Antibodies to MadCam

The present invention relates to antibodies including human antibodies and antigen-binding portions thereof that specifically bind to MadCAM, preferably human MadCAM and that function to inhibit MadCAM. The invention also relates to human anti-MadCAM antibodies and antigen-binding portions thereof. The invention also relates to antibodies that are chimeric, bispecific, derivatized, single chain antibodies or portions of fusion proteins. The invention also relates to isolated heavy and light chain immunoglobulins derived from human anti-MadCAM antibodies and nucleic acid molecules encoding such immunoglobulins. The present invention also relates to methods of making human anti-MadCAM antibodies, compositions comprising these antibodies and methods of using the antibodies and compositions for diagnosis and treatment. The invention also provides gene therapy methods using nucleic acid molecules encoding the heavy and/or light immunoglobulin molecules that comprise the human anti-MadCAM antibodies. The invention also relates to transgenic animals or plants comprising nucleic acid molecules of the invention.
ANTIBODIES TO MAdCAM

[0001] This application claims the benefit of United States Provisional Application 60/535,490, filed January 9, 2004.

BACKGROUND OF THE INVENTION

[0002] Mucosal addressin cell adhesion molecule (MAdCAM) is a member of the immunoglobulin superfamily of cell adhesion receptors. The selectivity of lymphocyte homing to specialized lymphoid tissue and mucosal sites of the gastrointestinal tract is determined by the endothelial expression of MAdCAM (Berlin, C. et al., Cell, 80:413-422(1994); Berlin, C., et al., Cell, 74:185-195 (1993); and Erle, D.J., et al., J. Immunol., 153: 517-528 (1994)). MAdCAM is uniquely expressed on the cell surface of high endothelial venules of organized intestinal lymphoid tissue, such as Peyer’s patches and mesenteric lymph nodes (Streeter et al., Nature, 331:41-6 (1988); Nakache et al., Nature, 337:179-81 (1989); Briskin et al., Am. J. Pathol. 151:97-110 (1997)), but also in other lymphoid organs, such as pancreas, gall bladder and splenic venules and marginal sinus of the splenic white pulp (Briskin et al(1997), supra; Kraal et al., Am. J. Path., 147: 763-771 (1995)).

[0003] While MAdCAM plays a physiological role in gut immune surveillance, it appears to facilitate excessive lymphocyte extravasation in inflammatory bowel disease under conditions of chronic gastrointestinal tract inflammation. TNFα and other pro-inflammatory cytokines increase endothelial MAdCAM expression and, in biopsy specimens taken from patients with Crohn’s disease and ulcerative
coliitis, there is an approximate 2-3 fold focal increase in MAdCAM expression at sites of inflammation (Briskin et al. (1997), Souza et al., Gut, 45:856-63 (1999); Arihiro et al., Pathol Int., 52:367-74 (2002)). Similar patterns of elevated expression have been observed in experimental models of colitis (Hesterberg et al., Gastroenterology, 111:1373-1380 (1997); Picarella et al., J. Immunol., 158: 2099-2106 (1997); Connor et al., J Leukoc Biol., 65:349-55 (1999); Kato et al., J Pharmacol Exp Ther., 295:183-9 (2000); Hokari et al., Clin Exp Immunol., 26:259-65 (2001); Shigematsu et al., Am J Physiol Gastrointest Liver Physiol., 281:G1309-15 (2001)). In other pre-clinical models for inflammatory conditions, such as insulin-dependent diabetes (Yang et al. Diabetes, 46:1542-7 (1997); Hänninen et al., J Immunol., 160:6018-25 (1998)), graft versus host disease (Fujisaki et al., Scand J Gastroenterol., 38:437-42 (2003), Murai et al., Nat Immunol., 4:154-60 (2003)), chronic liver disease (Hillan et al., Liver, 19:509-18 (1999); Grant et al., Hepatology, 33:1065-72 (2001)), inflammatory encephalopathy (Stalder et al., Am J Pathol., 153:767-83 (1998); Kanawar et al., Immunol Cell Biol., 78:641-5 (2000)), and gastritis (Barrett et al., J Leukoc Biol., 67:169-73 (2000); Hatanaka et al., Clin Exp Immunol., 130:183-9 (2002)), there is also reawakening of fetal MAdCAM expression and participation of activated α4β7+ lymphocytes in disease pathogenesis. In these inflammatory models as well as hapten-mediated (e.g., TNBS, DSS, etc.) or adoptive transfer (CD4+CD45Rbhigh) mouse colitic models, the rat anti-mouse MAdCAM monoclonal antibody (mAb), MECA-367, which blocks the binding of α4β7+ lymphocytes to MAdCAM, reduces the lymphocyte recruitment, tissue extravasation, inflammation and disease severity. Mouse monoclonal antibodies (mAbs) against human MAdCAM also have been reported (see, e.g., WO 96/24673 and WO 99/58573).

[0004] Given the role of MAdCAM in inflammatory bowel disease (IBD) and other inflammatory diseases associated with the gastrointestinal tract or other tissues, a means for inhibiting α4β7 binding and MAdCAM-mediated leukocyte recruitment is desirable. It further would be desirable to have such therapeutic means with advantageous properties including but not limited to the absence of unwanted interactions with other medications in patients and favorable physicochemical properties such as pK/pD values in humans, solubility, stability, shelf-life.
and in vivo half-life. A therapeutic protein, such as an antibody, would advantageously be free of unwanted post-translational modifications or aggregate formation. Accordingly, there is a critical need for therapeutic anti-MAdCAM antibodies.

SUMMARY OF THE INVENTION

[0005] The present invention provides an isolated antibody that specifically binds MAdCAM, wherein at least the CDR sequences of said antibody are human CDR sequences, or an antigen-binding portion of said antibody. In some embodiments the antibody is a human antibody, preferably an antibody that acts as a MAdCAM antagonist. Also provided are recombinations comprising said antibodies or portions.

[0006] The invention also provides a composition comprising the heavy and/or light chain of said anti-MAdCAM antagonist antibody or the variable region or other antigen-binding portion thereof or nucleic acid molecules encoding any of the foregoing and a pharmaceutically acceptable carrier. Compositions of the invention may further comprise another component, such as a therapeutic agent or a diagnostic agent. Diagnostic and therapeutic methods are also provided by the invention.

[0007] The invention further provides an isolated cell line, that produces said anti-MAdCAM antibody or antigen-binding portion thereof.

[0008] The invention also provides nucleic acid molecules encoding the heavy and/or light chain of said anti-MAdCAM antibody or the variable region thereof or antigen-binding portion thereof.

[0009] The invention provides vectors and host cells comprising said nucleic acid molecules, as well as methods of recombinantly producing the polypeptides encoded by the nucleic acid molecules.

[0010] Non-human transgenic animals or plants that express the heavy and/or light chain of said anti-MAdCAM antibody, or antigen-binding portion thereof, are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1 is an alignment of the predicted amino acid sequences of the heavy and kappa light chain variable regions of twelve human anti-MAdCAM
monoclonal antibodies with the germline amino acid sequences of the

[0012] Figure 1A shows an alignment of the predicted amino acid sequence of
the heavy chain for antibodies 1.7.2 and 1.8.2 with the germline human VH 3-15
gene product.

[0013] Figure 1B shows an alignment of the predicted amino acid sequence of
the heavy chain for antibody 6.14.2 with the germline human VH 3-23 gene
product.

[0014] Figure 1C shows an alignment of the predicted amino acid sequence of
the heavy chain for antibody 6.22.2 with the germline human VH 3-33 gene
product.

[0015] Figure 1D shows an alignment of the predicted amino acid sequence of
the heavy chain for antibody 6.34.2 with the germline human VH 3-30 gene
product.

[0016] Figure 1E shows an alignment of the predicted amino acid sequence of
the heavy chain for antibody 6.67.1 with the germline human VH 4-4 gene product.

[0017] Figure 1F shows an alignment of the predicted amino acid sequence of
the heavy chain for antibody 6.73.2 with the germline human VH 3-23 gene
product.

[0018] Figure 1G shows an alignment of the predicted amino acid sequence of
the heavy chain for antibody 6.77.1 with the germline human VH 3-21 gene
product.

[0019] Figure 1H shows an alignment of the predicted amino acid sequence of
the heavy chain for antibodies 7.16.6 and 7.26.4 with the germline human VH 1-18
gene product.

[0020] Figure 1I shows an alignment of the predicted amino acid sequence of the
heavy chain for antibody 7.20.5 with the germline human VH 4-4 gene product.

[0021] Figure 1J shows an alignment of the predicted amino acid sequence of the
heavy chain for antibody 9.8.2 with the germline human VH 3-33 gene product.

[0022] Figure 1K shows an alignment of the predicted amino acid sequence of
the light kappa chain for antibodies 1.7.2 and 1.8.2 with the germline human A3
gene product.
[0023] Figure 1L shows an alignment of the predicted amino acid sequence of the kappa light chain for antibody 6.14.2 with the germline human O12 gene product.

[0024] Figure 1M shows an alignment of the predicted amino acid sequence of the kappa light chain for antibody 6.22.2 with the germline human A26 gene product.

[0025] Figure 1N shows an alignment of the predicted amino acid sequence of the kappa light chain for antibody 6.34.2 with the germline human O12 gene product.

[0026] Figure 1O shows an alignment of the predicted amino acid sequence of the kappa light chain for antibody 6.67.1 with the germline human B3 gene product.

[0027] Figure 1P shows an alignment of the predicted amino acid sequence of the kappa light chain for antibody 6.73.2 with the germline human O12 gene product.

[0028] Figure 1Q shows an alignment of the predicted amino acid sequence of the kappa light chain for antibody 6.77.1 with the germline human A2 gene product.

[0029] Figure 1R shows an alignment of the predicted amino acid sequence of the kappa light chain for antibodies 7.16.6 and 7.26.4 with the germline human A2 gene product.

[0030] Figure 1S shows an alignment of the predicted amino acid sequence of the kappa light chain for antibody 7.20.5 with the germline human A3 gene product.

[0031] Figure 1T shows an alignment of the predicted amino acid sequence of the kappa light chain for antibody 9.8.2 with the germline human O18 gene product.

[0032] Figure 2 are CLUSTAL alignments of the predicted heavy and kappa light chain amino acid sequences of human anti-MAdCAM antibodies.

[0033] Figure 2A is a CLUSTAL alignment and radial tree of the predicted kappa light chain amino acid sequences, showing the degree of similarity between the anti-MAdCAM antibody kappa light chains.
[0034] Figure 2B is a CLUSTAL alignment and radial tree of the predicted heavy amino acid sequences, showing the degree of similarity between the anti-MAdCAM antibody heavy chains.

[0035] Figure 3 is an amino acid sequence CLUSTAL alignment of the 2 N-terminal domains of cynomolgus and human MAdCAM which form the $\alpha_4\beta_7$ binding domain. The $\beta$-strands are aligned according to Tan et al., Structure (1998) 6:793-801.

[0036] Figure 4 is a graph representing the dose effects of purified biotinylated 1.7.2 and 7.16.6 on the adhesion of human peripheral blood lymphocytes to sections of MAdCAM-expressing frozen human liver endothelium.

[0037] Figure 5 shows a two dimensional graphical representation based on the data captured in Table 7 of the diversity of MAdCAM epitopes to which the anti-MAdCAM antibodies, 1.7.2, 6.22.2, 6.34.2, 6.67.1, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2 bind. Anti-MAdCAM antibodies within the same circle show the same reactivity pattern, belong in the same epitope bin and are likely to recognize the same epitope on MAdCAM. Anti-MAdCAM antibody clones within overlapping circles are unable to bind simultaneously and are, therefore, likely to recognize an overlapping epitope on MAdCAM. Non-integrating circles represent anti-MAdCAM antibody clones with distinct spatial epitope separation.

[0038] Figure 6 shows sandwich ELISA data with anti-MAdCAM antibodies 1.7.2 and an Alexa 488-labelled 7.16.6, showing that two antibodies that are able to detect different epitopes on MAdCAM could be used to detect soluble MAdCAM for diagnostic purposes.

[0039] Figure 7 shows the effect of blocking an inhibitory anti-MAdCAM antibody (1 mg/kg) on the number of circulating peripheral $\alpha_4\beta_7^+$ lymphocytes, expressed as a fold increase over control IgG2a mAb or vehicle, using anti-MAdCAM mAb 7.16.6 in a cynomolgus monkey model.
DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Techniques

[0040] 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.

[0041] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0042] The term “polypeptide” encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

[0043] 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.

[0044] 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.

[0045] 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.

[0046] 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 α₄β₇ 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.

[0047] Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, or (5) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al., Nature, 354:105 (1991), which are each incorporated herein by reference.

[0048] Non-peptide analogs are commonly used in the pharmaceutical industry as drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed “peptide mimetics” or “peptidomimetics”. Fauchere, J. Adv. Drug Res., 15:29(1986); Veber and Freidinger, TINS, p.392(1985); and Evans et al., J. Med. Chem., 30:1229(1987), which are incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally
similar to a paradigm polypeptide (i.e., a polypeptide that has a desired biochemical property or pharmacological activity), such as a human antibody, but have one or more peptide linkages optionally replaced by a linkage such as: –CH₂NH–, –CH₂S–, –CH₂CH₂–, –CH=CH– (cis and trans), –COCH₂–, –CH(OH)CH₂–, and –CH₂SO–, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may also be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992), incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[0049] 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.

[0050] 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.

An “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. Antibody-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antibody-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.

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
ecompasses antibodies which have been raised in a transgenic mouse which
comprises some or all of the human immunoglobulin heavy and light chain loci.

[0057] 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.

[0058] In another aspect, the invention includes 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.

[0059] A “neutralizing antibody,” “an inhibitory antibody” or antagonist
antibody is an antibody that inhibits the binding of α4β7 or α4β7-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 α4β7 integrin or α4β7-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 α4β7-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 ≤1 μM, preferably ≤100 nM and most preferably ≤10 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 linearally 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.

[0065] 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 carboxyterminal direction, in accordance with standard usage and convention.

[0066] 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.

[0067] 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.

[0068] 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 polymucleotide 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.


[0070] “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 polymucleotide 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.

[0071] 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.
[0072] 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.

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0081] 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.

[0082] 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., $^3$H, $^{14}$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, $\beta$-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.

[0083] The term “agent” is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. The term “pharmaceutical agent or drug” as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)), incorporated herein by reference.

[0084] The term “anti-inflammatory” or “immuno-modulatory” agent is used herein to refer to agents that have the functional property of inhibiting inflammation, including inflammatory disease in a subject, including in a human. In various embodiments of this invention, the inflammatory disease may be, but is not limited to inflammatory diseases of the gastrointestinal tract including Crohn’s disease, ulcerative colitis, diverticula disease, gastritis, liver disease, primary biliary 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, miliary dermatitis, miliary 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), chronic pancreatitis, perineural inflammation, colitis (including amoebic colitis, infective colitis, bacterial colitis, Crohn’s colitis, ischemic colitis, ulcerative colitis, idiopathic proctocolitis, inflammatory bowel disease, pseudomembranous
colitis), 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, exudative diarrhea, gastritis, granulomatous hepatitis, granulomatous inflammation, cholecystitis, insulin-dependent diabetes mellitus, liver inflammatory diseases (liver fibrosis primary biliary cirrhosis, hepatitis, sclerosing cholangitis), lung inflammation (idiopathic pulmonary fibrosis, eosinophilic granuloma of the lung, pulmonary histiocytosis X, peribronchial inflammation, acute bronchitis), lymphogranuloma venereum, malignant melanoma, mouth/tooth disease (including gingivitis, periodontal disease), 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, graft versus host disease, acute burn injury, Behçet’s syndrome, Sjögren’s syndrome.

[0085] The terms patient and subject include human and veterinary subjects.

Human Anti-MAdCAM Antibodies and Characterization Thereof

[0086] In one embodiment, the invention provides anti-MAdCAM antibodies comprising human CDR sequences. In a preferred embodiment, the invention provides human anti-MAdCAM antibodies. In some embodiments, human anti-MAdCAM antibodies are produced by immunizing a non-human transgenic animal, e.g., a rodent, whose genome comprises human immunoglobulin genes so that the transgenic animal produces human antibodies. In some embodiments, the invention provides an anti-MAdCAM antibody that does not bind complement.
[0087] In a preferred embodiment, the anti-MAdCAM antibody is 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the anti-MAdCAM antibody comprises a light chain comprising an amino acid sequence selected from SEQ ID NO: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 58, 62, 66 or 68 (with or without the signal sequence) or the variable region of any one of said amino acid sequences, or one or more CDRs from these amino acid sequences. In another preferred embodiment, the anti-MAdCAM antibody comprises a heavy chain comprising an amino acid sequence selected from SEQ ID NO: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 52, 56, 60 or 64 (with or without the signal sequence) or the amino acid sequence of the variable region, or of one or more CDRs from said amino acid sequences. Also included in the invention are human anti-MAdCAM antibodies comprising the amino acid sequence from the beginning of the CDR1 to the end of the CDR3 of any one of the above-mentioned sequences. The invention further provides an anti-MAdCAM antibody comprising one or more FR regions of any of the above-mentioned sequences.

[0088] The invention further provides an anti-MAdCAM antibody comprising one of the afore-mentioned amino acid sequences in which one or more modifications have been made. In some embodiments, cysteines in the antibody, which may be chemically reactive, are substituted with another residue, such as, without limitation, alanine or serine. In one embodiment, the substitution is at a non-canonical cysteine. The substitution can be made in a CDR or framework region of a variable domain or in the constant domain of an antibody. In some embodiments, the cysteine is canonical.

[0089] In some embodiments, an amino acid substitution is made to eliminate potential proteolytic sites in the antibody. Such sites may occur in a CDR or framework region of a variable domain or in the constant domain of an antibody. Substitution of cysteine residues and removal of proteolytic sites may decrease the heterogeneity in the antibody product. In some embodiments, asparagine-glycine pairs, which form potential deamination sites, are eliminated by altering one or both of the residues. In some embodiments, an amino acid substitution is made to
add or to remove potential glycosylation sites in the variable region of an antibody
of the invention.

[0090] In some embodiments, the C-terminal lysine of the heavy chain of the
anti-MAdCAM antibody of the invention is cleaved. In various embodiments of
the invention, the heavy and light chains of the anti-MAdCAM antibodies may
optionally include a signal sequence.

[0091] In one aspect, the invention provides twelve inhibitory human anti-
MAdCAM monoclonal antibodies and the hybridoma cell lines that produce them.
Table 1 lists the sequence identifiers (SEQ ID NO:) of the nucleic acids encoding
the full-length heavy and light chains (including signal sequence), and the
corresponding full-length deduced amino acid sequences.

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>HUMAN ANTI-MAdCAM ANTIBODIES</th>
<th>SEQUENCE IDENTIFIER (SEQ ID NO:)</th>
<th>Full Length</th>
<th>Heavy DNA</th>
<th>Heavy Protein</th>
<th>Light DNA</th>
<th>Light Protein</th>
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<td></td>
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<td>46</td>
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</tbody>
</table>

[0092] In another aspect, the invention provides a modified version of certain of
the above-identified human anti-MAdCAM monoclonal antibodies. Table 2 lists
the sequence identifiers for the DNA and protein sequences of the modified antibodies.

Table 2

<table>
<thead>
<tr>
<th>Modified Monoclonal Antibody</th>
<th>HUMAN ANTI-MAdCAM ANTIBODIES</th>
<th>SEQUENCE IDENTIFIER (SEQ ID NO.:)</th>
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<tr>
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<td></td>
<td>Full Length</td>
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<tr>
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<td>Protein DNA</td>
</tr>
<tr>
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</tr>
<tr>
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<td>56</td>
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<tr>
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<tr>
<td>7.26.4-mod</td>
<td>41</td>
<td>42</td>
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</tbody>
</table>

Class and Subclass of anti-MAdCAM Antibodies

[0093] The antibody may be an IgG, an IgM, an IgE, an IgA or an IgD molecule. In a preferred embodiment, the antibody is an IgG class and is an IgG1, IgG2, IgG3 or IgG4 subclass. In a more preferred embodiment, the anti-MAdCAM antibody is subclass IgG2 or IgG4. In another preferred embodiment, the anti-MAdCAM antibody is the same class and subclass as antibody 1.7.2, 1.8.2, 7.16.6, 7.20.5, 7.26.4, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod which is IgG2, or 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1 or 9.8.2, which is IgG4.

[0094] The class and subclass of anti-MAdCAM antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are available commercially. ELISA, Western Blot as well as other techniques can determine the class and subclass. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various classes and subclasses of immunoglobulins, and determining the class and subclass of the antibodies as the class showing the highest sequence identity.
Species and Molecule Selectivity

[0095] In another aspect of the invention, the anti-MAdCAM antibody demonstrates both species and molecule selectivity. In one embodiment, the anti-MAdCAM antibody binds to human, cynomolgus or dog MAdCAM. In some embodiments, the anti-MAdCAM antibody does not bind to a New World monkey species such as a marmoset. Following the teachings of the specification, one may determine the species selectivity for the anti-MAdCAM antibody using methods well known in the art. For instance, one may determine species selectivity using Western blot, FACS, ELISA or immunohistochemistry. In a preferred embodiment, one may determine the species selectivity using immunohistochemistry.

[0096] In some embodiments, an anti-MAdCAM antibody that specifically binds MAdCAM has selectivity for MAdCAM over VCam, fibronectin or any other antigen that is at least 10 fold, preferably at least 20, 30, 40, 50, 60, 70, 80 or 90 fold, most preferably at least 100 fold. In a preferred embodiment, the anti-MAdCAM antibody does not exhibit any appreciable binding to VCam, fibronectin or any other antigen other than MAdCAM. One may determine the selectivity of the anti-MAdCAM antibody for MAdCAM using methods well known in the art following the teachings of the specification. For instance, one may determine the selectivity using Western blot, FACS, ELISA, or immunohistochemistry.

Binding Affinity of anti-MAdCAM antibodies to MAdCAM

[0097] In another aspect of the invention, the anti-MAdCAM antibodies specifically bind to MAdCAM with high affinity. In one embodiment, the anti-MAdCAM antibody specifically binds to MAdCAM with a $K_d$ of $3 \times 10^{-8}\text{M}$ or less, as measured by surface plasmon resonance, such as BIACore. In more preferred embodiments, the antibody specifically binds to MAdCAM with a $K_d$ of $1 \times 10^{-8}$ or less or $1 \times 10^{-9}\text{M}$ or less. In an even more preferred embodiment, the antibody specifically binds to MAdCAM with a $K_d$ of $1 \times 10^{-10}\text{M}$ or less. In other preferred embodiments, an antibody of the invention specifically binds to MAdCAM with a $K_d$ of $2.66 \times 10^{-10}\text{M}$ or less, $2.35 \times 10^{-11}\text{M}$ or less or $9 \times 10^{-12}\text{M}$
or less. In another preferred embodiment, the antibody specifically binds to MAdCAM with a $K_d$ or $1 \times 10^{-11}$ M or less. In another preferred embodiment, the antibody specifically binds to MAdCAM with substantially the same $K_d$ as an antibody selected from 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. An antibody with "substantially the same $K_d$" as a reference antibody has a $K_d$ that is $\pm$ 100 pM, preferably $\pm$ 50 pM, more preferably $\pm$ 20 pM, still more preferably $\pm$ 10 pM, $\pm$ 5 pM or $\pm$ 2 pM, compared to the $K_d$ of the reference antibody in the same experiment. In another preferred embodiment, the antibody binds to MAdCAM with substantially the same $K_d$ as an antibody that comprises one or more variable domains or one or more CDRs from an antibody selected from 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the antibody binds to MAdCAM with substantially the same $K_d$ as an antibody that comprises one of the amino acid sequences selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 52, 54, 56, 58, 62, 64, 66 or 68 (with or without the signal sequence), or the variable domain thereof. In another preferred embodiment, the antibody binds to MAdCAM with substantially the same $K_d$ as an antibody that comprises one or more CDRs from an antibody that comprises an amino acid sequence selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 52, 54, 56, 58, 62, 64, 66 or 68.

The binding affinity of an anti-MAdCAM antibody to MAdCAM may be determined by any method known in the art. In one embodiment, the binding affinity can be measured by competitive ELISAs, RIAs or surface plasmon resonance, such as BIAcore. In a more preferred embodiment, the binding affinity is measured by surface plasmon resonance. In an even more preferred embodiment, the binding affinity and dissociation rate is measured using a BIAcore. An example of determining binding affinity is described below in Example II.
Half-Life of Anti-MAdCAM Antibodies

[0099] According to another object of the invention, the anti-MAdCAM antibody has a half-life of at least one day in vitro or in vivo. In a preferred embodiment, the antibody or portion thereof has a half-life of at least three days. In a more preferred embodiment, the antibody or portion thereof has a half-life of four days or longer. In another embodiment, the antibody or portion thereof has a half-life of eight days or longer. In another embodiment, the antibody or antigen-binding portion thereof is derivatized or modified such that it has a longer half-life, as discussed below. In another preferred embodiment, the antibody may contain point mutations to increase serum half life, such as described WO 00/09560, published February 24, 2000.

[0100] The antibody half-life may be measured by any means known to one having ordinary skill in the art. For instance, the antibody half life may be measured by Western blot, ELISA or RIA over an appropriate period of time. The antibody half-life may be measured in any appropriate animal, such as a primate, e.g., cynomolgus monkey, or a human.

Identification of MAdCAM Epitopes Recognized by Anti-MAdCAM Antibody

[0101] The invention also provides a human anti-MAdCAM antibody that binds the same antigen or epitope as a human anti-MAdCAM antibody provided herein. Further, the invention provides a human anti-MAdCAM antibody that competes or cross-competes with a human anti-MAdCAM antibody. In a preferred embodiment, the human anti-MAdCAM antibody is 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the human anti-MAdCAM antibody comprises one or more variable domains or one or more CDRs from an antibody selected from 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In still another preferred embodiment, the human anti-MAdCAM antibody comprises one of the amino acid sequences selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46 48, 52, 54, 56, 58, 62, 64, 66 or 68 (with or
without the signal sequence), or a variable domain thereof. In another preferred embodiment, the human anti-MAdCAM antibody comprises one or more CDRs from an antibody that comprises one of the amino acid sequences selected from SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 52, 54, 56, 58, 62, 64, 66 or 68. In a highly preferred embodiment, the anti-MAdCAM antibody is another human antibody.

[0102] One may determine whether an anti-MAdCAM antibody binds to the same antigen as another anti-MAdCAM antibody using a variety of methods known in the art. For instance, one can use a known anti-MAdCAM antibody to capture the antigen, elute the antigen from the anti-MAdCAM antibody, and then determine whether the test antibody will bind to the eluted antigen. One may determine whether an antibody competes with an anti-MAdCAM antibody by binding the anti-MAdCAM antibody to MAdCAM under saturating conditions, and then measuring the ability of the test antibody to bind to MAdCAM. If the test antibody is able to bind to the MAdCAM at the same time as the anti-MAdCAM antibody, then the test antibody binds to a different epitope than the anti-MAdCAM antibody. However, if the test antibody is not able to bind to the MAdCAM at the same time, then the test antibody competes with the human anti-MAdCAM antibody. This experiment may be performed using ELISA, or surface plasmon resonance or, preferably, BIAcore. To test whether an anti-MAdCAM antibody cross-competes with another anti-MAdCAM antibody, one may use the competition method described above in two directions, i.e. determining if the known antibody blocks the test antibody and vice versa.

Light and Heavy Chain Gene Usage

[0103] The invention also provides an anti-MAdCAM antibody that comprises a light chain variable region encoded by a human \( \kappa \) gene. In a preferred embodiment, the light chain variable region is encoded by a human \( \kappa A2, A3, A26, B3, O12 \) or \( O18 \) gene family. In various embodiments, the light chain comprises no more than eleven, no more than six or no more than three amino acid substitutions from the germline human \( \kappa A2, A3, A26, B3, O12 \) or \( O18 \) sequence. In a preferred embodiment, the amino acid substitutions are conservative substitutions.
SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48 provide the amino acid sequences of the full-length kappa light chains of twelve anti-MAAdCAM antibodies, 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4 and 9.8.2. Figures 1K-1T are alignments of the amino acid sequences of the light chain variable domains of twelve anti-MAAdCAM antibodies with the germline sequences from which they are derived. Figure 2A shows an alignment of the amino acid sequences of the light chain variable domains of the kappa light chains of twelve anti-MAAdCAM antibodies to each other. Following the teachings of this specification, one of ordinary skill in the art could determine the differences between the germline sequences and the antibody sequences of additional anti-MAAdCAM antibodies. SEQ ID NOS: 54, 58, 62, 66 or 68 provide the amino acid sequences of the full length kappa light chains of five additional anti-MAAdCAM antibodies, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod, modified by amino acid substitution from their parent anti-MAAdCAM antibodies, 6.22.2, 6.34.2, 6.67.1, 6.77.1 or 7.26.4, respectively.

In a preferred embodiment, the VL of the anti-MAAdCAM antibody contains the same mutations, relative to the germline amino acid sequence, as any one or more of the VL of antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. The invention includes an anti-MAAdCAM antibody that utilizes the same human Vκ and human Jκ genes as an exemplified antibody. In some embodiments, the antibody comprises one or more of the same mutations from germline as one or more exemplified antibodies. In some embodiments, the antibody comprises different substitutions at one or more of the same positions as one or more of the exemplified antibodies. For example, the VL of the anti-MAAdCAM antibody may contain one or more amino acid substitutions that are the same as those present in antibody 7.16.6, and another amino acid substitution that is the same as antibody 7.26.4. In this manner, one can mix and match different features of antibody binding in order to alter, e.g., the affinity of the antibody for MAAdCAM or its dissociation rate from the antigen. In another embodiment, the mutations are made in the same position as those found in any one or more of the VL of antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6,
7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod, but conservative amino acid substitutions are made rather than using the same amino acid. For example, if the amino acid substitution compared to the germline in one of the antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod is glutamate, one may conservatively substitute aspartate.

Similarly, if the amino acid substitution is serine, one may conservatively substitute threonine.

[0106] In another preferred embodiment, the light chain comprises an amino acid sequence that is the same as the amino acid sequence of the VL of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another highly preferred embodiment, the light chain comprises amino acid sequences that are the same as the CDR regions of the light chain of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the light chain comprises an amino acid sequence with at least one CDR region of the light chain of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the light chain comprises amino acid sequences with CDRs from different light chains that use the same Vκ and Jκ genes. In a more preferred embodiment, the CDRs from different light chains are obtained from 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the light chain comprises an amino acid sequence selected from SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 58, 62, 64, 66 or 68 with or without the signal sequence. In another embodiment, the light chain comprises an amino acid sequence encoded by a nucleotide sequence selected from SEQ ID NOS: 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 53, 57, 61, 65 or 67 (with or without the signal sequence), or a nucleotide sequence that encodes an amino acid sequence having 1-11 amino acid insertions, deletions or substitutions therefrom. Preferably, the amino acid
substitutions are conservative amino acid substitutions. In another embodiment, the antibody or portion thereof comprises a lambda light chain.

[0107] The present invention also provides an anti-MAdCAM antibody or portion thereof that comprises a human VH gene sequence or a sequence derived from a human VH gene. In one embodiment, the heavy chain amino acid sequence is derived from a human VH 1-18, 3-15, 3-21, 3-23, 3-30, 3-33 or 4-4 gene family. In various embodiments, the heavy chain comprises no more than fifteen, no more than six or no more than three amino acid changes from germline human VH 1-18, 3-15, 3-21, 3-23, 3-30, 3-33 or 4-4 gene sequence.

[0108] SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42 and 46 provide the amino acid sequences of the full-length heavy chains of twelve anti-MAdCAM antibodies. Figures 1A-1J are alignments of the amino acid sequences of the heavy chain variable regions of twelve anti-MAdCAM antibodies with the germline sequences from which they are derived. Figure 2B shows the alignments of the amino acid sequences of the heavy chain variable regions of twelve anti-MAdCAM antibodies to each other. Following the teachings of this specification and the nucleotide sequences of the invention, one of ordinary skill in the art could determine the encoded amino acid sequence of the twelve anti-MAdCAM heavy chains and the germline heavy chains and determine the differences between the germline sequences and the antibody sequences. SEQ ID NOS: 52, 56, 60 and 64 provide the amino acid sequences of the full length heavy chains of anti-MAdCAM antibodies, 6.22.2-mod, 6.34.2-mod and 6.67.1-mod, modified by amino acid substitution from their parent anti-MAdCAM antibodies, 6.22.2, 6.34.2 and 6.67.1 respectively. One further modified anti-MAdCAM antibody, 7.26.4-mod, has a full length heavy chain amino acid sequence which is SEQ ID NO: 42.

[0109] In a preferred embodiment, the VH of the anti-MAdCAM antibody contains the same mutations, relative to the germline amino acid sequence, as any one or more of the VH of antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. Similar to that discussed above, the antibody comprises one or more of the same mutations from germline as one or more exemplified antibodies. In some embodiments, the antibody comprises different
substitutions at one or more of the same positions as one or more of the exemplified antibodies. For example, the VH of the anti-MAdCAM antibody may contain one or more amino acid substitutions that are the same as those present in antibody 7.16.6, and another amino acid substitution that is the same as antibody 7.26.4. In this manner, one can mix and match different features of antibody binding in order to alter, e.g., the affinity of the antibody for MAdCAM or its dissociation rate from the antigen. In another embodiment, an amino acid substitution compared to germline is made at the same position as a substitution from germline as found in any one or more of the VH of reference antibody 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod, but the position is substituted with a different residue, which is a conservative substitution compared to the reference antibody.

In another preferred embodiment, the heavy chain comprises an amino acid sequence that is the same as the amino acid sequence of the VH of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another highly preferred embodiment, the heavy chain comprises amino acid sequences that are the same as the CDR regions of the heavy chain of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the heavy chain comprises an amino acid sequence from at least one CDR region of the heavy chain of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.4, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the heavy chain comprises amino acid sequences with CDRs from different heavy chains. In a more preferred embodiment, the CDRs from different heavy chains are obtained from 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the heavy chain comprises an amino acid sequence selected from SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 52, 56, 60 or 64 with or without the signal sequence. In another embodiment, the heavy chain
comprises an amino acid sequence encoded by a nucleotide sequence selected from SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 51, 55, 59 or 63, or a nucleotide sequence that encodes an amino acid sequence having 1-15 amino acid insertions, deletions or substitutions therefrom. In another embodiment, the substitutions are conservative amino acid substitutions.

Methods of Producing Antibodies and Antibody-Producing Cell Lines

Immunization

[0111] In one embodiment of the instant invention, human antibodies are produced by immunizing a non-human animal comprising some or all of the human immunoglobulin heavy and light chain loci with an MAdCAM antigen. In a preferred embodiment, the non-human animal is a XENOMOUSE™ animal, which is an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. See, e.g., Green et al., Nature Genetics 7:13-21 (1994) and United States Patents 5,916,771, 5,939,598, 5,985,615, 5,998,208, 6,075,181, 6,091,001, 6,114,598 and 6,130,364. See also WO 91/10741, WO 94/02602, WO 96/34096 and WO 96/33735, WO 98/16654, WO 98/24893, WO 98/50433, WO 99/45031, WO 99/53049, WO 00 09560 and WO 00/037504. The XENOMOUSE™ animal produces an adult-like human repertoire of fully human antibodies and generates antigen-specific human mAbs. A second generation XENOMOUSE™ animal contains approximately 80% of the human antibody V gene repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and κ light chain loci. In other embodiments, XENOMOUSE™ mice contain approximately all of the human heavy chain and λ light chain locus. See Mendez et al., Nature Genetics 15:146-156 (1997), Green and Jakobovits, J. Exp. Med. 188:483-495 (1998), the disclosures of which are hereby incorporated by reference.

[0112] The invention also provides a method for making anti-MAdCAM antibodies from non-human, non-mouse animals by immunizing non-human transgenic animals that comprise human immunoglobulin loci. One may produce such animals using the methods described immediately above. The methods
disclosed in these documents can be modified as described in U.S. Patent 5,994,619 (the "'619 patent"), which is here incorporated by reference. The '619 patent describes methods for producing novel cultured inner cell mass (CICM) cells and cell lines, derived from pigs and cows, and transgenic CICM cells into which heterologous DNA has been inserted. CICM transgenic cells can be used to produce cloned transgenic embryos, fetuses, and offspring. The '619 patent also describes methods of producing transgenic animals that are capable of transmitting the heterologous DNA to their progeny. In a preferred embodiment, the non-human animals may be rats, sheep, pigs, goats, cattle or horses.

[0113] In another embodiment, the non-human animal comprising human immunoglobulin loci are animals that have a "minilocus" of human immunoglobulins. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of individual genes from the Ig locus. Thus, one or more VH genes, one or more DH genes, one or more JH genes, a \( \mu \) constant domain(s), and a second constant domain(s) (preferably a gamma constant domain(s) are formed into a construct for insertion into an animal. This approach is described, inter alia, in U.S. Patent No. 5,545,807, 5,545,806, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,591,669, 5,612,205, 5,721,367, 5,789,215, and 5,643,763, hereby incorporated by reference.

[0114] An advantage of the minilocus approach is the rapidity with which constructs including portions of the Ig locus can be generated and introduced into animals. However, a potential disadvantage of the minilocus approach is that there may not be sufficient immunoglobulin diversity to support full B-cell development, such that there may be lower antibody production.

[0115] To produce a human anti-MAdCAM antibody, a non-human animal comprising some or all of the human immunoglobulin loci is immunized with a MAdCAM antigen and an antibody or the antibody-producing cell is isolated from the animal. The MAdCAM antigen may be isolated and/or purified MAdCAM and is preferably a human MAdCAM. In another embodiment, the MAdCAM antigen is a fragment of MAdCAM, preferably the extracellular domain of MAdCAM. In another embodiment, the MAdCAM antigen is a fragment that comprises at least one epitope of MAdCAM. In another embodiment, the MAdCAM antigen is a cell
that expresses MAdCAM on its cell surface, preferably a cell that overexpresses MAdCAM on its cell surface.

[0116] Immunization of animals may be done by any method known in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, New York: Cold Spring Harbor Press (1990). Methods for immunizing non-human animals such as mice, rats, sheep, goats, pigs, cattle and horses are well known in the art. See, e.g., Harlow and Lane and United States Patent 5,994,619. In a preferred embodiment, the MAdCAM antigen is administered with an adjuvant to stimulate the immune response. Such adjuvants include complete or incomplete Freund’s adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks.

[0117] Example I provides a protocol for immunizing a XENOMOUSE™ animal with full-length human MAdCAM in phosphate-buffered saline.

*Production of Antibodies and Antibody-Producing Cell Lines*

[0118] After immunization of an animal with a MAdCAM antigen, antibodies and/or antibody-producing cells may be obtained from the animal. An anti-MAdCAM antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-MAdCAM antibodies may be purified from the serum.

[0119] In another embodiment, antibody-producing immortalized cell lines may be prepared from the immunized animal. After immunization, the animal is sacrificed and B cells are immortalized using methods well-known in the art. Methods of immortalizing cells include, but are not limited to, transfecting them with oncogenes, infecting them with an oncogenic virus and cultivating them under conditions that select for immortalized cells, subjecting them to carcinogenic or mutating compounds, fusing them with an immortalized cell, e.g., a myeloma cell,
and inactivating a tumor suppressor gene. See, e.g., Harlow and Lane, supra. In embodiments involving the myeloma cells, the myeloma cells do not secrete immunoglobulin polypeptides (a non-secretory cell line). After immortalization and antibiotic selection, the immortalized cells, or culture supernatants thereof, are screened using MAdCAM, a portion thereof, or a cell expressing MAdCAM. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay (RIA), preferably an ELISA. An example of ELISA screening is provided in PCT Publication No. WO 00/37504, herein incorporated by reference.

[0120] In another embodiment, antibody-producing cells may be prepared from a human who has an autoimmune disorder and who expresses anti-MAdCAM antibodies. Cells expressing the anti-MAdCAM antibodies may be isolated by isolating white blood cells and subjecting them to fluorescence-activated cell sorting (FACS) or by panning on plates coated with MAdCAM or a portion thereof. These cells may be fused with a human non-secretory myeloma to produce human hybridomas expressing human anti-MAdCAM antibodies. In general, this is a less preferred embodiment because it is likely that the anti-MAdCAM antibodies will have a low affinity for MAdCAM.

[0121] Anti-MAdCAM antibody-producing cells, e.g., hybridomas are selected, cloned and further screened for desirable characteristics, including robust cell growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas may be cultured and expanded in vivo in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture in vitro. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

[0122] Preferably, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma derived from the same species as the non-human animal. More preferably, the immunized animal is a XENOMOUSE™ animal and the myeloma cell line is a non-secretory mouse myeloma, such as the myeloma cell line is P3-X63-AG8-653 (ATCC). See, e.g., Example I.
[0123] Thus, in one embodiment, the invention provides methods for producing a cell line that produces a human monoclonal antibody or a fragment thereof directed to MADCAM comprising (a) immunizing a non-human transgenic animal described herein with MADCAM, a portion of MADCAM or a cell or tissue expressing MADCAM; (b) allowing the transgenic animal to mount an immune response to MADCAM; (c) isolating antibody-producing cells from transgenic animal; (d) immortalizing the antibody-producing cells; (e) creating individual monoclonal populations of the immortalized antibody-producing cells; and (f) screening the immortalized antibody-producing cells or culture supernatants thereof to identify an antibody directed to MADCAM.

[0124] In one aspect, the invention provides hybridomas that produce human anti-MADCAM antibodies. In a preferred embodiment, the hybridomas are mouse hybridomas, as described above. In another embodiment, the hybridomas are produced in a non-human, non-mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an anti-MADCAM antibody.

Nucleic Acids, Vectors, Host Cells and Recombinant Methods of Making Antibodies

20 Nucleic Acids

[0125] Nucleic acid molecules encoding anti-MADCAM antibodies of the invention are provided. In one embodiment, the nucleic acid molecule encodes a heavy and/or light chain of an anti-MADCAM immunoglobulin. In a preferred embodiment, a single nucleic acid molecule encodes a heavy chain of an anti-MADCAM immunoglobulin and another nucleic acid molecule encodes the light chain of an anti-MADCAM immunoglobulin. In a more preferred embodiment, the encoded immunoglobulin is a human immunoglobulin, preferably a human IgG. The encoded light chain may be a λ chain or a κ chain, preferably a κ chain.

[0126] In a preferred embodiment the nucleic acid molecule encoding the variable region of the light chain comprises the germline sequence of a human Vκ the A2, A3, A26, B3, O12 or O18 gene or a variant of said sequence. In a preferred embodiment, the nucleic acid molecule encoding the light chain
comprises a sequence derived from a human Jκ1, Jκ2, Jκ3, Jκ4 or Jκ5 gene. In a preferred embodiment, the nucleic acid molecule encoding the light chain encodes no more than eleven amino acid changes from the germline A2, A3, A26, B3, O12 or O18 Vκ gene, preferably no more than six amino acid changes, and even more preferably no more than three amino acid changes. In a more preferred embodiment, the nucleic acid encoding the light chain is the germline sequence.

The invention provides a nucleic acid molecule that encodes a variable region of the light chain (VL) containing up to eleven amino acid changes compared to the germline sequence, wherein the amino acid changes are identical to amino acid changes from the germline sequence from the VL of one of the antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. The invention also provides a nucleic acid molecule comprising a nucleotide sequence that encodes the amino acid sequence of the variable region of the light chain of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. The invention also provides a nucleic acid molecule comprising a nucleotide sequence that encodes the amino acid sequence of one or more of the CDRs of any one of the light chains of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In a preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of all of the CDRs of any one of the light chains of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of one of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 58, 62, 66, 68 or comprises a nucleotide sequence of one of SEQ ID NOS: 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 53, 57, 61, 65 or 67. In another preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of one or more of the CDRs of any one of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 58, 62, 66, 68 or comprises a nucleotide sequence of one or
more of the CDRs of any one of SEQ ID NOS: 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 53, 57, 61, 65, or 67. In a more preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of all of the CDRs of any one of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 58, 62, 66, 68 or comprises a the nucleotide sequence of all the CDRs of any one of SEQ ID NOS: 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 53, 57, 61, 65, or 67.

[0128] The invention also provides a nucleic acid molecule that encodes an amino acid sequence of a VL that has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a VL described above, particularly to a VL that comprises an amino acid sequence of one of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 58, 62, 66 or 68. The invention also provides a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of one of SEQ ID NOS: 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 53, 57, 61, 65 or 67.

[0129] In another embodiment, the invention provides a nucleic acid molecule that hybridizes under highly stringent conditions to a nucleic acid molecule encoding a VL as described above, particularly a nucleic acid molecule that comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 58, 62, 66 or 68. The invention also provides a nucleic acid molecule that hybridizes under highly stringent conditions to a nucleic acid molecule comprising a nucleotide sequence of one of SEQ ID NOS: 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 53, 57, 61, 65 or 67.

[0130] The invention also provides a nucleic acid molecule encoding a heavy chain variable region (VH) that utilizes a human VH 1-18, 3-15, 3-21, 3-23, 3-30, 3-33 or 4-4 VH gene. In some embodiments, the nucleic acid molecule encoding the VH gene further utilizes a human JH4 or JH6 family gene. In some embodiments, the nucleic acid molecule encoding the VH gene utilize the human JH4b or JH6b gene. In another embodiment, the nucleic acid molecule comprises a sequence derived from a human D 3-10, 4-23, 5-5, 6-6 or 6-19 gene. In an even more preferred embodiment, the nucleic acid molecule encoding the VH contains
no more than fifteen amino acid changes from the germline VH 1-18, 3-15, 3-21, 3-23, 3-30, 3-33 or 4-4 genes, preferably no more than six amino acid changes, and even more preferably no more than three amino acid changes. In a highly preferred embodiment, the nucleic acid molecule encoding the VH contains at least one amino acid change compared to the germline sequence, wherein the amino acid change is identical to an amino acid change from the germline sequence from the heavy chain of one of the antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In an even more preferred embodiment, the VH contains no more than fifteen amino acid changes compared to the germline sequences, wherein the changes are identical to those changes from the germline sequence from the VH of one of the antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod.

[0131] In one embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of the VH of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of one or more of the CDRs of the heavy chain of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In a preferred embodiment, the nucleic acid molecule comprises nucleotide sequences that encode the amino acid sequences of all of the CDRs of the heavy chain of 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of one of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 52, 56, 60 or 64 or that comprises a nucleotide sequence of one of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 51, 55, 59 or 63. In another preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of one or more of the CDRs of any one of SEQ
ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 52, 56, 60 or 64 or comprises a nucleotide sequence of one or more of the CDRs of any one of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 51, 55, 59 or 63. In a preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequences of all of the CDRs of any one of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 52, 56, 60 or 64 or comprises a nucleotide sequence of all of the CDRs of any one of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41 45, 51, 55, 59 or 63. In some embodiments the nucleic acid molecule comprises a nucleotide sequence encoding a contiguous region from the beginning of CDR1 to the end of CDR3 of a heavy or light chain of any of the above-mentioned anti-MAdCAM antibodies.

[0132] In another embodiment, the nucleic acid molecule encodes an amino acid sequence of a VH that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to one of the amino acid sequences encoding a VH as described immediately above, particularly to a VH that comprises an amino acid sequence of one of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 52, 56, 60 or 64. The invention also provides a nucleotide sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of one of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 51, 55, 59 or 63.

[0133] In another embodiment, the nucleic acid molecule encoding a VH is one that hybridizes under highly stringent conditions to a nucleotide sequence encoding a VH as described above, particularly to a VH that comprises an amino acid sequence of one of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 52, 56, 60 or 64. The invention also provides a nucleotide sequence encoding a VH that hybridizes under highly stringent conditions to a nucleic acid molecule comprising a nucleotide sequence of one of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 51, 55, 59 or 63.

[0134] The nucleotide sequence encoding either or both of the entire heavy and light chains of an anti-MAdCAM antibody or the variable regions thereof may be obtained from any source that produces an anti-MAdCAM antibody. Methods of isolating mRNA encoding an antibody are well-known in the art. See, e.g.,
Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). The mRNA may be used to produce cDNA for use in the polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In one embodiment of the invention, the nucleic acid molecules may be obtained from a hybridoma that expresses an anti-MAdCAM antibody, as described above, preferably a hybridoma that has as one of its fusion partners a transgenic animal cell that expresses human immunoglobulin genes, such as a XENOMOUSE™ animal, a non-human mouse transgenic animal or a non-human, non-mouse transgenic animal. In another embodiment, the hybridoma is derived from a non-human, non-transgenic animal, which may be used, e.g., for humanized antibodies.

[0135] A nucleic acid molecule encoding the entire heavy chain of an anti-MAdCAM antibody 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.

[0136] In a preferred embodiment, the nucleic acid encoding the variable region of the heavy chain encodes the variable region of amino acid sequences of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 52, 56, 60 or 64, and the nucleic acid molecule encoding the variable region of the light chains encodes the variable region of amino acid sequence of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 54, 58, 62, 66 or 68.

[0137] In one embodiment, a nucleic acid molecule encoding either the heavy chain of an anti-MAdCAM antibody or an antigen-binding portion thereof, or the light chain of an anti-MAdCAM antibody or an antigen-binding portion thereof may be isolated from a non-human, non-mouse animal that expresses human immunoglobulin genes and has been immunized with a MAdCAM antigen. In other embodiment, the nucleic acid molecule may be isolated from an anti-MAdCAM antibody-producing cell derived from a non-transgenic animal or from a human patient who produces anti-MAdCAM antibodies. mRNA from the anti-MAdCAM antibody-producing cells may be isolated by standard techniques, cloned and/or amplified using PCR and library construction techniques, and screened using standard protocols to obtain nucleic acid molecules encoding anti-MAdCAM heavy and light chains.

[0138] 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.

[0139] In another embodiment, the nucleic acid molecules of the invention may be used as probes or PCR primers for specific antibody sequences. For instance, a nucleic acid molecule probe may be used in diagnostic methods or a nucleic acid molecule PCR primer may be used to amplify regions of DNA that could be used, *inter alia*, to isolate nucleotide sequences for use in producing variable domains of
anti-MAdCAM antibodies. In a preferred embodiment, the nucleic acid molecules are oligonucleotides. In a more preferred embodiment, the oligonucleotides are from highly variable regions of the heavy and light chains of the antibody of interest. In an even more preferred embodiment, the oligonucleotides encode all or a part of one or more of the CDRs.

Vectors

[0140] 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.

[0141] To express the antibodies, or antibody portions of 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).

[0142] 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).

[0143] 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 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, are also well known in the art.

[0144] In addition to the antibody chain genes and regulatory sequences, the
recombinant expression vectors of 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. 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.

Non-Hybridoma Host Cells and Methods of Recombinantly Producing Protein

[0145] 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. 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 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.

[0146] 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 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, 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 include Schizosaccharomyces pombe, Saccharomyces cerevisiae and Pichia pastoris.

[0147] Further, expression of antibodies of 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 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.

[0148] 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 herein, or comprising the amino acid sequences provided herein are part of the instant invention, regardless of the glycosylation of the antibodies.

Transgenic Animals and Plants

[0149] The invention also provides transgenic non-human animals and transgenic plants comprising one or more nucleic acid molecules of the invention that may be used to produce antibodies of the invention. Antibodies can be produced 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 MA:dCAM 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 reference.

[0150] In another embodiment, non-human transgenic animals and transgenic plants are produced by introducing one or more nucleic acid molecules of 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).

In another embodiment, the transgenic non-human organisms may have a targeted disruption and replacement that encodes a heavy chain and/or a light chain of interest. In a preferred embodiment, the transgenic animals or plants comprise and express nucleic acid molecules encoding heavy and light chains that combine to bind specifically to MA:dCAM, preferably human MA:dCAM. In another embodiment, the transgenic animals or plants comprise nucleic acid molecules encoding a modified antibody such as a single-chain antibody, a chimeric antibody or a humanized antibody. The anti-MA:dCAM antibodies may be made in any transgenic animal. In a preferred embodiment, the non-human animals are 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.

Phage Display Libraries

[0151] The invention provides a method for producing an anti-MA:dCAM antibody or antigen-binding portion thereof comprising the steps of synthesizing a library of human antibodies on phage, screening the library with a MA:dCAM or a portion thereof, isolating phage that bind MA:dCAM, and obtaining the antibody from the phage. One method to prepare the library of antibodies comprises the...
steps of immunizing a non-human host animal comprising a human immunoglobulin locus with MAAdCAM or an antigenic portion thereof to create an immune response, extracting cells from the host animal the cells that are responsible for production of antibodies; isolating RNA from the extracted cells, reverse transcribing the RNA to produce cDNA, amplifying the cDNA using a primer, and inserting the cDNA into phage display vector such that antibodies are expressed on the phage. Recombinant anti-MAAdCAM antibodies of the invention may be obtained in this way.


[0153] In a preferred embodiment, to isolate human anti-MAAdCAM antibodies with the desired characteristics, a human anti-MAAdCAM antibody as described herein is first used to select human heavy and light chain sequences having similar
binding activity toward MAdCAM, using the epitope imprinting methods described in Hoogenboom et al., PCT Publication No. WO 93/06213. The antibody libraries used in this method are preferably scFv libraries prepared and screened as described in McCafferty et al., PCT Publication No. WO 92/01047, McCafferty et al., *Nature*, 348:552-554 (1990); and Griffiths et al., *EMBO J*, 12:725-734 (1993). The scFv antibody libraries preferably are screened using human MAdCAM as the antigen.

Once initial human VL and VH segments are selected, “mix and match” experiments, in which different pairs of the initially selected VL and VH segments are screened for MAdCAM binding, are performed to select preferred VL/VH pair combinations. Additionally, to further improve the quality of the antibody, the VL and VH segments of the preferred VL/VH pair(s) can be randomly mutated, preferably within the CDR3 region of VH and/or VL, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying VH and VL regions using PCR primers complimentary to the VH CDR3 or VL CDR3, respectively, which primers have been “spiked” with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode VH and VL segments into which random mutations have been introduced into the VH and/or VL CDR3 regions. These randomly mutated VH and VL segments can be rescreened for binding to MAdCAM.

Following screening and isolation of an anti-MAdCAM antibody of the invention from a recombinant immunoglobulin display library, nucleic acid encoding the selected antibody can be recovered from the display package (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can be further manipulated to create other antibody forms of the invention, as described below. To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into a mammalian host cells, as described above.
Class Switching

[0156] Another aspect of the instant invention is to provide a mechanism by which the class of an anti-MAdCAM antibody may be switched with another. In one aspect of the invention, a nucleic acid molecule encoding VL or VH is isolated using methods well-known in the art such that it does not include any nucleotide sequences encoding CL or CH. The nucleic acid molecule encoding VL or VH is then operatively linked to a nucleotide sequence encoding a CL or CH from a different class of immunoglobulin molecule. This may be achieved using a vector or nucleic acid molecule that comprises a CL or CH encoding sequence, as described above. For example, an anti-MAdCAM antibody that was originally IgM may be class switched to an IgG. Further, the class switching may be used to convert one IgG subclass to another, e.g., from IgG4 to IgG2. A preferred method for producing an antibody of the invention comprising a desired isotype or antibody subclass comprises the steps of isolating a nucleic acid encoding the heavy chain of an anti-MAdCAM antibody and a nucleic acid encoding the light chain of an anti-MAdCAM antibody, obtaining the variable region of the heavy chain, ligating the variable region of the heavy chain with the constant domain of a heavy chain of the desired isotype, expressing the light chain and the ligated heavy chain in a cell, and collecting the anti-MAdCAM antibody with the desired isotype.

Antibody Derivatives

[0157] One may use the nucleic acid molecules described above to generate antibody derivatives using techniques and methods known to one of ordinary skill in the art.

Humanized Antibodies

[0158] The immunogenicity of non-human antibodies can be reduced to some extent using techniques of humanization, potentially employing display techniques using appropriate libraries. It will be appreciated that murine antibodies or antibodies from other species can be humanized or primatized using techniques well known in the art. See, e.g., Winter and Harris, Immuno! Today, 14:43-46
(1993) and Wright et al., *Crit. Reviews in Immunol.*, 12125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the C_{H1}, C_{H2}, C_{H3}, hinge domains, and/or the framework domain with the corresponding human sequence (see WO 92/02190 and U.S. Patent Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,792, 5,714,350, and 5,777,085). In another embodiment, a non-human anti-MAdCAM antibody can be humanized by substituting the C_{H1}, hinge domain, C_{H2}, C_{H3}, and/or the framework domains with the corresponding human sequence of a anti-MAdCAM antibody of the invention.

*Mutated Antibodies*

10 In another embodiment, the nucleic acid molecules, vectors and host cells may be used to make mutated anti-MAdCAM antibodies. The antibodies may be mutated in the variable domains of the heavy and/or light chains to alter a binding property of the antibody. For example, a mutation may be made in one or more of the CDR regions to increase or decrease the K_{d} of the antibody for MAdCAM.

Techniques in site-directed mutagenesis are well-known in the art. See, e.g., Sambrook et al., and Ausubel et al., *supra*. In a preferred embodiment, mutations are made at an amino acid residue that is known to be changed compared to germline in a variable region of an anti-MAdCAM antibody. In a more preferred embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a variable region or CDR region of one of the anti-MAdCAM antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a variable region or CDR region whose amino acid sequence is presented in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 52, 54, 56, 58, 62, 64, 66 or 68, or whose nucleotide sequence is presented in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 51, 53, 55, 57, 61, 63, 65 or 67. In another embodiment, the nucleic acid molecules are mutated in one or more of the framework regions. A mutation may be made in a framework region or constant domain to increase the half-life of the anti-MAdCAM antibody. See, e.g., WO
00/09560, published February 24, 2000, herein incorporated by reference. In one embodiment, there may be one, three or five or ten point mutations and no more than fifteen point mutations. A mutation in a framework region or constant domain may also be made to alter the immunogenicity of the antibody, to provide a site for covalent or non-covalent binding to another molecule, or to alter such properties as complement fixation. Mutations may be made in each of the framework regions, the constant domain and the variable regions in a single mutated antibody. Alternatively, mutations may be made in only one of the framework regions, the variable regions or the constant domain in a single mutated antibody.

[0160] In one embodiment, there are no greater than fifteen amino acid changes in either the VH or VL regions of the mutated anti-MAdCAM antibody compared to the anti-MAdCAM antibody prior to mutation. In a preferred embodiment, there is no more than ten amino acid changes in either the VH or VL regions of the mutated anti-MAdCAM antibody, more preferably no more than five amino acid changes, or even more preferably no more than three amino acid changes. In another embodiment, there are no more than fifteen amino acid changes in the constant domains, more preferably, no more than ten amino acid changes, even more preferably, no more than five amino acid changes.

Modified Antibodies

[0161] In another embodiment, a fusion antibody or immunoadhesin may be made which comprises all or a portion of an anti-MAdCAM antibody linked to another polypeptide. In a preferred embodiment, only the variable regions of the anti-MAdCAM antibody are linked to the polypeptide. In another preferred embodiment, the VH domain of an anti-MAdCAM antibody are linked to a first polypeptide, while the VL domain of an anti-MAdCAM antibody are linked to a second polypeptide that associates with the first polypeptide in a manner in which the VH and VL domains can interact with one another to form an antibody binding site. In another preferred embodiment, the VH domain is separated from the VL domain by a linker such that the VH and VL domains can interact with one another (see below under Single Chain Antibodies). The VH-linker-VL antibody is then linked to the polypeptide of interest. The fusion antibody is useful to directing a polypeptide to a MAdCAM-expressing cell or tissue. The polypeptide may be a
therapeutic agent, such as a toxin, growth factor or other regulatory protein, or may be a diagnostic agent, such as an enzyme that may be easily visualized, such as horseradish peroxidase. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful if one wants to create a divalent or polyvalent antibody on a single polypeptide chain, or if one wants to create a bispecific antibody.

[0162] To create a single chain antibody, (scFv) the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence (Gly$_4$-Ser)$_3$, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see, e.g., Bird et al., Science, 242:423-426 (1988); Huston et al., Proc. Natl. Acad. Sci. USA, 85:5879-5883 (1988); McCafferty et al., Nature, 348:552-554 (1990)). The single chain antibody may be monovalent, if only a single VH and VL are used, bivalent, if two VH and VL are used, or polyvalent, if more than two VH and VL are used.


[0164] In another aspect, chimeric and bispecific antibodies can be generated. A chimeric antibody may be made that comprises CDRs and framework regions from different antibodies. In a preferred embodiment, the CDRs of the chimeric antibody comprises all of the CDRs of the variable region of a light chain or heavy chain of a human anti-MAdCAM antibody, while the framework regions are derived from one or more different antibodies. In a more preferred embodiment, the CDRs of the chimeric antibody comprise all of the CDRs of the variable regions of the light chain and the heavy chain of a human anti-MAdCAM antibody. The framework regions may be from another species and may, in a
preferred embodiment, be humanized. Alternatively, the framework regions may be from another human antibody.

[0165] A bispecific antibody can be generated that binds specifically to MAdCAM through one binding domain and to a second molecule through a second binding domain. The bispecific antibody can be produced through recombinant molecular biological techniques, or may be physically conjugated together. In addition, a single chain antibody containing more than one VH and VL may be generated that binds specifically to MAdCAM and to another molecule. Such bispecific antibodies can be generated using techniques that are well known for example, in connection with (i) and (ii) see, e.g., Fanger et al., *Immunol Methods* 4: 72-81 (1994) and Wright and Harris, *supra*. and in connection with (iii) see, e.g., Traunecker et al., *Int. J. Cancer (Suppl.)* 7: 51-52 (1992). In a preferred embodiment, the bispecific antibody binds to MAdCAM and to another molecule expressed at high level on endothelial cells. In a more preferred embodiment, the other molecule is VCAM, ICAM or L-selectin.

[0166] In various embodiments, the modified antibodies described above are prepared using one or more of the variable regions or one or more CDR regions from one of the antibodies selected from 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod or 7.26.4-mod. In another embodiment, the modified antibodies are prepared using one or more of the variable regions or one or more CDR regions whose amino acid sequence is presented in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 52, 54, 56, 58, 62, 64, 66 or 68 or whose nucleotide sequence is presented in SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 51, 53, 55, 57, 61, 63, 65 or 67.

**Derivatized and Labeled Antibodies**

[0167] An antibody or antibody portion of the invention can be derivatized or linked to another molecule (e.g., another peptide or protein). In general, the antibodies or portions thereof are derivatized such that the MAdCAM binding is not affected adversely by the derivatization or labeling. Accordingly, the antibodies and antibody portions of the invention are intended to include both
intact and modified forms of the human anti-MAdCAM antibodies described herein. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detection agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[0168] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, e.g., to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

[0169] Another type of derivatized antibody is a labeled antibody. Useful detection agents with which an antibody or antibody portion of the invention may be derivatized include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. An antibody may also be labeled with enzymes that are useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody is labeled with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be labeled with biotin, and detected through indirect measurement of avidin or streptavidin binding. An antibody may be labeled with a magnetic agent, such as gadolinium. An antibody may also be labeled with a predetermined polypeptide epitope recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary
antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0170] An anti-MAdCAM antibody may also be labeled with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect MAdCAM-expressing tissues by x-ray or other diagnostic techniques. Further, the radiolabel may be used therapeutically as a toxin for diseased tissue or MAdCAM expressing tumors. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionuclides -- $^3$H, $^{14}$C, $^{15}$N, $^{35}$S, $^{90}$Y, $^{99}$Tc, $^{111}$In, $^{125}$I, $^{131}$I.

[0171] An anti-MAdCAM antibody may also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups may be useful to improve the biological characteristics of the antibody, e.g., to increase serum half-life or to increase tissue binding. This methodology would also apply to any antigen-binding fragments or versions of anti-MAdCAM antibodies.

Pharmaceutical Compositions and Kits

[0172] In a further aspect, the invention provides compositions comprising an inhibitory human anti-MAdCAM antibody and methods for treating subjects with such compositions. In some embodiments, the subject of treatment is human. In other embodiments, the subject is a veterinary subject. In some embodiments, the veterinary subject is a dog or a non-human primate.

[0173] 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 of the invention and compositions comprising them, can be administered in combination with one or more other therapeutic, diagnostic or prophylactic agents. 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.
(Oprelevkin), IL-12, MIF/CD74 antagonists, CD40 antagonists, such as TNX-100/5-D12, OX40L antagonists, GM-CSF, pimecrolimus or rapamycin; the class of anti-TNFα agents such as infliximab, adalimumab, CDP-870, oncercept, 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 volatge senstize channel (α2δ) 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 or chemotherapeutic agents. Such additional agents may be included in the same composition or administered separately. In some embodiments, one or more inhibitory anti-MAdCAM antibodies of the invention can be used as a vaccine or as adjuvants to a vaccine. 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.

[0174] 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.

[0175] The compositions of 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.

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 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.

[0178] 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.


[0179] 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.

[0180] 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.

[0181] 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.

[0182] 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. 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.
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.

Gene Therapy

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.

Diagnostic Methods of Use

[0186] The anti-MAdCAM antibodies may be used to detect MAdCAM in a
biological sample in vitro or in vivo. The anti-MAdCAM antibodies may be used
in a conventional immunoassay, including, without limitation, an ELISA, an RIA,
FACS, tissue immunohistochemistry, Western blot or immunoprecipitation. The
anti-MAdCAM antibodies of the invention may be used to detect MAdCAM from
humans. In another embodiment, the anti-MAdCAM antibodies may be used to
detect MAdCAM from Old World primates such as cynomolgus and rhesus
monkeys, chimpanzees and apes. The invention provides a method for detecting
MAdCAM in a biological sample comprising contacting a biological sample with
an anti-MAdCAM antibody of the invention and detecting the antibody bound to
MAdCAM. In one embodiment, the anti-MAdCAM antibody is directly derivatized
with a detectable label. In another embodiment, the anti-MAdCAM antibody (the
first antibody) is unlabeled and a second antibody or other molecule that can bind
the anti-MAdCAM antibody is labeled. As is well known to one of skill in the art,
a second antibody is chosen that is able to specifically bind the specific species and
class of the first antibody. For example, if the anti-MAdCAM antibody is a human
IgG, then the secondary antibody may be an anti-human-IgG. Other molecules
that can bind to antibodies include, without limitation, Protein A and Protein G,
both of which are available commercially, e.g., from Pierce Chemical Co.

[0187] Suitable labels for the antibody or secondary have been disclosed supra,
and include various enzymes, prosthetic groups, fluorescent materials, luminescent
materials, magnetic agents and radioactive materials. Examples of suitable
enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or
acetylcholinesterase; examples of suitable prosthetic group complexes include
streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials
include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; an example of a magnetic agent includes gadolinium; and examples of suitable radioactive material include $^{125}\text{I}$, $^{131}\text{I}$, $^{35}\text{S}$ or $^{3}\text{H}$.

[0188] In an alternative embodiment, MAdCAM can be assayed in a biological sample by a competition immunoassay utilizing MAdCAM standards labeled with a detectable substance and an unlabeled anti-MAdCAM antibody. In this assay, the biological sample, the labeled MAdCAM standards and the anti-MAdCAM antibody are combined and the amount of labeled MAdCAM standard bound to the unlabeled antibody is determined. The amount of MAdCAM in the biological sample is inversely proportional to the amount of labeled MAdCAM standard bound to the anti-MAdCAM antibody.

[0189] One may use the immunoassays disclosed above for a number of purposes. In one embodiment, the anti-MAdCAM antibodies may be used to detect MAdCAM in cells in cell culture. In a preferred embodiment, the anti-MAdCAM antibodies may be used to determine the level of cell surface MAdCAM expression after treatment of the cells with various compounds. This method can be used to test compounds that may be used to activate or inhibit MAdCAM. In this method, one sample of cells is treated with a test compound for a period of time while another sample is left untreated, cell surface expression could then be determined by flow cytometry, immunohistochemistry, Western blot, ELISA or RIA. In addition, the immunoassays may be scaled up for high throughput screening in order to test a large number of compounds for either activation or inhibition of MAdCAM.

[0190] The anti-MAdCAM antibodies of the invention may also be used to determine the levels of MAdCAM on a tissue or in cells derived from the tissue. In a preferred embodiment, the tissue is a diseased tissue. In a more preferred embodiment, the tissue is inflamed gastrointestinal tract or a biopsy thereof. In a preferred embodiment of the method, a tissue or a biopsy thereof is excised from a patient. The tissue or biopsy is then used in an immunoassay to determine, e.g., MAdCAM levels, cell surface levels of MAdCAM, or localization of MAdCAM.
by the methods discussed above. The method can be used to determine if an
inflamed tissue expresses MAdCAM at a high level.

[0191] The above-described diagnostic method can be used to determine whether
a tissue expresses high levels of MAdCAM, which may be indicative that the
tissue will respond well to treatment with anti-MAdCAM antibody. Further, the
diagnostic method may also be used to determine whether treatment with anti-
MAdCAM antibody (see below) is causing a tissue to express lower levels of
MAdCAM and thus can be used to determine whether the treatment is successful.

[0192] The antibodies of the present invention may also be used in vivo to
localize tissues and organs that express MAdCAM. In a preferred embodiment,
the anti-MAdCAM antibodies can be used to localize inflamed tissue. The
advantage of the anti-MAdCAM antibodies of the present invention is that they
will not generate an immune response upon administration. The method comprises
the steps of administering an anti-MAdCAM antibody or a pharmaceutical
composition thereof to a patient in need of such a diagnostic test and subjecting the
patient to imaging analysis determine the location of the MAdCAM-expressing
tissues. Imaging analysis is well known in the medical art, and includes, without
limitation, x-ray analysis, gamma scintigraphy, magnetic resonance imaging
(MRI), positron emission tomography or computed tomography (CT). In another
embodiment of the method, a biopsy is obtained from the patient to determine
whether the tissue of interest expresses MAdCAM rather than subjecting the
patient to imaging analysis. In a preferred embodiment, the anti-MAdCAM
antibodies may be labeled with a detectable agent that can be imaged in a patient.
For example, the antibody may be labeled with a contrast agent, such as barium,
which can be used for x-ray analysis, or a magnetic contrast agent, such as a
gadolinium chelate, which can be used for MRI or CT. Other labeling agents
include, without limitation, radioisotopes, such as $^{99}$Tc. In another embodiment,
the anti-MAdCAM antibody will be unlabeled and will be imaged by
administering a second antibody or other molecule that is detectable and that can
bind the anti-MAdCAM antibody.

[0193] The anti-MAdCAM antibodies of the invention may also be used to
determine the levels of soluble MAdCAM present in donor blood, serum, plasma,
or other biofluid, including, but not limited to, stool, urine, sputum or biopsy sample. In a preferred embodiment, the biofluid is plasma. The biofluid is then used in an immunoassay to determine levels of soluble MAdCAM. Soluble MAdCAM could be a surrogate marker for ongoing gastrointestinal inflammation and the method of detection could be used as a diagnostic marker to measure disease severity.

[0194] The above-described diagnostic method can be used to determine whether an individual expresses high levels of soluble MAdCAM, which may be indicative that the individual will respond well to treatment with an anti-MAdCAM antibody. Further, the diagnostic method may also be used to determine whether treatment with anti-MAdCAM antibody (see below) or other pharmaceutical agent of the disease is causing an individual to express lower levels of MAdCAM and thus can be used to determine whether the treatment is successful.

Inhibition of α₄β₇/MAdCAM-dependent adhesion by anti-MAdCAM antibody:

[0195] In another embodiment, the invention provides an anti-MAdCAM antibody that binds MAdCAM and inhibits the binding and adhesion of α₄β₇-integrin bearing cells to MAdCAM or other cognate ligands, such as L-selectin, to MAdCAM. In a preferred embodiment, the MAdCAM is human and is either a soluble form, or expressed on the surface of a cell. In another preferred embodiment, the anti-MAdCAM antibody is a human antibody. In another embodiment, the antibody or portion thereof inhibits binding between α₄β₇ and MAdCAM with an IC₅₀ value of no more than 50 nM. In a preferred embodiment, the IC₅₀ value is no more than 5 nM. In a more preferred embodiment, the IC₅₀ value is less than 5 nM. In a more preferred embodiment, the IC₅₀ value is less than 0.05 μg/mL, 0.04 μg/mL or 0.03 μg/mL. In another preferred embodiment the IC₅₀ value is less than 0.5 μg/mL, 0.4 μg/mL or 0.3 μg/mL. The IC₅₀ value can be measured by any method known in the art. Typically, an IC₅₀ value can be measured by ELISA or adhesion assay. In a preferred embodiment, the IC₅₀ value is measured by adhesion assay using either cells or tissue which natively express MAdCAM or cells or tissue which have been engineered to express MAdCAM.
Inhibition of lymphocyte recruitment to gut-associated lymphoid tissue by anti-MAdCAM antibodies

[0196] In another embodiment, the invention provides an anti-MAdCAM antibody that binds natively expressed MAdCAM and inhibits the binding of lymphocytes to specialised gastrointestinal lymphoid tissue. In a preferred embodiment, the natively-expressed MAdCAM is human or primate MAdCAM and is either a soluble form, or expressed on the surface of a cell. In another preferred embodiment, the anti-MAdCAM antibody is a human antibody. In another embodiment, the antibody or portion thereof inhibits the recruitment of gut-trophic $\alpha_4\beta_7^{+}$ lymphocytes to tissues expressing MAdCAM with an IC$_{50}$ value of no more than 5 mg/kg. In a preferred embodiment, the IC$_{50}$ value is no more than 1 mg/kg. In a more preferred embodiment, the IC$_{50}$ value is less than 0.1 mg/kg. In one embodiment, the IC$_{50}$ value can be determined by measuring the dose effect relationship of recruitment of technetium-labeled peripheral blood lymphocytes to the gastrointestinal tract using gamma scintigraphy or single photon emission computed tomography. In an another embodiment, the IC$_{50}$ value can be determined by measuring the increase in gut-trophic $\alpha_4\beta_7^{+}$ lymphocytes, such as, but not limited to, CD$4^{+} \alpha_4\beta_7^{+}$ memory T-cells, in the peripheral circulation using flow cytometry as a function of the dose of anti-MAdCAM antibody.

[0197] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.
EXAMPLE 1:
Generation of anti-MAdCAM producing hybridomas

[0198] Antibodies of the invention were prepared, assayed and selected in accordance with the present Example.

5 Primary Immunogen Preparation:

[0199] Two immunogens were prepared for immunisation of the XenoMouse™ mice: (i) a MAdCAM-IgG1 Fc fusion protein and (ii) cell membranes prepared from cells stably transfected with MAdCAM.

(i) MAdCAM-IgG1 Fc Fusion Protein

Expression vector construction:

[0200] An EcoRI/BglII cDNA fragment encoding the mature extracellular, immunoglobulin-like domain of MAdCAM was excised from a pINCT 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:

MAdCAM-IgG1 Fc Fusion Protein:

MDFGGLALLLACGLLIGGSLQVKPLQVEPPEPVVAVALGASRQLTCLRLACADRGSVQWRGLDSLGAQSDTGRSVLTVRNASLSAAIGTRVCVGSCGGRFTPQHTVQLLLVYAFPDQLTVSPAALVPGDEVACTAHKVTPVDPNASLSFSLLVQGELEGAQALGPEVQEEEFPQGEDVLFVRTERWRLPLGTPVPAPLYCQATMRLPGLELSHRQAIVLHSPTSPDPEPDTSSPESPDPTSSQEPDTPDTTSQEPDPTSDPDTTSQEPDPTSDPDPTTPQGSSHTPRSPGSTRTRRPEIQPKSCDKTHCTPCCPAPELLGGPSVFLPPKPPTLDLMSRTPEVTVVTVDVHEDPEVFKNYVGDVEVHNAKTKRREEQNYSTRYVSSTVTLHQLNGKYECKVKSNALPALEKTSKAKGQPR

EPQVYTLPPSRDELTKNQVSLLTCVKGYPFSIDAVEWESNGQPENNYKATPPVLD
SDGSFFLYSKLTVDKSRWQGNNVFSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 107)

Underlined: signal peptide

5 Bold: MAdCAM extracellular domain

Recombinant Protein Expression/Purification:

[0201] 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.

15 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.

20 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.

(ii) Cell membranes stably expressing MAdCAM
[0202] A SacI/NotI fragment comprising nucleotides 645-1222 of the published MAAdCAM sequence (Shyjan AM, et al., *J Immunol.*, 156, 2851-7 (1996)) was PCR amplified from a colon cDNA library and cloned into SacI/NotI sites of pIND-Hygro vector (Invitrogen). A SacI fragment, comprising the additional 5’ coding sequence was sub-cloned into this construct from pCDNA3.1 MAAdCAM-IgG1 Fc, to generate the full length MAAdCAM cDNA. A KpnI/NotI fragment containing the MAAdCAM cDNA was then cloned into corresponding sites in a pEF5FRTV5GWCAT vector (Invitrogen) and replacing the CAT coding sequence. The cDNA insert was sequence verified and used in transfections to generate single stably expressing clones in FlpIn NIH 3T3 cells (Invitrogen) by Flp recombinase technology, according to the manufacturer’s instructions. Stably expressing clones were selected by their ability to support the binding of a α4β7+ JY human B lymphoblastoid cell line (Chan BM, et al, *J. Biol. Chem.*, 267:8366-70 (1992)), outlined below. Stable clones of CHO cells expressing MAAdCAM were prepared in the same way, using FlpIn CHO cells (Invitrogen).

[0203] MAAdCAM-expressing FlpIn NIH-3T3 cells were grown in Dulbecco’s modified Eagles Medium (Gibco), containing 2 mM L-glutamine, 10% Donor calf serum (Gibco) and 200 μg/mL Hygromycin B (Invitrogen) and expanded in roller bottles. MAAdCAM-expressing FlpIn CHO cells were grown in Ham’s F12/Dulbecco’s modified Eagles Medium (Gibco), containing 2 mM L-glutamine, 10% Donor calf serum (Gibco) and 350 μg/mL Hygromycin B (Invitrogen) and expanded in roller bottles. Cells were harvested by use of a non-enzymatic cell dissociation solution (Sigma) and scraping, washing in phosphate buffered saline by centrifugation. Cell membranes were prepared from the cell pellet by two rounds of polytron homogenization in 25 mM Bis Tris pH 8, 10 mM MgCl2, 0.015% (w/v) aprotinin, 100 U/mL bacitracin and centrifugation. The final pellet was resuspended in the same buffer, and 50x10⁶ cell equivalents aliquoted into thick-walled eppendorfs and spun at >100,000g to generate cell membrane pellets for XenoMouse mice immunisations. Supernatant was decanted and membranes were stored in eppendorfs at -80°C until required. Confirmation of protein expression in the cell membranes was determined by SDS-PAGE and Western
blotting with a rabbit anti-peptide antibody raised against the N-terminal residues of MAdCAM ([(C]-KPLQVEPPEP).

**Immunization and hybridoma generation:**

[0204] Eight to ten week old XENOMOUSE™ mice were immunized intraperitoneally or in their hind footpads with either the purified recombinant MAdCAM-IgG1 Fc fusion protein (10 µg/dose/mouse), or cell membranes prepared from either stably expressing MAdCAM-CHO or NIH 3T3 cells (10x10^6 cells/dose/mouse). This dose was repeated five to seven times over a three to eight week period. Four days before fusion, the mice received a final injection of the extracellular domain of human MAdCAM in PBS. Spleen and lymph node lymphocytes from immunized mice were fused with the non-secretory myeloma P3-X63-Ag8.653 cell line and were subjected to HAT selection as previously described (Galfre and Milstein, *Methods Enzymol.* 73:3-46 (1981)). A panel of hybridomas all secreting MAdCAM specific human IgG2κ and IgG4κ antibodies were recovered and sub-cloned. Twelve hybridoma sub-clones, 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4 and 9.8.2, producing monoclonal antibodies specific for MAdCAM were recovered and detected with assays described below. The parental lines 1.7, 1.8, 6.14, 6.22, 6.34, 6.67, 6.73, 6.77, 7.16, 7.20, 7.26 and 9.8, from which the sub-clone hybridoma lines, 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4 and 9.8.2, were derived all had anti-MAdCAM activity.

**ELISA assays:**

[0205] Detection of antigen-specific antibodies in mouse serum and hybridoma supernatant was determined by ELISA as described (Coligan et al., Unit 2.1 “Enzyme-linked immunosorbent assays,” in *Current Protocols in Immunology* (1994)) using MAdCAM-IgG1 Fc fusion protein to capture the antibodies. For animals that were immunised with MAdCAM-IgG1 Fc fusion protein, antibodies were screened for non-specific reactivity against human IgG1 and for the ability to bind to FpIn CHO MAdCAM cells by flow cytometry.

[0206] In a preferred ELISA assay, the following techniques are used:
[0207] ELISA plates were coated overnight at 4°C with 100 µL/well of
MAdCAM-IgG1 Fc fusion (4.5 µg/mL) in plate containing buffer (100 mM sodium
carbonate/bicarbonate buffer pH 9.6). After incubation, coating buffer was
removed and the plate blocked with 200 µL/well blocking buffer (5% BSA, 0.1%
Tween 20, in phosphate buffered saline) and incubated at room temperature for 1
hour. Blocking buffer was removed and 50 µL/well of hybridoma supernatant or
other serum or supernatant (e.g., positive control) added for 2 hours at room
temperature. After incubation the plate was washed with PBS (3 x 100 µL/well)
and the binding of the hybridoma mAb detected with HRP-conjugated secondary
antibodies (i.e. 1:1000 mouse anti-human IgG2-HRP (SB Cat. No. 9060-05) for
IgG2 antibodies or 1:1000 mouse anti-human IgG4-HRP (Zymed Cat. No. 3840)
for IgG4 antibodies) diluted in PBS. The plates were incubated at room
temperature for 1 hour, washed in PBS (3 x 100 µL/well) and finally developed
with 100 µL OPD (o-phenylenediamine (DAKO S2405) + 5 µL 30% H2O2/12
mL). The plates were allowed to develop 10-20 mins, stopping the reaction with
100 µL 2M H2SO4. The plates were read at 490 nm.

Adhesion assays:

[0208] Antibodies that demonstrated binding to MAdCAM-IgG1 Fc fusion
protein by ELISA, were assessed for antagonist activity in an adhesion assays with
α4β7+ JY cells and either (i) MAdCAM-IgG1 Fc fusion protein or (ii) MAdCAM-
CHO cells.

(i) MAdCAM-IgG1 Fc fusion assay

[0209] 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 8x10^5 cells/mL) and 2x10^6
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 1-2x10^5/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 2x10^6 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 μM) 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 μg/mL (1 μg/mL final) in 0.1 mg/mL BSA (Sigma#A3059) in PBS; for full IC_{50} curves, the antibodies were diluted in 0.1 mg/mL BSA/ PBS, with 3 μg/mL (1 μg/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 μL 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 1x10^6/mL in 1 mg/mL BSA/PBS. 100 μL 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 Victor^2 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 IC_{50} 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 IC_{50} value <0.1 μg/mL, were considered to have potent antagonist activity and
were progressed to the MAdCAM-CHO adhesion assay. All twelve of the tested Abs showed potent antagonist activity (Table 3). Monoclonal antibodies 1.7.2, 1.8.2, 7.16.6, 7.20.5 and 7.26.4 were derived from IgG₂κ lineages, and monoclonal antibodies 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1 and 9.8.2 were derived from IgG₄κ lineages.

(ii) MAdCAM-CHO cell adhesion assay.

[0211] JY cells were cultured as above. MAdCAM-expressing CHO cells were generated with the pEF5FRT MAdCAM cDNA construct and using the Flp recombinase technology (Invitrogen) as described above. Single stable clones of MAdCAM-expressing CHO cells were selected based on their ability to support the adhesion of JY cells and the binding, by flow cytometry, of the rabbit anti-peptide antibody, raised against the N-terminus of MAdCAM and described above. MAdCAM-expressing CHO cells were cultured in a DMEM/F12 media (Gibco # 21331-020) containing 2 mM L-glutamine, 10% fetal bovine serum (Gibco) and 350 μg/mL Hygromycin B (Invitrogen), splitting 1:5 every 2/3 days. For the adhesion assay, MAdCAM-expressing CHO cells were seeded at 4x10⁴ cells/well in 96 well black plates–clear bottom (Costar # 3904) in 200 μL culture medium and cultured overnight at 37°C/5% CO₂.

[0212] The following day, hybridoma supernatant or purified monoclonal antibody was diluted from a starting concentration of 30 μg/mL (equivalent to a final concentration of 10 μg/mL) in 1 mg/mL BSA/PBS, as described above. For the MAdCAM CHO plates, the plate contents were flicked out and 50 μL of antibodies/controls were added to each well and the plate incubated at 37°C for 20 min. The final well of the row was used for determining total binding, so 0.1 mg/mL BSA in PBS was used. Calcein AM-loaded JY cells, to a final concentration of 1x10⁶/mL in 1 mg/mL BSA/PBS, were prepared as above, then 100 μL added to the plate after the 20 min incubation period with the antibody. The plate was then incubated at 37°C for 45 min, then washed on a Tecan plate washer (PW 384) and fluorescence measured using the Wallac plate reader as described above. 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. Antibodies that were able to inhibit the binding of JY cells to MAdCAM CHO cells with an IC$_{50}$ value <1 µg/mL were considered to have potent antagonist activity. As before, the IC$_{50}$ value is defined as the anti-MAdCAM antibody concentration at which the adhesion response had decreased to 50% of the response in the absence of anti-MAdCAM antibody. The IC$_{50}$ potencies for 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4 and 9.8.2 in this assay are described below in Table 3.

Table 3. IC$_{50}$ values of exemplified anti-MAdCAM antibodies

<table>
<thead>
<tr>
<th>Clone</th>
<th>MAdCAM IgG1 Fc fusion Mean IC50 (µg/mL) n</th>
<th>MAdCAM FplIn CHO Assay Mean IC50 (µg/mL) n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7.2</td>
<td>0.030 ± 0.011 6</td>
<td>0.502 ± 0.280 9</td>
</tr>
<tr>
<td>1.8.2</td>
<td>0.027 ± 0.011 4</td>
<td>0.424 ± 0.107 8</td>
</tr>
<tr>
<td>7.16.6</td>
<td>0.019 ± 0.009 7</td>
<td>0.389 ± 0.093 16</td>
</tr>
<tr>
<td>7.20.5</td>
<td>0.025 ± 0.027 7</td>
<td>0.387 ± 0.202 9</td>
</tr>
<tr>
<td>7.26.4</td>
<td>0.021 ± 0.040 4</td>
<td>0.574 ± 0.099 15</td>
</tr>
<tr>
<td>6.14.2</td>
<td>0.011 ± 0.005 4</td>
<td>0.291 ± 0.096 6</td>
</tr>
<tr>
<td>6.22.2</td>
<td>0.018 ± 0.011 4</td>
<td>0.573 ± 0.168 7</td>
</tr>
<tr>
<td>6.34.2</td>
<td>0.013 ± 0.008 4</td>
<td>0.285 ± 0.073 7</td>
</tr>
<tr>
<td>6.67.1</td>
<td>0.013 ± 0.070 4</td>
<td>0.298 ± 0.115 8</td>
</tr>
<tr>
<td>6.73.2</td>
<td>0.020 ± 0.010 4</td>
<td>0.369 ± 0.103 8</td>
</tr>
<tr>
<td>6.77.1</td>
<td>0.022 ± 0.004 4</td>
<td>0.520 ± 0.100 4</td>
</tr>
<tr>
<td>9.8.2</td>
<td>0.020 ± 0.050 4</td>
<td>0.440 ± 0.342 8</td>
</tr>
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</table>

[0213] To measure the antagonist potency of anti-MAdCAM mAbs in flow-based assays, under sheer stress conditions that are designed to mimic the microvascular environment on the high endothelial venules which serve the gut associated lymphoid tissue, CHO cells expressing MAdCAM were plated in glass microslides (50 x 4 mm) and allowed to adhere to form a confluent monolayer (ca. 2.5 x 10$^5$ cells). The cells were then incubated with affinity-purified mAb over a range of concentrations (0.1-10 µg/mL) for 20 mins at 37°C, before being connected to the flow assay system. An isotype matched IgG2 or IgG4 mAb (10
μg/mL) was used as a negative control. Normal donor peripheral blood lymphocytes (PBLs) were perfused over the cell monolayer at a constant shear stress of 0.05 Pa. Experiments were videoed and total adhesion of lymphocytes (rolling + firm adhesion) was calculated. All of the tested monoclonal antibodies were shown to be potent antagonists under the conditions described.

(iii) Stamper-Woodruff assays

[0214] To visualise MAdCAM⁺ vessels, biotinylated anti-MAdCAM mAb was generated on 1-2 mg of affinity-purified protein, using a 20 molar excess of biotin-NHS (Pierce) in phosphate buffer saline, according to manufacturer's instructions. The reaction was allowed to sit at room temperature (30 min), and desalted with a PD-10 (Pharmacia) column and the protein concentration determined.

[0215] Normal liver lymph node was removed from a donor organ, snap-frozen in liquid nitrogen and stored at -70°C until use. 10 μm cryostat sections were cut, air-dried on poly-L lysine coated slides, and fixed in acetone prior to the assay.

Sections were blocked using an avidin-biotin blocking system (DAKO), and then incubated with biotinylated anti-MAdCAM mAb over a range of concentrations (1-50 μg/mL) at room temperature (2 hrs). An isotype matched IgG₂ or IgG₄ mAb (50 μg/mL) was used as a negative control and a blocking anti-β7 antibody (50 μg/mL) as a positive control.

[0216] Peripheral blood lymphocytes, taken from normal donors, were labeled with a mouse anti-human CD2 mAb (DAKO) to allow subsequent visualisation of adherent cells. 5x10⁵ PBLs were added to each lymph node section and incubated for 30 mins before being gently rinsed off to avoid detachment of adherent cells. Sections were then re-fixed in acetone, and re-incubated with biotinylated anti-MAdCAM mAb (10 μg/mL), followed by biotinylated goat-anti-mouse mAb (to recognise CD2 labeled PBLs and unstained MAdCAM⁺ vessels) and then streptABcomplex/HRP (DAKO). Finally MAdCAM⁺ vessels & CD2 labeled PBLs were visualised by addition of DAB substrate (DAKO) to the sections, with a brown reaction product showing areas of positive staining. Lymphocyte adhesion was quantified by counting the number of lymphocytes adhering to 50 MAdCAM-1⁺ vessels of portal tracts, veins or sinusoids. Data, expressed as mean values, were then normalised to percent adhesion, using the adhesion of PBLs in
the absence of any antibody taken as 100%. The data were compiled on the basis of n=3 different PBL donors and for different liver lymph node donors. Representative data for biotinylated purified monoclonal antibodies 1.7.2 and 7.16.6 are depicted in Figure 4 compared to a blocking anti-β7 antibody control.

Selectivity assays:

[0217] VCAM and fibronectin are close structural and sequence homologues to MAAdCAM. Affinity-purified anti-MAAdCAM mAbs were assessed for MAAdCAM-specificity by determining their ability to block the binding of α4β1+/α5β1+ Jurkat T-cells (ATCC) to their cognate cell adhesion molecule. 100μL of a 4.5μg/mL solution of Fibronectin cell binding fragment (110 Kd, Europa Bioproducts Ltd, Cat. No. UBF4215-18) or VCAM (Panvera) in Dulbecco’s PBS was adsorbed to 96 well Black Microfluor “B” u-bottom (Dynex #7805) plates overnight at 4°C. The 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 Jurkat T cells were counted using tryptan blue exclusion and loaded with Calcein AM dye as previously described for JY cells above. The antibodies to be tested, were diluted from a top concentration of 10 μg/mL in 0.1 mg/ml BSA in PBS. The final well of the row was used for determining total binding, so 0.1mg/ml BSA in PBS was used. Echistatin (Bachem, Cat. No. H-9010) prepared in PBS was used at a top concentration of 100 nM to block the α5β1/Fibronectin interaction. An anti-CD106 mAb (Clone 51-10C9, BD Pharmingen Cat. No. 555645) at a top concentration of 1 μg/mL was used to block the α4β1/VCAM interaction.

[0218] After blocking, the plate contents were flicked out and 50 μL of antibodies/controls were added to each well and the plate incubated at 37°C for 20 min. Calcein-loaded Jurkat T cells were washed once as before, resuspending the final cell pellet to 1x10⁶/mL in 1 mg/mL BSA/PBS. 100 μL 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 Skatrap 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, the degree of inhibition is expressed below pictorially, in Table 4 (- negligible inhibition of adhesion, *** complete inhibition of adhesion). All mAbs exemplified are potent and selective anti-MAdCAM antagonists, demonstrating substantially greater than 100 fold selectivity for MAdCAM over VCAM and fibronectin.

Table 4. Comparative selectivity of anti-MAdCAM antibody for MAdCAM over other cell adhesion molecules, Fibronectin and VCAM

<table>
<thead>
<tr>
<th>Clone</th>
<th>Inhibition in ( \alpha_5\beta_1 )/Fibronectin assay (10 ( \mu )g/mL)</th>
<th>Inhibition in ( \alpha_4\beta_1 )/VCAM assay (10 ( \mu )g/mL)</th>
<th>Inhibition in ( \alpha_4\beta_7 )/MAdCAM assay (0.1 ( \mu )g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7.2</td>
<td>-</td>
<td>-</td>
<td>***</td>
</tr>
<tr>
<td>1.8.2</td>
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<td>-</td>
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<tr>
<td>7.16.6</td>
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<td>7.20.5</td>
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<tr>
<td>7.26.4</td>
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<td>6.14.2</td>
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<td>6.22.2</td>
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<td>6.34.2</td>
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<tr>
<td>6.67.1</td>
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<td>6.73.2</td>
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<tr>
<td>6.77.1</td>
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<tr>
<td>9.8.2</td>
<td>-</td>
<td>-</td>
<td>***</td>
</tr>
</tbody>
</table>

[0219] Hybridomas 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:
<table>
<thead>
<tr>
<th>Hybridoma</th>
<th>Deposit No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7.2</td>
<td>03090901</td>
</tr>
<tr>
<td>1.8.2</td>
<td>03090902</td>
</tr>
<tr>
<td>6.14.2</td>
<td>03090903</td>
</tr>
<tr>
<td>6.22.2</td>
<td>03090904</td>
</tr>
<tr>
<td>6.34.2</td>
<td>03090905</td>
</tr>
<tr>
<td>6.67.1</td>
<td>03090906</td>
</tr>
<tr>
<td>6.73.2</td>
<td>03090907</td>
</tr>
<tr>
<td>6.77.1</td>
<td>03090908</td>
</tr>
<tr>
<td>7.16.6</td>
<td>03090909</td>
</tr>
<tr>
<td>7.20.5</td>
<td>03090910</td>
</tr>
<tr>
<td>7.26.4</td>
<td>03090911</td>
</tr>
<tr>
<td>9.8.2</td>
<td>03090912</td>
</tr>
</tbody>
</table>

**EXAMPLE II:**

**Determination of Affinity Constants (K_d) of Fully Human Anti-MAdCAM Monoclonal Antibodies by BIAcore**

[0220] We performed affinity measures of purified antibodies by surface plasmon resonance using the BIAcore 3000 instrument, following the manufacturer’s protocols.

**Protocol 1**

[0221] To perform kinetic analyses, a high density mouse anti-human (IgG₂ and IgG₄) antibody surface over a CM5 BIAcore sensor chip was prepared using routine amine coupling. Hybridoma supernatants were diluted 10, 5, 2-fold in HBS-P (10 mM HEPES pH 7.4, 150 mM NaCl, 0.005% Surfactant P20) running buffer containing 100 µg/mL BSA and 10 mg/mL carboxymethyl dextran or used neat. Each mAb was captured onto a separate surface using a 1 min contact time and a 5 min wash for stabilization of the mAb baseline. MAdCAM-IgG₁ Fc (141 nM) fusion protein was then injected at over all surfaces for one minute, followed by a 3 min dissociation. The data were normalized for the amount of antibody captured on each surface and evaluated with global fit Langmuir 1:1, using
baseline drift models available on the BIAevaluation software provided by BIAcore.

Protocol 2

[0222] Affinity-purified mAb were immobilized onto the dextran layer of a CM5 biosensor chip using amine coupling. Chips were prepared using pH 4.5 acetate buffer as the immobilization buffer and protein densities of 2.5-5.5 kRU were achieved. Samples of MAdCAM-IgG1 Fc fusion protein in running buffer were prepared at concentrations ranging from 0.2-55 nM (a 0 nM solution comprising running buffer alone was included as a zero reference). Samples were randomized and injected in duplicate for 3 min each across 4 flow cells using HBS-EP (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% Surfactant P20) as running buffer. A flow rate of 100 µL/min was used to minimize mass transport limitations. Dissociation of MAdCAM-IgG1 Fc fusion protein was monitored for 180 mins, the surface regenerated by a 6 sec injection of 25 mM H₃PO₄ (50 µL/min), or 10 mM (6.22.2), 20 mM (6.67.1, 6.73.2, 6.77.1) to 25 mM (6.34.2) and 45 mM NaOH (6.14.2) and the data analysed using the BIAevaluation (v3.1) software package.

[0223] Table 5 lists affinity measurements for representative anti-MAdCAM antibodies of the present invention:
Table 5. Determination of affinity constant, $K_d$, by surface plasmon resonance (BIAcore)

<table>
<thead>
<tr>
<th>CLONE</th>
<th>Protocol 1</th>
<th>Protocol 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{on}$ (1/Ms)</td>
<td>$k_{off}$ (1/s)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.7.2</td>
<td>$2.4 \times 10^5$</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>1.8.2</td>
<td>$2.9 \times 10^5$</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>7.16.6</td>
<td>$1.5 \times 10^6$</td>
<td>$2.2 \times 10^6$</td>
</tr>
<tr>
<td>7.20.5</td>
<td>$4.5 \times 10^5$</td>
<td>$1.9 \times 10^5$</td>
</tr>
<tr>
<td>7.26.4</td>
<td>$9.6 \times 10^5$</td>
<td>$2.6 \times 10^4$</td>
</tr>
<tr>
<td>6.14.2</td>
<td>$1.3 \times 10^5$</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>6.22.2</td>
<td>$1.5 \times 10^6$</td>
<td>$1.4 \times 10^6$</td>
</tr>
<tr>
<td>6.34.2</td>
<td>$1.2 \times 10^6$</td>
<td>$1.9 \times 10^5$</td>
</tr>
<tr>
<td>6.67.1</td>
<td>$5.9 \times 10^5$</td>
<td>$1 \times 10^6$</td>
</tr>
<tr>
<td>6.73.2</td>
<td>$1.4 \times 10^5$</td>
<td>$1.3 \times 10^4$</td>
</tr>
<tr>
<td>6.77.1</td>
<td>$1.5 \times 10^5$</td>
<td>$1 \times 10^5$</td>
</tr>
<tr>
<td>9.8.2</td>
<td>$2.3 \times 10^6$</td>
<td>$2.3 \times 10^4$</td>
</tr>
</tbody>
</table>

IgG2
IgG4

[0224] The kinetic analyses indicate that the antibodies prepared in accordance with the invention possess high affinities and strong binding constants for the extracellular domain of MAdCAM.

EXAMPLE III:

Identification of epitope selectivity and species cross-reactivity of anti-MAdCAM mAbs

[0225] Antibodies recognize surface-exposed epitopes on antigens as regions of linear (primary) sequence or structural (secondary) sequence. Luminex epitope binning, BIAcore binning and species immunohistochemical analysis were used in concert, in order to define the functional epitope landscape of the anti-MAdCAM antibodies.
Luminex-based Epitope Binning:

[0226] MxhlG 2,3,4-conjugated beads (Calbiochem M1 1427) were coupled to the primary unknown anti-MAdCAM antibody. We added 150 μL of primary unknown antibody dilution (0.1 μg/mL diluted in hybridoma medium) to the well of a 96-well tissue culture plate. The bead stock was gently vortexed and diluted in supernatant to a concentration of 0.5 x 10^5 beads/mL. The beads were incubated in the supernatant on a shaker overnight in the dark at 4°C.

[0227] Each well of a 96-well microtiter filter plate (Millipore # MABVN1250) was pre-wetted by adding 200 μL wash buffer (PBS containing 0.05% Tween20) and removed by aspiration. Next, 50 μL/well of the 0.5 x 10^5 beads/mL stock was added to the filter plate, and the wells washed with wash buffer (2 x100 μL/well). 60 μL/well of MAdCAM-IgG1 Fc antigen diluted in hybridoma medium (0.1 μg/mL) was added. The plates were covered and incubated at room temperature with gentle shaking for one hour. The wells were washed twice by addition of 100 μL/well wash buffer followed by aspiration. Next, we added 60 μL/well of secondary unknown anti-MAdCAM antibody diluted in hybridoma medium (0.1 μg/mL). The plates were shaken at room temperature in the dark for two hours. Next, the wells were washed twice by addition of 100 μL/well wash buffer followed by aspiration. Next, 60 μL/well of biotinylated MxhlG 2,3,4 (0.5 μg/mL) was added. The plates were shaken at room temperature in the dark for one hour. The wells were washed twice by addition of 100 μL/well wash buffer followed by aspiration. To each well, 60 μL of 1 μg/mL MxhlG 2,3,4 Streptavidin-PE (Pharmacia #554061) diluted in hydridoma medium was added. The plates were shaken at room temperature in the dark for twenty minutes. The wells were washed twice by addition of 100 μL/well wash buffer followed by aspiration. Next, each well was resuspended in 80 μL blocking buffer (PBS with 0.5% bovine serum albumin, 0.1% TWEEN and 0.01% Thimerosal) carefully pipetted up and down to resuspend the beads.

[0228] Using Luminex 100 and its accompanying software (Luminex® Corporation) the plates were read to determine luminescence readings. Based on the luminescence data obtained for the various anti-MAdCAM antibodies tested,
the anti-MAdCAM antibodies were grouped according to their binding specificities. The anti-MAdCAM antibodies that were tested fall into a series of epitope bins, represented in Table 8.

BIAcore binning:

[0229] In a similar method to that described above, BIAcore can also be used to determine the epitope exclusivity of the anti-MAdCAM antibodies exemplified by this invention. Nine anti-MAdCAM antibody clones, 6.22.2, 6.34.2, 6.67.1, 6.77.1, 7.20.5, 9.8.2, 1.7.2, 7.26.4 and 7.16.6, were immobilized onto the dextran layer of separate flow cells of a CM5 biosensor chip using amine coupling. The immobilization buffer was either 10 mM acetate buffer pH 4.5 (clones 6.22.2, 6.34.2, 7.20.5, 9.8.2, 1.7.2, 7.26.4 and 7.16.6) or 10 mM acetate buffer pH 5.5 (clones 6.67.1 and 6.77.1). A protein density of approximately 3750 RU was achieved in all cases. Deactivation of unreacted N-hydroxysuccinimide esters was performed using 1 M ethanolamine hydrochloride, pH 8.5. [0230] MAdCAM-

IgG1 Fc fusion protein was diluted to a concentration of 1.5 μg/mL (approximately 25 nM) in HBS-EP running buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Polysorbate 20). It was then injected across the first flow cell, in a volume of 50 μL at a rate of 5 μL/min. After the injection was complete, the first antibody probe was added to the same flow cell. All test antibodies were diluted to a concentration of approximately 20 μg/mL in HBS-EP, and also injected in a volume of 50 μL at a flow rate of 5 μL/min. When no binding of the test antibody was observed, the next test clone was injected immediately afterwards. When binding did occur, the sensor surface was regenerated to remove both the MAdCAM-IgG1 Fc fusion protein and the test antibody. A variety of regeneration solutions were used depending upon the immobilized antibody and the test antibody present. A summary of the regeneration conditions used is depicted in Table 6.
Table 6. Summary of regeneration conditions used to perform BIAcore epiope mapping

<table>
<thead>
<tr>
<th>Immobilised antibody</th>
<th>Antibody probe to be removed</th>
<th>Regeneration solution</th>
<th>Injection volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.16.6</td>
<td>6.22.2</td>
<td>40 mM Phosphoric Acid</td>
<td>20 µL</td>
</tr>
<tr>
<td></td>
<td>6.34.2</td>
<td>40 mM Phosphoric Acid</td>
<td>40 µL</td>
</tr>
<tr>
<td></td>
<td>7.20.5</td>
<td>40 mM Phosphoric Acid</td>
<td>20 µL</td>
</tr>
<tr>
<td>6.77.1</td>
<td>9.8.2</td>
<td>40 mM Phosphoric Acid</td>
<td>10 µL</td>
</tr>
<tr>
<td></td>
<td>1.7.2</td>
<td>40 mM Phosphoric Acid</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>7.16.6</td>
<td>40 mM Phosphoric Acid</td>
<td>10 µL</td>
</tr>
<tr>
<td>1.7.2</td>
<td>6.77.1</td>
<td>25 mM Phosphoric Acid</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>9.8.2</td>
<td>25 mM Phosphoric Acid</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>7.20.5</td>
<td>25 mM Phosphoric Acid</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>6.22.2</td>
<td>25 mM Phosphoric Acid</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>6.34.2</td>
<td>25 mM Sodium Hydroxide</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>6.67.1</td>
<td>25 mM Sodium Hydroxide</td>
<td>5 µL</td>
</tr>
<tr>
<td>6.22.2</td>
<td>9.8.2</td>
<td>25 mM Sodium Hydroxide</td>
<td>20 µL</td>
</tr>
<tr>
<td></td>
<td>7.26.4</td>
<td>25 mM Sodium Hydroxide</td>
<td>5 µL</td>
</tr>
<tr>
<td>6.34.2</td>
<td>9.8.2</td>
<td>25 mM Sodium Hydroxide</td>
<td>70 µL</td>
</tr>
<tr>
<td></td>
<td>1.7.2</td>
<td>40 mM Sodium Hydroxide</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>7.26.4</td>
<td>40 mM Sodium Hydroxide</td>
<td>5 µL</td>
</tr>
<tr>
<td>6.67.1</td>
<td>9.8.2</td>
<td>40 mM Sodium Hydroxide</td>
<td>5 µL</td>
</tr>
<tr>
<td></td>
<td>1.7.2</td>
<td>40 mM Sodium Hydroxide</td>
<td>5 µL</td>
</tr>
<tr>
<td>7.20.5</td>
<td>9.8.2</td>
<td>25 mM Phosphoric Acid</td>
<td>5 µL</td>
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<td>1.7.2</td>
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<td>5 µL</td>
</tr>
<tr>
<td>7.26.4</td>
<td>9.8.2</td>
<td>40 mM Sodium Hydroxide</td>
<td>20 µL</td>
</tr>
<tr>
<td></td>
<td>6.22.2</td>
<td>75 mM Phosphoric Acid</td>
<td>20 µL</td>
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<td>7.20.5</td>
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<td>20 µL</td>
</tr>
<tr>
<td></td>
<td>7.16.6</td>
<td>75 mM Phosphoric Acid</td>
<td>20 µL</td>
</tr>
<tr>
<td>9.8.2</td>
<td>9.8.2</td>
<td>25 mM Phosphoric Acid</td>
<td>15 µL</td>
</tr>
<tr>
<td></td>
<td>6.22.2</td>
<td>25 mM Phosphoric Acid</td>
<td>10 µL</td>
</tr>
<tr>
<td></td>
<td>7.20.5</td>
<td>25 mM Phosphoric Acid</td>
<td>20 µL</td>
</tr>
<tr>
<td></td>
<td>7.16.6</td>
<td>25 mM Phosphoric Acid</td>
<td>10 µL</td>
</tr>
</tbody>
</table>

(Flow rate was 50 µL/min during all regeneration procedures)

[0231] After regeneration, MA
cAM-IgG Fc fusion protein was bound again and further test antibodies were injected. These procedures were carried out until the entire panel of clones had been injected over the surface of the immobilised antibody, with bound MA
cAM-IgG Fc fusion protein. A new flow cell with a different immobilised antibody and bound MA
cAM was then used for probing
with the nine test clones. Anti-MAdCAM antibodies 1.7.2 and 1.8.2 were 
expected to recognise the same MAdCAM epitope, based on the close primary 
amino acid sequence homology of their heavy and kappa light chains, SEQ ID 
NOS: 2, 4, 6, 8 respectively. Accordingly, only 1.7.2 was assessed though the 
BIAcore response matrix. Antibodies 6.14.2 and 6.73.2 were omitted from this 
analysis, but all other combinations of anti-MAdCAM antibody pairs were tested 
in this way. An arbitrary level of 100 RU was chosen as the threshold between 
binding/non-binding and a response matrix, (Table 7), was created based on 
whether binding was observed.

Table 7: **BIAcore epitope binning response matrix**

<table>
<thead>
<tr>
<th>Immobilised antibody</th>
<th>Secondary antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.22.2 6.34.2 6.67.1 6.77.1 7.20.5 9.8.2 1.7.2 7.26.4 7.16.6</td>
</tr>
<tr>
<td>6.22.2</td>
<td>- - - - x x x x</td>
</tr>
<tr>
<td>6.34.2</td>
<td>- - - - x x x x</td>
</tr>
<tr>
<td>6.67.1</td>
<td>- - - - x x x -</td>
</tr>
<tr>
<td>6.77.1</td>
<td>- - - - x x - -</td>
</tr>
<tr>
<td>7.20.5</td>
<td>- - - - x x x x</td>
</tr>
<tr>
<td>9.8.2</td>
<td>x x x x x x - -</td>
</tr>
<tr>
<td>1.7.2</td>
<td>x x x x x x - x</td>
</tr>
<tr>
<td>7.26.4</td>
<td>x x - - x x - x</td>
</tr>
<tr>
<td>7.16.6</td>
<td>x x - - x - - -</td>
</tr>
</tbody>
</table>

Response matrix for all combinations of antibody pairs. - indicates no binding of the antibody probe, x indicates binding was observed (above a chosen threshold level of 100 RU).

[0232] The matrix diagonal in Table 7 (shaded grey) holds the binding data for identical probe pairs. In all instances, except for the two clones 7.16.6 and 9.8.2, the antibodies were self-blocking. Antibodies 7.16.6 and 9.8.2 do not cross compete. The lack of self-blocking could be due to a mAb-induced conformational change in the fusion protein that permits additional binding of the mAb to a second site on MAdCAM-IgFc.

Grouping the clones that show the same reactivity pattern gives rise to at least six different epitope bins, as shown in the graphical representation, Figure 5).
Further precise identification of the MA
dCAM epitope sequences with which an anti-
MACM antibody interacts can be determined by any of a num-
ber of methods, including, but not limited to, Western analysis of spotted peptide
library arrays (Reineke et al., Curr. Topics in Microbiol. and Immunol 243: 23-36
(1999), M. Famulok, E-L Winnacker, C-H Wong eds., Springer-Verlag, Berlin),
phage or bacterial flagellin/fliC expression library display, or simple MALDI-TOF
analysis of bound protein fragments following limited proteolysis.

Immunohistochemical assays:

OCT or sucrose-embedded frozen tissue specimens of ileum (Peyer’s
patches), mesenteric lymph node, spleen, stomach, duodenum, jejunum and colon
were used as a positive staining controls for the anti-MA
cAM mAbs. For staining
human sections with human IgG2 mAbs, biotinylated derivatives of the anti-
MA
cAM mAbs were generated. 10 μm frozen tissue sections were cut onto poly
L-lysine coated slides, placed directly into 100% acetone 4°C (10 min), then 3%
hydrogen peroxide in methanol (10 min), washing between steps with PBS. The
slides were blocked with Biotin Blocking System (DAKO Cat. No. X0590), prior
to incubation with the primary antibody (1:100 - 1:1000) in PBS (1 hr), washed
with PBS-Tween 20 (0.05%) and then binding developed with HRP-Streptavidin
(BD Bioscience Cat. No.550946, 30 min) and DAB substrate (Sigma Cat. No.
D5905). For IgG4 mAbs, an HRP-conjugated, mouse anti-human IgG4 (Zymed
Cat. No. 3840) secondary was used. The slides were counterstained with Mayer’s
Haemalum (1 min), washed and then mounted in DPX.

Binding affinity was compared for a number of species (mouse, rat,
rabbit, dog, pig, cynomolagus and human tissue). There was no reactivity for rat,
rabbit and pig tissue by immunohistochemistry and no cross-reactivity of the anti-
MA
cAM antibodies for recombinant mouse MA
cAM, when analyzed by
ELISA. The data for human, cynomolagus and dog tissue are presented in table
form, Table 8 below:
Table 8. Pattern of cross reactivity of anti-MAdCAM antibodies to MAdCAM species orthologues

<table>
<thead>
<tr>
<th>CLONE</th>
<th>Luminex BIN</th>
<th>human ileum</th>
<th>cyno ileum</th>
<th>marmoset ileum</th>
<th>dog ileum</th>
</tr>
</thead>
<tbody>
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<td>1.7.2</td>
<td>3a</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1.8.2</td>
<td>3a</td>
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<td></td>
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<tr>
<td>7.16.6</td>
<td>3b</td>
<td></td>
<td></td>
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<td>7.20.5</td>
<td>2b</td>
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<td>3b</td>
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</tr>
<tr>
<td>6.22.2</td>
<td>2</td>
<td></td>
<td></td>
<td>n.d</td>
<td></td>
</tr>
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<td>6.34.2</td>
<td>6</td>
<td></td>
<td></td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>6.67.1</td>
<td>5</td>
<td></td>
<td></td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>6.73.2</td>
<td>3</td>
<td></td>
<td></td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>6.77.1</td>
<td>1</td>
<td></td>
<td></td>
<td>n.d</td>
<td></td>
</tr>
<tr>
<td>9.8.2</td>
<td>3a</td>
<td></td>
<td></td>
<td>n.d</td>
<td></td>
</tr>
</tbody>
</table>

IgG2: Binding
IgG4: No Binding
n.d: not determined

[0236] Anti-MAdCAM binding to specialised endothelial structures and lymphoid tissue is indicated by the shading, according to the key. The epitope bin based on Luminex epitope analysis and the pattern of MAdCAM cross-reactivity are indicated for each antibody. Luminex epitope binning data for anti-MAdCAM antibodies 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.3 and 6.77.1 (italics) were derived from separate experiments than that for 1.7.2, 1.8.2, 7.16.6, 7.20.5, 7.26.4 and 9.8.2 (bold type), as indicated by the difference in font character.

[0237] All anti-MAdCAM antibodies tested had the ability to recognize a human MAdCAM epitope expressed on vascular endothelial compartments of the gastrointestinal tract. Apart from 1.7.2 and 1.8.2, all other anti-MAdCAM antibodies tested were able to specifically bind the vascular endothelial compartments of the cynomolgus gastrointestinal tract. Certain other anti-MAdCAM antibodies, namely 6.14.2 and 6.67.1 also had the ability to specifically recognize the dog MAdCAM orthologue as well as cynomolgus MAdCAM.
Generation of a functionally active chimeric cynomolgus/human MAdCAM-expressing CHO cell line:

[0238] The differences in binding affinity of certain anti-MAdCAM antibodies for human and cynomolgus MAdCAM led us to determine whether a structural basis for this observation could be made. [0239] Based on the published amino acid sequence for Macaque MAdCAM (Shyjan AM, et al., *J Immunol.*, 156, 2851-7 (1996)), primers were designed to PCR amplify the cynomolgus MAdCAM $\alpha_4\beta_7$ binding domain sequence. Total RNA was prepared from frozen excised cynomolgus mesenteric lymph node (ca. 200 mg) using the Trizol method (Invitrogen) according to the manufacturer’s instructions. 1-2 $\mu$g was oligo-dT primed and reverse transcribed with AMV reverse transcriptase (Promega). A proportion of the reverse transcribed product was subjected to PCR with forward 5'-AGC ATG GAT CGG GGC CTG GCC-3' (SEQ ID NO: 67) and reverse 5'-GTG CAG GAC CGG GAT GGC CTG-3' (SEQ ID NO: 68) primers with GC-2 polymerase in 1M GC melt (Clontech) and at an annealing temperature of 62°C. An RT-PCR product of the appropriate size was excised and purified from a 1% agarose gel after electrophoresis, then TOPO-TA cloned (Invitrogen) between EcoRI sites of pCR2.1. The insert was sequence confirmed. The nucleotide and predicted translated amino acid sequences are shown in SEQ ID NOS 49 and 50, respectively.

[0240] The predicted human and cynomolgus MAdCAM amino acid sequences for the $\alpha_4\beta_7$ binding domain show a high degree of sequence identity (90.8%) when aligned (Figure 3 provides this sequence alignment). To generate a functionally active cynomolgus MAdCAM-expressing cell line, which mimicked the anti-MAdCAM binding pattern represented by Table 8, a SacI fragment corresponding to the cynomolgus $\alpha_4\beta_7$ binding domain sequence in pCR2.1, was subcloned directly into the C-terminal human MAdCAM pIND-Hygro construct containing carboxyl-terminal mucin stalk and transmembrane domain, described above. The sequence and orientation was verified, then a KpnI/NotI fragment was cloned into pEF5FRTV5GWCAT vector (Invitrogen), replacing the CAT coding sequence and used in transfections to generate single stably expressing clones in Flp In CHO cells (Invitrogen), according to the manufacturer’s instructions.
The binding of anti-MAdCAM antibody clones to the CHO cells expressing cynomolgus/human MAdCAM chimera was assessed by flow cytometry and the functional activity of anti-MAdCAM antibodies was determined using a very similar JY cell adhesion assay as that described above. The binding and functional activity of anti-MAdCAM antibodies are expressed in Table 9.
Table 9. Correlation between the functional activity in the cynomolgus/human MAdCAM-CHO/JY adhesion assay and human and cynomolgus/human MAdCAM CHO cell binding, as measured by FACS, for a range of anti-MAdCAM antibodies.

<table>
<thead>
<tr>
<th>CLONE</th>
<th>Functional IC&lt;sub&gt;50&lt;/sub&gt; (μg/mL)</th>
<th>FACS binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>human</td>
</tr>
<tr>
<td>1.7.2</td>
<td>inactive</td>
<td></td>
</tr>
<tr>
<td>1.8.2</td>
<td>inactive</td>
<td></td>
</tr>
<tr>
<td>7.16.6</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>7.20.5</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>7.26.4</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>6.14.2</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>6.22.2</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>6.34.2</td>
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<td></td>
</tr>
<tr>
<td>6.67.1</td>
<td>0.75</td>
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</tr>
<tr>
<td>6.73.2</td>
<td>inactive</td>
<td></td>
</tr>
<tr>
<td>6.77.1</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>9.8.2</td>
<td>0.83</td>
<td></td>
</tr>
</tbody>
</table>

IgG2 | No Binding 
IgG4 | Binding

Taken together, there is a good correlation between the ability of a given anti-MAdCAM antibody to bind human or cynomolgus MAdCAM, as detected by immunohistochemistry (Table 8), with recombinant cell-based binding and functional activity (Table 9). Anti-MAdCAM antibodies 1.7.2, 1.8.2 and 6.73.2, for instance, demonstrated a consistent lack of binding to cynomolgus tissue and cells expressing a chimeric cynomolgus/human MAdCAM protein. Anti-MAdCAM antibodies 1.7.2, 1.8.2 and 6.73.2 also did not have the ability to detect functional blocking activity in the cynomolgus/human MAdCAM/JY adhesion assay.

Similar approaches could be used to define the epitope of the anti-MAdCAM antibodies 6.14.2 and 6.67.1 that recognise dog MAdCAM.
EXAMPLE IV:

Use of anti-MAdCAM mAbs
in the detection of circulating soluble MAdCAM as a method of disease diagnosis

5  [0245] Anti-MAdCAM antibodies can be used for the detection of circulating soluble MAdCAM (sMAdCAM). Detection of sMAdCAM in clinical plasma, serum samples or other biofluid, such as, but not limited to, stool, urine, sputum, is likely to be a useful surrogate disease biomarker for underlying disease, including, but not limited to, inflammatory bowel disease.

10  [0246] Based on the epitope binning data (Tables 7 and 8), anti-MAdCAM antibodies 1.7.2 and 7.16.6 appear to recognise different epitopes on human MAdCAM. ELISA plates were coated overnight at 4°C with 100 μL/well of a 50 μg/mL solution of 1.7.2 in phosphate buffered saline (PBS). After incubation the plate was blocked for 1.5 hours with a PBS blocking buffer containing 10% milk (200 μL/well). After incubation the plate was washed with PBS (2 x 100 μL/well) and serial dilutions of MAdCAM-IgG1-Fc fusion protein, from a top concentration of 50 μg/mL down to approximately 5 ng/mL in PBS, to a final volume of 100 μL, were added to the plate for incubation of 2 hours at room temperature. In a similar approach the MAdCAM-IgG1-Fc protein can be diluted in plasma or serum, or some other such relevant biofluid and used to determine the expression of soluble MAdCAM in a clinical sample, as described below. As a negative control, only buffer was added to the wells containing the primary anti-MAdCAM antibody. After this time, the plate was washed with PBS (3 x 100 μL/well) and the plate then incubated in the dark with an Alexa488-labelled 7.16.6 (100 μL, 5 μg/mL).

20  The Alexa488-labelled 7.16.6 was generated using a commercially available kit (Molecular Probes, A-20181), following Manufacturer’s protocols.

[0247] The plate was washed with PBS containing 0.05% Tween-20, and binding of labeled 7.16.6 to captured soluble MAdCAM determined by measuring the fluorescence (Wallac Victor2 1420 Multilabel Reader, excitation λ485nm, emission λ535nm count from top, 3 mm from bottom of plate, for 0.1 sec with normal emission aperture). When fluorescence is plotted as a function of the concentration of MAdCAM-IgG1-Fc fusion protein, Figure 6, it indicates that

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1.7.2 and a labeled 7.16.6 can be used for diagnostic purposes to determine the level of circulating soluble MAdCAM expressed in a biofluid or clinical sample. This sandwich ELISA approach is not restricted to the use of 1.7.2 and 7.16.6, but any combination of anti-MAdCAM antibodies that recognise different epitopes on MAdCAM, as outlined by the data and interpretation of table 7 and Figure 5. Similar strategies could be applied to the development of similar assays, such as immunohistochemistry and Western Blot, with the other anti-MAdCAM antibodies described, using different partners, variants, labels, etc.

EXAMPLE V:

Amino acid structure of anti-MAdCAM mAbs prepared in accordance to the invention

[0248] In the following discussion, structural information related to the anti-MAdCAM mAbs prepared in accordance with the invention is provided.

[0249] To analyze structures of mAbs produced in accordance with the invention, we cloned the genes encoding the heavy and light chain fragments out of the specific hybridoma clone. Gene cloning and sequencing was accomplished as follows:

[0250] Poly(A)+ mRNA was isolated from approximately 2x10⁵ hybridoma cells derived from immunized XenoMouse mice using Fast-Track kit (Invitrogen). The generation of random primed cDNA was followed by PCR. Human VH or Vκ family specific primers (Marks et al., ‘Oligonucleotide primers for polymerase chain reaction amplification of human immunoglobulin variable gene and design of family-specific oligonucleotide probes’; Eur. J. Immunol., 21, 985-991 (1991)) or a universal human VH primer, MG-30 (5′-CAG GTG CAG CTG GAG CAG TCI GG-3 (SEQ ID NO: 108) was used in conjunction with primers specific for the human Cγ2, MG40-d (5′-GCT GAG GGA GTA GAG TCC TGA GGA-3 (SEQ ID NO: 109) or Cγ4 constant region, MG-40d (5′GCT GAG GGA GTA GAG TCC TGA GGA CTG T -3 (SEQ ID NO: 110), or Cκ constant region (hκP2; as previously described in Green et al., 1994). Sequences of the human mAb-derived heavy and kappa chain transcripts from hybridomas were obtained by direct sequencing of PCR products generated from poly (A+) RNA using the
primers described above. PCR products were cloned into pCR2.1 using a TOPO-TA cloning kit (Invitrogen) and both strands were sequenced using Prism dye terminator sequencing kits and an ABI 377 sequencing machine. All sequences were analysed by alignments to the ‘V BASE sequence directory’ (Tomlinson, et al, *J. Mol. Biol.*, 227, 776–798 (1992); *Hum. Mol. Genet.*, 3, 853–860 (1994); *EMBO J.*, 14, 4628–4638 (1995).)

Further each of the antibodies, 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod, were subjected to full length DNA sequencing. For such, total RNA was isolated from approximately 3-6x10^6 hybridoma cells using an RNaseasy kit (Qiagen). The mRNA was reverse transcribed using oligo-dT and an AMV-based reverse transcriptase system (Promega). V BASE was used to design 5’ specific amplification primers, containing an optimal Kozak sequence and ATG start codon (underlined) and 3’ reverse primers for the specific heavy and kappa chains as depicted in Table 10.

**Table 10:** PCR primer pairs for cDNA amplification from anti-MAdCAM mAb-expressing hybridomas and primers used in the construction of modified versions of anti-MAdCAM antibodies.

<table>
<thead>
<tr>
<th>Primer Pair</th>
<th>Oligo sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH1-18</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCATCCTT 3’ (SEQ ID NO: 70)</td>
</tr>
<tr>
<td>VH3-15</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 71)</td>
</tr>
<tr>
<td>VH3-21</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 72)</td>
</tr>
<tr>
<td>VH3-23</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 73)</td>
</tr>
<tr>
<td>VH3-30</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 74)</td>
</tr>
<tr>
<td>VH3-33</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 75)</td>
</tr>
<tr>
<td>VH4-4</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 76)</td>
</tr>
<tr>
<td>A2A3</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 77)</td>
</tr>
<tr>
<td>A26</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 78)</td>
</tr>
<tr>
<td>B3</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGACCTGGAGCTT 3’ (SEQ ID NO: 79)</td>
</tr>
<tr>
<td>O12</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGAGCTGGAGCTT 3’ (SEQ ID NO: 80)</td>
</tr>
<tr>
<td>O18</td>
<td>5’ TATCTAAGCTTCTAGACTCGAGGCCACCATGGAGCTGGAGCTGGAGCTT 3’ (SEQ ID NO: 81)</td>
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<tr>
<td>Oligo sequence</td>
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</tr>
<tr>
<td>------------------------</td>
<td>---</td>
</tr>
<tr>
<td>RevIgG2</td>
<td>5'-TTCTCTGATCAGATATTCCATTACATTCACCTACCAGGACACGAGGAGG 3' (SEQ ID NO: 82)</td>
</tr>
<tr>
<td>RevIgG4</td>
<td>5'-TTCTTTGATCAGATTCTTCACATACCCTACCCCTGTTGACG 3' (SEQ ID NO: 83)</td>
</tr>
<tr>
<td>RevKappa</td>
<td>5'-TTCTCTGATCAGATATTCCATTACATTCACCTACCAGGACACGAGGAGG 3' (SEQ ID NO: 84)</td>
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<tr>
<td>6.22.2VK_FL1</td>
<td>5'-GGA TCT GGG ACA GAT TTC ACC ACC ACC AAT AGC CTG GAA GCA GC-3' (SEQ ID NO: 85)</td>
</tr>
<tr>
<td>6.22.2VK_R1</td>
<td>5'-GCT TCC AGG CTA TTG ATG GTG AGT AAA TCT GCC CAA GAT CC-3' (SEQ ID NO: 86)</td>
</tr>
<tr>
<td>6.22.2VH_FL1</td>
<td>5'-GCA GGC TCT GTA ACC TCT AGT AGC GC-3' (SEQ ID NO: 87)</td>
</tr>
<tr>
<td>6.22.2VH_R1</td>
<td>5'-GCT ACT GAA GGT GAG TCA TCC AGA GGC TGC-3' (SEQ ID NO: 88)</td>
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<tr>
<td>6.22.2VH_CS*</td>
<td>5'-CGG AGG TGC TCC TAG AGC AGG GGC-3' (SEQ ID NO: 89)</td>
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<tr>
<td>6.34.2VK_FL1</td>
<td>5'-GCA AGT CAG AGT ATT AGT AGG TAT TTA AAT TGG TAT CAG CAG AAA CC-3' (SEQ ID NO: 90)</td>
</tr>
<tr>
<td>6.34.2VK_R1</td>
<td>5'-GCT TCC TGC TGA TAC CAA TTT AAA TAG CTA CTA ATA CTC TGA CTT GC-3' (SEQ ID NO: 91)</td>
</tr>
<tr>
<td>6.34.2VK_F2</td>
<td>5'-CCA TCA GTT CTC TGC AAC CTG AGG ATT TGG CAA CTT ACT ACT GTC ACC-3' (SEQ ID NO: 92)</td>
</tr>
<tr>
<td>6.34.2VK_R3</td>
<td>5'-GCT GAC AGT AGT AGT AGG TTG CAA AAT CTC CAG GGT GCA GAG AAC TGA TGG-3' (SEQ ID NO: 93)</td>
</tr>
<tr>
<td>6.34.2VH_FL6.34</td>
<td>5'-GCA AAT GAA CAG CCT GCC GGC TGA GCA GAC CAC G-3' (SEQ ID NO: 94)</td>
</tr>
<tr>
<td>.2VH_R1</td>
<td>5'-GCT GTC CTC AGG GCG CAG GCT GGT CAT TCT GC-3' (SEQ ID NO: 95)</td>
</tr>
<tr>
<td>6.67.1VK_F1</td>
<td>5'-CAA TAA GAA CAA CTA TCC AGC TTG TTA CCA ACA GAA ACC AGG ACA GCC-3' (SEQ ID NO: 96)</td>
</tr>
<tr>
<td>6.67.1VK_R1</td>
<td>5'-GCG TGT CCT GGT TCT TGT TGT TAC CAA GCT AGG TAG TTC TTA TTG-3' (SEQ ID NO: 97)</td>
</tr>
<tr>
<td>6.67.1VH_FL1</td>
<td>5'-CCC TCA GGG GTC GAG TCA CCA TGT CAG TAG ACA CTT CCA AGA ACC-3' (SEQ ID NO: 98)</td>
</tr>
<tr>
<td>6.67.1VH_R1</td>
<td>5'-GCT TCT TGG AGG TGT CTA CTG ACA TGG TCC CTC GAC CCC TGA GGG-3' (SEQ ID NO: 99)</td>
</tr>
<tr>
<td>6.67.1VH_CS*</td>
<td>5'-ATT CTA GAG CAG GGC GGC AGG-3' (SEQ ID NO: 100)</td>
</tr>
<tr>
<td>6.77.1VK_F1</td>
<td>5'-CCA TCT CCT GCA AGT CTA GTC AGA GCC TCC-3' (SEQ ID NO: 101)</td>
</tr>
<tr>
<td>6.77.1VK_R1</td>
<td>5'-GGA GGC TCT GAC TAG ACT TGC AGG AGT TGG-3' (SEQ ID NO: 102)</td>
</tr>
<tr>
<td>6.77.1VH_F2</td>
<td>5'-GTT TTA TTA CTA GCA AGT TAT ACA GCT GTT GTC CAG TTT TGG CC-3' (SEQ ID NO: 103)</td>
</tr>
<tr>
<td>6.77.1VH_R2</td>
<td>5'-GCC CAA AAC TGA ACA TAA GCT GTA TAC TTC TGG GCA TGC AGT AAT AAA CC-3' (SEQ ID NO: 104)</td>
</tr>
<tr>
<td>7.26.4K_FL1</td>
<td>5'-CTT GCA AGT CTA GTC AGA GCC TCC-3' (SEQ ID NO: 105)</td>
</tr>
<tr>
<td>7.26.4K_R1</td>
<td>5'-GGA GGC TCT GAC TAG ACT TGC AGG-3' (SEQ ID NO: 106)</td>
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</tbody>
</table>

[0252] The primers pairs were used to amplify the cDNAs using Expand High Fidelity Taq polymerase (Roche), and the PCR products cloned into pCR2.1 TOPO-TA (Invitrogen) for subsequent sequencing. Heavy and kappa light chain sequence verified clones were then cloned into pE6.1 and pE12.1 vectors (LONZA) using XbaI/EcoRI and HindIII/EcoRI sites respectively.

Gene Utilization Analysis
Table 11 displays the heavy and kappa light chain gene utilization for each hybridoma outlined in the invention.

Table 11: Heavy and Kappa light chain Gene Utilization

<table>
<thead>
<tr>
<th>CLONE</th>
<th>Heavy Chain</th>
<th>Kappa light Chain</th>
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<tbody>
<tr>
<td></td>
<td>VH</td>
<td>D</td>
</tr>
<tr>
<td>1.7.2</td>
<td>VH3-15</td>
<td>D6-19</td>
</tr>
<tr>
<td>1.8.2</td>
<td>VH3-15</td>
<td>D6-19</td>
</tr>
<tr>
<td>7.16.6</td>
<td>VH1-18</td>
<td>D6-6</td>
</tr>
<tr>
<td>7.20.5</td>
<td>VH4-4</td>
<td>D3-10</td>
</tr>
<tr>
<td>7.26.4</td>
<td>VH1-18</td>
<td>D6-6</td>
</tr>
<tr>
<td>6.14.2</td>
<td>VH3-23</td>
<td>D5-5</td>
</tr>
<tr>
<td>6.22.2</td>
<td>VH3-33</td>
<td>D5-12</td>
</tr>
<tr>
<td>6.34.2</td>
<td>VH3-30</td>
<td>D4-23</td>
</tr>
<tr>
<td>6.67.1</td>
<td>VH4-4</td>
<td>D3-10</td>
</tr>
<tr>
<td>6.73.2</td>
<td>VH3-23</td>
<td>D6-19</td>
</tr>
<tr>
<td>6.77.1</td>
<td>VH3-21</td>
<td>D6-19</td>
</tr>
<tr>
<td>9.8.2</td>
<td>VH3-33</td>
<td>D3-10 or D3-16</td>
</tr>
</tbody>
</table>

5 Sequence Analysis

[0254] To further examine antibody structure predicted amino acid sequences of the antibodies were obtained from the cDNAs obtained from the clones.

[0255] Sequence identifier numbers (SEQ ID NO:) 1-48 and 51-68 provide the nucleotide and amino acid sequences of the heavy and kappa light chains of the anti-MAdCAM antibodies 1.7.2 (SEQ ID NOS 1-4), 1.8.2 (SEQ ID NOS 5-8), 6.14.2 (SEQ ID NOS 9-12), 6.22.2 (SEQ ID NOS 13-16), 6.34.2 (SEQ ID NOS 17-20), 6.67.1 (SEQ ID NOS 21-24), 6.73.2 (SEQ ID NOS 25-28), 6.77.1 (SEQ ID NOS 29-32), 7.16.6 (SEQ ID NOS 33-36), 7.20.5 (SEQ ID NOS 37-40), 7.26.4 (SEQ ID NOS 41-44), 9.8.2 (SEQ ID NOS 45-48) and the modified anti-MAdCAM antibodies 6.22.2-mod (SEQ ID NOS 51-54), 6.34.2-mod (SEQ ID NOS 55-58), 6.67.1-mod (SEQ ID NOS 59-62) and 6.77.1-mod (SEQ ID NOS 63-66) and 7.26.4-mod (SEQ ID NOS 41-42, 67-68). For each anti-MAdCAM antibody sequence cloned, the sequences of the signal peptide sequence (or the bases encoding the same) are indicated in lower case and underlined.
[0256] Figures 1A-1J provide sequence alignments between the predicted heavy chain amino acid sequences of antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4 and 9.8.2 and the amino acid sequence of the respective germline gene products. The positions of the CDR1, CDR2 and CDR3 sequences of the antibodies are underlined, differences between the expressed sequence the corresponding germline sequence are indicated in bold and where there are additions in the expressed sequence compared to the germline these are indicated as a (-) in the germline sequence.

[0257] Figures 1K-1T provide sequence alignments between the predicted kappa light chain amino acid sequences of the antibodies 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4 and 9.8.2 and the amino acid sequence of the respective germline gene products. The positions of the CDR1, CDR2 and CDR3 sequences of the antibodies are underlined, differences between the expressed sequence the corresponding germline they are indicated in bold and where there are additions in the expressed sequence compared to the germline these are indicated as a (-) in the germline sequence.

Presence of post-translational modification: glycosylation and deamidation:

[0258] The effect of some of the changes in the expressed anti-MAdCAM antibody sequence, compared with the derived germline sequence, is to introduce residues that potentially could be subject to N-linked glycosylation (Asn-X-Ser/Thr) and/or deamidation (Asn-Gly) (see Table 12). The nucleic acid sequences encoding the kappa light chain variable domain amino acid sequences of the anti-MAdCAM antibodies 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.26.4 and 9.8.2, (SEQ ID NOS: 16, 20, 24, 28, 32, 44 and 48) and the heavy chain variable domain of antibody 6.14.2, (SEQ ID NO: 10), predict the presence of N-linked glycosylation. The presence of this post-translational modification was investigated using a combination of SDS-PAGE and Pro-Q® Emerald 488 Glycoprotein (Molecular Probes) staining with mAbs 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.26.4 and 9.8.2.

[0259] Briefly, approximately 2 µg of reduced anti-MAdCAM antibody was loaded onto a 4-12% SDS-polyacrylamide gel using a MOPS buffer. Following electrophoresis, the gel was fixed in 50% MeOH, 5% acetic acid and washed in 3% acetic acid. Any carbohydrates on the gel were then oxidised with periodic acid.
and stained using Pro-Q® Emerald 488 Glycoprotein Stain Kit (Molecular
Probes). After a final wash step, glycoprotein staining was visualised using a
fluorescence scanner set at a wavelength of 473 nm.

After glycoprotein staining, the gel was stained for total protein using
SYPRO Ruby protein gel stain and analysed using a fluorescence scanner set at a
wavelength of 473 nm. The kappa light chains of anti-MAdCAM antibodies,
6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.26.4 and 9.8.2, all stained positively for the
presence of glycosylation. As an additional confirmation, anti-MAdCAM
antibody 7.26.4, was subjected to tryptic/chymotryptic digestion, the LC-MS/MS
analysis confirmed the presence of a modified tryptic peptide and provided
additional confirmation of kappa light chain glycosylation.

Specific Asn-Gly sequences in the CDR1 regions of anti-MAdCAM
antibodies, 1.7.2, 1.8.2, 6.22.2 and 7.20.5, render these regions sensitive to
deamidation. Deamidation at neutral pH introduces a negative charge and can also
lead to β-isomerisation, which could affect the properties of an antibody. For anti-
MAdCAM antibodies 1.7.2, 1.8.2 and 7.20.5, the presence of deamidated Asn-
isoaspartate residues was assessed by mass spectroscopy following trapping the
isoaspartate side chain with MeOH.

In brief, for the anti-MAdCAM antibody 1.7.2, the status of the
tryptic/Asp-N peptide SSSQLLQSNQNYL (SEQ ID NO: 69) (1573.7 Da) was
selected for monitoring by LC-MS/MS. Anti-MAdCAM antibody 1.7.2 was
reduced in 10 mM DTT, alkylated in 5 mM Na iodoacetate and subsequently
buffer exchanged into trypsin digestion buffer (50mM Tris-HCl, 1mM CaCl₂, pH
7.6). The antibody was then mixed with sequencing grade modified trypsin
(Promega) in a protease:protein ratio of 1:20. Protein was digested in trypsin for
15 hours at 30°C, and the resulting peptides separated by HPLC using a C-18 RPC
on an Ettan LC system. The 33 Asn-containing peptide (4032 Da) was collected
from the column and diluted in Asp-N digestion buffer (50 mM sodium phosphate
buffer, pH 8.0). Endoproteinase Asp-N (Roche) was then added at an approximate
peptide:enzyme ratio of 10:1.

Acetyl chloride (100 µL) was added to a sample of methanol (1 mL, -
20°C), the mixture warmed to room temperature. The tryptic+Asp-N digest was
dried in a Speed-Vac and then 5 µL of the methanol/acetyl chloride was added (45 min, room temp), then dried again in a Speed-Vac. The resulting residue was reconstituted in 0.1% TFA and peptides were analysed initially on the Voyager-DE STR MALDI-TOF mass spectrometer using either the nitrocellulose thin layer sample preparation method or reverse phase purification using C18 ZipTips (Millipore) followed by droplet mixing with α-cyano matrix. The methylated peptide mixture was also analysed using LC-MS/MS on a Deca XP Plus Ion Trap Mass Spectrometer as above. The elution was plumbed straight into the Ion Trap MS and peptides were subsequently analysed by MS and MS/MS. The MS was set to analyse all ions between 300 and 2000 Da. The strongest ion in any particular scan was then subjected to MS/MS analysis.

Table 12. Post-translational modification of anti-MAdCAM antibodies

<table>
<thead>
<tr>
<th>CLONE</th>
<th>Heavy Chain</th>
<th></th>
<th>Kappa light chain</th>
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</thead>
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<tr>
<td></td>
<td>Glycosylation (NXS/T)</td>
<td>Confirmed</td>
<td>Glycosylation (NXS/T)</td>
</tr>
<tr>
<td>1.7.2</td>
<td>TPNNSAMT</td>
<td>N.D</td>
<td>CKSNQSLLY</td>
</tr>
<tr>
<td>1.8.2</td>
<td></td>
<td></td>
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<tr>
<td>7.16.6</td>
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<td></td>
<td></td>
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<tr>
<td>7.20.5</td>
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</tr>
<tr>
<td>7.26.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6.14.2</td>
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<td>6.22.2</td>
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<td>9.8.2</td>
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<td>IgG4</td>
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</tbody>
</table>

Mutagenesis studies:

[0264] The primary amino acid sequence of the anti-MAdCAM antibodies exemplified in this invention can be modified, by site-directed mutagenesis, to remove potential sites of post-translational modification (e.g., glycosylation, deamidation) or to alter the isotype background, or to engineer other changes which may improve the therapeutic utility. As an example, PCR was used to engineer changes to the anti-MAdCAM antibodies 6.22.2, 6.34.2, 6.67.1, 6.77.1 and 7.26.4,
to revert certain framework sequences to germline, to remove potential
glycosylation sites and/or to change the isotype background to a human IgG2.
pCR2.1 TOPO-TA cloned cDNAs (100 ng), corresponding to heavy chain
nucleotide SEQ ID NOS: 13, 17, 21 and 29, and kappa light nucleotide SEQ ID
NOS: 15, 19, 23, 31 and 43, were used as a template in a series of PCRs using
overlap-extension and a panel of primer sets described in Table 10.

[0265] 6.22.2 Heavy chain: PCR primer sets 6.22.2_VH_F1 and 6.22.2VH_CS*
(1) and VH3-33 and 6.22.2_VH_R1 (2) were used to generate separate PCR
products (1) and (2), using an Expand Taq polymerase and a pCR2.1 TOPO-TA
cDNA template (100 ng) represented by nucleotide sequence SEQ ID NO: 13.
Products (1) and (2) were purified and combined in a third PCR step (ca. 50 ng
each) along with VH3-33 and VK6.22.2_CS* primers, to generate the modified
6.22.2 heavy chain V-domain. This modified version contains a His/Phe mutation
in FR1 and introduces an XbaI restriction site to enable in frame cloning into a
pEE6.1 derived vector, termed pEE6.1CH, which contains the corresponding
human IgG2 constant domain. The final PCR fragment was cloned into the XbaI
site of pEE6.1CH, checked for orientation and the insert full sequence verified.
The nucleotide sequence for the modified 6.22.2 heavy chain is found in SEQ ID
NO: 51 and the corresponding amino acid sequence in SEQ ID NO: 52. The
changes in the nucleotide and amino acid sequences compared with the parent are
indicated.

[0266] 6.22.2 kappa light chain: PCR primer sets 6.22.2_VK_F1 and revKappa
(1), and A26 and 6.22.2_VK_R1 (2) were used to generate separate PCR products
(1) and (2), using an Expand Taq polymerase and a pCR2.1 TOPO-TA cDNA
template (100 ng) represented by nucleotide sequence SEQ ID NO: 15. Products
(1) and (2) were purified and combined in a third PCR step (ca. 50 ng each) along
with A26 and revKappa primers, to generate the modified 6.22.2 kappa light chain
V-domain. This modified version contains Asn/Asp and Gly/Ser changes to the
FR3 sequence. The resultant PCR product was cloned into pEE12.1 using
HindIII/EcoR1 sites and fully sequence verified. The nucleotide sequence for the
modified 6.22.2 kappa light chain is found in SEQ ID NO: 53 and the
corresponding amino acid sequence in SEQ ID NO: 54. The changes in the nucleotide and amino acid sequences compared with the parent are indicated.

[0267] 6.34.2 Heavy chain: PCR primer sets 6.34.2_VH_F1 and 6.22.2VH_CS* (1) and VH3-30 and 6.34.2_VH_R1 (2) were used to generate separate PCR products (1) and (2), using an Expand Taq polymerase and a pCR2.1 TOPO-TA cDNA template (100 ng) represented by nucleotide sequence SEQ ID NO: 17. Products (1) and (2) were purified and combined in a third PCR step (ca. 50 ng each) along with VH3-30 and VK6.22.2_CS* primers, to generate the modified 6.34.2 heavy chain V-domain. This modified version contains a Ser/Arg mutation in FR3 and introduces an XbaI restriction site to enable in frame cloning into a pEE6.1 derived vector, termed pEE6.1CH, which contains the corresponding human IgG2 constant domain. The final PCR fragment was cloned into the XbaI site of pEE6.1CH, checked for orientation and the insert full sequence verified. The nucleotide sequence for the modified 6.34.2 heavy chain is found in SEQ ID NO: 55 and the corresponding amino acid sequence in SEQ ID NO: 56. The changes in the nucleotide and amino acid sequences compared with the parent are indicated.

[0268] 6.34.2 kappa light chain: PCR primer sets O12 and 6.34.2_VK_R1 (1), 6.34.2_VK_F1 and 6.34.2_VK_R2 (2), as well as 6.34.2_VK_F2 and revKappa (3) were used to generate separate PCR products (1), (2) and (3), using an Expand Taq polymerase and a pCR2.1 TOPO-TA cDNA template (100 ng) represented by nucleotide sequence SEQ ID NO: 19. Products (1), (2) and (3) were purified and (1) and (2) were combined in a third PCR step (ca. 50 ng each), along with O12 and 6.34.2_VK_R2 primers, to generate the PCR product (4). PCR products (2) and (3) were combined in a fourth PCR step (ca. 50 ng each), along with 6.34.2_VK_F1 and revKappa, to generate the PCR product (5). PCR products (4) and (5) were purified and combined together (ca.50 ng each) with primers O12 and revKappa to generate the modified 6.34.2 kappa light chain V-domain. This modified version contains an Asn/Ser change in CDR1, a Phe/Tyr change in FR2 and Arg-Thr/Ser-Ser, Asp/Glu and Ser/Tyr changes to the FR3 sequence. The resultant PCR product was cloned into pEE12.1 using HindIII/EcoR1 sites and fully sequence verified. The nucleotide sequence for the modified 6.34.2 kappa
light chain is found in SEQ ID NO: 57 and the corresponding amino acid sequence in SEQ ID NO: 58. The changes in the nucleotide and amino acid sequences compared with the parent are indicated.

[0269] 6.67.1 Heavy chain: PCR primer sets 6.67.1_VH_F1 and 6.67.1VH_CS* (1) and VH4-4 and 6.67.1_VH_R1 (2) were used to generate separate PCR products (1) and (2), using an Expand Taq polymerase and a pCR2.1 TOPO-TA cDNA template (100 ng) represented by nucleotide sequence SEQ ID NO: 21. Products (1) and (2) were purified and combined in a third PCR step (ca. 50 ng each) along with VH4-4 and VK6.67.1_CS* primers, to generate the modified 6.67.1 heavy chain V-domain. This modified version contains an Ile-Leu-Ala/Met-Ser-Val conversion in FR3 and introduces an XbaI restriction site to enable in frame cloning into a pEE6.1 derived vector, termed pEE6.1CH, which contains the corresponding human IgG2 constant domain. The final PCR fragment was cloned into the XbaI site of pEE6.1CH, checked for orientation and the insert full sequence verified. The nucleotide sequence for the modified 6.67.1 heavy chain is found in SEQ ID NO: 59 and the corresponding amino acid sequence in SEQ ID NO: 60. The changes in the nucleotide and amino acid sequences compared with the parent are indicated.

[0270] 6.67.1 kappa light chain: PCR primer sets 6.67.1_VK_F1 and revKappa (1), and B3 and 6.67.1_VK_R1 (2) were used to generate separate PCR products (1) and (2), using an Expand Taq polymerase and a pCR2.1 TOPO-TA cDNA template (100 ng) represented by nucleotide sequence SEQ ID NO: 23. Products (1) and (2) were purified and combined in a third PCR step (ca. 50 ng each) along with B3 and revKappa primers, to generate the modified 6.67.1 kappa light chain V-domain. This modified version contains a Thr/Asn change in CDR1 and an Arg/Gly change in FR2. The resultant PCR product was cloned into pEE12.1 using HindIII/EcoR1 sites and fully sequence verified. The nucleotide sequence for the modified 6.67.1 kappa light chain is found in SEQ ID NO: 61 and the corresponding amino acid sequence in SEQ ID NO: 62. The changes in the nucleotide and amino acid sequences compared with the parent are indicated.

[0271] 6.77.1 Heavy chain: PCR primer sets VH 3-21 and 6.22.2VH_CS* were used to generate a single PCR product using an Expand Taq polymerase and
a pCR2.1 TOPO-TA cDNA template (100 ng) represented by nucleotide sequence SEQ ID NO: 29. The PCR products were digested with XbaI, gel purified and cloned into the XbaI site of pEE6.1CH, checking for orientation. The insert was fully sequence verified. The nucleotide sequence for the modified 6.77.1 heavy chain is found in SEQ ID NO: 63 and the corresponding amino acid sequence in SEQ ID NO: 64. The changes in the nucleotide and amino acid sequences compared with the parent are indicated.

**0272** 6.77.1 kappa light chain: PCR primer sets A2 and 6.77.1_VK_R1 (1), 6.77.1_VK.VK_F1 and 6.77.1_R2 (2), as well as 6.77.1_VK_F2 and revKappa (3) were used to generate separate PCR products (1), (2) and (3), using an Expand Taq polymerase and a pCR2.1 TOPO-TA cDNA template (100 ng) represented by nucleotide sequence SEQ ID NO: 31. Products (1), (2) and (3) were purified and, (1) and (2) were combined in a third PCR step (ca. 50 ng each) along with A2 and 6.77.1_VK.R2 primers, to generate PCR product (4). PCR product (2) and (3) were combined in a fourth PCR step (ca. 50 ng each) along with 6.77.1_VK.F1 and revKappa primers, to generate PCR product (5). PCR products (4) and (5) were purified and combined together (ca. 50 ng each) with primers A2 and JK2 to generate the modified 6.77.1 kappa light chain V-domain. This modified version contains an Asn/Lys change in CDR1, a Ser/Tyr change in FR3 and a Cys/Ser residue change in CDR3 sequence. The resultant PCR product was cloned into pEE12.1 using HindIII/EcoR1 sites and fully sequence verified. The nucleotide sequence for the modified 6.77.1 kappa light chain is found in SEQ ID NO: 65 and the corresponding amino acid sequence in SEQ ID NO: 66. The changes in the nucleotide and amino acid sequences compared with the parent are indicated.

**0273** 7.26.4 kappa light chain: PCR primer sets 7.26.4_VK_F1 and revKappa (1), and A2 and 7.26.4_VK_R1 (2) were used to generate separate PCR products (1) and (2), using an Expand Taq polymerase and a pCR2.1 TOPO-TA cDNA template (100 ng) represented by nucleotide sequence SEQ ID NO: 43. Products (1) and (2) were purified and combined in a third PCR step (ca. 50 ng each) along with A2 and revKappa primers, to generate the modified 7.26.4 kappa light chain V-domain. This modified version contains an Asn/Ser change in CDR1. The resultant PCR product was cloned into pEE12.1 using HindIII/EcoR1 sites and
fully sequence verified. The nucleotide sequence for the modified 7.26.4 kappa light chain is found in SEQ ID NO: 67 and the corresponding amino acid sequence in SEQ ID NO: 68. The changes in the nucleotide and amino acid sequences compared with the parent are indicated.

[0274] A functional eukaryotic expression vector for each of the modified versions of 6.22.2, 6.34.2, 6.67.1, 6.77.1 and 7.26.4, referred to as 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod, and representing respectively the heavy chain nucleotide sequences SEQ ID NOS: 51, 55, 59, 63 and 41, and corresponding amino acid sequences SEQ ID NOS: 52, 56, 60, 64 and 42, as well as the kappa light chain nucleotide sequences SEQ ID NOS: 53, 57, 61, 65 and 67, and the corresponding amino acid sequences SEQ ID NOS: 54, 58, 62, 66 and 68 were assembled as follows: The heavy chain cDNA inserts corresponding to 6.22.2-mod, 6.34.2-mod, 6.67.1-mod and 6.77.1-mod were excised from the pEE6.1CH vector with NotI/SalI, the parental version of the heavy chains of 7.26.4 was excised from the pEE6.1 vector with NotI/SalI, and the purified fragments were cloned into identical sites into the corresponding pEE12.1 vector containing the modified versions of the kappa light chain sequences 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod. The sequences of the vectors were confirmed, and purified amounts used in transient transfections with HEK 293T cells. Briefly, 9x10^6 HEK 293T cells, seeded in a T165 flask the day before transfection and washed into Optimem, were transiently transfected with vector cDNAs corresponding to 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod (40 µg) using Lipofectamine PLUS (Invitrogen) according to manufacturer’s instructions. The cells were incubated for 3 hrs, then the transfection media replaced with DMEM (Invitrogen 21969-035) media containing 10% ultra-low IgG fetal calf serum (Invitrogen 16250-078) and L-Glutamine (50 mL). The media supernatant was harvested 5 days later, filter sterilised and the anti-MAdCAM antibody purified using protein G sepharose affinity chromatography, in a similar manner as to that described above. The amount of antibody recovered (20-100 µg) was quantified by a Bradford assay.

[0275] The anti-MAdCAM activity of affinity purified antibody corresponding to 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod was assessed in
the MAdCAM-IgG1-Fc fusion assay as described previously. The IC\textsubscript{50} values of these anti-MADCAM antibodies compared with the parental anti-MAdCAM antibodies from which they were derived are presented in Table 13. There was minimal effect of the amino acid substitutions described above on the activity of the modified anti-MAdCAM antibodies compared with their parents was minimal. The antibodies also maintained their binding to CHO cells expressing recombinant human MAdCAM or the cynomolgus/human MAdCAM chimera.

Table 13. Activity of modified versions of anti-MAdCAM antibodies, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod compared with their parents.

<table>
<thead>
<tr>
<th>CLONE</th>
<th>MAdCAM IgG1 Fc fusion Assay Mean IC\textsubscript{50} (\mu g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parent</td>
</tr>
<tr>
<td>6.22.2</td>
<td>0.018</td>
</tr>
<tr>
<td>6.34.2</td>
<td>0.013</td>
</tr>
<tr>
<td>6.67.1</td>
<td>0.013</td>
</tr>
<tr>
<td>6.77.1</td>
<td>0.022</td>
</tr>
<tr>
<td>7.26.4</td>
<td>0.021</td>
</tr>
</tbody>
</table>

**EXAMPLE VI**

Increase in β\textsuperscript{7}\textsuperscript{+} lymphocytes in the peripheral circulation by blocking anti-MAdCAM antibodies

[0276] An assay was developed to identify and correlate a mechanistic effect of an anti-MAdCAM antibody and its circulating level in blood. An inhibitory anti-MAdCAM antibody should have the effect of inhibiting the recruitment of leukocytes expressing the α\textsubscript{4}β\textsubscript{7} integrin to the gastrointestinal tract. Classes of α\textsubscript{4}β\textsubscript{7} integrin-bearing leukocytes should, therefore, be restricted to the peripheral circulation [0277] This was demonstrated with a fully human anti-human MAdCAM mAb 7.16.6, in cynomolgus.

[0278] Purified anti-human MAdCAM mAb 7.16.6 (1 mg/kg) or vehicle (20 mM NaAcetate, 0.2 mg/mL polysorbate 80, 45 mg/mL mannitol, and 0.02 mg/mL
EDTA at pH 5.5) were assessed in a similar manner by intravenous administration via the saphenous vein to two groups of cynomolgus monkeys (n=4/group). At day 3 post-dosing blood samples were collected in EDTA tubes by femoral venipuncture. LPAM specific antibodies, which crossreact with the cynomolgus α4β7 integrin, are not commercially available, so an anti-β7 antibody (recognising α4β7 and α6β7 integrin) was used instead. Antibodies (30 μL), according to the following table, table 15, were added to tubes containing 100 μL of cynomolgus blood, mixed by gentle vortexing and incubated for 20-30 mins at 4°C.

Table 15. Antibodies (BD Pharmingen) used in immunophenotyping of cynomolgus blood

<table>
<thead>
<tr>
<th>Catalogue Number</th>
<th>Antibody or Isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>555748</td>
<td>mIgG1, k-FITC</td>
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<tr>
<td>555844</td>
<td>mIgG2a, k-PE</td>
</tr>
<tr>
<td>559425</td>
<td>mIgG1 - PerCP</td>
</tr>
<tr>
<td>555751</td>
<td>mIgG1, k-APC</td>
</tr>
<tr>
<td>555728</td>
<td>CD 28-FITC</td>
</tr>
<tr>
<td>555945</td>
<td>β7-PE</td>
</tr>
<tr>
<td>558814</td>
<td>CD 95-APC</td>
</tr>
<tr>
<td>550631</td>
<td>CD 4-PerCP</td>
</tr>
</tbody>
</table>

To each tube, 1 mL of 1:10 FACslyse solution (BD # 349202) was added, mixed by gentle vortex and incubated at room temperature for approximately 12 minutes in the dark until red blood cell lysis was complete. Then 2 mL of BD stain buffer (# 554656) was added to each tube, mixed and centrifuged at 250 x g for 6-7 mins at room temperature. The supernatant was decanted and the pellet resuspended in 3 mL of stain buffer, mixed again and centrifuged at 250 x g for 6-7 mins at room temperature. Cytofix buffer (BD # 554655), containing w/v paraformaldehyde (100 μL) was added to the cell pellets from monkey peripheral blood and mixed thoroughly by low/moderate speed of vortexer. The samples were kept at 4°C in the dark until they acquired on the FACSCalibur. Just prior to acquisition, PBS (100 μL) was added to all tubes immediately before acquisition.

The absolute cell numbers of CD4+β7+CD95loCD28+ (naïve), CD4+β7+CD95hiCD28+ (central memory), CD4+β7-CD95hiCD28+ (central memory), CD4+β7+CD95hiCD28- (effector memory) were acquired by appropriate
gating and quadrant analyses. Other T cell subsets for example, CD8⁺ T central memory cell (β7⁻CD8⁺CD28⁻CD95⁻) and any other leukocytes bearing a MAdCAM ligand, may also be analyzed by this method with the appropriate antibodies. Compared with the vehicle control, anti-MAdCAM mAb 7.16.6 caused an approximate 3 fold increase in the levels of circulating CD4⁺β7⁺CD95hiCD28⁺ central memory T cells, as shown in Figure 7. There were no effects on the population of circulating CD4⁺β7⁻CD95hiCD28⁺ central memory T cells, indicating that the effect of anti-MAdCAM mAb 7.16.6 is specific for gut homing T cells. The effects of anti-MAdCAM mAb 7.16.6, in cynomolgus, on populations of circulating (α4β7)⁺ lymphocytes indicates that this is a robust surrogate proof of mechanism biomarker, particularly in the context of practical application in a clinical setting.

Sequences

[0280] SEQ ID NO: 1-48 and 51-68 provide nucleotide and amino acid sequences of the heavy and kappa light chains for twelve human anti-MAdCAM antibodies, nucleotide and amino acid sequences of cynomolgus MAdCAM α4β7 binding domain sequences and nucleotide and amino acid sequences of five modified human anti-MAdCAM antibodies.


[0282] SEQ ID NO: 49-50 provide the nucleotide and amino acid sequences of a cynomolgus MAdCAM α4β7 binding domain.

[0283] SEQ ID NO: 51-68 provide the heavy and kappa light chain nucleotide and amino acid sequences for the modified monoclonal anti-MAdCAM antibodies: 6.22.2 (SEQ ID NO: 51-54), modified 6.34.2 (SEQ ID NO: 55-58), modified 6.67.1 (SEQ ID NO: 59-62), modified 6.77.1 (SEQ ID NO: 63-66) and the kappa
SEQ ID NOS: 70-106 and 108-110 provide various primer sequences.
What is Claimed is:

1. A human monoclonal antibody or an antigen-binding portion thereof that specifically binds to Mucosal Adressin Cell Adhesion Molecule (MAdCAM).

2. The human monoclonal antibody or antigen-binding portion according to claim 1, wherein said antibody or portion possesses at least one of the following properties:
   (a) binds to human cells;
   (b) has a selectivity for MAdCAM over VCAM or fibronectin of at least 100 fold;
   (c) binds to human MAdCAM with a $K_d$ of $3 \times 10^{-10}$ M or less; or
   (d) inhibits the binding of $\alpha_4\beta_7$ expressing cells to human MAdCAM.
   (e) inhibits the recruitment of lymphocytes to gastrointestinal lymphoid tissue.

3. The human monoclonal antibody or antigen-binding portion according to claim 2, wherein said antibody or portion binds human MAdCAM with a $K_d$ of $3 \times 10^{-10}$ M or less and inhibits $\alpha_4\beta_7$ binding to human MAdCAM.

4. A hybridoma cell line that produces the human monoclonal antibody according to claim 1, wherein the hybridoma is selected from the group consisting of 1.7.2 (ECACC Accession No. 03090901), 1.8.2 (ECACC Accession No. 03090902), 6.14.2 (ECACC Accession No. 03090903), 6.22.2 (ECACC Accession No. 03090904), 6.34.2 (ECACC Accession No. 03090905), 6.67.1 (ECACC Accession No. 03090906), 6.73.2 (ECACC Accession No. 03090907), 6.77.1 (ECACC Accession No. 03090908), 7.16.6 (ECACC Accession No. 03090909), 7.20.5 (ECACC Accession No. 03090910), 7.26.4 (ECACC Accession No. 03090911), and 9.8.2 (ECACC Accession No. 03090912).
5. The human monoclonal antibody produced by the hybridoma cell line according to claim 4 or an antigen-binding portion of said monoclonal antibody.

6. The human monoclonal antibody according to claim 5, wherein the heavy chain C-terminal lysine is cleaved.

7. The human monoclonal antibody or antigen-binding portion thereof according to either of claims 1 or 5, wherein said antibody or antigen-binding portion inhibits binding of human MAdCAM to α4β7, and wherein the antibody or portion thereof has at least one of the following properties:

(a) cross-competes with a reference antibody for binding to MAdCAM;

(b) competes with a reference antibody for binding to MAdCAM;

(c) binds to the same epitope of MAdCAM as a reference antibody;

(d) binds to MAdCAM with substantially the same Kₐ as a reference antibody;

(e) binds to MAdCAM with substantially the same off rate as a reference antibody;


8. A monoclonal antibody that specifically binds MAdCAM, wherein the antibody is selected from the group consisting of:
(a) an antibody comprising 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 amino acid sequences set forth in SEQ ID NO: 6 and SEQ ID NO: 8, without the signal sequences;

(c) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 10 and SEQ ID NO: 12, without the signal sequences;

(d) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 14 and SEQ ID NO: 16, without the signal sequences;

(e) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 18 and SEQ ID NO: 20, without the signal sequences;

(f) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 22 and SEQ ID NO: 24, without the signal sequences;

(g) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 26 and SEQ ID NO: 28, without the signal sequences;

(h) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 30 and SEQ ID NO: 32, without the signal sequences;

(i) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 34 and SEQ ID NO: 36, without the signal sequences;

(j) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 38 and SEQ ID NO: 40, without the signal sequences;

(k) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 42 and SEQ ID NO: 44, without the signal sequences;

(l) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 46 and SEQ ID NO: 48, without the signal sequences;

(m) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 52 and SEQ ID NO: 54, without the signal sequences;

(n) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 56 and SEQ ID NO: 58, without the signal sequences;

(o) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 60 and SEQ ID NO: 62, without the signal sequences;

(p) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 64 and SEQ ID NO: 66, without the signal sequences; and
35 (q) an antibody comprising the amino acid sequences set forth in SEQ ID NO: 42 and SEQ ID NO: 68, without the signal sequences.

9. A monoclonal antibody or an antigen-binding portion thereof, wherein the heavy chain of said antibody or portion thereof comprises the heavy chain CDR1, CDR2 and CDR3 or wherein the light chain comprises the light chain CDR1, CDR2 and CDR3 of a monoclonal antibody selected from the group consisting of: 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod.

10. The monoclonal antibody or antigen-binding portion according to claim 9, wherein said antibody or portion comprises a heavy chain that utilizes a human VH 1-18 gene, a human VH 3-15 gene, a human VH 3-21 gene, a human VH 3-23 gene, a human VH 3-30 gene, a human VH 3-33 gene or a human VH 4-4 gene.

11. The monoclonal antibody or an antigen-binding portion thereof according to claim 10, wherein said antibody or portion comprises a light chain that utilizes a human V\textsubscript{\text{K}} A2 gene, a human V\textsubscript{\text{K}} A3 gene, a human V\textsubscript{\text{K}} A26 gene, a human V\textsubscript{\text{K}} B3 gene, a human V\textsubscript{\text{K}} O12 gene or a human V\textsubscript{\text{K}} O18 gene.

12. The human monoclonal antibody or antigen-binding portion according to claim 1, wherein the heavy chain variable region, the light chain variable region or both are at least 90% identical in amino acid sequence to the corresponding region or regions of a monoclonal antibody selected from the group consisting of: monoclonal antibody 1.7.2, monoclonal antibody 1.8.2, monoclonal antibody 6.14.2, monoclonal antibody 6.22.2, monoclonal antibody 6.34.2, monoclonal antibody 6.67.1, monoclonal antibody 6.73.2, monoclonal antibody 6.77.1, monoclonal antibody 7.16.6, monoclonal antibody 7.20.5, monoclonal antibody 7.26.4 monoclonal antibody 9.8.2, monoclonal antibody 6.22.2-mod, monoclonal antibody 6.34.2-mod, monoclonal antibody 6.67.1-mod, monoclonal antibody 6.77.1-mod and monoclonal antibody 7.26.4-mod.
13. A monoclonal antibody or an antigen-binding portion thereof that specifically binds MAdCAM, wherein:

(a) the heavy chain comprises the heavy chain CDR1, CDR2 and CDR3 amino acid sequences of a reference antibody selected from the group consisting of: 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod

(b) the light chain comprises the light chain CDR1, CDR2 and CDR3 amino acid sequences of a reference antibody selected from the group consisting of: 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod

(c) the antibody comprises a heavy chain of (a) and a light chain of (b); and

(d) the antibody of (c) wherein the heavy chain and light chain CDR amino acid sequences are selected from the same reference antibody.

14. The monoclonal antibody or antigen-binding portion according to claim 13, wherein the heavy chain, the light chain or both comprise the amino acid sequence from the beginning of the CDR1 through the end of the CDR3 of the heavy chain, the light chain or both, respectively, of the reference antibody.

15. The monoclonal antibody or antigen-binding portion according to claim 13, wherein said antibody comprises:

(a) a heavy chain comprising the heavy chain variable region amino acid sequence of an antibody selected from the group consisting of: 1.7.2 (SEQ ID NO: 2); 1.8.2 (SEQ ID NO: 6); 6.14.2 (SEQ ID NO: 10); 6.22.2 (SEQ ID NO: 14); 6.34.2 (SEQ ID NO: 18); 6.67.1 (SEQ ID NO: 22); 6.73.2 (SEQ ID NO: 26); 6.77.1 (SEQ ID NO: 30); 7.16.6 (SEQ ID NO: 34); 7.20.5 (SEQ ID NO: 38); 7.26.4 (SEQ ID NO: 42); and 9.8.2 (SEQ ID NO: 46); 6.22.2-mod (SEQ ID NO: 52); 6.34.2-mod (SEQ ID NO: 56); 6.67.1-mod (SEQ ID NO: 60); 6.77.1-mod (SEQ ID NO: 64); and 7.26.4-mod (SEQ ID NO: 42);
(b) a light chain comprising the light chain variable region amino acid sequence of an antibody selected from the group consisting of: 1.7.2 (SEQ ID NO: 4); 1.8.2 (SEQ ID NO: 8); 6.14.2 (SEQ ID NO: 12); 6.22.2 (SEQ ID NO: 16); 6.34.2 (SEQ ID NO: 20); 6.67.1 (SEQ ID NO: 24); 6.73.2 (SEQ ID NO: 28); 6.77.1 (SEQ ID NO: 32); 7.16.6 (SEQ ID NO: 36); 7.20.5 (SEQ ID NO: 40); 7.26.4 (SEQ ID NO: 44); and 9.8.2 (SEQ ID NO: 48); 6.22.2-mod (SEQ ID NO: 54); 6.34.2-mod (SEQ ID NO: 58); 6.67.1-mod (SEQ ID NO: 62); 6.77.1-mod (SEQ ID NO: 66); and 7.26.4-mod (SEQ ID NO: 68); or

(c) the heavy chain of (a) and the light chain of (b).

16. The monoclonal antibody according to any one of claims 1-3 and 5-15 that is an immunoglobulin G (IgG), an IgM, an IgE, and IgA or an IgD molecule, a humanized antibody, a chimeric antibody or a bispecific antibody.

17. The antigen-binding portion according to any one of claims 1-3, 5-7 and 9-16 which is an Fab fragment, an F(ab')2 fragment, an Fv fragment or a single chain antibody.

18. A pharmaceutical composition comprising an effective amount of the monoclonal antibody or antigen-binding portion thereof according to any one of claims 1-3 and 5-17 and a pharmaceutically acceptable carrier.

19. 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 one of claims 1-3 and 5-17 wherein said antibody or antigen-binding portion inhibits binding of MAAdCAM to α4β7.

20. The method of claim 19, wherein the inflammatory disease is inflammatory disease of the gastrointestinal tract.

21. The method of claim 20, wherein the inflammatory disease of the gastrointestinal tract is selected from the group consisting of inflammatory bowel disease, Crohn's disease, ulcerative colitis, diverticula disease, gastritis, liver disease, primary biliary sclerosis and sclerosing cholangitis.
22. The method of claim 20, wherein the inflammatory bowel
disease is Crohn's disease, ulcerative colitis or both.

23. The method of claim 20, wherein the inflammatory diseases are
insulin-dependent diabetes and graft versus host disease.

24. An isolated cell line that produces the monoclonal antibody or
antigen-binding portion according to any one of claims 1-3 and 5-17 or the heavy
chain or light chain of said antibody or of said portion thereof.

25. The cell line according to either of claims 4 or 24 that produces
an antibody selected from the group consisting of: 1.7.2, 1.8.2, 6.14.2, 6.22.2,
6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4 and 9.8.2, or an antibody
comprising the amino acid sequences of one of said antibodies.

26. The cell line according to claim 25 that produces a monoclonal
antibody selected from the group consisting of: 6.22.2-mod, 6.34.2-mod, 6.67.1-
mod, 6.77.1-mod and 7.26.4-mod or an antibody comprising the amino acid
sequences of one of said antibodies.

27. An isolated nucleic acid molecule comprising a nucleotide
sequence that encodes the heavy chain or an antigen-binding portion thereof or the
light chain or an antigen-binding portion thereof of an antibody according to any
one of claims 1-3 and 5-17.

28. A vector comprising the nucleic acid molecule according to
claim 27, wherein the vector optionally comprises an expression control sequence
operably linked to the nucleic acid molecule.

29. A host cell comprising the vector according to claim 28 or the
nucleic acid molecule according to claim 27.

30. A host cell according to claim 29 comprising a nucleic acid
molecule encoding the heavy chain or an antigen-binding portion thereof and a
nucleic acid molecule encoding the light chain or an antigen-binding portion thereof of an antibody or antigen-binding portion according to any one of claims 1-3 and 5-17.

31. A method for producing a human monoclonal antibody or antigen-binding portion thereof that specifically binds MA发展战略, comprising culturing the host cell according to claim 29 or 30 or the cell line according to either of claims 4 or 24 under suitable conditions and recovering said antibody or antigen-binding portion.

32. A non-human transgenic animal or transgenic plant comprising (a) nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof; (b) a nucleic acid molecule encoding the light chain or an antigen-binding portion thereof; or (c) both (a) and (b) of an antibody according to any one of claims 1-3 or 5-17, wherein the non-human transgenic animal or transgenic plant expresses said heavy chain or light chain or both.

33. A method of isolating an antibody or antigen-binding portion thereof that specifically binds to MA发展战略, comprising the step of isolating the antibody from the non-human transgenic animal or transgenic plant according to claim 32.

34. A method of treating a subject in need thereof with a human antibody or antigen-binding portion thereof that specifically binds to MA发展战略 and inhibits binding to α4β7 comprising the steps of:

(a) administering an effective amount of an isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof, an isolated nucleic acid molecule encoding the light chain or an antigen-binding portion thereof, or nucleic acid molecules encoding the light chain and the heavy chain or antigen-binding portions thereof; and

(b) expressing the nucleic acid molecule.

35. A method for producing a human monoclonal antibody that specifically binds MA发展战略, comprising the steps of:
(a) immunizing a non-human transgenic animal that is capable of producing human antibodies with MAdCAM, with an immunogenic portion of MAdCAM or a with cell or tissue expressing MAdCAM; and

(b) allowing the transgenic animal to mount an immune response to MAdCAM.

36. A human monoclonal antibody produced by the method according to claim 35.

37. A method of inhibiting $\alpha_4 \beta_7$ binding to cells expressing human MAdCAM comprising contacting the cells with the monoclonal antibody according to any one of claims 1-3 and 5-17 or an antigen-binding portion thereof.

38. A method for inhibiting MAdCAM-mediated leukocyte-endothelial cell adhesion comprising contacting the endothelial cells with the monoclonal antibody according to any one of claims 1-3 and 5-17 or an antigen-binding portion thereof.

39. A method for inhibiting MAdCAM-mediated leukocyte adhesion, migration and infiltration into tissues comprising the step of contacting the endothelial cells with the monoclonal antibody according to any one of claims 1-3 and 5-17 or an antigen-binding portion thereof.

40. A method for inhibiting $\alpha_4 \beta_7$/MAdCAM-dependent cellular adhesion comprising the step of contacting cells expressing human MAdCAM with the monoclonal antibody according to any one of claims 1-3 and 5-17 or antigen-binding portion thereof.

41. A method for inhibiting the MAdCAM-mediated recruitment of lymphocytes to gastrointestinal lymphoid tissue comprising the step of contacting cells expressing human MAdCAM with the monoclonal antibody according to any one of claims 1-3 and 5-17 or antigen-binding portion thereof.

42. A monoclonal antibody or an antigen-binding portion thereof that specifically binds MAdCAM, wherein said antibody or portion thereof
comprises one or more of an FR1, FR2, FR3 or FR4 amino acid sequence of a human monoclonal antibody selected from the group consisting of: 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod.

43. The human monoclonal antibody or antigen-binding portion according to claim 1, wherein the antibody comprises:

(a) a heavy chain amino acid sequence that is at least 90% identical to the heavy chain amino acid sequence of a monoclonal antibody selected from the group consisting of: 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod;

(b) a light chain amino acid sequence that is at least 90% identical to the light chain amino acid sequence of a monoclonal antibody selected from the group consisting of: 1.7.2, 1.8.2, 6.14.2, 6.22.2, 6.34.2, 6.67.1, 6.73.2, 6.77.1, 7.16.6, 7.20.5, 7.26.4, 9.8.2, 6.22.2-mod, 6.34.2-mod, 6.67.1-mod, 6.77.1-mod and 7.26.4-mod;

(c) both (a) and (b); or

(d) either (a), (b) or (c), with or without the signal sequence.

44. A method for diagnosing a disorder characterized by circulating soluble human MAdCAM comprising the steps of: (1) contacting a biological sample with the monoclonal antibody according to any one of claims 1-3 and 5-17 or antigen-binding portion and (2) detecting binding.

45. A method for detecting inflammation in a subject comprising the steps of: (1) administering to said subject the monoclonal antibody or antigen-binding portion according to any one of claims 1-3 and 5-17 wherein said antibody or portion thereof is detectably labeled and (2) detecting binding.

46. A diagnostic kit comprising the monoclonal antibody according to any one of claims 1-3 and 5-17 or antigen-binding portion.
47. The pharmaceutical composition according to claim 18 further comprising one or more additional anti-inflammatory or immunomodulatory agents.

48. The pharmaceutical composition according to claim 47, wherein the one or more additional anti-inflammatory or immunomodulatory agents are selected from the group consisting of: corticosteroids, aminosalicylates, azathioprine, methotrexate, cyclosporin, FK506, IL-10, GM-CSF, rapamycin, anti-TNFα agents and adhesion molecule antagonists.

49. A vaccine comprising an effective amount of the human antibody thereof according to any one of claims 1-3 and 5-17 or antigen-binding portion and a pharmaceutically acceptable carrier.

50. The vaccine according to claim 49, wherein the vaccine is mucosal.

51. A method of detecting the effect of administration of an inhibitory anti-MAdCAM antibody or antigen-binding portion thereof to a subject comprising the steps of:

(a) administering to a subject a human monoclonal antibody that specifically binds to MAdCAM; and

(b) determining whether there is an increase in the levels of circulating α4β7-expressing leukocytes.

52. The method according to claim 51, wherein said leukocytes are lymphocytes.

53. The method according to claim 51, wherein said increase in the levels of circulating α4β7-expressing leukocytes is determined by FACS analysis.
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<th>CDR3</th>
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Figure 2B

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6.73.2
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6.22.2
6.34.2
9.8.2
7.20.5
6.67.1

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Figure 4
Figure 5

6.22.2
6.34.2
7.20.5

6.67.1
6.77.1

7.26.4

1.7.2

7.16.6

9.8.2
Figure 6
Figure 7
Key:
Signal sequence: underlined lower case
Amino acid changes in modified anti-MAdCAM antibodies sequence compared to parent: underlined upper case

SEQ ID NO. 1
1.7.2 Heavy Chain Nucleotide Sequence

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SEQ ID NO. 2
1.7.2 Predicted Heavy Chain Protein Sequence

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151  CSRSSTSEST ALCGLVLYDF PEPVTVSWSM GALTSGVNTF PAVLQSSGLY
201  SLSSTVYTPS SNFGTQTYTCV NVDHKPSNTK VDKTVERKKC VBCPGCPFP
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301  HNATKXPRQG QPNSTFRVVS VTLTVHQQWIL NGKBYKKCVS NKGKLPAKLEK
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SEQ ID NO. 3
1.7.2 Kappa Light Chain Nucleotide Sequence

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101  CCCCCTGAGA GCCTGGCTCC ATCTCTCAGA GCTCTAGCTA GAGCCCTCTGT
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25  CTGACAGTGG CAGTGGCAAG TGGATGAGTC GTAGGAGTCA TGCCAAGCTCT
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50  GCCCAATGTA CAGTGAGAAG TGATAAGAG CCTCAACTGC GGTAACTCC
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SEQ ID NO. 4
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2/34
SEQ ID NO. 5
1.8.2 Heavy Chain Nucleotide Sequence

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atggagtgg  gcgtgacgctg  gattttcctt  gtgtgctattt  taaaaggtgt

5
ccagtgtGAG  GTGAGGCCTGG  TGGAGCTTGG  GGGGAGGTTG  GGGAAAGCGT

10
GGGGGTCCCT  TAGACTCTCC  TTGTTAGTCT  TGGAGTACAC  TTACCTAACC

15
GCCTCGATGA  TCGTGGTCCG  CGAGCTCTCA  GGGAAAGGCG  GGGAAAGGT

20
CGGAGCGGCT  GGAGACCGCG  GGGAGCGGCG  GGGGAGGCGG  GGGGAGGCTG

25
TGCTTCAAGA  GCAGCTGGCG  GGGAGCGGCG  GGGGAGGCGG  GGGGAGGCTG

30
GCAGCTGGCG  GGGAGCGGCG  GGGGAGGCGG  GGGGAGGCTG  GGGGAGGCTG

SEQ ID NO. 6
1.8.2 Predicted Heavy Chain Protein Sequence

1
mefqswfl  aail kgvge E  VQLVESGGGL  VKPQGSLRQLS  CVSGQPTFTP

5
AWMNIVRQAP  GPKLSWVGR1  KRKTGDTTGD  YAAVPVKRTF  ISRDSKMTL

10
YLVMSNLKTBE  DTVAYVHQGQ  TLVTVVSAAT  KGPSVFPFLAP

15
CRSTSGSESTAA  ALSNLVKDYF  PEFPFTVWSNS  GALTSGVTHTF  PAVLQGGSLY

20
SLSSVTVPS  SNFQGTYTCT  NVDHPQNKT  VDKTVERKCC  WECPPCAPP

25
VAGPSVLPFP  PKPXDLMWLS  RPEPVHTCVVY  DVSHSDEFFVQ  FNMWVDGVVF

30
HNAKTFKPRRE  QPNSTFRPVS  VLTQVHQDWNL  NG keyHCKVKS  NKGLPAPIEK

35
TISSTKGKPR  EQPVYTLPS  RVEEMTNQVS  LTCLVKGFPF  SDIADVbens

40
GQPENNYKTT  PMMLDSDGSP  PLYSKLTVDK  SRWQQGNYVS  CSVMEHEALHN

45
HYTQKSLSL  PGK
SEQ ID NO. 7
1.8.2 Kappa Light Chain Nucleotide Sequence

1  atgaggtctcc ctcgtcagct cctgggctg ctaatgctct gggctcttgt
5  atccagtggt gatatgtgta tgtgacagtct ctcgatacgc tctgggtcttg
10  cccctggaga ggcggccctc attctctgca gttgctagtc gagccctcctg
15  caaagtagtg gatgtcaacta tttggtattg tacctgcaag agccagggca
20  gatccgccag ctcctgactt attgggttgc taaatggggc tccgggtgctc
25  ctgacaggttt cagtgacagt ggtcagacca cagatattac actgaaatct
30  acgagagttg gaaggtggga tgttggttgt tattactgca tgcaagctct
35  acgaactatc acctctggcc aagggaggag ctagagatt aacggagactg
40  tggccagcacc atctgcttct aatctccgag cattggatga gcagttgaaa
45  ctgccagctg ctcgctgttg gttcgcctgt gaaataactc atcagggaga
50  gccaagagta cagtgagagc tggataacgc cctccaatgc gtaactccgc
55  agagagattg cagggcactg gactggcaag gacgaccaag acacgcctca cagctccgc
60  acgacccctga gctgacgca aacagactac gaagaaacca aagtctacgc
65  ctgcaagactc acccatcgag ccctgagcct gcggctcaca aagagcttca
70  acgggggaga gttgtagag

SEQ ID NO. 8
1.8.2 Predicted Kappa Light Chain Protein Sequence

20  mrlpaulgl glmlwvsgsgq divmtqsplds lpvtpgepas iscrssqsl
51  qsnfynlydw ylqepqspqo lliylgsnra svgwpfsgss gsgtdplkki
101  srveadevgv yvnqoalqti tfqgqtrleq krtvaaapsvif ipfpsdeqdlk
151  sgatasvvcnl nfnfpreakv qwkvdnalqs gnsqyvtelq dskdeskyls
202  stlrlskady ekhekvyacev thqglsspvt kspnrgec
SEQ ID NO. 9
6.14.2 Heavy Chain Nucleotide Sequence

1  atggagttg ggctggagct ggttctttctt gttgctattt taaaaggtgt
51  ccagtgcttgg GTCGAGCAGTT CAGGACATGT GGGGGCTGG GGGGACGCTG
10  GGGGTCCTCCT GAGACTTCTCC TGTGAGGCTT CTGGAGCTAC TTCTACCAT
150  TCTGCGTATGA CACTGCTCTT CTTCAAGGC AACACTGGGT CTCCGGCCTG
20  CTCGCGACAG CACTCTCTCC GCCGCCCTCC GCCGCCCTCC GCCGCCCTCC
25  AGCACTTCAAG

SEQ ID NO. 10
6.14.2 Predicted Heavy Chain Protein Sequence

35  mefqlswhfl vailkgvqrq VQLLESGGGL VQPGGLRLLS CAASGTLTFN
51  SAmtnwvrap GKGLEWSTT GSGGTTYYA DSVKGFTPS SRSKMTLTYL
100  QMNLRALREDT AVYVCAGAAQ GGTGTPYKZT QGGTVLTVS ASTKQPSVF
150  LACPSRSTSE TTAALGCLKV DYPPEPVTVS WSGALGTSGV HTFPAVLQSS
200  GLYELSLSVT USPSSSLTTK YTTCNVNKPS NTKVDRKVS XGYPCSCP
250  APEPLGGSVP FLFPFPEPTD LMISRTPETV CVVVDVQED PQVNPWNYVD
300  GVEVHNATKT PREEQPNSTY RVSVLVTVNH QDNNLNGKEY CKVSNKLPLS
350  SIEKTIKSQA QPQEPQYTT LPSPQEPMTK NVQLSCTLVK GYPSSDIAVE
400  WESNGQPENN YKTTPPVLDS DGSPFLYSLR TVDJKRQBEVG NVFSCSVYME
450  ALHNYHTQKS LSLSLGK
SEQ ID NO. 11
6.14.2 Kappa Light Chain Nucleotide Sequence

1  atggacatga gggtccccgc tcagctcctg gggtctctgc tachtggtct
51  ccaaggaggc agatgtgaca tccagatgac cccagctccag tccctccctgt
5  ctagactctgt agggagcaga tgcaccatca ctggcgagcg aagtaagagc
15  attaagcaagt atttaaatgg gtaatcacag aaccagagga aagcaccctaa
20  aagttctgtgt cccgagttta aagttgagct ccacaggttt cccatctgtg
25  tgaagccccgt ggtttggcag acagctttca cctgttctct caacagctctg
30  caacagagat ctttcagcttc attactactgt cattagagtt caagtcccccc
35  tattaccttc gcccagcgga cagcaactgga gatacagaga actgtggtctg
40  caccagctgt ccctcatcttc cccgccctctcg atgagcgatt gaaactcggga
45  actggcctctg tgtgtgcctt gctgaataac ttcctcctca gaggagccaa
50  aagacatgtg gagaatgcctt acgccctcctc atcggtatac tccagagcaga
55  gtaagcccccgg gatacagcgc aagagacggc cttacagccct cagcagcccc
60  ctgacccctga gcaagacgaa tgtcagagaa cacaagctct ccgctccgca
65  aagccttcag cgcgcctcag gtcgcccctt cacaagagcc ttcaacaggg
70  gagaaggtttta g

SEQ ID NO. 12
6.14.2 Predicted Kappa Light Chain Protein Sequence

20  mdsrvpeaqll glllwrlrga rcdoqmtqsp sslaavsgdr vtitcrasrs
51  isslymvyqq kpklakpvliv ffivsslsqsv pssflsgsgg tdfiltissl
101  qepdfatyyc qonyippiop qgqtreirr tvapaqvfif ppsdeqlksg
151  tasvvcvlmn fyprekqvq kvdnlqosgn sqevesqds kdstyslsst
202  ltlslkadyek hvkyczvevth qglsspvtks fnrgec
25
SEQ ID NO. 13
6.22.2 Heavy Chain Nucleotide Sequence

1  atggagtggg ggtgtcgtct cggtttcctc gtggctcaatt taagagctgt
5  gctg
10  ggtgtcctgc gacagttaag tctgagcagc agatgtgccg ccagccggtg
tacattcctc ccagctgagt tctgaggatg ggtgtcctga
15  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct
20  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct
25  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct
30  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct

SEQ ID NO. 14
6.22.2 Predicted Heavy Chain Protein Sequence

1  mfglswvfll valrrgvcgq vqlvvesgqv vqpgsrlrs ccagshfpsi
5  qmhydrvqap kgklemvaai wqkqknfkk vaqktqrpit rsndkntyl
10  gqmslrvaat ayvavcardpp yyq0mvdwog gttvysvss tkpgsvppla
15  gqadctgctgt tccacatgcc cagttggtgc ggtggtgcct cagttggtgc
tacattcctc ccagctgagt tctgaggatg ggtggtgcct
20  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct
25  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct
30  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct
35  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct
40  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct
45  ggtggtgcct cagttggtgc ggtggtgcct cagttggtgc ggtggtgcct

SEQ ID NO. 15
6.22.2 Kappa Light Chain Nucleotide Sequence

```
   1  atgttcgccat  cacaactcat  tgggtttctg  ctgtctggg  ttccagcttc
  51  cagggstgAA  ATGTGCTGTA  CTGAGTCTCC  AGACTTCACG  TCGTGACTC
  10  CAAAGAGAAA  AGTACCACATC  ACCGCCCGGQ  CCAATGACAG  AAATGGTAGT
  151  AGCTTCACTCT  GGTACCAAGCA  GAAACCAAGAT  CAGTCCTCCA  AAACCTCTCAT
  201  CAAAGTATGCT  TCCCAAGTCC  TGTACGGGSGT  CCCCTCGAGG  TCCAGTGCGC
  251  GTGGATCTTG  GACAATTTTC  ACCCTCACCA  TCAATGACCT  GGAAGCTGAA
  301  GATGGCTGCAA  CTATTACTTG  TCATGAAAGT  GCTCGTTTAC  CAGTCACTGT
  351  CGGCGGAGGG  ACCAAAGTGG  AGATCAAAAG  AACTGGCTGT  GCACCATCTG
  401  TCTTCATCTT  CCCGCAACCT  GATGAGAAGT  TGAATCTGCG  AAATGGCTCTT
  451  GTTGTGTGGA  TCGTTGATTA  CTTCCTATCCC  AGGAGGCGCA  AGTACGCTTG
  501  GAAGGTCGAT  AACGCCCTCC  AATCGGGTAA  CTCCCAAGGA  AGTGTCACCG
  551  ACCAGGAGAG  CAGAGCCGGC  ACCCACAGGC  TCCAGCCACG  CAGGACCCTG
  601  AGCAAAGCAG  AACTACGAGA  ACACAAGCTC  TAGCCCTGGG  AGGTCAACCA
  651  TCAGGGCCTG  AGCTCAGCCC  TCACAAGAGAG  CTTCACACCG  GGAGGCTGTT
  701  AGTGA
```

SEQ ID NO. 16
6.22.2 Predicted Kappa Light Chain Protein Sequence

```
   20  mlpsqligfl’llwvpasrgE  IVLTQSFPDFQ  SVTPKEKVTI  TCRASQIYGS
  51  SLHNYQQKPD  GSPKLLIIYX  SQFGSFGPSR  FGSQGQGTF  TLTVGQGAE
  101  DAATTTYCHQS  GRLPLFPGQG  TKVEKIKRTV  APSFVPFPFS  DEQLKSQGAS
  151  VVCLLNYFPFP  REAKVQWKVD  NALQSGNSQG  SVTEQDSKSK  TYSLSLLTL
  201  SKADYBKHKV  YACEVTQHQL  SSPVTRSFN  RGC

8/34
SEQ ID NO. 17
6.34.2 Heavy Chain Nucleotide Sequence

1  atggagtttg  gcttgagctg  ggttttcctc  gttgctcttt  taagagttgt
51  ccagttcAG  GTGCAAGCTG3  TTGAGTCTG3  GGGAGGCGTG3  GTCCAGCTTG
10  GAGGTCCTCT  GAGACTCCTC  TTGCAAGCTC  ACTCAGTACG  CTAAGTACG
15  TTCCCCGTCTG  CCCGTGCTTC  CGAGCAAGAC  TCCAGAAGAC  AGCGAGCGC
20  AACTCGAGCC  GTCGACTACG  CTTCCGCGCC  TGCTGCAACG  TGCTGACACG
25  CTCTGACGAC  GCTATGCAAG  CCACTTCACG  AGGTTCAGTG  AGTCCAGCG
30  CCTCCAGGCG  GCTAGAAGCG  AGCTCAAGGCG  AGGTCAAGCG  AGGTCAAGCG

SEQ ID NO. 18
6.34.2 Predicted Heavy Chain Protein Sequence

1  mefqlswwf1  vallrgvcQQ  VQLVESGGGV  VQPRGSLRLS  CAAGGFTFSS
35  YGMHHRVQAQ  GKGLWAVVI  SNGMNKYYA  DSVKGRFTIS  RDNKNTLYL
10  QMNLSAEKT  AVYVYCARST  AITYYYGMG  VWQQGTTTIV  SAASTKQPSV
15  FLAPCSTST  SESTALGCL  VLYDDPPPBT  VSNMGLATLS  GUVFPAPLQ
20  SGLYSLSV SVTVPSSSLGT  KTYTNCVDHK  PSTTKVDKRV  ESKYGPPCS
25  CAFPEFLLGP  SVFLLPPPKP  DTLMISETPE  VTCCVVDVQ  EDBPQFQNY
30  VDQVBRHNAK  TKPRESQNS  TTRYVSVLTV  LHQDMLNKE  YKCKVDNKL
35  FSSIEKKTSS  AKQPREBEPQ  YTLPLPSEQEM  TKNQVSTCLL  VQIPPSDIA
40  VEWESNQOPE  NNYXTTTPPYL  DSDGSPFLYS  RLTVDKSRKQ  BGNVPSCSVM
45  HEALHNNHTQ  KSLSLGLK
SEQ ID NO. 19
6.34.2 Kappa Light Chain Nucleotide Sequence

1  atggacatga gggctcccccgc tcagctcctg gggctccctg tactcttggct
51  cggaggtgccc agatgtgACA TCCAGATAGC CCACTCCTCA TCCTCCTCTG
5  CTGATCTGCT CGGAGCAGAC GTCACCATCA CCAGGCGGGC AAGTCACGAT
15  ATATTAGACT ATTAAAAATG GTTTCGAGCA AAGACGGGGA AGGCCCCAAC
20  GCTTCTGATC TATGCTGAC TCGGGTTGAA GCGTGGGCTC CCATCAGCGT
25  TCAAGCTGAG TGAGGTCCGG ACAGATTCTCA CTCTCACTCA CAGGACCTTG
30  CAACTGATGG ATTTTGCAAC TTACTCTCTG CACAGAGGTA ACAGTCGCCAC
35  ATTCTACTTTC GCAGGCTCGGA CCAGATGGGA TATCAAGCAG ACTGGTGCTG
40  CACCACTCTGCT CTCTGATTCC CGCCCATCTG ATGAGCAGTTG GAATCTCTGA
45  ACTGCTCTCTG TTTTCTGCCG CACAGATAAC TTCTATCACCA GAGGAGCGAA
50  AGTCAAGTGG AGAGGATGTA AGCAGCTCTCA ATCCGTGAAAC TCCCCAGAGA
55  GTGTCACAGGA CAGGACAGCG AAGACAGCGA CCTACACGGT CAGCACCCACC
60  CTGACGCTGGA GCAAGAGCAGA CTCAAGAGAA CACAAAGCTC AGCCCTCCGA
65  AGTCAAGCAGT CAGGCTCCTGA GCTGCGCCCTG CACAAAGAGC TCTCAACAGGG
70  GAGAGTCTTA GTGA

SEQ ID NO. 20
6.34.2 Predicted Kappa Light Chain Protein Sequence

20  1  mdrvpaqlIl qllllwlrfa rcDlGmTQsP SSLSASVGDR VTITCRASQN
51  ISYLYWWFQQ KPGKAPKLLI YAASGLKQGV P5RFSGGSGS TDFTLITRTL
5  QPDDFATSC HQQSLPFTP GPQTVKDIK TVAPTSPVFIF PSDEQKLSG
10  TASSVCLNNN FPYREAKVQW KVNDNLQSGN SQESVTEQDS KSTSTRSLST
15  LTILSKADYEH HKVYACEVTH QGLSSPVTKS FHRESC
20  25
SEQ ID NO. 21
6.67.1 Heavy Chain Nucleotide Sequence

1  atgaacacc tggcctcttt ctctctgtctg gtggcagcct ccgatggttg
51  cctgtcctcag gtgacgctgct aagagtcgggc ccaggactgt gtaagccctt
100  cggagccctc gtccctcacc tcgacgtcct cggccgactc ctgatagagt
150  aacttttggg ctaatgctca aggccgcttg aacctctcct gctatctcag
200  gacccgctgg gacgggtggt gacggctgggc aagaggtgtc tggcctcctg
250  agaagccaga gttgctaccc tggccctct gctatctcct gccgctgggc
300  atgacgtgag ccctcagcca ccactacaca cagaaagcag tctgggttaa
350  1401  tctgggttaa tga

SEQ ID NO. 22
6.67.1 Predicted Heavy Chain Protein Sequence

1  mhklwfflli vasprwvlsq vqlqesgpgl vksbhtslt ctvsgdsiss
35  nywswikopa gkglewigmri ytsogtnsnf slkrgvtila dtsoqflsk
100  lsvttad7ta vycardrit iirgilpssf dywqoqtlvt vssastkgps
150  vplapcsrcs tsestalgcg lkvdypppev tvswgsgalt svhtpfvav
200  qssulglsrs vtvpsisig ttkyttcvnvd kpsntvkudr vspsyppcpp
250  scppepllgd psvfplppkp kdtlmsrto evtcvvdvs qedepvqvfnw
300  yvdgvevhnna ktktppqfkn sytryvvsvlt vlhqdwlngk eyckkvsnk
350  lfsesiexltis kakqreqepq vylppqsepq mtkqvsltcc lvkgyfpsidi
400  avenwegnoqp bnygktpppv ldsgsffly srltvdkserw qegnynfcs
450  mhealhnhym qkstlsllgk

11/34
SEQ ID NO. 23
6.67.1 Kappa Light Chain Nucleotide Sequence

1 atggtgttgc agacccaggt cttcatttct ctgttgcctct ggtatctcttg
51 tgcttacgsg gacatctgta tgacccagtc tccagactcc ctggtctggt
5 101 ctctgggcca gaggccacc acatcactgca agttccagcca gagtgtttta
15 201 tacagctccct aaattgtcctc tttactgsgc atctataccg gataatgsgg
25 301 tccctgaccg attcagttgc agggtgcttg ggcagaggtt catctcacc
30 401 atcagcagcgg tgcaggtcca agatgtggca gttatatctc gttcacaata
45 501 tttatatatt cctccctctca ctttcggscc cggccacag gttcagatca
55 601 aaggaactgt ggctgcacc a c cctcctcc acatctctca tctccgcccc acatcagtga
65 701 agcctgggac gcacctggac gctagaccaac gcagactcag gagacaccaag
75 801 agtcctacgcc tctcagtcga ccactcgagg cttgagctcg cccctcgaca

SEQ ID NO. 24
6.67.1 Predicted Kappa Light Chain Protein Sequence

20 1 mvlgqtqyfis lllwiesysg divmtqspds lavslgerat incksqsyl
51 ysnnkktyla wyyqqkprqpp rlliywasir eygypdfsqs sgsxtftlt
101 isslqadvva yyfcoqyqysr ppltfpqgtk veikrtvaaep svfipfssde
15 201 qlksstasvvy cllnnytpre akvqvkvdna losngsqsves tqsdsksdy
25

12/34
SEQ ID NO. 25
6.73.2 Heavy Chain Nucleotide Sequence

1  atggagtttg_ggctagctgg_gctttttctt_gtgctatat ttaaaggtgt
51  ccagtgcGAG_GTGCAGCTGT_TGGAGTCTGG_GGAGACTTG_GTCCAGCTG
101  GGGGGTCCTT_CAGACTCTCC_TGGCAAGCTT_GTGGATTCAC_CTTTGAAGAT
151  TATGCGAGTA_ACTGGTGGCC_AAGGAGCCA_GGAGAAGCTC_TGGAGTGGT
201  CTACGTATTG_ATGTTGTCTG_GTGTTACTAC_ATACTACCA_GATCCGCTGA
251  AAGGCGCCTT_CACACATCGG_GAAGCAACTG_CGCTCTCTCT_CTTCTCTTCT
301  GCTGGGAACCT_GCGGCGGTG_ATGAGGCTCT TCCTCCCTCT_CTCTCCAGCT
351  CAGAGCTTCC_TGCCGGTCG_GTCGGTGATG_GGACGGGAGG_CCGCTTTCTC
401  GTGGCCACTG_TGCAGCTGTC_CGCTCTCGAG_AGAGTTGAGA_GGGCTGCTC
451  TACAGACTCTT_CAGACTCTCT_TCCCGGCGCC_GTGGCTACTA_CTACATGGG
501  TAGAGCTTCC_TCAGGACGGC_TGCTGTCTAC_AGGACTCTCT CTCTTCCTTT
551  GTGAGAGCCT_AAGGCGGCTG_ATGCTGCTCT TACAGCTCCT TCCTCCCTCT
601  CTACAGCTCTT_CAGAGCTTGA_CTCCGGGCGG_AGGCCTGGA_GGGCTGCTC
651  TAGAGCTTCC TCAGGAGGCTC_TCTCTCTGTT GGGCAGGAGG_CCGCTTTCTC
701  GACAGACAGA_GCTGCTGCTC_TGAGTGTGAC_GGAGCCCGGA_AGAGCTGCTC
751  CCATCGTACC_CAGACAGGAG_GCTTCTCGTG_GGAGGCGGAG_TCTCTGCTTC
801  CCGCAGGAAAC_CCCGAGGAGA_TGACGATGAT_AGCCAGAGA_AGAGCTGCTC
851  GTGGCCACTG_TGCAGCTGTC_CGCTCTCGAG_AGAGTTGAGA_GGGCTGCTC
901  TAGAGCTTCC TCAGGAGGCTC_TCTCTCTGTT GGGCAGGAGG_CCGCTTTCTC
951  CCGAGAGGAG_GAGTGTGAC_GGAGCGGAGG_GGAGCTGCTG_CTCTTCCTTT
1001  ACCAGGGACTG_GGAGGAGGAGA_AGAGTTGAGA_GGGCTGCTC
1051  GTGGCCACTG_TGCAGCTGTC_CGCTCTCGAG_AGAGTTGAGA_GGGCTGCTC
1101  CTCCGGGCGG_AGGGCTGGA_GGGCTGCTC
1151  CAGACAGGAG_GCTTCTCGTG_GGAGGCGGAG_TCTCTGCTTC
1201  ATGGCAAGAG_GAGTGTGAC_GGAGCGGAGG_GGAGCTGCTC
1251  CTCCGGGCGG_AGGGCTGGA_GGGCTGCTC
1301  CCCTCTCTCT_CTCTTGCTTG_TCTCTCTCT_CTCTTGCTTG
1351  GTGGCCACTG_TGCAGCTGTC_CGCTCTCGAG_AGAGTTGAGA_GGGCTGCTC
1401  TAGAGCTTCC TCAGGAGGCTC_TCTCTCTGTT GGGCAGGAGG_CCGCTTTCTC

SEQ ID NO. 26
6.73.2 Predicted Heavy Chain Protein Sequence

1  meflaslfl1 vaikygygev VQLESQGDL VQPGGLSRLS CAAAGPTTFS
35  YAMNVRQAP GGLLENVSI SGRQGTCTYA DSVKGRFTTS RDNSKNTLYL
101  QMNLSAEDA AYYVCAAKAY AGSELYXYXG MDVWQOQDTV TVSASSKTGP
151  SYPFLAPSCR GSTENTPAALG CLVQDGPPRP VTVSKNGAL TSGVHTFPFAV
201  LQSSGYLSLS SVTVPRPSSL GKTGYTNVD HKPSNKTVDK REVSKYGGPC
251  PSCPPAFSLG GSVFLPPPK PDDLMISRT PEVTCCVVDV SQEDPVEQFN
301  WYDYDVENVH AKTRKPRQF NSTYRVVSVL TVLHKDVLNM KEYCKKVSNK
351  GLRSLNBKTI SKAKQGQPQF QYVTLLLLPSB EMTKNQVSIT CLVQGYPFSD
401  IAVNGNQG PENNYKTPPP VLDSGDFSSL YSRLTVDKSR WQEGNVFSCS
451  VMHEALHNHY TQKSLSSLG K
SEQ ID NO. 27
6.73.2 Kappa Light Chain Nucleotide Sequence

1  atggacatga_gggtccccgc_tcaagctctgt_gggtcctgct_tacctctggt
t  5
  51  cccagagggc_agatgGACA_TCCAGATGAC_CCAATCTCCA_TCTTCCCTGT
  10
  101  GTKCATCTGT_AGUGACAGAG_GTCACCTTCA_GTGCCCGGGC_AAGTCAGAC
  15
  151  ATTACCAACT_ATTTAATTG_GTCATAAGAG_AACCCAGGGA_AGGCCCATAA
  20
  201  GCTCTGACTC_TATGCTGGGG_CAGTTGAGCC_AAGAGGGGTC_CCATCAAGGT
  25
  251  GCTCTGAGAG_GGGTAGCCAG_GGACTCTGGG_ACAGATTCTAC_GTCTCAGACT
  30
  301  CAACCTGAAG_ATTTTGGCAAC_TTAAGCTCTG_CGAGATAGAT_ACAGTAATCC
  35
  351  TCCCGAGTGC_GGTTTTGGCC_AGGGACCCAC_GTGGGATATC_AAAGGGACTG
  40
  401  TGGCTGACCC_ATCTGACTTC_ATCTGCGGGA_CATTGGATGA_GGAGTTGAAA
  45
  451  TCTGAAACGG_CCTCTGTGTGT_GTGCTGGTCTG_ATCTACTTCT_ATTCCAGAGA
  50
  501  GGCCAAAGTA_CATGGGAAGG_TGGATAACGC_CCTGAAATCG_GTGAACCGCC
  55
  551  AGGAGAAGGT_GCACAGCACG_AGACAGCAAG_GACACACCTA_GACGCTCAGC
  60
  601  AGCACCCCTGA_GGCTGAGCAG_AAGAGACTAC_GAGAAACACA_AAGTCTACGC
  65
  651  CTGCAGAAGTC_ACCCATCAGG_GCCTGAGGCTC_GGCCGTCACA_AAGAGCTTCA
  70
  701  AGAGGGAGGA_GTGGTTAGGA_A

SEQ ID NO. 28
6.73.2 Predicted Kappa Light Chain Protein Sequence

20  mdmrvpagl_q1lllwrlota_rcDIQMTQSP_SSSSASVGDTR_VTFTCRASQN
25
  251  ITNYLYNQGG_KPGKAPKLIL_AAYSSLPRGQ_PSFRGSSGGQ_TDFTLPLTSL

SEQ ID NO. 29
6.77.1 Heavy Chain Nucleotide Sequence

1  atggactgct ggctcgcctg ggttctcctg gttgtctatg taggaaggtg
5  ccaggttagGC GTGACACTGCG TGGAGTCTGG GGGAGGCCTG GTCAAGCCGTG
10  GGGGTCCTCT GAGACTCTCC TGGCAGCCTC CTGGATTACG CTTCAGTACG
15  TAGAGCAGCAG CTATTGTCAG TGCCCTACTA CCCTCTCTCAT ATGGGCTACG
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25  CAGCAAGACG CATTCGAGCG TGGAGTGCAG CAACAGACAC CAAACAGACG
30  TTGGGCAAGG CCGCTGCTGG TGGAGGGCGA AGAGGAAAGC ATGGGACACG
35  CAGGACACTG GGGAGGCCTG GTCAAGCCGTG

SEQ ID NO. 30
6.77.1 Predicted Heavy Chain Protein Sequence

1  melglrrwvfl vailegvgcE VQLVESGGGL VKPGSSGLSLS CAASGPTFSS
5  YSMNWVQAP GKLW BLEWSSS SSSSSYYYA DSNVKRPTIS RDNKAKSYLYL
10  QMNILRAEDT AVYYCARGDY SSNSSYYLYY GMDVWQCGTT VTGSSASTGK
15  PSVGFLAPCP RSTSGSSTAA GCILIVDYPFR PTGVSNSGAR LTVQGVHTTPA
20  VLSQQGLYLSSL SAVTVTVPSS QGKTCTCNV DHKPSNTKVD KRRFSKYGPP
25  GQPSGVRFFP KPQDTLMISR TPEVTCVDV VSGQDPERQVF
30  NWYDGVVEH NAKTPKREQQ FNSTYRVVSV LTVLVQDWLGN GKEYKKCQSVN
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40  DIAVWBNESNG QPPENNYKTTP PVLSDSOGSF LYSRSLVSDK RKQEGNMSER
45  SVMHEALNH YTQKSLSSSL GK
SEQ ID NO. 31
6.77.1 Kappa Light Chain Nucleotide Sequence

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1  atgaggtctcc  cttgctcagct  cctggggtcg  ctaatgtcct  ggataccttg
51  atccagtgca  GATATTGTGA  TGACCCAGAC  TCCACTCTCT  CTGTCGGTCA
101  CTCCTGGACA  GCCGGCCCTCC  ATCTCTGGCA  ACTCTAGTCA  GAGCCCTCTG
151  CTTAGTGTGATG  GAAAGACCTA  TTGTGAATGG  TACCTGCGAGA  AGCCCGGCCA
201  GCTTCCCACAG  CTCCTGGACT  ATGAAAGTTTC  CAACCGGGTC  TCTGGAGTGC
251  CAGACAGGGT  CAGTGGCCAGC  GGGTCAGGGA  CAGATTCCAC  ACTGAAGACT
301  AGCCGGAGTG  AGGCTGAGGA  TGTTGGGGTT  TATTTCTGCA  TGCAAGATAT
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601  AGCAGCCACCC  TGAGCTGAGG  CAAAGCAGACG  TACGGGAAAC  ACAAGGTCTA
651  CGCCTGGCGAA  GTCAACCATCC  AGGGCTGGAG  CTGGCCCCGT  ACAAGAGACG
701  TCAACAGGGG  AGAGTGTATT  TGA
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SEQ ID NO. 32
6.77.1 Predicted Kappa Light Chain Protein Sequence

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51  LSDDKTYLNW  YLQKPQFPQ  LLYEVSNRF  SGVDRFSGS  GSGTDFTKLI
101  SRVEAEDVGV  YSMCQ55QLM  CSFGQGKLE  IKTVAAPSVP  FIFPPSDEQL
151  KSQTASVVC  LNNFYPFPRAK  VQNKVDNALQ  SNSQESVTVE  QDSKDSSTYL
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### SEQ ID NO. 33

7.16.6 Heavy Chain Nucleotide Sequence

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7.16.6 Predicted Heavy Chain Protein Sequence

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SEQ ID NO. 35
7.16.6 Kappa Light Chain Nucleotide Sequence

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10  cccctggca gcccctgctc atctgagctc aagtctgtgct agcttcccttg
15  gctctgatgg atggagaatttc attttgggagc ttacgggtga
20  cagatagttt ccagccagcg ggttgagggag cagatttcac atgaaatcact
25  gctgtggctgc accatctctct tttattttcc cgcattaagatg cagcattcgt
30  gatctgagaatttc cctgggctagt gcgcctgatag accgctgaat atc
35  agccgggttg aagctggaaa cagcattgtaa aatgctgaa
40  cagctgctgg ctagctctctt tttttacagc ctgcttcggc ggggttggc
45  aagcagcagc gccaccaggc gacgccggct cagctgctgg cagctgctgg
50  aggctctggaa cagctgctgg cagctgctgg cagctgctgg cagctgctgg
55  cccctgatgg atttgggagc ttacgggtga
60  cagctgctgg ctagctctctt tttttacagc ctgcttcggc ggggttggc
65  aagcagcagc gccaccaggc gacgccggct cagctgctgg cagctgctgg
70  tcaacagggag aagtgttagc tga

SEQ ID NO. 36
7.16.6 Kappa Light Chain Protein Sequence

20  mrlpqaglgl lmlwipgssa divmrtgpaas lovtfqdpas isckssqslf
5  htdcgtytlyw ylqkgpqqpg lliyevsnrf sgvpdrfsgs sggtddlklk
10  srmvetexvii yycmhnqipl wtffgqgkvdb ikrtvapasv fippdsxb ql
15  kgstasvqvcn lnnyppeak vqknvqnaalq sgnqsgsvse qdskeqstyl
20  sstltlslknd yekkvyvacet vteqglsspy takrnrgec
SEQ ID NO. 37
7.20.5 Heavy Chain Nucleotide Sequence

1  atgaacaccc tgggtttctt cctcttgctg gtggcaagctg ccagatgggt
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101  CCGGAGACCT GTCCCTCAAC TGGACTGTCT GTGGTAGTCT CATCAGTATG
151  TACCACTGGA ACTGGAATCG GAGCAGCCGC GGGAGAGGAC CAGAGATGGA
201  TGGGCGTATC TATACAGTGA GAGACCACTA CAGACACTCA TCTCCAGGAG
251  GTGGCTCTGC CATGTGATAC GACACGTGCA AAGACAGCTA TCTCCAGCCT
301  TGGACCTTGG CATGACGCGT TCTCGACGAG GGTGTCACAC CAGAAAGCGC
351  GCATTCCGCT GCCGACGACG CGGGCCGGAT TCTCCAGCCT CAGGATCAGC
401  GTGGGGACGC TGTTCAAGGC TCTCCAGCCT TCTCCAGCCT CAGGATCAGC
451  AGTGGGGGTG TCCTCCAGCCT TCTCCAGCCT CAGGATCAGC
501  TACCACTGGA ACTGGAATCG GAGCAGCCGC GGGAGAGGAC CAGAGATGGA
551  TGGGCGTATC TATACAGTGA GAGACCACTA CAGACACTCA TCTCCAGGAG
601  GTGGCTCTGC CATGTGATAC GACACGTGCA AAGACAGCTA TCTCCAGCCT
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701  GCATTCCGCT GCCGACGACG CGGGCCGGAT TCTCCAGCCT CAGGATCAGC
751  AGTGGGGGTG TCCTCCAGCCT TCTCCAGCCT CAGGATCAGC
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901  GTGGCTCTGC CATGTGATAC GACACGTGCA AAGACAGCTA TCTCCAGCCT
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1001  GCATTCCGCT GCCGACGACG CGGGCCGGAT TCTCCAGCCT CAGGATCAGC
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1151  TGGGCGTATC TATACAGTGA GAGACCACTA CAGACACTCA TCTCCAGGAG
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3251  TGGGCGTATC TATACAGTGA GAGACCACTA CAGACACTCA TCTCCAGGAG
3301  GTGGCTCTGC CATGTGATAC GACACGTGCA AAGACAGCTA TCTCCAGCCT
3351  TGGACCTTGG CATGACGCGT TCTCGACGAG GGTGTCACAC CAGAAAGCGC
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SEQ ID NO. 38
7.20.5 Predicted Heavy Chain Protein Sequence

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35  YHWNWRQAF AKGIGKOOQ IYSGCTNYP SLKSRVTEML DTSNVPNGL
101  LSSVTAADTA VYVYCEGVR YYVASGYY GLDWWGGDDT TTSSSAGTSG
151  PSFPPLACPS RSTSRSAAL CGLVKNDFPV PTVTSWNSQG LTSGVHTFFPA
201  VLQSSGLYSL SVTVTPPSN PSTQYTCNV DHKPSNKTVD KTVREKCCVE
251  CPFCAPFPPA GPSPFLFPFP PDKTLMIERT PENVTCVVDV SEDPVPVFN
301  WYVDGVSIVI AKTKPREEQP NSFRVVSVL TVVHQDXLNG KEYKCKVSNK
351  GLARPARIKTI SRTKQPPRBP QVYTLPPPES EMNKTVQVSL CLVKGFYPSD
401  IAVNEWSNQG PENNYKTTTP MLSDGSFPL YSKLTVDSR WQQGNVFSCT
451  VMHERALHNHY TQKSLSLSPG K
SEQ ID NO. 39
7.20.5 Kappa Light Chain Nucleotide Sequence

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250  tctgctggcagtt  caggtgacag  ggtctagagcag  gatagatttc  acgtgaaatct  
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400  tgcctgccacc  atctgtctctc  atcttcccggc  catctctgatg  acgggtggtaaa  
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SEQ ID NO. 40
7.20.5 Predicted Kappa Light Chain Protein Sequence

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200  stltltskady  ekhkyacev  thqglsspvvt  ksfnpgec
SEQ ID NO. 41
7.26.4 Heavy Chain Nucleotide Sequence

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101  GGGCCCTCAG GAGGCTCTCC TGAGGAGGCT CTGGTACAC CTGGTACCAC
151  TATGGTGATCG ACTGGTGACG AACAGGGGCT GGAACAGAGC TTGGGATGAT
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251  AGGGCAAGAT CACATAGTCC ACAAGACAT CACAGACAGC AGCCTCAATG
301  GAGCTGAAAGA GGCTGCAAGTC TGAAGACAGC GCGTTAGATC ACTGGTGGAG
351  AGAGGACAGAC GTGCGCTGAC GAGGTTAGA GAACAGGCTC AGGACCATCG
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601  CGATTTCCGAC GACCTCTACG CCTGAGAGAC GCTGAGGAGC TCACGTCCAG
651  CAACTGCGCA ACGAGAGAAT ACCAGAGATT GATAAGACCA AAGCCAGACA
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751  CGCCGGGCCCC AAGGGCCCTG GCTGAGGAGC AGGAGGAGGCA CTTCCGCGCC
801  AAACAAAGCA CAGCCCTTGAC TGGTTAGATG GCTGGATGGCC GATCCGGGCC
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1151  AGCTGCGCTG GATGAGGGCG CTACACAGCTG AGCGGGGCGA CGAGGGCGA
1201  GTGAGGCGGCG TTGGAGGTGA CACGATCCAG TAAGGCGGAG AAGGACAGGC
1251  TCCACAGTGCT GCCAGGCGCC GCCCTGATTT CTCCTACAG AGCGGTGCA
1301  TGGACAAAGAG CAGGGCCGAG CAGGGCCGAG CTCCTGCTAG CTCCTGAGAT
1351  CATGAGGCTC TGCCACAGCA TACACAGGCA AGAGGGGCTT CTTCCGCTCC
1402  GGGTAAGATGA

SEQ ID NO. 42
7.26.4 Predicted Heavy Chain Protein Sequence

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101  ELRSLRSDDT AUYV-CAGEEG BSGDGYDYM CVWGGCQVTV VSSASTKFGS
151  VFLAPACCSR TSESTRAALC LVKIDPEPPV TVSNWSSQAT SGGHITTPAVL
201  QSSGLYLSLS VUTVPPSSNG TGTYTCDVNDH KPSNTKVQDT VERKCCVSCP
251  PCAPPAPVAPG SVFLLPPKPKP DTLMISRTPE VTCVVVDVSDH EDPBVPQNYW
301  VDGVENRNK TKKBREEQPSN TFRVSVLTV VHQDMLNGKB YKCKVSNKGL
351  PAPIKTITSK TKQGFPRPPOV YTLPPSREEM TKNQVSSLCL VKGYFODSIDA
401  VVWSSNQDPE NNYKTTTMLR DSDGSFPLYS KILVDKSRWQ QGNVFSCECVS
451  HESLHNNHTQK KSLLSLGK

21/34
SEQ ID NO. 43
7.26.4 Kappa Light Chain Nucleotide Sequence

1  atgagacctc  ctgcctagct  cctgggctg  ctaatgcctc  gataacctgg
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101  CCCCCGGAACA  GCGGCGCCCTC  ATCTTCTGCA  AGTCTAATCA  GAGCCCTCTG
151  TATAGTGATG  GAAGAGCCCTA  TTGTGTGTTG  TACCTGCGAG  AGCCAGCGCA
201  GCCCTCCACAG  CTCCTGAGCT  AAGAAGTTTC  CAAACGATTC  CTGGAGGCTG
251  CAGATAGGTTT  CAGTGCGACG  GGGTCAGGGAG  CAGATTCCAC  ACTGAAATCT
301  AGCCCGGTTGG  AGGCAGGAGA  TGTTGGGTTT  TATTACTGCA  TGCAAAGTTAT
351  ACGCTTTCCG  TGGACGTGCTG  GCCAAGGGAGC  CAAGGAGCAA  ATCAAACGAA
401  CTGTGCTGTCG  ACCATCGCTC  TCTCATCTCC  CCGGACTCTG  TGAAGAGTCTG
451  AAATCGGAAA  ATGGCCCGTCG  CTGAAATACCT  CTGTTAGCCAG
501  AGAGCGCCAAA  GTACAGTGGA  AGGTGGATAAA  CCTGCTCCAA  TGGGTTAACT
551  CCCAGGGAGA  TCTCACGAGG  CAGGACAGCA  AGGACAGCAC  CTACGAGCTC
601  AAGCAGCACCC  TGACGCTGAG  CAAAGCGAGC  TACGGAGAAC  ACAAGTCTA
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701  TCAACAGGGG  AGAAGTGTAG  TGA

SEQ ID NO. 44
7.26.4 Predicted Kappa Light Chain Protein Sequence

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101  SVRBAEDVGV  YYCMQ5QXLP  NTFTGQGNTKE  IKTVAAPSV  FIFPPSDEQL
151  KOSHTASVVCL  LNNFYPBRAK  VQKVDNALQL  SGNQSMSVTE  QDSKDSYSL
201  SSSLTSKAD  YEBHKIVYACE  VTHQGSLSPV  TKSFRNGECC

22/34
SEQ ID NO. 45
9.8.2 Heavy Chain Nucleotide Sequence

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51  ccagtggtcag gtgcagctgg tggagctctg gggagccgtg gccagccttg
101  gaggtgcctct gagactcccc gttgagcgtct cttggtctat cttcgtagg
151  tatggtcttgc actggtgctgc cgcagctttc gacagacttt gacagcggc
201  ggctghtacg tctccctgct gtgccgacgt gctgctatgt cttctctctt
251  gcgtgcacaa ctccaccttt ggcagtgttt gtaacagagt gacagacagt
301  acaggtgttg ggacagcaac tgcagcagcc ggctgtctgg ctgactttca
351  atcgcacagg cagcagccgg cggcgtctgt gcgtgcacag gacagagttg
401  ttctccccaa ctagttccgg cagcagccgg cggcgtctgt gcgtgcacag
451  ctctctctct cctctctctc gctgctgtgc gcgtgcacag gacagagttg

SEQ ID NO. 46
9.8.2 Predicted Heavy Chain Protein Sequence

1  mefglaswfl valrrgvcq VQLVESGGGGV VQPGRSLRLS CAAAGPTFSS
51  ygmhvwqvpap ggkglewnvay wedygnvwaqv ysvkgrfptis rdnskntyl
101  qmnslraedt avyycaqagv hfaqtmqctltg ltvssastkg psvpplacs
151  rtsrgsttal cglvkydppe pvteswnsga ltsqvhtpepa vlqsgsryssl
201  ssytvpgss sgtktgtnvnr dhwhntkvdv krveskygpp cpsscpaefl
251  ggpsvlfpfp kpkkdltmisk tpevctccvd vsqepdpeqvfq wynvkdvvevh
301  naatkprseq fnstyrvvsv ltvldwqdlwn gkeykckvsn kglssieuik
351  iskakqoppr pqvytylppsq bembknqvel tclvkgfyyp fiaqewnbsng
401  qpenykytpp pvlvdsdgsff lysrltvdkrs rwqsgnvfsc svmhealnhn
451  ytqksslssl gk
SEQ ID NO. 47
9.8.2 Kappa Light Chain Nucleotide Sequence

1  atggacatga  ggttccctgc  tcagctcctg  gggctcctgc  tgcctcctgct
51  ctcagtcgca  ggtggcaaat  gtcgacatcc  gattgacccag  tctccatctct

5  ccctgtctgc  aatcgtgagga  gacagagtca  ccacactttg  cagcaaaac  cagggagaac
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50  ctgccaagctc  accoatcagg  gccctgcttc  gccggcata  aagagcttca
55  acagggaga  gtttagtga

SEQ ID NO. 48
9.8.2 Predicted Kappa Light Chain Protein Sequence

20  mdmrvpagll  gilllwslva  garcdiqmtq  spsslssavg  drvtitcqas
51  qdisnlywyy  qokpgrakpl  liydasnlbet  gypsrfsstg  gstdfpttis
10  slqpdiaaty  scqsdnlsli  tftqqgtrlhi  krvtapssvf  ifpssdeqlk
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20  stltlksady  ekkhtvacev  thqglsspvv  ksfmrgec
25
SEQ ID NO. 49
Nucleotide Sequence of cynomolgus MAdCAM α4β7 binding domain

1  ATGGATCGGG GCCTGGCCCT CTCCTCTTTG GGTCACTC
5  GCCGGGCTGCG GGCACGTCCT TTCAGGGAAG GCTGAGCACCC
10  CAGCGCTGCCT GCCTGGACCG CGGGCCACGG CTGACATTGG GGGCCCTTGA
15  CGCAGCTCCG GGGCGGTGCG ATGCGGACGC GGGGGAGAGC GTGGCTACGC
20  TGCCCAACGC TCTGTGTGCG GGGGCAGGCA CCGCTGTGTG TGACACCTCC
25  TGGGCGGCGC GCACCTTCCA GCCACCGTGCG CCGCTCTTGA TGACACCTCC
30  GCCGCAACAG CTGACCAATCT CCCGCGACGC CTCCTGCGCT GTGGACCGGC
35  AGCTGCGCCT TACGCGTCAC AAACTCAGGC CTGAGGACGC CAACTGCCCT
40  TCGTCTCTCC TCTGTGTGCG GGAAGGAGA CTGAGGCAAG CCGGAGCTCT
45  GGCGCCGCAG TGATGAAAGG AGAGGAGGCC CGAGGAGGAG GGGAGCGGTC
50  AGCTGCGCCT TACGCGTCAC AAACTCAGGC CTGAGGACGC CAACTGCCCT
55  TGGGCGGCGC GCACCTTCCA GCCACCGTGCG CCGCTCTTGA TGACACCTCC
60  GCCGCAACAG CTGACCAATCT CCCGCGACGC CTCCTGCGCT GTGGACCGGC
65  CAGCAGCGGC CAGCCATCC CGGTCTCGCA C

SEQ ID NO. 50
Amino acid sequence of cynomolgus MAdCAM α4β7 binding domain

20  MDRLLALLLA GLLGLLQVGC GQSSLQVKPLQ VEPPEPVVAV ALGASRQLTC
25  RLDCADDGAT VQVWGLDTSL QAVQSADGRS VLTVNRASLGA AAGTRVCGS
### SEQ ID NO. 51
Modified 6.22.2 Heavy Chain Nucleotide Sequence

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Modified 6.22.2 Heavy Chain Amino Acid Sequence

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Modified 6.22.2 Kappa Light Chain Nucleotide Sequence

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51  caggggtgAA  ATTTGCTGGA CTCAGTCTCC AGACTTTCAG TCTGTGACTC
101 CAAAAGAGAA AGTCACCCATC ACCTGCGCGG CCAGTCAGAG AATTGTTAGT
151 AGCTTACACT GGTACCAAGCA GAAACCAGAT CAAGTCTCCA AACTCCTCAT
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251 GTGGATCTGG TCCAGATTTCC ACACTTCCACA TCAATATCGCT GGAAGCTGGA
301 GATGCTCGAA TCTTTATTQG TCATCATGAG ATGCTGTCTTAC CGCTCATT
351 CGGCGGGAGG ACCAAGTGAG AGATCAACAGG AACTGTGGCT GCACCATCTG
401 TCTTTCATCTT CCCGCAATCT CGTGAACAGT TGAAATCTGG AACTGCCCTCT
451 GTTGTGTGTCG TGCTGAAATA CTGCTATCCG AGAGAGGCA AAGTACAGTG
501 GAAGGTGGAT AAGCCTTCCTC AATCGGATTAA CTGCCAGAGA AGTGTCCACAG
551 AGCAGGACAG CAGGGACGAC ACCTACAGCG TOAAGCGACG CCTGAGGCTG
601 AGCAAAAGCG ACTACQAGAA ACAAAAGTG TACGCTGCG AAGTACACCA
651 TCAGGCCCTG AGCTCGCCCG TCACAAGAGG ATTTCAACAGG GGAAGAGTGTT
701 AGTGA

SEQ ID NO. 54
Modified 6.22.2 Kappa Light Chain Amino Acid Sequence

1   mlpsglfll llwpaergE IVLTQSYPDFQ SVTPKKEVTI TCRASQRIGS
51  STLHWQKPD QSPPLLIKYA SQSFSGVPSR FSGSSGTDTP TLTINSLEAE
101 DAAATYCHQS GRLPLTFGGG TKVEIKRTVA APSVFIPFPPS DRLKSGTAS
151 VVCLLNNFYP REAKVQOKV DNLQSGNSQE SYTEQDSKDS TYSLSSTLTR
201 SKADYKHKV YACEVTHQGL SSPVTKSFNR GEC
SEQ ID NO. 55
Modified 6.34.2 Heavy Chain Nucleotide Sequence

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SEQ ID NO. 56
Modified 6.34.2 Heavy Chain Amino Acid Sequence

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40  ewesngpbn  nkyitppmlld  bggsfflys  ltvdkrsrwq  gqvnpscsgvm
45  balhnhnytqk  slslspgk
**SEQ ID NO. 57**
Modified 6.34.2 Kappa Light Chain Nucleotide Sequence

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Modified 6.34.2 Kappa Light Chain Amino Acid Sequence

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Modified 6.67.1 Heavy Chain Nucleotide Sequence

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Modified 6.67.1 Kappa Light Chain Nucleotide Sequence

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10  CGG AGG GCC ACC ATCA ACTG C A GTCC AGC C A GAG TTT TG TCT
15  A TCA CAG GCC TGCA CTC GCA AGA TG TGC CA GTT T A TT CTT CTA
20  TTAT AGTATT CTCC C C TCA CCTG C CGG AGGG ACAG AG GTGG AGAT CA
25  A C GAA ACT GT GGG TGCC C ACC TGT TCTT C TA T CTT CCG CC A ATCGA TGAG
30  CAG T TGA AAA AT CTGG A CTG C T C TGT TGT G TGCTG CTG A A T A ACT CTA
35  G GGC AAGG AG CAC AAT TAC AGT GAGG TT GTAA AC CC CGT CAT CAC GGC
40  CAGA CTCC AA GA GAGT GT TC AAG AAC G AAGA CAG A CAC TAC
45  AGC T TCA GC GAC C C TGCA GCC AA A CAG C AGA C AGA C AC A A C AC A
50  AGT CTAC GC TC GCAG TCA C C AT CAG G G TGT ACC C C A A C A C AA

SEQ ID NO. 62
Modified 6.67.1 Kappa Light Chain Amino Acid Sequence

1  mvlgtqvfis lllwlgayg DIVMTQPDS LA VSLGERAT INC KSSQGVL
5  YSSNN K YLA W YQQ RPQPP K L L IYW A SIR E YGV PDRF SQG SS G T P L L T
10  ISSLQ AEDVA VYFCQQY Y SI P PL TFQG GTK VE I KRT VAA P SVFI PPS DE
15  QLQS T A SVV CLINNFY PR K A VQ W K V D N A L Q SGNSQ E SV T EQSDK D STY
20  SLSTLTL SK ADYBH K V YA CE V T HQGL SS PVTKSF N RG E C
SEQ ID NO. 63
Modified 6.77.1 Heavy Chain Nucleotide Sequence

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5   1 atggaaactgg ggcctcggtg ggtttctctt gtggcataat tagaaggtgt
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SEQ ID NO. 64
Modified 6.77.1 Heavy Chain Protein Sequence

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SEQ ID NO. 66
Modified 6.77.1 Kappa Light Chain Amino Acid Sequence

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15  ksgtasvvcvl lnnpfpreak vqkwvdmalq ssnsqesvtr qdskd转型升级
20  sltltskaad yekhvvyace vthqglsspv tksfnrgec
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SEQ ID NO. 67
Modified 7.26.4 Kappa Light Chain Nucleotide Sequence

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atccagtgcc GATATTGAGA TGACCCAGAC TCCACTCTCT CTTGCCCGCA

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50
CGCCCTCGAA GTCACCACATC AGGCGCTGAG CTCGCGCGTC ACAAGAGGCT

CGCCCTCGAA GTCACCACATC AGGCGCTGAG CTCGCGCGTC ACAAGAGGCT

55
TCAAACGGGG AGATGTTTAG TGA

TCAAACGGGG AGATGTTTAG TGA

SEQ ID NO. 68
Modified 7.26.4 Kappa Light Chain Amino Acid Sequence

mrplaqllgllmlwipgssa DIVMTQTPLS LSVTPGQPAS IECKSSQSL

5
YSDGKTYLFW YLQKFGQPPF LLYYEVEHNF SGVPDRFGGS GSGTDPFLKI

10
SRVBAEDVGV YYMQSIIQLP WTFTGQQTVTVE IKRTVAVPFS VIFPPSDEQL

15
KSQTASVACL LNHYFPRKEA VQWKVDNALQ SGNSQESVTE QDSKDSSTYSL

20
SSTLTLSKAD YEHKHYACE VTHQGLSSFP TKSFNREGC

30

35
IN THE UNITED STATES RECEIVING OFFICE
OF THE PATENT COOPERATION TREATY

Applicants : PFIZER INC. et al.
Filed : Herewith
For : ANTIBODIES TO MadCAM

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INDICATIONS RELATING TO THE EXPERT SOLUTION IN RESPECT OF DEPOSITED BIOLOGICAL MATERIAL REFERRED TO IN THE DESCRIPTION

Sir:

The indications relating to deposited biological material are all contained in the description. The following additional indications are not required to be part of the description and should be treated as "separate indications." They relate only to the expert solution.

The additional indications made below relate to the deposited biological material referred to as Hybridoma 1.7.2 in the description on page 81, line 2.

The deposit was made in:

European Collection of Cell Cultures (ECACC)
Health Protection Agency
Porton Down
Salisbury, Wiltshire SP4 0JG
UNITED KINGDOM

on 09 September 2003 (09.09.2003), under Deposit Number 03090901.
The additional indications are:

For CA (Canada) designation:

In respect of the designation of Canada, samples of the deposited biological material will be made available until the grant of the Canadian patent, or until the date on which the application is refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, as provided in Rules 107 and 108 of the Patent Rules under the Canadian Patent Act, only by the issue of a sample to an independent expert nominated by the Commissioner (Rule 104(4)).

For EP (European Patent) designation:

In respect of the designation of the EPO, samples of the deposited biological material will be made available until the publication of the mention of the grant of the European patent, or until 20 years from the date of filing if the application is refused or withdrawn or is deemed to be withdrawn, as provided in Rule 28(3) of the Implementing Regulations under the EPC, only by the issue of a sample to an expert nominated by requester (Rule 28(4) EPC).

For FI (Finland) designation:

In respect of the designation of Finland, until the publication of the mention of grant by the National Board of Patents and Registration, or for 20 years from the date of filing if the application has been finally decided upon without resulting in the grant of a patent by the National Board of Patents and Registration, the furnishing of samples of the deposited biological material will be made available only to an expert in the art.

For GB (United Kingdom) designation:

In respect of the designation of the United Kingdom, the applicant(s) hereby give(s) notice of my/our intention that the furnishing of samples of the deposited biological material will be made available only to an expert in the art.

For IS (Iceland) designation:
In respect of the designation of Iceland, until a patent has been granted by the Icelandic Patent Office, or a final decision taken by the Icelandic Patent Office if the application has been finally decided upon without resulting in the grant of a patent, the furnishing of samples of the deposited biological material will only be effected to an expert in the art.

For SE (Sweden) designation:

In respect of the designation of Sweden, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of samples of the deposited biological material will be made available only to an expert in the art.

For SG (Singapore) designation:

Applicant(s) hereby give notice of my/our intention that samples of the above-identified culture shall be available only to experts in accordance with paragraph 3 of the Fourth Schedule to the Patents Rules 1995.