	n
7.16.6	7.3	3.3 x 10 ⁵	2.42 x 10 ⁻⁶ *	24.7	7.6	6
7.16.6_S	23.2	1.83 x 10 ⁵	4.27 x 10 ⁻⁶ *	34.2	7.4	5
7.16.6_V	162	1.53 x 10 ⁵	2.49 x 10 ⁻⁵	41.2	20.9	5
7.16.6_VS	90.8	2.40 x 10 ⁵	2.18 x 10 ⁻⁵	58.2	51.2	5
7.16.6_L	79.5	1.52 x 10 ⁵	1.21 x 10 ⁻⁵	35.6	10	5
7.16.6_LVS	119	1.94 x 10 ⁵	2.31 x 10 ⁻⁵	45.8	16.4	5

The results show that the variants still bind MAdCAM with high affinity, sufficiently high for therapeutic use.

5

7.16.6, 7.16.6_V, 7.16.6_S, 7.16.6_VS, 7.16.6_L and 7.16.6_LVS also bound to CHO cells expressing MAdCAM, as detected by flow cytometry.

The K_d values shown in Table 2 were determined by BIAcore, essentially as described in Example II in WO 2005/067620, with the following modifications:

10

Sample injections

Flow cells used: Either 2 with 1 as a reference, or 4 with 3 as a reference

Flow rate: 100µL/min

15

Injection time: 2 minutes

Wait after injection: 60 minutes (=dissociation time)

Injection cycles: Triplicate of all concentrations, plus six injections of buffer blank

Regeneration Method

20

Solution/flow rate/injection time:

Immobilised antibody	Regeneration solution	Regeneration flow rate, µL/min	Injection time (vol)
7.16.6	50mM Phosphoric Acid	50	12 seconds (10µL)
7.16.6_V	60mM Phosphoric Acid	50	54 seconds (45µL)
7.16.6_S	4M Magnesium Chloride	100	15 seconds (25µL)
7.16.6_VS	50mM Phosphoric Acid	50	12 seconds (10µL)
7.16.6_L	7.5mM Sodium Hydroxide	50	6 seconds (5µL)
7.16.6_LVS	5mM Sodium Hydroxide	50	6 seconds (5µL)

Stabilisation time after regeneration: 5 minutes

25

Example 3: Immunohistochemistry staining of cynomolgus tissues with variants of 7.16.6

Immunohistochemistry was essentially performed as described in Example III in WO 2005/067620. The results are summarized in Table 3 below.

5

Table 3: Summary of staining of normal cynomolgus tissues with anti-hMAAdCAM 1/500 dilution (ca. 0.3 µg/mL), 1 hour incubation at room temperature).

Tissue	Staining Intensity Clone 7.16.6	Staining Pattern	Comparative intensity of various clones with clone 7.16.6					
			7.16.6 LVS	7.16.6 L	7.16.6	7.16.6 S	7.16.6 VS	Neg. Control
Positive staining								
Ileum/Peyer's Patch (PP)	2-3+	HEV (PP), Vasc. endothelium (lamina propria-LP)	=	^	=	=	=	<<<
	3+	vasc.endothelium (LP)	=	=	=	=	=	<<<
Cecum								
Spleen	2-4+	Sinus-lining cells (marginal zone of white pulp)	<	=	=	V	V	<<<
	4+	HEV	=	=	=	=	=	<<<
Mesenteric LN								
Axillary LN	trace	Dendritic-like cells around lymphoid follicles	=	=	=	=	=	<<<
		Vas endothelium (venules of exocrine lobules)	=	=	V	=	=	<<<
Pancreas	1+							
Negative Staining								
Kidney*	0	---	=	=	=	=	=	=
Heart (ventricle)	0	---	=	=	=	=	=	=
Thyroid	0	---	=	=	=	=	=	=

• Strong staining in tubules attributable to non-specific binding of the secondary antibody and interpreted as background noise.

Example 4: Characterisation of modified variants of 6.22.2, 6.34.2, 6.67.1, 6.77.1 and 9.8.2

Using similar methods as described here in Example 2 and referred to in WO2005/067620, some of the significant potential T cell epitopes in 6.22.2, 6.34.2, 6.67.1, 6.77.1 and 9.8.2 described in Table 1 were modified to germline by substitution or deletion to generate purified recombinant 6.22.2-mod_V (SEQ ID No. 2), 6.34.2-mod_SSQ (SEQ ID No. 8), 6.67.1-mod_Y (SEQ ID No. 14), 6.77.1-mod_ΔAS (SEQ ID No. 22) and 9.8.2-mod_ΔRGAYH,D (SEQ ID No. 36) chain variants. The IC₅₀ potencies of the following mAbs referred to as 6.22.2-mod_V (SEQ ID Nos. 2 and 4), 6.34.2-mod_SSQ (SEQ ID Nos. 6 and 8), 6.67.1-mod_Y (SEQ ID Nos. 14 and 20), 6.77.1-mod_ΔAS (SEQ ID Nos. 22 and 24) and 9.8.2-mod_ΔRGAYH,D (SEQ ID Nos. 36 and 38) in the MAdCAM-IgG₁ Fc fusion: JY cell binding assay are represented in Table 4 compared with their parental counterpart 6.22.2, 6.34.2, 6.67.1, 6.77.1 and 9.8.2 mAbs. All modified variants maintained similar antagonist potency to their parental mAbs.

Table 4. Comparison of IC₅₀ potencies of the 6.22.2, 6.34.2, 6.67.1, 6.77.1 and 9.8.2 modified mAbs, 6.22.2-mod_V, 6.34.2-mod_SSQ, 6.67.1-mod_Y, 6.77.1-mod_ΔAS and 9.8.2-mod_ΔRGAYH,D respectively in the MAdCAM-IgG₁ Fc fusion: JY cell binding assay

	IC ₅₀ (μg/mL)				IC ₅₀ (μg/mL)		
		S.D	n			S.D	n
6.22.2	0.018	0.0109	4	6.22.2-mod_V	0.076	0.033	7
6.34.2	0.013	0.0079	4	6.34.2-mod_SSQ	0.086	0.04	11
6.67.1	0.013	0.0071	4	6.67.1-mod_Y	0.063	0.025	6
6.77.1	0.022	0.0045	4	6.77.1-mod_ΔAS	0.0485	0.0135	7
9.8.2	0.02	0.0047	4	9.8.2_ΔRGAYH,D	0.072	0.03	6

Sequences:

The sequences of the signal peptide sequence (or the bases encoding the same) are indicated in lower case and underlined. The variable regions are in upper case. The constant regions are in upper case and underlined in the protein sequences. Bold are residues modified from the originally disclosed sequences (WO2005/067620).

SEQ ID NO: 1

6.22.2-mod_V Heavy Chain Nucleotide Sequence

```

10      1   atggagtttg ggctgagctg ggttttcctc gttgctcttt taagaggtgt
      51   ccagtgtCAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCCAGCCTG
     101   GGAGGTCCTT GAGACTCTCC TGTGCAGCGT CTGGATTACAC CTTCAGTAGC
     151   GATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT
     201   GGCAGTGATA TGGTATGATG GAAGTAATAA ATATTATGCA GACTCCGTGA
    15    251   AGGGCCGATF CACCATCTCC AGAGACAATT CCAAGAACAC GCTGTATCTG
      301   CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTATATT ACTGTGCGAG
      351   AGATCCCGGC TACTATTACG GTATGGACGT CTGGGGCCAA GGGACCACGG
      401   TCACCGTCTC CTCAGCTTCC ACCAAGGGCC CATCCGTCTT CCCCCTGGCG
      451   CCCTGCTCTA GAAGCACCTC CGAGAGCACA GCGGCCCTGG GCTGCCTGGT
    20    501   CAAGGACTAC TTCCCCGAAC CGGTGACGGT GTCGTGGAAC TCAGGCGCTC
      551   TGACCAGCGG CGTGCACACC TTCCCAGCTG TCCTACAGTC CTCAGGACTC
      601   TACTCCCTCA GCAGCGTGGT GACCGTGCCC TCCAGCAACT TCGGCACCCA
      651   GACCTACACC TGCAACGTAG ATCACAAGCC CAGCAACACC AAGGTGGACA
      701   AGACAGTTGA GCGCAAATGT TGTGTCGAGT GCCCACCCTG CCCAGCACCA
    25    751   CCTGTGGCAG GACCGTCAGT CTTCCTCTTC CCCCCAAAAC CCAAGGACAC
      801   CCTCATGATC TCCCGGACCC CTGAGGTCAC GTGCGTGGTG GTGGACGTGA
      851   GCCACGAAGA CCCCAGGTC CAGTTCAACT GGTACGTGGA CGGCGTGGAG
      901   GTGCATAATG CCAAGACAAA GCCACGGGAG GAGCAGTTCA ACAGCACGTT
      951   CCGTGTGGTC AGCGTCCTCA CCGTTGTGCA CCAGGACTGG CTGAACGGCA
    30   1001   AGGAGTACAA GTGCAAGGTC TCCAACAAAG GCCTCCCAGC CCCCATCGAG
     1051   AAAACCATCT CAAAACCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC
     1101   CCTGCCCCCA TCCCGGGAGG AGATGACCAA GAACCAGGTC AGCCTGACCT
     1151   GCCTGGTCAA AGGCTTCTAC CCCAGCGACA TCGCCGTGGA GTGGGAGAGC
     1201   AATGGGCAGC CGGAGAACAA CTACAAGACC ACACCTCCCA TGCTGGACTC
    35   1251   CGACGGCTCC TTCTTCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT
     1301   GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC
     1351   AACCAC TACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGATAG

```

SEQ ID NO: 2

40 6.22.2-mod_V Predicted Heavy Chain Protein Sequence

```

      1   mefglswvfl vallrgvqcQ VQLVESGGGV VQPGRSLRLS CAASGFTFSS
      51   DGMHWVRQAP GKGLEWVAVI WYDGSNKYYA DSVKGRFTIS RDNSKNTLYL
     101   QMNSLRAEDT AVYYCARDPG YYYGMDVWGQ GTTVTVSSAS TKGPSVFPLA
     151   PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL
    45   201   YSLSSVVTVP SSNFGTQTYT CNVDHKPSNT KVDKTVERKC CVECPCPCAP
     251   PVAGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE
     301   VHNAKTKPRE EQFNSTFRVV SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE
     351   KTISKTKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES
     401   NGQPENNYKT TTPMLDSDGS FFLYSLKLTVD KSRWQQGNVF SCSVMHEALH
    50   451   NHYTQKSLSL SPGK

```

SEQ ID NO: 3

6.22.2-mod Kappa Light Chain Nucleotide Sequence

```

5      1  atgttgccat cacaactcat tggggtttctg ctgctctggg ttccagcttc
      51  caggggtGAA ATTGTGCTGA CTCAGTCTCC AGACTTTCAG TCTGTGACTC
     101  CAAAAGAGAA AGTCACCATC ACCTGCCGGG CCAGTCAGAG AATTGGTAGT
     151  AGCTTACACT GGTACCAGCA GAAACCAGAT CAGTCTCCAA AACTCCTCAT
     201  CAAGTATGCT TCCCAGTCCT TCTCAGGGGT CCCCTCGAGG TTCAGTGGCA
10    251  GTGGATCTGG GACAGATTTT ACCCTCACCA TCAATAGCCT GGAAGCTGAA
      301  GATGCTGCAA CTTATTACTG TCATCAGAGT GGTGCTTTAC CGCTCACTTT
      351  CGGCGGAGGG ACCAAGGTGG AGATCAAACG AACTGTGGCT GCACCATCTG
      401  TCTTTCATCTT CCCGCCATCT GATGAGCAGT TGAAATCTGG AACTGCCTCT
      451  GTTGTGTGCC TGCTGAATAA CTTCTATCCC AGAGAGGCCA AAGTACAGTG
15    501  GAAGGTGGAT AACGCCCTCC AATCGGGTAA CTCCCAGGAG AGTGTACAG
      551  AGCAGGACAG CAAGGACAGC ACCTACAGCC TCAGCAGCAC CCTGACGCTG
      601  AGCAAAGCAG ACTACGAGAA ACACAAAGTC TACGCCTGCG AAGTACCCCA
      651  TCAGGGCCTG AGCTCGCCCG TCACAAAGAG CTTCAACAGG GGAGAGTGTT
      701  AGTGA
20

```

SEQ ID NO: 4

6.22.2-mod Predicted Kappa Light Chain Amino Acid Sequence

```

      1  mlpsqligfl llwvpasrgE IVLTQSPDFQ SVTPKEKVTI TCRASQRIGS
     51  SLHWYQQKPD QSPKLLIKYA SQSFSGVPSR FSGSGSGTDF TLTINSLEAE
25    101  DAATYYCHQS GRLPLTFGGG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS
     151  VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTTL
     201  SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

```

SEQ ID NO. 5

6.34.2-mod Heavy Chain Nucleotide Sequence

```

1   atggagtttg ggctgagctg ggttttcctc gttgctcttt taagaggtgt
5   51   ccagtgtCAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCCAGCCTG
101  GGAGGTCCCT GAGACTCTCC TGTGCAGCCT CTGGATTACAC CTTCAGTAGC
151  TATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT
201  GGCAGTTATA TCAAATGATG GAAATAATAA ATACTATGCA GACTCCGTGA
251  AGGGCCGATT CACCATCTCC AGAGACAATT CCAAAAACAC GCTGTATCTG
301  CAAATGAACA GCCTGCGCGC TGAGGACACG GCTGTGTATT ACTGTGCGAG
10  351  AGATAGTACG GCGATAACCT ACTACTACTA CGGAATGGAC GTCTGGGGCC
401  AAGGGACCAC GGTCAACGTC TCCTCAGCTT CCACCAAGGG CCCATCCGTC
451  TTCCCCCTGG CGCCCTGCTC TAGAAGCACC TCCGAGAGCA CAGCGGCCCT
501  GGGCTGCCTG GTCAAGGACT ACTTCCCCGA ACCGGTGACG GTGTCGTGGA
551  ACTCAGGCGC TCTGACCAGC GGCGTGCACA CCTTCCCAGC TGTCTACAG
15  601  TCCTCAGGAC TCTACTCCCT CAGCAGCGTG GTGACCGTGC CCTCCAGCAA
651  CTTCCGACAC CAGACCTACA CCTGCAACGT AGATCACAAG CCCAGCAACA
701  CCAAGGTGGA CAAGACAGTT GAGCGCAAAT GTTGTGTCTGA GTGCCCACCG
751  TGCCCAGCAC CACCTGTGGC AGGACCGTCA GTCTTCTCTT TCCCCCAA
801  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACGTGCGTGG
20  851  TGGTGGACGT GAGCCACGAA GACCCCGAGG TCCAGTTCAA CTGGTACGTG
901  GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCACGGG AGGAGCAGTT
951  CAACAGCACG TTCCGTGTGG TCAGCGTCCT CACCGTTGTG CACCAGGACT
1001 GGCTGAACGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGGCCTCCCA
1051 GCCCCATCG AGAAAACCAT CTCCAAAACC AAAGGGCAGC CCCGAGAACC
25  1101 ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC AAGAACCAGG
1151 TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ACCCCAGCGA CATCGCCGTG
1201 GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACACCTCC
1251 CATGCTGGAC TCCGACGGCT CCTTCTTCTT CTACAGCAAG CTCACCGTGG
1301 ACAAGAGCAG GTGGCAGCAG GGAACGTCT TCTCATGCTC CGTGATGCAT
30  1351 GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCTCCGGG
1401 TAAATGATAG

```

SEQ ID NO. 6

35 6.34.2-mod Predicted Heavy Chain Amino Acid Sequence

```

1   mefglswvfl vallrgvqcQ VQLVESGGGV VQPGRSLRLS CAASGFTFSS
51  YGMHWVRQAP GKGLEWVAVI SNDGNNKYA DSVKGRFTIS RDNSKNTLYL
101 QMNSLRAEDT AVYYCARDST AITYYYGMD VWGQGTTVTV SSASTKGPSV
151 FPLAPCSRST SESTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ
40  201  SSGLYSLSSV VTVPSNFGT QTYTCNV DHK PSNTKVDKTV ERKCCVECPP
251 CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV
301 DGVEVHNAKT KPREEQFNST FRVSVLTVV HQDWLNGKEY KCKVSNKGLP
351 APIEKTISKI KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
401 EWESNGQOPEN NYKTTTPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
45  451  EALHNHYTQK SLSLSPGK

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SEQ ID NO: 7

5 6.34.2-mod_SSQ Kappa Light Chain Nucleotide Sequence

```

      1  atggacatga gggccccgc tcagctcctg gggctcctgc tactctggct
     51  ccgagggtgcc agatgtGACA TCCAGATGAC CCAGTCTCCA TCCTCCCTGT
    101  CTGCATCTGT CGGAGACAGA GTCACCATCA CTTGCCGGGC AAGTCAGAGT
    151  ATTAGTAGCT ATTTAAATTG GTATCAGCAG AAACCAGGGA AAGCCCCTAA
    201  GCTCCTGATC TATGCTGCAT CCAGTTTGAG TCAGGGGGTC CCATCACGGT
    251  TCAGTGGTAG TGGATCTGGG ACAGATTTCa CTCTCACCAT CAGTTCTCTG
    301  CAACCTGAGG ATTTTGCAAC TTACTACTGT CACCAGAGTT ACAGTCTCCC
    351  ATTCACTTTC GGCCCTGGGA CCAAAGTGGA TATCAAACGA ACTGTGGCTG
    401  CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA
    451  ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA
    501  AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA
    551  GTGTCACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC
    601  CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT ACGCCTGCGA
    651  AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG
    701  GAGAGTGTTA GTGA

```

SEQ ID NO: 8

6.34.2-mod_SSQ Predicted Kappa Light Chain Protein Sequence

```

    25  1  mdmrvpagll gl111wlrge rcDIQMTQSP SSLSASVGDR VTITCRASQS
      51  ISSYLNWYQQ KPGKAPKLLI YAASSLSQGV PSRFSGSGSG TDFTLTISL
    101  QPEDFATYYC HQSYSLPFTF GPGTKVDIKR TVAAPS VFIF PPSDEQLKSG
    151  TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYLSLST
    201  LTLISKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC

```

SEQ ID NO: 9

6.34.2-mod_QT Kappa Light Chain Nucleotide Sequence

```

5      1   atggacatga ggggtccccgc tcagctcctg ggggtcctgc tactctgget
      51   ccgagggtgcc agatgtGACA TCCAGATGAC CCAGTCTCCA TCCTCCCTGT
     101   CTGCATCTGT CGGAGACAGA GTCACCATCA CTTGCCGGGC AAGTCAGAGT
     151   ATTAGTAGCT ATTTAAATTG GTATCAGCAG AAACCAGGGA AAGCCCCTAA
     201   GCTCCTGATC TATGCTGCAT CCGGTTTGAA GCGTGGGGTC CCATCACGGT
10    251   TCAGTGGTAG TGGATCTGGG ACAGATTTCA CTCTCACCAT CAGTTCTCTG
     301   CAACCTGAGG ATTTTGCAAC TTACTACTGT CAGCAGAGTT ACAGTACTCC
     351   ATTCACTTTC GGCCCTGGGA CCAAAGTGGA TATCAAACGA ACTGTGGCTG
     401   CACCATCTGT CTTTCATCTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA
     451   ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA
15    501   AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA
     551   GTGTCACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC
     601   CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT ACGCCTGCGA
     651   AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG
     701   GAGAGTGTTA GTGA
20

```

SEQ ID NO: 10

6.34.2-mod_QT Predicted Kappa Light Chain Protein Sequence

```

      1   mdmrvpaql1 gl111wlrga rcDIQMTQSP SSLSASVGDR VTITCRASQS
     51   ISSYLNWYQQ KPGKAPKLLI YAASGLKRGV PSRFSGSGSG TDFTLTISSL
25    101   QPEDFATYYC QSYSTPFTF GPGTKVDIKR TVAAPSVFIF PPSDEQLKSG
     151   TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYLSLST
     201   LTLSKADYEK HKVYACEVTH QGLSSPVTKS FNRGEC

```

SEQ ID NO: 11

6.34.2-mod_SSQ,QT Kappa Light Chain Nucleotide Sequence

```

1   atggacatga gggccccgc tcagctcctg gggctcctgc tactctggct
5   51   cccgaggtgcc agatgtGACA TCCAGATGAC CCAGTCTCCA TCCTCCCTGT
101  CTGCATCTGT CGGAGACAGA GTCACCATCA CTTGCCGGGC AAGTCAGAGT
151  ATTAGTAGCT ATTTAAATTG GTATCAGCAG AAACCAGGGA AAGCCCCTAA
201  GCTCCTGATC TATGCTGCAT CCAGTTTGAG TCAGGGGGTC CCATCACGGT
251  TCAGTGGTAG TGGATCTGGG ACAGATTTC ACTCTACCAT CAGTTCTCTG
10  301  CAACCTGAGG ATTTTGCAAC TTACTACTGT CAGCAGAGTT ACAGTACTCC
351  ATTCACTTTC GGCCCTGGGA CCAAAGTGGA TATCAAACGA ACTGTGGCTG
401  CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA
451  ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA
501  AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA
15  551  GTGTACAGA GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC
601  CTGACGCTGA GCAAAGCAGA CTACGAGAAA CACAAAGTCT ACGCCTGCGA
651  AGTCACCCAT CAGGGCCTGA GCTCGCCCGT CACAAAGAGC TTCAACAGGG
701  GAGAGTGTTA GTGA

```

20 SEQ ID NO: 12

6.34.2-mod_SSQ,QT Predicted Kappa Light Chain Protein Sequence

```

1   mdmrvpaql1 gl111wlrga rcDIQMTQSP SSSLASVGDR VTITCRASQS
51  ISSYLNWYQQ KPGKAPKLLI YAASSLSQGV PSRFSGSGSG TDFTLTISL
101 QPEDFATYYC QSYSTPPTF GPGTKVDIKR TVAAPSVFIF PPSDEQLKSG
25  151  TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYLSST
201  LTLISKADYEK HKVYACEVTH QGLSPVTKS FNRGEC

```

SEQ ID NO:13

6.67.1-mod Heavy Chain Nucleotide Sequence

```

1   atgaaacacc tgtggttctt cctcctgctg gtggcagctc ccagatgggt
5   51   cctgtccCAG GTGCAGCTGC AGGAGTCGGG CCCAGGACTG GTGAAGCCTT
    101  CGGAGACCCT GTCCCTCACC TGCACGTGCT CTGGTGACTC CATCAGTAGT
    151  AACTATTGGA GCTGGATCCG GCAGCCCGCC GGGAAAGGAC TGGAGTGGAT
    201  TGGGCGTATC TATACCAGTG GGGGCACCAA CTCCAACCCC TCCCTCAGGG
    251  GTCGAGTCAC CATGTCTAGTA GACACGTCCA AGAACCAGTT CTCTCTGAAA
10   301  CTGAGTTCTG TGACCGCCGC GGACACGGCC GTGTATTACT GTGCGAGAGA
    351  TCGTATTACT ATAATTCGGG GACTTATTCC ATCCTTCTTT GACTACTGGG
    401  GCCAGGGAAC CTTGGTCACC GTCTCCTCAG CTTCACCAA GGGCCCATCC
    451  GTCTTCCCCC TGGCGCCCTG CTCTAGAAGC ACCTCCGAGA GCACAGCGGC
    501  CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCAGTG ACGGTGTCGT
15   551  GGAATCAGG CGCTCTGACC AGCGGCGTGC ACACCTTCCC AGCTGTCCTA
    601  CAGTCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG
    651  CAACTTCGGC ACCCAGACCT ACACCTGCAA CGTAGATCAC AAGCCCAGCA
    701  ACACCAAGGT GGACAAGACA GTTGAGCGCA AATGTTGTGT CGAGTGCCCA
    751  CCGTGCCAG CACCACCTGT GGCAGGACCG TCAGTCTTCC TCTTCCCCCC
20   801  AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACGTGCG
    851  TGGTGGTGGA CGTGAGCCAC GAAGACCCCG AGGTCCAGTT CAACTGGTAC
    901  GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAGCCAC GGGAGGAGCA
    951  GTTCAACAGC ACGTTCCGTG TGGTCAGCGT CCTCACCGTT GTGCACCAGG
25   1001  ACTGGCTGAA CGGCAAGGAG TACAAGTGCA AGGTCTCAA CAAAGGCCTC
    1051  CCAGCCCCCA TCGAGAAAAC CATCTCCAAA ACCAAAGGGC AGCCCCGAGA
    1101  ACCACAGGTG TACACCCTGC CCCCATCCCG GGAGGAGATG ACCAAGAACC
    1151  AGGTCAGCCT GACCTGCCTG GTCAAAGGCT TCTACCCAG CGACATCGCC
    1201  GTGGAGTGGG AGAGCAATGG GCAGCCGAG AACAAC TACA AGACCACACC
    1251  TCCCATGCTG GACTCCGACG GCTCCTTCTT CCTCTACAGC AAGCTCACCG
30   1301  TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
    1351  CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
    1401  GGGTAAATGA TAG

```

SEQ ID NO. 14

6.67.1-mod Predicted Heavy Chain Amino Acid Sequence

```

1   mkhlwfflll vaaprwlslQ VQLQESGPGI VKPSETLSLT CTVSGDSISS
35  51   NYWSWIRQPA GKGLEWIGRI YTSGGTNSNP SLRGRVTMSV DTSKNQFSLK
    101  LSSVTAADTA VYYCARDRII IIRGLIPSFF DYWGQGTILVT VSSASTKGPS
40  151  VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
    201  QSSGLYSLSS VVTVPSSNFG TQTYTCNVDH KPSNTKVDKT VERKCCVECP
    251  PCPAPPVAGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVQFNWY
    301  VDGVEVHNAK TKPREEQFNS TFRVVSVLTV VHQDWLNGKE YKCKVSNKGL
    351  PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
45  401  VEWESNGQPE NNYKTTPPML DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
    451  HEALHNHYTQ KSLSLSPGK

```

SEQ ID NO. 15

6.67.1-mod_Y Kappa Light Chain Nucleotide Sequence

```

      1   atggtggttgc agacccaggt cttcatttct ctggttgcctct ggatctctctgg
5      51   tgccctacggg GACATCGTGA TGACCCAGTC TCCAGACTCC CTGGCTGTGT
      101   CTCTGGGCGA GAGGGCCACC ATCAACTGCA AGTCCAGCCA GAGTGTTTTA
      151   TACAGCTCCA ACAATAAGAA CTACTTAGCT TGGTACCAAC AGAAACCAGG
      201   ACAGCCTCCT AAATTGCTCA TTTACTGGGC ATCTATACGG GAATATGGGG
      251   TCCCTGACCG ATTCACTGGC AGCGGGTCTG GGACAGATT T CACTCTCACC
10      301   ATCAGCAGCC TGCAGGCTGA AGATGTGGCA GTTTATTATT GTCAACAATA
      351   TTATAGTATT CCTCCCCTCA CTTTCGGCGG AGGGACCAAG GTGGAGATCA
      401   AACGAACTGT GGCTGCACCA TCTGTCTTCA TCTTCCCGCC ATCTGATGAG
      451   CAGTTGAAAT CTGGAAGTGC CTCTGTTGTG TGCCTGCTGA ATAACCTCTA
      501   TCCCAGAGAG GCCAAAGTAC AGTGGAAGGT GGATAACGCC CTCCAATCGG
15      551   GTAACCTCCA GGAGAGTGTG ACAGAGCAGG ACAGCAAGGA CAGCACCTAC
      601   AGCCTCAGCA GCACCCTGAC GCTGAGCAAA GCAGACTACG AGAAACACAA
      651   AGTCTACGCC TCGGAAGTCA CCCATCAGGG CCTGAGCTCG CCCGTCACAA
      701   AGAGCTTCAA CAGGGGAGAG TGTTAGTGA

```

20 SEQ ID NO: 16

6.67.1-mod_Y Predicted Kappa Light Chain Amino Acid Sequence

```

      1   mvlqtqvffis lllwisgayg DIVMTQSPDS LAVSLGERAT INCKSSQSVL
25      51   YSSNNKNYLA WYQQKPGQPP KLLIYWASIR EYGVDPDRFSG SGSGTDFTLT
      101   ISSLQAEDVA VYYCQYYSI PPLTFGGGTK VEIKRTVAAP SVFIFPPSDE
      151   QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY
      201   SLSSTLTLSK ADYEKHKVYA CEVTHQGLSS PVTKSFNERGE C

```

SEQ ID NO: 17

6.67.1-mod_TΔP Kappa Light Chain Nucleotide Sequence

```

5      1   atggtgttgc agacccaggt cttcatttct ctggtgctct ggatctctgg
      51   tgcctacggg GACATCGTGA TGACCCAGTC TCCAGACTCC CTGGCTGTGT
     101   CTCTGGGCGA GAGGGCCACC ATCAACTGCA AGTCCAGCCA GAGTGTTTTA
     151   TACAGCTCCA ACAATAAGAA CTACTTAGCT TGGTACCAAC AGAAACCAGG
     201   ACAGCCTCCT AAATTGCTCA TTTACTGGGC ATCTATACGG GAATATGGGG
10    251   TCCCTGACCG ATTCAGTGGC AGCGGGTCTG GGACAGATT T CACTCTCACC
      301   ATCAGCAGCC TGCAGGCTGA AGATGTGGCA GTTTATT TCT GTCAACAATA
      351   TTATAGTACC CCT---CTCA CTTTCGGCGG AGGGACCAAG GTGGAGATCA
      401   AACGAACTGT GGCTGCACCA TCTGTCTTCA TCTTCCCGCC ATCTGATGAG
      451   CAGTTGAAAT CTGGAAGTGC CTCTGTTGTG TGCCTGCTGA ATAACTTCTA
15    501   TCCAGAGAG GCCAAAGTAC AGTGAAGGT GGATAACGCC CTCCAATCGG
      551   GTAAC TCCA GGAGAGTGTC ACAGAGCAGG ACAGCAAGGA CAGCACCTAC
      601   AGCCTCAGCA GCACCCTGAC GCTGAGCAAA GCAGACTACG AGAAACACAA
      651   AGTCTACGCC TGCGAAGTCA CCCATCAGGG CCTGAGCTCG CCCGTCACAA
      701   AGAGCTTCAA CAGGGGAGAG TGT TAGTGA
20

```

SEQ ID NO: 18

6.67.1-mod_TΔP Predicted Kappa Light Chain Amino Acid Sequence

```

25    1   mvlqtqvfis lllwisgayg DIVMTQSPDS LAVSLGERAT INCKSSQSVL
      51   YSSNNKNYLA WYQQKPGQPP KLLIYWASIR EYGV PDRFSG SGSGTDFTLT
     101   ISSLQAEDVA VFYFCQYYST P-LTFGGGTK VEIKRTVAAP SVFIFPPSDE
     151   QLKSGTASVV CLLNNFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY
     201   SLSSTLTLSK ADYEKHKVYA CEVTHQGLSS PVT KSFNRGE C

```

SEQ ID NO: 19

6.67.1-mod_Y,TΔP Kappa Light Chain Nucleotide Sequence

```

5      1   atggtggtgc agaccaggt cttcatttct ctggtgctct ggatctctgg
      51   tgcctacggg GACATCGTGA TGACCCAGTC TCCAGACTCC CTGGCTGTGT
     101   CTCTGGGCGA GAGGGCCACC ATCAACTGCA AGTCCAGCCA GAGTGTTTTTA
     151   TACAGCTCCA ACAATAAGAA CTACTTAGCT TGGTACCAAC AGAAACCAGG
     201   ACAGCCTCCT AAATTGCTCA TTTACTGGGC ATCTATACGG GAATATGGGG
10    251   TCCCTGACCG ATTCAGTGGC AGCGGGTCTG GGACAGATTT CACTCTCACC
     301   ATCAGCAGCC TGCAGGCTGA AGATGTGGCA GTTTATTATT GTCAACAATA
     351   TTATAGTACC CCT--CTCA CTTTCGGCGG AGGGACCAAG GTGGAGATCA
     401   AACGAAGTGT GGCTGCACCA TCTGTCTTCA TCTTCCCGCC ATCTGATGAG
     451   CAGTTGAAAT CTGGAAGTGC CTCTGTTGTG TGCCTGCTGA ATAACTTCTA
15    501   TCCCAGAGAG GCCAAAGTAC AGTGGAAGGT GGATAACGCC CTCCAATCGG
     551   GTAAGTCCCA GGAGAGTGTG ACAGAGCAGG ACAGCAAGGA CAGCACCTAC
     601   AGCCTCAGCA GCACCTGAC GCTGAGCAAA GCAGACTACG AGAAACACAA
     651   AGTCTACGCC TGCGAAGTCA CCCATCAGGG CCTGAGCTCG CCCGTCACAA
20    701   AGAGCTTCAA CAGGGGAGAG TGTTAGTGA

```

SEQ ID NO: 20

6.67.1-mod_Y,TΔP Predicted Kappa Light Chain Amino Acid Sequence

```

25      1   mvlqtqvfis lllwisgayg DIVMTQSPDS LAVSLGERAT INCKSSQSVL
      51   YSSNNKNYLA WYQOKPGQPP KLLIYWASIR EYGVPRDFSG SGSGTDFTLT
     101   ISSLQAEDVA VYQCQYYST P-LTFGGGTK VEIKRTVAAP SVFLFPPSDE
     151   QLKSGTASVV CLLNMFYPRE AKVQWKVDNA LQSGNSQESV TEQDSKDSTY
     201   SLSSTLTLSK ADYEKHKVYA CEVTHQGLSS PVTKSFNRGE C
30

```

SEQ ID NO: 21

6.77.1-mod_ΔS Heavy Chain Nucleotide Sequence

```

5      1   atggaactgg ggctccgctg ggttttcctt gttgctatatt tagaaggtgt
      51   ccagtgtGAG GTGCAGCTGG TGGAGTCTGG GGGAGGCCTG GTCAAGCCTG
     101   GGGGGTCCCT GAGACTCTCC TGTGCAGCCT CTGGATTACAC CTTCAGTAGC
     151   TATAGCATGA ACTGGGTCCG CCAGGCTCCA GGAAGGGGC TGGAGTGGGT
     201   CTCATCCATT AGTAGTAGTA GTAGTTACAT ATACTACGCA GACTCAGTGA
    10   251   AGGGCCGATT CACCATCTCC AGAGACAACG CCAAGAACTC ACTGTATCTG
     301   CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCGAG
     351   AGATGGGTAT AGCAGTGGCT GG---TACTA CTACTACTAC GGTATGGACG
     401   TCTGGGGCCA AGGGACCACG GTCACCGTCT CCTCAGCTTC CACCAAGGGC
     451   CCATCCGTCT TCCCCCTGGG GCCCTGCTCT AGAAGCACCT CCGAGAGCAC
    15   501   AGCGGCCCTG GGCTGCCCTG TCAAGGACTA CTTCCCGGAA CCGGTGACGG
     551   TGTCTGGGAA CTCAGGCGCT CTGACCAGCG GCGTGACACAC CTTCCAGCT
     601   GTCCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC
     651   CTCCAGCAAC TTCGGCACCC AGACCTACAC CTGCAACGTA GATCACAAGC
     701   CCAGCAACAC CAAGGTGGAC AAGACAGTTG AGCGCAAATG TTGTGTCGAG
    20   751   TGCCCAACCGT GCCCAGCACC ACCTGTGGCA GGACCGTCAG TCTTCCTCTT
     801   CCCCCAAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA
     851   CGTGCGTGGT GGTGGACGTG AGCCACGAAG ACCCCGAGGT CCAGTTCAAC
     901   TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCACGGGA
     951   GGAGCAGTTC AACAGCACGT TCCGTGTGGT CAGCGTCCTC ACCGTTGTGC
    25   1001  AGCAGGACTG GCTGAACGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA
     1051  GGCCTCCAG CCCCATCGA GAAAACCATC TCCAAAACCA AAGGGCAGCC
     1101  CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA
     1151  AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA CCCCAGCGAC
     1201  ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC
    30   1251  CACACCTCCC ATGCTGGACT CCGACGGCTC CTTCTTCCTC TACAGCAAGC
     1301  TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC
     1351  GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT
     1401  GTCTCCGGGT AAATGATAG

```

35

SEQ ID NO: 22

6.77.1-mod_ΔS Predicted Heavy Chain Protein Sequence

```

40      1   melglrwvfl vailegvqcE VQLVESGGGL VKPGGSLRLS CAASGFTFSS
      51   YSMNWVRQAP GKGLEWVSSI SSSSSYIYYA DSVKGRFTIS RDNAKNSLYL
     101   QMNSLRAEDT AVYYCARDGY SSGW-YYYYY GMDVWQGTT VTVSSASTKG
     151   PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA
     201   VLQSSGLYSL SSVVTVPSSN FGTQTYTCNV DHKPSNTKVD KTVRKCCVE
    45   251   CPPCPAPPVA GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVQFN
     301   WYVDGVEVHN AKTKPREEQF NSTFRVSVL TVVHQDWLNG KEYKCKVSNK
     351   GLPAPIEKTI SKTKGQPREP QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD
     401   IAVEWESNGQ PENNYKTPP MLDSGGSFFL YSKLTVDKSR WQQGNVFSCS
     451   VMHEALHNNHY TQKSLSLSPG K
    50

```

SEQ ID NO: 23

6.77.1-mod Kappa Light Chain Nucleotide Sequence

```

5      1  atgagggctcc ctgctcagct cctggggctg ctaatgctct ggatacctgg
      51  atccagtgca GATATTGTGA TGACCCAGAC TCCACTCTCT CTGTCCGTCA
     101  CTCTGGACA GCCGGCCTCC ATCTCCTGCA AGTCTAGTCA GAGCCTCCTG
     151  CTTAGTGATG GAAAGACCTA TTTGAATTGG TACCTGCAGA AGCCCGGCCA
     201  GCCTCCACAG CTCCTGATCT ATGAAGTTTC CAACCGGTTT TCTGGAGTGC
10    251  CAGACAGGTT CAGTGGCAGC GGGTCAGGGA CAGATTTTCA ACTGAAAATC
     301  AGCCGGGTGG AGGCTGAGGA TGTGGGGTT TATTACTGCA TGCAAAGTAT
     351  ACAGCTTATG TGCAGTTTTC GCCAGGGGAC CAAGCTGGAG ATCAAACGAA
     401  CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG
     451  AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT TCTATCCCAG
15    501  AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT
     551  CCCAGGAGAG TGTACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC
     601  AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA
     651  CGCCTGCGAA GTCACCCATC AGGGCCTGAG CTCGCCCCGTC ACAAAGAGCT
     701  TCAACAGGGG AGAGTGTTAG TGA
20

```

SEQ ID NO. 24

6.77.1-mod Predicted Kappa Light Chain Amino Acid Sequence

```

25      1  mrlpagllgl lmlwipgssa DIVMTQTPLS LSVTPGQPAS ISCKSSQSLI
      51  LSDGKTYLNW YLQKPGQPPQ LLIYEVSNR FSGVPDRFSGS GSGTDFTLKI
     101  SRVEAEDVGV YSCMQSIQLM SSFGQGTKLE IKRTVAAPSV FIFPPSDEQL
     151  KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDYSTYSL
     201  SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC
30

```

SEQ ID NO. 25

7.16.6 Heavy Chain Nucleotide Sequence

```

1   atggactgga cctggagcat ccttttcttg gtggcagcag caacaggtgc
5   51   ccactccCAG GTTCAGCTGG TGCAGTCTGG AGCTGAGGTG AAGAAGCCTG
    101  GGGCCTCAGT GAAGGTCTCC TGCAAGGCTT CTGGTTACAC CTTTACCAGC
    151  TATGGTATCA ACTGGGTGCG ACAGGCCCCCT GGACAAGGGC TTGAGTGGAT
    201  GGGATGGATC AGCGTTTACA GTGGTAACAC AAACATATGCA CAGAAGGTCC
    251  AGGGCAGAGT CACCATGACC GCAGACACAT CCACGAGCAC AGCCTACATG
10   301  GACCTGAGGA GCCTGAGATC TGACGACACG GCCGTGTATT ACTGTGCGAG
    351  AGAGGGTAGC AGCTCGTCCG GAGACTACTA TTACGGTATG GACGTCTGGG
    401  GCCAAGGGAC CACGGTCACC GTCTCCTCAG CCTCCACCAA GGGCCCATCG
    451  GTCTTCCCCC TGGCGCCCTG CTCCAGGAGC ACCTCCGAGA GCACAGCGGC
    501  CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCAGGTG ACGGTGTCGT
15   551  GGAACCTCAG CGCTCTGACC AGCGGCGTGC ACACCTTCCC AGCTGTCCTA
    601  CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG
    651  CAACCTCGGC ACCCAGACCT ACACCTGCAA CGTAGATCAC AAGCCCAGCA
    701  ACACCAAGGT GGACAAGACA GTTGAGCGCA AATGTTGTGT CGAGTGCCCA
    751  CCGTGCCAG CACCACCTGT GGCAGGACCG TCAGTCTTCC TCTTCCCCCC
20   801  AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACGTGCG
    851  TGGTGGTGGA CGTGAGCCAC GAAGACCCCG AGGTCCAGTT CAACTGGTAC
    901  GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAAGCCAC GGGAGGAGCA
    951  GTTCAACAGC ACGTTCCGTG CGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGGCCTC
25   1001  ACTGGCTGAA CGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGGCCTC
    1051  CCAGCCCCCA TCGAGAAAAC CATCTCCAAA ACCAAAGGGC AGCCCCGAGA
    1101  ACCACAGGTG TACACCCTGC CCCCATCCCG GGAGGAGATG ACCAAGAACC
    1151  AGGTACAGCT GACCTGCCTG GTCAAAGGCT TCTACCCAG CGACATCGCC
    1201  GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAACTACA AGACCACACC
    1251  TCCCATGCTG GACTCCGACG GCTCCTTCTT CCTCTACAGC AAGCTCACCG
30   1301  TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
    1351  CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
    1401  GGGTAAATGA

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35

SEQ ID NO. 26

7.16.6 Predicted Heavy Chain Protein Sequence

```

1   mdwtwsilfl vaaatgahsQ VQLVQSGAEV KKPGASVKVS CKASGYTFTS
40   51   YGINWVRQAP GQGLEWMGWI SVYSGNTNYA QKVQGRVTMT ADTSTSTAYM
    101  DLRLSLRSDDT AVYYCAREGS SSSGDYYYGM DVWGQGTITV VSSASTKGPS
    151  VFPLAPCSRS TSESTAALGC LVKDYPPEPV TVSWNSGALT SGVHTFPAVL
    201  QSSGLYSLSV VVTVPSSNFG TOTYTCNVDH KPSNTKVDKT VERKCCVECP
    251  PCPAPPVAGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVQFNWY
45   301  VDGVEVHNAK TKPREEQFNS TFRVSVLTV VHQDWLNGKE YKCKVSNKGL
    351  PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
    401  VEWESNGQPE NNYKTTTPML DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
    451  HEALHNHYTQ KSLSLSPGK

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SEQ ID NO: 27

7.16.6_L Heavy Chain Nucleotide Sequence

```

1      atggactgga cctggagcat ccttttcttg gtggcagcag caacaggtgc
5      51      ccactccCAG GTTCAGCTGG TGCAGTCTGG AGCTGAGGTG AAGAAGCCTG
      101      GGGCCTCAGT GAAGGTCTCC TGCAAGGCTT CTGGTTACAC CTTTACCAGC
      151      TATGGTATCA ACTGGGTGCG ACAGGCCCTT GGACAAGGGC TTGAGTGGAT
      201      GGGATGGATC AGCGTTTACA GTGGTAACAC AAATATGCA CAGAAGCTTC
      251      AGGGCAGAGT CACCATGACC GCAGACACAT CCACGAGCAC AGCCTACATG
10     301      GACCTGAGGA GCCTGAGATC TGACGACACG GCCGTGTATT ACTGTGCGAG
      351      AGAGGGTAGC AGCTCGTCCG GAGACTACTA TTACGGTATG GACGTCCTGGG
      401      GCCAAGGGAC CACGGTCACC GTCTCCTCAG CCTCCACCAA GGGCCCATCG
      451      GTCTTCCCCC TGGCGCCCTG CTCCAGGAGC ACCTCCGAGA GCACAGCGGC
      501      CTCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACC GGTTGTCGT
15     551      GGAAGTCAGG CGCTCTGACC AGCGGCGTGC ACACCTTCCC AGCTGTCCTA
      601      CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG
      651      CAAGTTCGGC ACCCAGACCT ACACCTGCAA CGTAGATCAC AAGCCCAGCA
      701      ACACCAAGGT GGACAAGACA GTTGAGCGCA AATGTTGTGT CGAGTGCCCA
      751      CCGTGCCCGA CACCACCTGT GGCAGGACCG TCAGTCTTCC TCTTCCCCCC
20     801      AAAACCCAAG GACACCCTCA TGATCTCCCG GACCCCTGAG GTCACGTGCG
      851      TGGTGGTGGG CGTGAGCCAC GAAGACCCCG AGGTCCAGTT CAACTGGTAC
      901      GTGGACGGCG TGGAGGTGCA TAATGCCAAG ACAAGCCAC GGGAGGAGCA
      951      GTTCAACAGC ACGTTCCGTG TGGTCAGCGT CCTCACCGTT GTGCACGAG
25     1001     ACTGGCTGAA CGGCAAGGAG TACAAGTGCA AGGTCTCCAA CAAAGGCCTC
      1051     CCAGCCCCCA TCGAGAAAAC CATCTCCAAA ACCAAAGGGC AGCCCCGAGA
      1101     ACCACAGGTG TACACCCTGC CCCCATCCCG GGAGGAGATG ACCAAGAACC
      1151     AGGTGAGCCT GACCTGCCTG GTCAAAGGCT TCTACCCAG CGACATCGCC
      1201     GTGGAGTGGG AGAGCAATGG GCAGCCGGAG AACAAC TACA AGACCACACC
      1251     TCCCATGCTG GACTCCGACG GCTCCTTCTT CCTCTACAGC AAGCTCACCG
30     1301     TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
      1352     CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCCTCT CCCTGTCTCC
      1401     GGGTAAATGA

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35

SEQ ID NO. 28

7.16.6_L Predicted Heavy Chain Protein Sequence

```

1      mdwtwsilfl vaaatgahsQ VQLVQSGAEV KKPGASVKVS CKASGYTFTS
40     51      YGINWVRQAP GQGLEWMGWI SVYSGNTNYA QKLQGRVTMT ADTSTSTAYM
      101      DLRLSRSDDT AVYYCAREGS SSSGDYYYGM DVWGQGT'TVT VSSASTKGPS
      151      VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
      201      QSSGLYSLSS VVTVPSSNFG TQTYTCNVDH KPSNTKVDKT VERKCCVECP
25     251     PCPAPPVAGP SVFLFPPKPK DTLNISRTPE VTCVVVDVSH EDPEVQFNWY
45     301      VDGVEVHNAK TKPREEQFNS TFRVVSIVLT VHQDWLNGKE YKCKVSNKGL
      351      PAPIEKTISK TKGQPREPQV YTLPPSREEM TKNQVSLTCL VKGFYPSDIA
      401      VEWESNGQPE NNYKTTTPML DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
      452      HEALHNHYTQ KSLSLSPGK

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SEQ ID NO: 29

7.16.6_V Kappa Light Chain Nucleotide Sequence

```

5      1   atgagggctcc ctgctcagct cctgggggctg ctaatgctct ggatacctgg
      51   atccagtgca GATATTGTGA TGACCCAGAC TCCACTCTCT CTGTCCGTCA
     101   CCCCTGGACA GCCGGCCTCC ATCTCCTGCA AGTCTAGTCA GAGCCTCCTG
     151   CATACTGATG GAACGACCTA TTTGTATTGG TACCTGCAGA AGCCAGGCCA
     201   GCCTCCACAG CTCCTGATCT ATGAAGTTTC CAACCGGTTT TCTGGAGTGC
10    251   CAGATAGGTT CAGTGGCAGC GGGTCAGGGA CAGATTTTCA ACTGAAAATC
     301   AGCCGGGTGG AGGCTGAGGA TGTGGGGTT TATTACTGCA TGCAAAATAT
     351   ACAGCTTCCG TGGACGTTCC GCCAAGGGAC CAAGGTGGAA ATCAAACGAA
     401   CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG
     451   AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT TCTATCCCAG
15    501   AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT
     551   CCCAGGAGAG TGTCACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC
     601   AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA
     651   CGCCTGCGAA GTCACCCATC AGGGCCTGAG CTCGCCCCGTC ACAAAGAGCT
     701   TCAACAGGGG AGAGTGTTAG TGA
20

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SEQ ID NO: 30

7.16.6_V Predicted Kappa Light Chain Protein Sequence

```

      1   mrlpaqlgl lmlwipgssa DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL
     51   HTDGTTYLYW YLQKPGQPPQ LLIYEVS NRF SGVPDRFSGS GSGTDFTLKI
25    101   SRVEAEDVG V YYCMQNIQLP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL
     151   KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL
     201   SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC

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SEQ ID NO: 31

7.16.6_S Kappa Light Chain Nucleotide Sequence

```

      1   atgaggctcc ctgctcagct cctgggggctg ctaatgctct ggatacctgg
5      51   atccagtgcg GATATTGTGA TGACCCAGAC TCCACTCTCT CTGTCCGTCA
      101  CCCCTGGACA GCCGGCCTCC ATCTCCTGCA AGTCTAGTCA GAGCCTCCTG
      151  CATACTGATG GAACGACCTA TTTGTATTGG TACCTGCAGA AGCCAGGCCA
      201  GCCTCCACAG CTCCTGATCT ATGAAGTTTC CAACCGGTTT TCTGGAGTGC
10     251  CAGATAGGTT CAGTGGCAGC GGGTCAGGGA CAGATTTTCA ACTGAAAAATC
      301  AGCCGGGTGG AGGCTGAGGA TGTGGGATT TATTACTGCA TGCAAAGTAT
      351  ACAGCTTCCG TGGACGTTCT GCCAAGGGAC CAAGGTGGAA ATCAAACGAA
      401  CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG
      451  AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT TCTATCCCAG
      501  AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT
15     551  CCCAGGAGAG TGTACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC
      601  AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA
      651  CGCCTGCGAA GTCACCCATC AGGGCCTGAG CTCGCCCCGTC ACAAAGAGCT
      701  TCAACAGGGG AGAGTGTTAG TGA

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20 SEQ ID NO: 32

7.16.6_S Predicted Kappa Light Chain Protein Sequence

```

      1   mrlpaqlllgl lmlwipgssa DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL
      51   HTDGTITYLYW YLQKPGQPPQ LLIYEVSNR FSGVPDRFSGS GSGTDFTLKI
      101  SRVEAEDVGI YYCMQSIQLP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL
25     151  KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL
      201  SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC

```

SEQ ID NO: 33

7.16.6_VS Kappa Light Chain Nucleotide Sequence

```

1   atgaggctcc ctgctcagct cctggggctg ctaatgctct ggatacctgg
5   51   atccagtgca GATATTGTGA TGACCCAGAC TCCACTCTCT CTGTCCGTCA
    101  CCCCTGGACA GCCGGCCTCC ATCTCCTGCA AGTCTAGTCA GAGCCTCCTG
    151  CATACTGATG GAACGACCTA TTTGTATTGG TACCTGCAGA AGCCAGGCCA
    201  GCCTCCACAG CTCCTGATCT ATGAAGTTTC CAACCGGTTT TCTGGAGTGC
    251  CAGATAGGTT CAGTGGCAGC GGGTCAGGGA CAGATTTTAC ACTGAAAATC
10   301  AGCCGGGTGG AGGCTGAGGA TGTGGGGTT TATTACTGCA TGCAAAGTAT
    351  ACAGCTTCCG TGGACGTTCC GCCAAGGGAC CAAGGTGGAA ATCAAACGAA
    401  CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG
    451  AAATCTGGAA CTGCCTCTGT TGTGTGCCTG CTGAATAACT TCTATCCCAG
    501  AGAGGCCAAA GTACAGTGGG AGGTGGATAA CGCCCTCCAA TCGGGTAACT
15   551  CCCAGGAGAG TGTACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC
    601  AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA
    651  CGCCTGCGAA GTCACCCATC AGGGCCTGAG CTCGCCCGTC ACAAAGAGCT
    701  TCAACAGGGG AGAGTGTAG TGA

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20

SEQ ID NO: 34

7.16.6_VS Predicted Kappa Light Chain Protein Sequence

```

1   mrlpaqllg lmlwipgssa DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL
25  51   HTDGTTYLYW YLQKPGQPPQ LLIYEVSNRFG SGVPDRFSGS GSGTDFTLKI
    101  SRVEAEDVGV YYCMQSIQLP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL
    151  KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL
    201  SSTLTLSKAD YEKHKVYACE VTHQGLSSPV TKSFNRGEC

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30

SEQ ID NO: 35

9.8.2_ARGAYH, D Heavy Chain Nucleotide Sequence

```

1   atggagtttg ggctgagctg ggttttcctc gttgctcttt taagaggtgt
5   51   ccagtgtCAG GTGCAGCTGG TGGAGTCTGG GGGAGGCGTG GTCCAGCCTG
    101  GGAGGTCCCT GAGACTCTCC TGTGCAGCGT CTGGATTACAC CTTCAGTAGC
    151  TATGGCATGC ACTGGGTCCG CCAGGCTCCA GGCAAGGGGC TGGAGTGGGT
    201  GGCAGTTATA TGGTATGATG GAAGTAATGA ATACTATGCA GACTCCGTGA
    251  AGGGCCGATT CACCATCTCC AGAGACAATT CCAAGAACAC GCTGTATCTG
10   301  CAAATGAACA GCCTGAGAGC CGAGGACACG GCTGTGTATT ACTGTGCG--
    351  ----- ---TTTGA CTGTTGCTCTT GGTGCTCTTT taagaggtgt
    401  CCTCAGCTTC CACCAAGGGC CCATCCGTCT TCCCCCTGGC GCCCTGCTCC
    451  AGGAGCACCT CCGAGAGCAC AGCCGCCCTG GGCTGCCTGG TCAAGGACTA
    501  CTTCCCCGAA CCGGTGACGG TGTCTGTGAA CTCAGGCGCC CTGACCAGCG
15   551  GCGTGCACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
    601  AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACGA AGACCTACAC
    651  CTGCAACGTA GATCACAAGC CCAGCAACAC CAAGGTGGAC AAGAGAGTTG
    701  AGTCCAAATA TGGTCCCCCA TGCCCATCAT GCCCAGCACC TGAGTTCCTG
    751  GGGGGACCAT CAGTCTTCCT GTTCCCCCA AAACCCAAGG AACTCTCAT
20   801  GATCTCCCGG ACCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCAGG
    851  AAGACCCCGA GGTCCAGTTC AACTGGTACG TGGATGGCGT GGAGGTGCAT
    901  AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTACCGTGT
    951  GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
25   1001 ACAAGTGCAA GGTCTCCAAC AAAGGCCTCC CGTCTCCAT CGAGAAAACC
    1051 ATCTCCAAAG CCAAAGGGCA GCCCCGAGAG CCACAGGTGT ACACCCTGCC
    1101 CCCATCCCAG GAGGAGATGA CCAAGAACCA GGTGAGCCTG ACCTGCCTGG
    1151 TCAAAGGCTT CTACCCAGC GACATCGCCG TGGAGTGGGA GAGCAATGGG
    1201 CAGCCGGAGA ACAACTACAA GACCACGCCT CCCGTGCTGG ACTCCGACGG
    1251 CTCCTTCTTC CTCTACAGCA GGCTAACCGT GGACAAGAGC AGGTGGCAGG
30   1301 AGGGGAATGT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCAC
    1351 TACACACAGA AGAGCCTCTC CCTGTCTCTG GGTAAATGA

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SEQ ID NO: 36

9.8.2_ARGAYH, D Predicted Heavy Chain Protein Sequence

```

35   1   mefglswvfl vallrgvqcQ VQLVESGGGV VQPGSRSLRLS CAASGFTFSS
    51   YGMHWVRQAP GKGLEWVAVI WYDGSNEYA DSVKGRFTIS RDNSKNTLYL
    101  QMNSLRAEDT AVYYCA---- -FDYWGQGT VTVSSASTKG PSVFPLAPCS
    151  RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
40   201  SSVVTVPSST LGTKYTCNV DHKPSNTKVD KRVESKYGPP CPSCPAPEFL
    251  GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSQEDPEVQF NWYVDGVEVH
    301  NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KGLPSSIEKT
    351  ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL TCLVKGFYPS DIAVEWESNG
    401  QPENNYKTP PVLDSDGSFF LYSRLTVDKS RWQEGNVFSC SVMHEALHNNH
45   451  YTQKSLSLSL GK

```

SEQ ID NO: 37

9.8.2-mod Kappa Light Chain Nucleotide Sequence

```

1   atggacatga gggtcctgc tcagtcctg gggtcctgc tgctctggct
5   51   ctgagtcgca ggtgccagat gtGACATCCA GATGACCCAG TCTCCATCCT
    101  CCCTGTCTGC ATCTGTAGGA GACAGAGTCA CCATCACTTG CCAGGCGAGT
    151  CAGGACATTA GCAACTATTT AAATTGGTAT CAGCAGAAAC CAGGGAAAGC
    201  CCCTAAGCTC CTGATCTACG ATGCATCCAA TTTGGAAACA GGGGTCCCAT
    251  CAAGGTTTCA TGGAAGTGGA TCTGGGACAG ATTTTACTTT CACCATCAGC
    301  AGCCTGCAGC CTGAAGATAT TGCAACATAT TCCTGTCAAC ACTCTGATAA
10  351  TCTCATCACC TTCGGCCAGG GGACACGACT GGAGATTAAA CGAACTGTGG
    401  CTGCACCATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA GTTGAAATCT
    451  GGAAGTGCCT CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC
    501  CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG
    551  AGAGTGTACAG AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC
15  601  ACCCTGACGC TGAGCAAGGC AGACTACGAG AAACACAAAG TCTACGCCTG
    651  CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA
    701  GGGGAGAGTG TTAGTGA

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SEQ ID NO: 38

9.8.2-mod Predicted Kappa Light Chain Protein Sequence

```

25  1   mdmrvpaql1 gl111wlsva garcDIQMTQ SPSSLSASVG DRVTITCQAS
    51   QDISNYLNWY QQKPGKAPKL LIYDASNLET GVPSRFSGSG SGTDFTFITIS
    101  SLQPEDIATY SCQHSDNLIT FGQGTRLEIK RTVAAPSVFI FPPSDEQLKS
    151  GTASVVCLLN NFYPREAKVQ WKVDNALQSG NSQESVTEQD SKDSTYSLSS
    201  TLTLISKADYE KHKVYACEVT HQGLSSPVTK SFNRGEC

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30

Claims

1. A monoclonal antibody that specifically binds MAdCAM, or antigen-binding portion thereof, selected from the group consisting of:
 - 5 (a) an antibody comprising the variable region of the heavy chain of 6.22.2-mod_V (SEQ ID NO: 2);
 - (b) an antibody comprising the variable region of the light chain of 6.34.2-mod_SSQ, 6.34.2-mod_QT, or 6.34.2-mod_SSQ,QT (SEQ ID NOs: 8, 10, 12);
 - (c) an antibody comprising the variable region of the light chain of 6.67.1-mod_Y, 6.67.1-mod_TΔP, or 6.67.1-mod_Y,TΔP (SEQ ID NOs: 16, 18, 20);
 - 10 (d) an antibody comprising the variable region of the heavy chain of 6.77.1-mod_ΔS (SEQ ID NO: 22);
 - (e) an antibody comprising the variable region of the heavy chain of 7.16.6_L (SEQ ID NO: 28); or
 - (f) an antibody comprising the variable region of the light chain of 7.16.6_V, 7.16.6_S, or 7.16.6_VS (SEQ ID NOs: 30, 32, 34);
 - (g) an antibody comprising the variable region of the light chain of 7.16.6_V, 7.16.6_S, or 7.16.6_VS (SEQ ID NOs: 30, 32, 34) and the variable region of the heavy chain of 7.16.6_L (SEQ ID NO: 28); or
 - 15 (h) an antibody comprising the variable region of the heavy chain of 9.8.2_ΔRGAYH,D (SEQ ID NO: 36).
 - (i) an antibody comprising the variable region of the light chain of 9.8.2-mod (SEQ ID NO: 38).
2. The antibody or portion according to claim 1, selected from the group consisting of:
 - 20 (a) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 3;
 - (b) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NO: 5 and SEQ ID NOs: 7, 9 or 11;
 - (c) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in
 - 25 SEQ ID NO: 13 and SEQ ID NOs: 15, 17, or 19;
 - (d) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NO: 21 and SEQ ID NO: 23;
 - (e) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NOs: 25 or 27 and SEQ ID NOs: 29, 31, or 33; or
 - 30 (f) an antibody comprising the variable regions encoded by the nucleic acid sequences set forth in SEQ ID NO: 35 and SEQ ID NO: 37.
3. The monoclonal antibody or portion according to claim 1 or claim 2, wherein the antibody is selected from the group consisting of:
 - 35 (a) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 2 and SEQ ID NO: 4, without the signal sequences;
 - (b) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 6 and SEQ ID NOs: 8, 10, or 12, without the signal sequences;
 - (c) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO:
 - 40 14 and SEQ ID NOs: 16, 18, or 20, without the signal sequences;

- (d) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 22 and SEQ ID NO: 24, without the signal sequences;
- (e) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NOs: 26 or 28 and SEQ ID NOs: 30, 32 or 34, without the signal sequences; or
- 5 (f) an antibody comprising the variable regions of the amino acid sequences set forth in SEQ ID NO: 36 and SEQ ID NO: 38, without the signal sequences.
4. The antibody or portion according to any of claims 1 to 3, selected from the group consisting of:
- (a) an antibody encoded by the nucleic acid sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 3;
- 10 (b) an antibody encoded by the nucleic acid sequences set forth in SEQ ID NO: 5 and SEQ ID NOs: 7, 9 or 11;
- (c) an antibody encoded by the nucleic acid sequences set forth in SEQ ID NO: 13 and SEQ ID NOs: 15, 17, or 19;
- 15 (d) an antibody encoded by the nucleic acid sequences set forth in SEQ ID NO: 21 and SEQ ID NO: 23;
- (e) an antibody encoded by the nucleic acid sequences set forth in SEQ ID NOs: 25 or 27 and SEQ ID NOs: 29, 31, or 33; or
- (f) an antibody encoded by the nucleic acid sequences set forth in SEQ ID NO: 35 and SEQ ID NO: 37.
- 20 5. A monoclonal antibody or portion of any of claims 1 to 4, wherein the heavy chain C-terminal lysine is cleaved.
- 25 6. Use of the antibody or antigen-binding portion of any of claims 1 to 5 as a medicament.
7. Use of the antibody of any of claims 1 to 5 for the manufacture of a medicament for the treatment of a disease involving MAdCAM-mediated adhesion of leukocytes.
- 30 8. The use of claim 7, wherein the disease is an inflammatory disease.
9. The use of claim 7, wherein the disease is selected from inflammatory bowel disease, liver fibrosis, graft versus host disease, uveitis, encephalitis, insulin-dependent diabetes,
- 35 10. The use of claim 9, wherein inflammatory bowel disease is Crohn's disease or ulcerative colitis.
11. A pharmaceutical composition comprising the antibody or antigen binding portion of any of claims 1 to 5 and a pharmaceutically acceptable carrier or excipient.

12. A method of treating inflammatory disease in a subject in need thereof, comprising the step of administering to said subject the monoclonal antibody or antigen-binding portion thereof according to any of claims 1 to 5.