ANTI-MAdCAM ANTIBODY COMPOSITIONS

CROSS-REFERENCE TO RELATED PATENTS AND PATENT APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/752,712, filed March 8, 2005; U.S. Provisional Patent Application Serial No. 60/762,456, filed January 26, 2006; U.S. Provisional Patent Application Serial No. 60/659,766, filed March 8, 2005; U.S. Provisional Patent Application No. 60/728,165, filed October 19, 2005, all of which are incorporated by reference herein in their entireties.

BACKGROUND

The invention relates to antibody compositions and methods of stabilizing antibodies, and to pharmaceutically acceptable compositions comprising anti-MAdCAM antibodies, and methods of reducing anti-MAdCAM antibody instability.

Mucosal addressin cell adhesion molecule (MAdCAM) is a member of the immunoglobulin superfamily of cell adhesion receptors. 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. Antibodies that inhibit the binding of $\alpha_4\beta_7^+$ lymphocytes to MAdCAM have been shown to reduce lymphocyte recruitment, tissue extravasation, inflammation and disease severity in animal models.

Antibodies that bind to and inhibit the activity of MAdCAM have been reported in the literature. For example, International Patent Application Number PCT/US2005/000370 reports several human monoclonal antibodies to MAdCAM, including an antibody having the heavy and light chain amino acid sequences of antibody 7.16.6. A hybridoma cell line producing antibody 7.16.6 was deposited in the European Collection of Cell Cultures (ECACC), H.P.A. at CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG on 9 September 2003 with Deposit No. 03090909.

One possible mode of administering such MAdCAM antibodies is parenteral. Anti-MAdCAM compositions, as with all protein compositions, are subject to concerns regarding chemical and physical degradation of the antibody in the composition over time. In general, anti-MAdCAM antibody compositions must exhibit acceptable chemical and physical stability under the expected range of storage and use conditions, i.e., the anti-MAdCAM antibody compositions must have a sufficient shelf life and yet remain biologically active. The present application discloses novel anti-MAdCAM antibody compositions that exhibit improved chemical and/or physical stability relative to anti-MAdCAM compositions previously disclosed in the literature.

SUMMARY

In one aspect, the present invention provides a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence

shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a chelating agent.

The present invention also provides a method for preparing a stable liquid pharmaceutical composition comprising mixing at least one monoclonal anti-MAdCAM antibody with a pharmaceutically acceptable chelating agent in an amount which reduces instability of the antibody, wherein when the composition is stored for a period of about 26 weeks at a temperature of about 40°C; the decrease between an aggregate chromatogram peak area for the stable liquid pharmaceutical composition comprising monoclonal anti-MAdCAM antibodies and the chelating agent; and an aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent that is stored for a period of about 26 weeks at a temperature of about 40°C is at least about 2%.

The present invention also provides a method for stabilizing at least one monoclonal anti-MAdCAM antibody in a liquid pharmaceutical composition comprising forming a liquid composition comprising the at least one antibody and a pharmaceutically acceptable chelating agent, wherein when the composition is stored for a period of about 26 weeks at a temperature of about 40°C; the decrease between an aggregate chromatogram peak area for the stable liquid pharmaceutical composition comprising at least one monoclonal anti-MAdCAM antibody and the chelating agent; and an aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent that is stored for a period of about 26 weeks at a temperature of about 40°C is at least about 2%.

The present invention also provides a method for the treatment of an inflammatory disease in a subject, comprising administering to the subject a liquid pharmaceutical composition comprising: a therapeutically effective amount of monoclonal anti-MAdCAM antibody 7.16.6; and a pharmaceutically acceptable chelating agent.

The present invention also provides a kit for preparing a liquid composition of a stabilized antibody comprising: a first container comprising at least one monoclonal anti-MAdCAM antibody 7.16.6 in solution, and a second container comprising a pharmaceutically acceptable chelating agent.

The present invention also provides an article of manufacture comprising a container which holds a mixture of at least one monoclonal anti-MAdCAM antibody 7.16.6 and a pharmaceutically acceptable chelating agent.

The present invention also provides a liquid pharmaceutical composition comprising at least one monoclonal anti-MAdCAM antibody and a pharmaceutically acceptable chelating agent, wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar and the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and wherein the molar ratio of antibodies to chelating agent ranges from about 0.00001 to about 450.

The present invention also provides a liquid pharmaceutical composition comprising at least one human monoclonal anti-MAdCAM antibody, wherein the antibody binds to human MAdCAM; and a chelating agent.

The present invention also provides a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that is at least about 10 mg/ml.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph that illustrates the percent aggregation in various test compositions differing in mAb concentration after storage at 40ºC for up to 26 weeks by size exclusion chromatography (SEC);

Figure 2 is a graph that illustrates the percent aggregation in various test compositions differing in EDTA concentration after storage at 40°C for up to 26 weeks, by SEC;

Figure 3 is a graph that illustrates the percent aggregation in various test compositions differing in PS80 concentration after storage at 40ºC for up to 26 weeks by SEC;

Figure 4 is a graph that illustrates the percent aggregation in various test compositions differing in buffer species after storage at 40ºC for up to 26 weeks by SEC;

Figure 5 is a graph that illustrates the percent aggregation in various test compositions differing in stabilizer/tonicifier species after storage at 40ºC for up to 26 weeks by SEC;

Figure 6 is a graph that illustrates the percent aggregation in various test compositions differing in surfactant species after storage at 40ºC for up to 26 weeks by SEC;.

DETAILED DESCRIPTION OF THE INVENTION

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, 2d 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). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of subjects.

Definitions:

In order to aid the reader in understanding the following detailed description, the following definitions are provided:

As used herein, the term "composition" as it relates to an anti-MAdCAM antibody is meant to describe the antibody in combination with a pharmaceutically acceptable excipient comprising a chelating agent. For example, the compositions of the invention have an improved shelf life and/or stability as compared to art recognized compositions comprising an anti-MAdCAM antibody.

As used herein, the term "antibody" refers to an intact antibody or an antigen-binding portion that competes with the intact antibody for specific binding. See generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989). Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some embodiments, antigen-binding portions include Fab, Fab', $F(ab')_2$, Fd, 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 antibody that is sufficient to confer specific antigen binding to the polypeptide. From N-terminus to C-terminus, both the mature light and heavy chain variable domains comprise the regions 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)), Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987), or Chothia et al., Nature 342:878-883 (1989).

As used herein, an antibody that is referred to by number has the same heavy and light chain amino acid sequences as a monoclonal antibody that is obtained from the hybridoma of the same number. For example, monoclonal antibody 7.16.6 has the same heavy and light chain amino acid sequences as one obtained from hybridoma 7.16.6. Thus, reference to antibody 7.16.6 includes the antibody which has the heavy and light chain amino acid sequences shown in SEQ ID NOS. 2 and 4. It also includes an antibody lacking a terminal lysine on the heavy chain, as this is normally lost in a proportion of antibodies during manufacture.

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

As used herein, an Fd fragment means an antibody fragment that consists of the V_H and C_H 1 domains; an Fv fragment consists of the V_L and V_H domains of a single arm of an antibody; and a dAb fragment (Ward et al., Nature 341:544-546 (1989)) consists of a V_H domain.

The term "or an antigen-binding portion thereof" when used with the term "antibody" 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, at least 20, at least 50, or at least 70, 80, 90, 100, 150 or 200 amino acids long.

As used herein, the term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts or lacking a C-terminal lysine. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations, which typically include different antibodies, directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler, et al., Nature 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson, et al., Nature 352:624-628 (1991) and Marks, et al., J. Mol. Biol. 222:581-597 (1991), for example.

As used herein, the terms "isolated antibody" or "purified antibody" refers to an antibody that by virtue of its origin or source of derivation has one to four of the following: (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, an antibody that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates is isolated and purified from its naturally associated components. An antibody may also be rendered substantially free of naturally associated components by isolation and purification, using protein purification techniques well known in the art. Examples of isolated/purified antibodies include an anti-MAdCAM antibody that has been affinity purified using MAdCAM, an anti-MAdCAM antibody that has been synthesized by a hybridoma or other cell line in vitro, and a human anti-MAdCAM antibody derived from a transgenic mouse.

An antibody is "substantially pure," "substantially homogeneous," or "substantially purified" when at least about 60 to 75% of a sample exhibits a single species of antibody. The antibody may be monomeric or multimeric. A substantially pure antibody will typically comprise about 50%, 60%, 70%, 80% or 90% w/w of an antibody sample, more usually about 95%, and preferably will be over 99% pure. Antibody purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of an antibody 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 achieved by using HPLC or other means well known in the art for purification.

As used herein, the term "human antibody" is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

As used herein, the term "recombinant human antibody" is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library, antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

As used herein, the term "polynucleotide" 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.

As used herein, the term "isolated polynucleotide" means a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin or source of derivation, the "isolated polynucleotide" has one to three of the following: (1) is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

As used herein, the term "naturally occurring nucleotides" includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" as used herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche et al., Nucl. Acids Res. 14:9081 (1986); Stec et al., J. Am. Chem. Soc. 106:6077 (1984); Stein et al., Nucl. Acids Res. 16:3209 (1988); Zon et al., Anti-Cancer Drug Design

6:539 (1991); Zon et al., Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); U.S. Patent No. 5,151,510; Uhlmann and Peyman, Chemical Reviews 90:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

"Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. The term "expression control sequence" as used herein means polynucleotide sequences that 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.

As used herein, the term "vector" means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, the vector is a plasmid, i.e., a circular double stranded DNA loop into which additional DNA segments may be ligated. In some embodiments, the vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. In some embodiments, the 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). In other embodiments, the 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").

As used herein, the terms "recombinant host cell" (or simply "host cell") means a cell into which a recombinant expression vector has been introduced. It should be understood that "recombinant host cell" and "host cell" mean not only the particular subject cell but also 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.

As used herein, the terms "is capable of specifically binding" refers to when an antibody binds to an antigen with a dissociation constant that is ≤ 1 μM, preferably ≤ 1 nM and most preferably ≤ 10 pM.

As used herein, the terms "selectively hybridize" mean 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. One example of "high stringency" or "highly stringent" conditions is the incubation of 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 42ºC for 12-16 hours, followed by twice washing at 55ºC using a wash buffer of 1X SSC, 0.5% SDS. See also Sambrook et al., supra, pp. 9.50-9.55.

The term "percent sequence identity" in the context of nucleic acid sequences means the percent of residues when a first contiguous sequence is compared and aligned for maximum correspondence to a second contiguous sequence. 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 that 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 10.0, Genetics Computer Group (GCG), Madison, Wisconsin. 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 nucleic acid 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 GCG Version 6.1, herein incorporated by reference.

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

The term "substantial similarity" or "substantial sequence similarity," when referring to a nucleic acid or fragment thereof, means that when optimally aligned with appropriate nucleotide

insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

As applied to polypeptides, the term "substantial identity", "percent identity" or "% identical" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, as supplied with the programs, share at least 70%, 75% or 80% sequence identity, preferably at least 90% or 95% sequence identity, and more preferably at least 96%, 97%, 98% or 99% sequence identity. In certain embodiments, 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 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. 243:307-31 (1994). 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; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

Sequence identity for polypeptides, is typically measured using sequence analysis software. Protein analysis software matches 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, as specified with the programs, 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 mutant thereof. See, e.g., GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, see GCG Version 6.1. (University of Wisconsin WI) 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, Methods Enzymol. 183:63-98 (1990); Pearson, Methods Mol. Biol. 132:185-219 (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, as supplied with the programs. See, e.g., Altschul et al., J. Mol. Biol. 215:403-410 (1990); Altschul et al., Nucleic Acids Res. 25:3389-402 (1997). 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.

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, which includes treatment or prophylactic prevention of inflammatory diseases. It is to be noted that dosage values may vary with the 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. Likewise, 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, the ability of the antibody or antibody portion to elicit a desired response in the individual, and the desired route of administration of the antibody composition. 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.

As used herein, the term "saccharide" refers to a class of molecules that are derivatives of polyhydric alcohols.

As used herein, the term "subject" for purposes of treatment includes any subject, and preferably is a subject who is in need of the treatment of an inflammatory disease. For purposes of prevention, the subject is any subject, and preferably is a subject that is at risk for, or is predisposed to, developing an inflammatory disease. The term "subject" is intended to include living organisms, e.g., prokaryotes and eukaryotes. Examples of subjects include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In specific embodiments of the invention, the subject is a human.

As used herein, the term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic disease or condition. Those in need of treatment include those already with the disease as well as those prone to have the disease or those in whom the disease is to be prevented.

When introducing elements of the present invention or the preferred embodiment(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "comprise", "comprises", "including" and "having" are

intended to be inclusive and mean that there may be additional elements other than the listed elements.

Anti-MAdCAM antibodies:

In accordance with the present invention, it has been discovered that the stability of certain monoclonal anti-MAdCAM antibodies that are described herein can be improved in solution by mixing the anti-MAdCAM antibodies with a pharmaceutically acceptable chelating agent, such as ethylenediaminetetraacetic acid ("EDTA").

While not wishing to be bound by theory, it is believed that the presence of a chelating agent in the composition of the present invention help to improve stability of the antibody polypeptide by reducing the incidence of one or more of the following: anti-MAdCAM antibody aggregation, fragmentation, oxidation, freeze/thaw instability, discoloration, and/or deamidation. The present invention comprises anti-MAdCAM antibody compositions having improved chemical and/or physical stability as compared to previously disclosed antibody compositions.

Therefore, in certain aspects, the present invention provides a liquid pharmaceutical composition comprising a pharmaceutically acceptable chelating agent, such as EDTA and at lease one monoclonal anti-MAdCAM antibody or an antigen-binding portion thereof. In still other aspects, the aforementioned liquid anti-MAdCAM antibody compositions comprising a chelating agent can include additional pharmaceutically acceptable excipients, including, but not limited to, one or more excipients that are chosen from buffers, tonicity agents, surfactants, and mixtures thereof.

The present invention provides novel compositions comprising anti-MAdCAM antibodies. As used herein, the phrase "anti-MAdCAM antibody" refers to any antibody, or any portion thereof, that is capable of binding to any portion of a MAdCAM polypeptide that may be present within or isolated from any animal. In certain embodiments, the MAdCAM polypeptide is a human MAdCAM polypeptide.

Suitable anti-MAdCAM antibodies for use with the present invention may be chosen from polyclonal or monoclonal antibodies. In certain aspects, the monoclonal anti-MAdCAM antibody can be a murine, chimeric, humanized or human antibody. In further embodiments, the monoclonal anti-MAdCAM antibody is a human monoclonal anti-MAdCAM antibody.

In certain embodiments, the anti-MAdCAM antibodies which are suitable for use with the present invention include those anti-MAdCAM antibodies and methods to prepare them that are described in International Application Number PCT/US2005/000370, filed 7 January 2005 and published 28 July 2005. In other embodiments, the anti-MAdCAM antibodies which are suitable for use with the present invention include those anti-MAdCAM monoclonal antibodies having the heavy and light chain amino acid sequences of the antibody designated 7.16.6 in International Application Number PCT/US2005/000370.

In addition, such anti-MAdCAM antibodies may be chosen based on differences in the amino acid sequences in the constant region of their heavy chains. For example, the anti-MAdCAM antibodies may be chosen from the IgG class, which have "gamma" type heavy chains. 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 commercially available. The class and subclass can be determined by ELISA, or Western Blot as well as other techniques. 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 class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

The anti-MAdCAM antibody can be an IgG, an IgM, an IgE, an IgA, or an IgD molecule. In further embodiments, the anti-MAdCAM antibody is an IgG and is an IgG1, IgG2, IgG3 or IgG4 subclass. However, as it will be appreciated; it is generally not desirable to kill MAdCAM expressing cells. Rather, one generally desires to simply inhibit MAdCAM binding with its ligands to mitigate T cell down regulation. One of the major mechanisms through which antibodies kill cells is through fixation of complement and participation in CDC. The constant region of an antibody plays an important role in connection with an antibody's ability to fix complement and participate in CDC. Thus, generally one selects the isotype of an antibody to either provide the ability of complement fixation, or not. In the case of the present invention, generally, as mentioned above, it is generally not preferred to utilize an antibody that kills the cells. There are a number of isotypes of antibodies that are capable of complement fixation and CDC, including, without limitation, the following: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, and human IgG3. In contrast, preferred isotypes which are not capable of complement fixation and CDC include, without limitation, human IgG2 and human IgG4. In addition to heavy chain sequence differences, the IgG antibodies differ within their subclass based on the number of disulfide bonds and length of the hinge region. For example, the IgG2 subclass has several differences distinct from the other subclasses. The IgG2 and IgG4 subclasses are known to have 4 disulfide bonds within their hinge region, while IgG1 has 2 and IgG3 has 11 disulfide bonds. Other differences for IgG2 antibodies include their reduced ability to cross the placenta and the inability of IgG2 antibodies to bind to lymphocyte Fc receptors. Thus, in certain embodiments, the anti-MAdCAM antibody is subclass IgG2 or IgG4. In another preferred embodiment, the anti-MAdCAM antibody is subclass IgG2.

In some embodiments, the antibody is a single-chain antibody (scFv) in which a V_L and V_H domains are paired to form a monovalent molecules 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)). In some embodiments, the antibodies are diabodies, i.e., are bivalent antibodies in which V_H and V_L 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)). In

some embodiments, 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. In such embodiments, the CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently.

In another embodiment, the anti-MAdCAM antibody has selectivity (or specificity) for MAdCAM that is at least 100 times greater than its selectivity for any other polypeptide. In some embodiments, the anti-MAdCAM antibody does not exhibit any appreciable specific binding to any other protein other than MAdCAM. One can 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 can determine the selectivity using Western blot, FACS, ELISA, or RIA. Thus, in some embodiments, the monoclonal anti-MAdCAM antibody is capable of specifically binding to MAdCAM.

In some embodiments, the C-terminal lysine of the heavy chain of the anti-MAdCAM antibody of the invention is not present. In various embodiments of the invention, the heavy and light chains of the anti-MAdCAM antibodies may optionally include a signal sequence.

Table 2 lists the sequence identifiers (SEQ ID NOS) of the nucleic acids that encode the heavy and light chains and the corresponding predicted amino acid sequences for the anti-MAdCAM monoclonal antibody 7.16.6. While DNA sequences encoding a signal polypeptide are shown in the sequence identifiers (SEQ ID NOS), the antibody typically does not comprise a signal polypeptide because the signal polypeptide is generally eliminated during post-translational modifications. In various embodiments of the invention, one or both of the heavy and light chains of the anti-MAdCAM antibodies includes a signal sequence (or a portion of the signal sequence). In other embodiments of the invention, neither the heavy nor light chain of the anti-MAdCAM antibodies includes a signal sequence.

Table 1:

HUMAN ANTI-MAdCAM ANTIBODY 7.16.6				
MAb	SEQUENCE IDENTIFIER (SEQ ID NOS:)			
	Heavy		Light	
	DNA	Amino Acid	DNA	Amino Acid
7.16.6	1	2	3	4

In some embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes the V_L amino acid sequence of monoclonal antibody 7.16.6 (SEQ ID NO: 4), or a portion thereof. In some embodiments, said portion comprises at least the CDR3 region. In some embodiments, the nucleic acid encodes the amino acid sequence of the light chain CDRs of said antibody. In some embodiments, said portion is a contiguous portion comprising CDR1-CDR3.

In still other embodiments, the nucleic acid molecule encodes a V_L amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to a V_L amino acid sequence of antibody 7.16.6, or an amino acid sequence of SEQ ID NO: 4. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleic acid sequence encoding the light chain amino acid sequence of SEQ ID NO: 4.

In further embodiments, the nucleic acid molecule comprises a nucleotide sequence that encodes at least a portion of the V_H amino acid sequence of 7.16.6 (SEQ ID NO: 2) or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative amino acid substitutions. In various embodiments the sequence encodes one or more CDR regions, preferably a CDR3 region, all three CDR regions, a contiguous portion including CDR1-CDR3, or the entire V_H region.

In some embodiments, the nucleic acid molecule encodes a V_H amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H amino acid sequence of SEQ ID NO: 2. Nucleic acid molecules of the invention include nucleic acids that hybridize under highly stringent conditions, such as those described above, to a nucleotide sequence encoding the heavy chain amino acid sequence of SEQ ID NO: 2.

In other aspects, the present invention provides a liquid pharmaceutical composition comprising at least one purified human antibody that binds to MAdCAM, wherein the antibody comprises a heavy chain amino acid sequence with at least 95% sequence identity to SEQ ID NO: 2 and a light chain amino acid sequence with at least 95% sequence identity to SEQ ID NO: 4. In other aspects, the antibody comprises a heavy chain amino acid sequence with at least 99% sequence identity to SEQ ID NO: 2 and a light chain amino acid sequence with at least 99% sequence identity to SEQ ID NO: 4. In still other aspects, the antibody comprises a heavy chain amino acid sequence that comprises the variable region of SEQ ID NO: 2 and a light chain amino acid sequence that comprises the variable region SEQ ID NO: 4. In further aspects, the antibody comprises a heavy chain amino acid sequence comprising SEQ ID NO: 2 and a light chain amino acid sequence comprising SEQ ID NO: 4.

In one embodiment of the present invention, the anti-MAdCAM antibody specifically binds to a conformational epitope on human MAdCAM.

Preparation of the Monoclonal Anti-MAdCAM Antibody Compositions:

The anti-MAdCAM antibody typically is formulated as a pharmaceutical composition for parenteral administration to a subject. In one embodiment, the pharmaceutical composition is a liquid composition. In another embodiment, the pharmaceutical composition is an liquid composition.

In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising at least one anti-MAdCAM antibody and a pharmaceutically acceptable chelating agent. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising at least one anti-MAdCAM antibody and EDTA. In another embodiment, the

invention is directed to a liquid pharmaceutical composition comprising at least one anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent, and a pharmaceutically acceptable buffer. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising at least one anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent, and histidine. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, EDTA, and histidine. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, DTPA, and histidine.

In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent, and a pharmaceutically acceptable tonicity agent. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent, and trehalose. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, EDTA, and trehalose. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, DTPA, and trehalose.

In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent, and a pharmaceutically acceptable surfactant. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, EDTA, and a pharmaceutically acceptable surfactant. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, DTPA, and a pharmaceutically acceptable surfactant. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent selected from the group consisting of EDTA and DTPA, and polysorbate 80.

In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, a pharmaceutically acceptable buffer, and a pharmaceutically acceptable surfactant. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, histidine, and a pharmaceutically acceptable surfactant. In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising anti-MAdCAM antibody, histidine, and polysorbate 80.

In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent, a pharmaceutically acceptable buffer, and a pharmaceutically acceptable surfactant.

In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent, a pharmaceutically acceptable buffer, and a pharmaceutically acceptable tonicity agent.

In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody, a pharmaceutically acceptable chelating agent, a pharmaceutically acceptable buffer, a pharmaceutically acceptable surfactant, and a pharmaceutically acceptable tonicity agent.

In another embodiment, the invention is directed to a liquid pharmaceutical composition comprising an anti-MAdCAM antibody and histidine.

The term "pharmaceutical composition" refers to preparations which are in such form as to permit the biological activity of the active ingredients to be effective. "Pharmaceutically acceptable excipients" (vehicles, additives) are those, which can reasonably (i.e., safely) be administered to a subject to provide an effective dose of the active ingredient employed. The term "excipient" or "carrier" as used herein refers to an inert substance, which is commonly used as a diluent, vehicle, preservative, binder or stabilizing agent for drugs. As used herein, the term "diluent" refers to a pharmaceutically acceptable (safe and non-toxic for administration to a human) solvent and is useful for the preparation of the liquid compositions herein. Exemplary diluents include, but are not limited to, sterile water and bacteriostatic water for injection (BWFI).

The anti-MAdCAM antibody present in the liquid pharmaceutical composition can be as previously described in this application. In one embodiment, the liquid pharmaceutical composition comprises an anti-MAdCAM antibody comprising a V_L amino acid sequence that is 90%, 95%, or 99% identical to a V_L amino acid sequence shown in SEQ ID NO: 4, and further comprises a V_H amino acid sequence that is 90%, 95%, or 99% identical to a V_H amino acid sequence shown in SEQ ID NO: 2. In another embodiment, the liquid pharmaceutical composition comprises an anti-MAdCAM antibody that is monoclonal anti-MAdCAM antibody 7.16.6.

The concentration of the anti-MAdCAM antibody in the liquid pharmaceutical compositions of the present invention is generally at least about 0.1 milligram per milliliter (mg/ml) or higher, at least about 1.0 mg/ml or higher, at least about 10 mg/ml or higher, at least about 50 mg/ml or higher, at least about 75 mg/ml or higher, at least about 100 mg/ml or higher, or at least about 200 mg/ml or higher. In certain embodiments, the concentration of the anti-MAdCAM antibody generally ranges from about 1 mg/ml to about 200 mg/ml, from about 2 mg/ml to about 100 mg/ml, from about 5 mg/ml to about 90 mg/ml, from about 10 mg/ml to about 80 mg/ml, from about 50 mg/ml to about 90 mg/ml, from about 60 mg/ml to about 80 mg/ml, from about 65 mg/ml to about 85 mg/ml, or is about 75 mg/ml. In one embodiment, the concentration of the anti-MAdCAM4 antibody in the liquid pharmaceutical composition ranges from about 50 mg/ml to about 100 mg/ml.

As used herein, the terms "chelating agent" generally refers to an excipient that can form at least one bond (e.g., covalent, ionic, or otherwise) to a metal ion. A chelating agent is typically a multidentate ligand that can be used in selected liquid compositions as a stabilizer to complex with species, which might promote instability. Often, compounds that can act as a chelating agent will have electron-rich functional groups. Suitable electron-rich functional

groups include carboxylic acid groups, hydroxy groups and amino groups. Arrangement of these groups in aminopolycarboxylic acids, hydroxypolycarboxylic acids, hydroxyaminocarboxylic acids, and the like, result in moieties that have the capacity to bind metal.

However, the present invention is not intended to be limited to chelating agents that enhance antibody stability primarily by the chelating agent's ability to form bonds with a metal ion. Therefore, the present invention is not intended to be limited by the specific mechanism by which the chelating agent stabilizes the compositions of the present invention and the excipients termed chelating agents herein may achieve their antibody stability enhancing properties primarily through mechanisms that are altogether unrelated to the chelating agent's ability to form bonds with a metal ion.

Chelating agents that are suitable for use in the present invention, include, but are not limited to, aminopolycarboxylic acids, hydroxyaminocarboxylic acids, N-substituted glycines, 2-(2-amino-2-oxocthyl) aminoethane sulfonic acid (BES), deferoxamine (DEF), citric acid, niacinamide, and desoxycholates. Examples of suitable aminopolycarboxylic acids include ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetic acid 5 (DTPA), nitrilotriacetic acid (NTA), N-2-acetamido-2-iminodiacetic acid (ADA), bis(aminoethyl)glycolether, N,N,N',N'-tetraacetic acid (EGTA), trans-diaminocyclohexane tetraacetic acid (DCTA), glutamic acid, and aspartic acid. Examples of suitable hydroxyaminocarboxylic acids include N- hydroxyethyliminodiacetic acid (HIMDA), N,N-bis-hydroxyethylglycine (bicine) and N-(trishydroxymethylmethyl) 10 glycine (tricine). An example of a suitable N-substituted glycine is glycylglycine. An example of a suitable desoxycholate is sodium desoxycholate.

Chelating agents used in the invention can be present, where possible, as the free acid or free base form of the compound (e.g., referred to interchangeably herein as "EDTA" or "edetate") or as a corresponding salt form (e.g., the corresponding acid addition salt or base addition salt, such as disodium edetate). Suitable acid addition salts, e.g., include alkali metal salts (e.g., sodium or potassium salts), alkaline earth metal salts (e.g., calcium), and salts can be prepared using other weakly bound metal ions. As is known in the art, the nature of the salt and the number of charges to be neutralized will depend on the number of carboxyl groups present and the pH at which the stabilizing chelating agent is supplied. As is also known in the art, chelating agents have varying strengths with which particular target ions are bound. By way of further illustration, suitable salts of EDTA include dipotassium edetate, disodium edetate, edetate calcium disodium, sodium edetate, trisodium edetate, and potassium edetate; and a suitable salt of deferoxamine (DEF) is deferoxamine mesylate (DFM).

Chelating agents used in the invention can be present as an anhydrous, solvated or hydrated form of the compound or corresponding salt. Where the chelating agent is in a solvated or hydrated form, it can be present in varying states of solvation or hydration (including, e.g., anhydrous, hydrated, dihydrated, and trihydrated forms). By way of further

illustration, a suitable hydrate of EDTA is disodium EDTA dihydrate; and suitable forms of citric acid include anhydrous citric acid, citric acid monohydrate, and trisodium citrate-dihydrate.

Suitable chelating agents used in an antibody composition of the present invention also include, for example, those that bind to metal ions in solution to render them unable to react with available O_2, thereby minimizing or preventing generation of hydroxyl radicals which are free to react with and degrade the antibody. Other chelating agents such as DFM can lower the formation of reduced oxygen species, reduce acidic species (e.g., deamidation) formation and/or reduce antibody fragmentation. In still other embodiments, the chelating agent can reduce or prevent aggregation of the antibodies in the compositions described herein. Such chelating agents can reduce or prevent degradation of an antibody that is formulated without the protection of a chelating agent.

When a concentration of a chelating agent is referred to, it is intended that the recited concentration represents the molar concentration of the free acid or free base form of the chelating agent. For example, the concentration of chelating agent in certain liquid pharmaceutical compositions generally ranges from about 0.3 micromolar to about 50 millimolar, from about 3.0 micromolar to about 10.0 millimolar, from about 30 micromolar to about 5.0 millimolar, from about 0.1 millimolar to about 1 millimolar. In specific embodiments, the concentration of chelating agent in the liquid pharmaceutical composition can be about 3 micromolar, about 13 micromolar, about 27 micromolar, about 0.27 millimolar, about 1 millimolar or about 2.7 millimolar. In one embodiment, the concentration of chelating agent is about 0.27 millimolar. Unless stated otherwise, the concentrations listed herein are those concentrations at ambient conditions, (i.e., at 25°C and atmospheric pressure).

In one embodiment, the chelating agent is selected from the group consisting of EDTA, DTPA, DFM, and mixtures thereof. In another embodiment, the chelating is agent is DFM. In another embodiment, the chelating agent is EDTA. In another embodiment, the chelating agent is DTPA. In another embodiment, the liquid pharmaceutical composition comprises EDTA in an amount ranging from about 0.3 micromolar to about 50 millimolar, and in some embodiments, from about 0.1 millimolar to about 1.0 millimolar. In another embodiment, the liquid pharmaceutical composition comprises EDTA in an amount of about 0.27 millimolar.

As noted above, the liquid pharmaceutical compositions of the present invention optionally may further comprise a pharmaceutically acceptable buffer in addition to a chelating agent. As used herein, the term "buffer" refers to an added composition that allows a liquid antibody composition to resist changes in pH. In certain embodiments, the added buffer allows a liquid antibody composition to resist changes in pH by the action of its acid-base conjugate components.

Examples of suitable buffers include, but are not limited to, acetate (e.g., sodium acetate), succinate (e.g., sodium succinate), gluconate, citrate, and other organic acid buffers, including, but not limited to, buffers such as amino acids (e.g., histidine), acetic acid, phosphoric acid and phosphates, ascorbate, tartartic acid, maleic acid, glycine, lactate, lactic acid, ascorbic acid, imidazoles, carbonic acid and bicarbonates, succinic acid, sodium benzoic

acid and benzoates, gluconate, edetate (EDTA), acetate, malate, imidazole, tris, phosphate, and mixtures thereof.

In one embodiment, the buffer is histidine. The histidine starting material used to prepare the liquid pharmaceutical compositions of the present invention can exist in different forms. For example, the histidine can be an enantiomeric (e.g., L- or D-enantiomer) or racemic form of histidine, a free acid or free base form of histidine, a salt form (e.g., a monohydrochloride, dihydrochloride, hydrobromide, sulfate, or acetate salt) of histidine, a solvated form of histidine, a hydrated form (e.g., monohydrate) of histidine, or an anhydrous form of histidine. The purity of histidine base and/or salt used to prepare the liquid pharmaceutical compositions generally can be at least about 98%, at least about 99%, or at least about 99.5%. As used herein, the term "purity" in the context of histidine refers to chemical purity of histidine as understood in the art, e.g., as described in The Merck Index, 13th ed., O'Neil et al. ed. (Merck & Co., 2001).

When a concentration of a buffer is referred to, it is intended that the recited concentration represent the molar concentration of the free acid or free base form of the buffer. For example, the concentration of the buffer when present in certain liquid pharmaceutical compositions can range from about 0.1 millimolar (mM) to about 100 mM. In one embodiment, the concentration of the buffer is from about 1 mM to about 50 mM. In another embodiment, the concentration of the buffer is from about 5 mM to about 20 mM. In various embodiments, the concentration of the buffer is about 1 mM, about 5 mM, about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM or about 100 mM. In one embodiment, the concentration of histidine in the pharmaceutical composition is about 10mM. In one embodiment, the pharmaceutical composition contains about 10mM of L-histidine (in base form).

In general, the buffer is used to maintain an acceptable pH level (which can affect antibody stability) in the liquid pharmaceutical composition. The liquid pharmaceutical composition typically is buffered to maintain a pH in the range of from about 4 to about 8; from about 4.5 to about 7; or from about 5.2 to about 5.8. Ranges intermediate to the above-recited pH's are also intended to be part of this invention. For example, ranges of values using a combination of any of the above-recited values as upper and/or lower limits are intended to be included. In one embodiment, the liquid pharmaceutical composition is buffered to maintain a pH of about 5.5.

As noted above, the liquid pharmaceutical compositions of the present invention optionally may further comprise a pharmaceutically acceptable tonicity agent in addition to a chelating agent. As used herein, the terms "tonicity agent" or "tonicifier" refers to an excipient that can adjust the osmotic pressure of a liquid antibody composition. In certain embodiments, the tonicity agent can adjust the osmotic pressure of a liquid antibody composition to isotonic so that the antibody composition is physiologically compatible with the cells of the body tissue of the subject. In still other embodiments, the "tonicity agent" may contribute to an

improvement in stability of any of the anti-MAdCAM antibodies described herein. An "isotonic" composition is one that has essentially the same osmotic pressure as human blood. Isotonic compositions generally have an osmotic pressure from about 250 to 350 mOsm. The term "hypotonic" describes a composition with an osmotic pressure below that of human blood. Correspondingly, the term "hypertonic" is used to describe a composition with an osmotic pressure above that of human blood. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

The tonicity agent used to prepare the liquid pharmaceutical compositions of the present invention can exist in different forms. For example, the tonicity agent can be in an enantiomeric (e.g., L- or D-enantiomer) or racemic form; isomers such as alpha or beta, including alpha, alpha or beta, beta, or alpha, beta or beta, alpha; a free acid or free base form; a hydrated form (e.g., monohydrate), or an anhydrous form.

In one embodiment, the tonicity agent is a saccharide. Saccharides are commonly referred to as carbohydrates and may contain different amounts of sugar (saccharide) units, e.g., monosaccharides, disaccharides and polysaccharides. Saccharides that are suitable for use as a tonicity agent in the present invention, include, but are not limited to, saccharides selected from the group consisting of fructose, glucose, mannose, sorbose, xylose, lactose, maltose, sucrose, dextran, pullulan, dextrin, cyclodextrins, soluble starch, hydroxyethyl starch, water-soluble glucans, and mixtures thereof. In one embodiment, the tonicity agent is sucrose.

In another embodiment, the tonicity agent is a polyol. As used herein, the term "polyol" refers an excipient with multiple hydroxyl groups, and includes sugars (reducing and nonreducing sugars), sugar alcohols and sugar acids. In one embodiment, the polyol has a molecular weight that is less than about 600 kD (e.g., in the range from about 120 to about 400 kD). A "reducing sugar" is one which contains a hemiacetal group that can reduce metal ions or react covalently with lysine and other amino groups in proteins and a "nonreducing sugar" is one which does not have these properties of a reducing sugar. Polyols that are suitable for use as a tonicity agent in the present invention, include, but are not limited to, polyols selected from the group consisting of mannitol, trehalose, sorbitol, erythritol, isomalt, lactitol, maltitol, xylitol, glycerol, lactitol, propylene glycol, polyethylene glycol, inositol, and mixtures thereof. In one embodiment, the tonicity agent is a non-reducing sugar selected from the group consisting of sucrose, trehalose, sorbose, melezitose, raffinose, and mixtures thereof. In another embodiment, the tonicity agent is a non-reducing sugar selected from the group consisting of trehalose and sucrose, and mixtures thereof. In another embodiment, the tonicity agent is mannitol. In another embodiment, the tonicity agent is D-mannitol. In another embodiment, the tonicity agent is trehalose. In another embodiment, the tonicity agent is α α-trehalose dihydrate. In another embodiment, the tonicity agent is a salt, such as sodium chloride.

The concentration of the tonicity agent when present in the liquid pharmaceutical composition can range from about 1.0 millimolar to about 600 millimolar, from about 10 millimolar to about 400 millimolar, or from about 100 millimolar to about 300 millimolar. In one embodiment, the concentration of the tonicity agent ranges from about 200 millimolar to about

250 millimolar. In another embodiment, the concentration of the tonicity agent in the liquid pharmaceutical composition is about 222 millimolar, about 238 millimolar, or about 247 millimolar.

In yet another embodiment, the tonicity agent is mannitol and is present in the liquid pharmaceutical composition at a concentration of 247 millimolar. In another embodiment, the tonicity agent is trehalose and is present in the liquid pharmaceutical composition at a concentration of 222 millimolar. In another embodiment, the tonicity agent is trehalose and is present in the liquid pharmaceutical composition at a concentration of 238 millimolar.

As noted above, the liquid pharmaceutical compositions of the present invention optionally may further comprise a pharmaceutically acceptable surfactant in addition to a chelating agent. As used herein, the term "surfactant" refers to an excipient that can alter the surface tension of a liquid antibody composition. In certain embodiments, the surfactant reduces the surface tension of a liquid antibody composition. In still other embodiments, the "surfactant" may contribute to an improvement in stability of any of the anti-MAdCAM antibodies described herein. For example, the surfactant may reduce aggregation of the formulated antibody and/or minimize the formation of particulates in the composition and/or reduces adsorption. The surfactant may also improve stability of the antibody during and after a freeze/thaw cycle.

Suitable surfactants include polysorbate surfactants, poloxamer 18, triton surfactants such as Triton X-100®, polysorbate surfactants such as Tween 20® and Tween 80®, sodium dodecyl sulfate, sodium laurel sulfate, sodium octyl glycoside, lauryl-sulfobetaine, myristyl-sulfobetaine, linoleyl-sulfobetaine, stearyl-sulfobetaine, lauryl-sarcosine, myristyl-sarcosine, linoleyl-sarcosine, stearyl-sarcosine, linoleyl-betaine, myristyl-betaine, cetyl-betaine, lauroamidopropyl-betaine, cocamidopropyl-betaine, linoleamidopropyl-betaine, myristamidopropyl-betaine, palmidopropyl-betaine, isostearamidopropyl-betaine, myristamidopropyl-dimethylamine, palmidopropyl-dimethylamine, isostearamidopropyl-dimethylamine, sodium methyl cocoyl-taurate, disodium methyl oleyl-taurate, dihydroxypropyl peg 5 linoleammonium chloride, polyethylene glycol, polypropylene glycol, and mixtures thereof.

In one embodiment, the surfactant is a polysorbate surfactant comprising at least one excipient that is selected from the group consisting of polysorbate 20, polysorbate 21, polysorbate 40, polysorbate 60, polysorbate 61, polysorbate 65, polysorbate 80, polysorbate 81, polysorbate 85, and mixtures thereof. In another embodiment, the liquid pharmaceutical composition comprises polysorbate 80.

The concentration of the surfactant when present in the liquid pharmaceutical composition can range from about 0.005 millimolar to about 10 millimolar, from about 0.007 millimolar to about 5.0 millimolar, or from about 0.01 millimolar to about 1.0 millimolar. In another embodiment, the concentration of the surfactant is from about 0.05 millimolar to about 1.0 millimolar. In another embodiment, the liquid pharmaceutical composition contains about 0.30 millimolar polysorbate 80.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a chelating agent.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a chelating agent; and wherein the composition is substantially free of chloride ions or acetate ions or both.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one human monoclonal anti-MAdCAM antibody, wherein the antibody binds to human MAdCAM; and a chelating agent.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one human monoclonal anti-MAdCAM antibody, wherein the antibody binds to human MAdCAM; and a chelating agent; and wherein the composition is substantially free of chloride ions or acetate ions or both.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that is at least about 50 mg/ml, at least about 60 mg/ml, at least about 70 mg/ml, at least about 80 mg/ml, at least about 90 mg/ml, or at least about 100 mg/ml.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that ranges from about 10 mg/ml to about 200 mg/ml.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a

pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that ranges from about 15 mg/ml to about 200 mg/ml.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that ranges from about 20 mg/ml to about 200 mg/ml.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that ranges from about 50 mg/ml to about 200 mg/ml.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that ranges from about 100 mg/ml to about 200 mg/ml.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM; and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that ranges from about 65 mg/ml to about 85 mg/ml.

In one embodiment, the present invention encompasses a liquid pharmaceutical composition comprising at least one antibody comprising an amino acid sequence that is at least 95% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 95% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MADCAM; and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that is about 75 mg/ml.

In one embodiment, the liquid pharmaceutical composition comprises from about 0.01 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and from about 0.3 micromolar to about 50 millimolar of chelating agent.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and from about 30 micromolar to about 5.0 millimolar of chelating agent.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and about 0.27 millimolar of chelating agent.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and from about 0.3 micromolar to about 50 millimolar of EDTA.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and from about 30 micromolar to about 10.0 millimolar of EDTA.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and from about 0.1 millimolar to about 1.0 millimolar of EDTA.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and about 0.27 millimolar of EDTA.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and from about 30 micromolar to about 5.0 millimolar of DTPA.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and from about 30 micromolar to about 5.0 millimolar of deferoxamine.

In one embodiment, the liquid pharmaceutical composition comprises from about 0.01 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; and from about 1 mM to about 100 mM of histidine.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 30 micromolar to about 5.0 millimolar of chelating agent; and from about 1 mM to about 100 mM of histidine.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 30 micromolar to about 5.0 millimolar of chelating agent; and from about 10 millimolar to about 400 millimolar of trehalose.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 30 micromolar to about 5.0 millimolar of chelating agent; from about 10 millimolar to about 400 millimolar of trehalose; and from about 1 mM to about 100 mM of histidine.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 30 micromolar to about 5.0 millimolar of chelating agent; from about 10 millimolar to about 400 millimolar of trehalose; from about 1 mM to about 100 mM of histidine; and from about 0.005 millimolar to about 10 millimolar of polysorbate 80.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 30 micromolar to about 5.0 millimolar of EDTA; from about 10 millimolar to about 400 millimolar of a tonicity agent; from about 1 mM to about 100 mM of a buffer; and from about 0.005 millimolar to about 10 millimolar of a surfactant.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 30 micromolar to about 5.0 millimolar of EDTA; from about 10 millimolar to about 400 millimolar of a tonicity agent; from about 1 mM to about 100 mM of histidine; and from about 0.005 millimolar to about 10 millimolar of a surfactant.

In another embodiment, the liquid pharmaceutical composition comprises from about 0.1 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 30 micromolar to about 5.0 millimolar of EDTA; from about 10 millimolar to about 400 millimolar of trehalose; from about 1 mM to about 100 mM of histidine; and from about 0.005 millimolar to about 10 millimolar of a surfactant.

In certain aspects of the present invention, the liquid anti-MAdCAM antibody compositions comprise from about 10 mg/ml to about 200 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 1 mM to about 100 mM of histidine; from about 0.005 millimolar to about 10 millimolar of polysorbate 80; from about 30 micromolar to about 5.0 millimolar of EDTA; and from about 10 millimolar to about 400 millimolar of trehalose.

In other aspects of the present invention, the liquid anti-MAdCAM antibody compositions comprise from about 50 mg/ml to about 100 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 5 mM to about 30 mM of histidine; from about 0.01 millimolar to about 1.0 millimolar of polysorbate 80; from about 30 micromolar to about 5.0 millimolar of EDTA; and from about 100 millimolar to about 300 millimolar of trehalose.

In other aspects of the present invention, the liquid anti-MAdCAM antibody compositions comprise from about 65 mg/ml to about 85 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; from about 5 mM to about 15 mM of histidine; from about 0.05 millimolar to about 0.5 millimolar of polysorbate 80; from about 0.1 millimolar to about 1 millimolar of EDTA; and from about 200 millimolar to about 250 millimolar of trehalose.

In other aspects of the present invention, the liquid anti-MAdCAM antibody compositions comprise about 75 mg/ml of monoclonal anti-MAdCAM antibody 7.16.6; about 20 mM of histidine; about 0.3 millimolar of polysorbate 80; about 0.27 millimolar of EDTA; and about 238 millimolar of trehalose.

In another embodiment, the invention is directed to a stable liquid pharmaceutical composition comprising an anti-MAdCAM antibody and a pharmaceutically acceptable chelating agent, wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar and the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.00001 to about 450; from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or
is about 1.9.

In another embodiment, the invention is directed to a stable liquid pharmaceutical composition comprising anti-MAdCAM antibody 7.16.6 and a pharmaceutically acceptable chelating agent, wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar and the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and wherein the molar ratio of antibody to chelating agent ranges from about 0.00001 to about 450; from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

In another embodiment, the invention is directed to a stable liquid pharmaceutical composition comprising anti-MAdCAM antibody 7.16.6, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine ranges from about 1 millimolar to about 100 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.00001 to about 450; from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 1; or is about 0.5.

In another embodiment, the invention is directed to a stable liquid pharmaceutical composition comprising anti-MAdCAM antibody 7.16.6, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine ranges from about 5 millimolar to about 30 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.0001 to about 100; from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

In another embodiment, the invention is directed to a stable liquid pharmaceutical composition comprising anti-MAdCAM antibody 7.16.6, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of

histidine ranges from about 5 millimolar to about 20 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.005 to about 50; from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

In another embodiment, the invention is directed to a stable liquid pharmaceutical composition comprising anti-MAdCAM antibody 7.16.6, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine ranges from about 5 millimolar to about 20 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

In another embodiment, the invention is directed to a stable liquid pharmaceutical composition comprising anti-MAdCAM antibody 7.16.6, a pharmaceutically acceptable chelating agent, and histidine; wherein the molar concentration of the antibody ranges from about 0.0006 millimolar to about 1.35 millimolar, the molar concentration of the chelating agent ranges from about 0.003 millimolar to about 50 millimolar, and the molar concentration of histidine is about 20 millimolar; and wherein the molar ratio of antibody to chelating agent ranges from about 0.001 to about 10; from about 0.01 to about 5; from about 0.1 to about 3; or is about 1.9.

Methods of Producing Anti-MAdCAM Antibodies and Antibody Producing Cell Lines:

Antibodies in accordance with the invention can be prepared through the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted, but that is rendered deficient in the production of endogenous, murine, antibodies. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are discussed below.

It is possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. In particular, however, one embodiment of transgenic production of mice and antibodies therefrom is disclosed in International Application Number PCT/US2005/000370. Through use of such technology, antibodies that bind to MAdCAM and hybridomas producing such antibodies can be prepared.

Human antibodies avoid certain of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a subject that receives administration of such antibodies.

For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin

gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen (e.g., MAdCAM) challenge. See, e.g., Jakobovits et al, Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggermann et al., Year in Immuno., 7:33 (1993); and Duchosal et al., Nature 355:258 (1992). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., J. Mol. Biol., 227:381 (1991); Marks et al., J. MoL Biol., 222:581-597 (1991); Vaughan et al., Nature Biotech 14:309 (1996)).

In some embodiments, human anti-MAdCAM antibodies can be produced by immunizing a non-human transgenic animal, e.g., XENOMOUSE™ mice, whose genome comprises human immunoglobulin genes so that the recombinant mouse produces human antibodies. XENOMOUSE™ mice are engineered mouse strains that comprise large fragments of human immunoglobulin heavy chain and light chain loci and are deficient in mouse antibody production. XENOMOUSE™ mice produce an adult-like human repertoire of fully human antibodies and generate antigen-specific human antibodies. In some embodiments, the XENOMOUSE™ mice contain approximately 80% of the human antibody V gene repertoire through introduction of megabase sized, germline configuration yeast artificial chromosome (YAC) fragments of the human heavy chain loci and kappa light chain loci. In other embodiments, XENOMOUSE™ mice further contain approximately all of the lambda light chain locus. See, e.g., Green et al., Nature Genetics 7:13-21 (1994) and U.S. Patents 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598, 6,130,364, 6,162,963 and 6,150,584. See also WO 91/10741, WO 94/02602, WO 96/34096, 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.

In some embodiments, the non-human animal comprising human immunoglobulin genes are animals that have a human immunoglobulin "minilocus". In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of individual genes from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant domain, and a second constant domain (preferably a gamma constant domain) are formed into a construct for insertion into an animal. This approach is described, inter alia, in U.S. Patent Nos. 5,545,807, 5,545,806, 5,569,825, 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.

Therefore, in some embodiments, human antibodies can be produced by immunizing a non-human animal comprising in its genome some or all of human immunoglobulin heavy chain and light chain loci with a MAdCAM antigen.

In some embodiments, the MAdCAM antigen is isolated and/or purified MAdCAM. In a preferred embodiment, the MAdCAM antigen is human MAdCAM. In some embodiments, the MAdCAM antigen is a fragment of MAdCAM. In some embodiments, the MAdCAM fragment comprises at least one epitope of MAdCAM. In other embodiments, the MAdCAM antigen is a cell that expresses or overexpresses MAdCAM or an immunogenic fragment thereof on its surface. In still other embodiments, the MAdCAM antigen is a MAdCAM fusion protein.

MAdCAM can be purified from natural sources using known techniques. In addition, recombinant MAdCAM protein is commercially available.

In a preferred embodiment, the non-human animal is a XENOMOUSE™ animal (Abgenix Inc., Fremont, CA). Another non-human animal that may be used is a transgenic mouse produced by Medarex (Medarex, Inc., Princeton, NJ).

Immunization of animals can be 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, supra, and U.S. Patent 5,994,619. In a preferred embodiment, the MAdCAM antigen is administered with an adjuvant to stimulate the immune response. Exemplary 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 can involve two or more administrations of the polypeptide, spread out over several weeks.

After immunization of an animal with a MAdCAM antigen, antibodies and/or antibody-producing cells can be obtained from the animal. In some embodiments, 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.

In some embodiments, antibody-producing immortalized cell lines are prepared from cells isolated from the immunized animal. After immunization, the animal is sacrificed and lymph node and/or splenic B cells are immortalized. Methods of immortalizing cells include, but are not limited to, transfecting them with oncogenes, infecting them with an oncogenic virus, 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 a preferred embodiment, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma cell line from the same species as the non-human animal. In a more preferred embodiment, the immunized animal is a XENOMOUSE™ animal and the myeloma cell line is a non-secretory mouse myeloma. If fusion with myeloma cells is used, the myeloma cells preferably do not secrete immunoglobulin polypeptides (a non-secretory cell line). Immortalized cells 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. An example of ELISA screening is provided in WO 00/37504.

Anti-MAdCAM antibody-producing cells, e.g., hybridomas, are selected, cloned and further screened for desirable characteristics, including robust growth, high antibody production

and desirable antibody characteristics, as discussed further below. Hybridomas can be 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.

As will be appreciated, antibodies in accordance with the present invention can be recombinantly expressed in cell lines other than hybridoma cell lines. Nucleic acid sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of a suitable mammalian or nonmammalian host cells.

The present invention also encompasses nucleic acid molecules encoding anti-MAdCAM antibodies. In some embodiments, different nucleic acid molecules encode a heavy chain and a light chain of an anti-MAdCAM immunoglobulin. In other embodiments, the same nucleic acid molecule encodes a heavy chain and a light chain of an anti-MAdCAM immunoglobulin. In one embodiment, the nucleic acid encodes an anti-MAdCAM antibody of the invention.

A nucleic acid molecule encoding the heavy or entire light chain of an anti-MAdCAM antibody or portions thereof can be isolated from any source that produces such antibody. In various embodiments, the nucleic acid molecules are isolated from a B cell isolated from an animal immunized with anti-MAdCAM or from an immortalized cell derived from such a B cell that expresses 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 3rd Ed. Vol.3 (1989). The mRNA may be used to produce cDNA for use in the polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In a preferred embodiment, the nucleic acid molecule is isolated from a hybridoma that has as one of its fusion partners a human immunoglobulin-producing cell from a non-human transgenic animal. In an even more preferred embodiment, the human immunoglobulin producing cell is isolated from a XENOMOUSE™ animal. In another embodiment, the human immunoglobulin-producing cell is from a non-human, non-mouse transgenic animal, as described above. In another embodiment, the nucleic acid is isolated from a non-human, non-transgenic animal. The nucleic acid molecules isolated from a non-human animal may be used, e.g., for humanized antibodies.

In some embodiments, a nucleic acid encoding a heavy chain of an anti-MAdCAM antibody of the invention can comprise a nucleotide sequence encoding a V_H domain of the invention joined in-frame to a nucleotide sequence encoding a heavy chain constant domain from any source. Similarly, a nucleic acid molecule encoding a light chain of an anti-MAdCAM antibody of the invention can comprise a nucleotide sequence encoding a V_L domain of the invention joined in-frame to a nucleotide sequence encoding a light chain constant domain from any source.

In a further aspect of the invention, nucleic acid molecules encoding the variable domain of the heavy (V_H) and light (V_L) chains are "converted" to full-length antibody genes. In one embodiment, nucleic acid molecules encoding the V_H or V_L domains are converted to full-length antibody genes by insertion into an expression vector already encoding heavy chain

constant (C_H) or light chain (C_L) constant domains, respectively, such that the V_H segment is operatively linked to the C_H segment(s) within the vector, and the V_L segment is operatively linked to the C_L segment within the vector. In another embodiment, nucleic acid molecules encoding the V_H and/or V_L domains are converted into full-length antibody genes by linking, e.g., ligating, a nucleic acid molecule encoding a V_H and/or V_L domains to a nucleic acid molecule encoding a C_H and/or C_L domain using standard molecular biological techniques. Nucleic acid sequences of human heavy and light chain immunoglobulin constant domain 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.

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

In some embodiments, the anti-MAdCAM antibodies, or antigen-binding portions of the invention are expressed by inserting DNAs encoding partial or full-length light and heavy chains, obtained as described above, into expression vectors such that the genes are operatively linked to necessary expression control sequences such as 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 vectors. 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 complementry restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

A convenient vector is one that encodes a functionally complete human C_H or C_L immunoglobulin sequence, with appropriate restriction sites engineered so that any V_H or V_L sequence can easily be 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 domain, and also at the splice regions that occur within the human C_H exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector also can 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 immunoglobulin chain. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

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 retroviruses (such as 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. Patent No. 5,168,062, U.S. Patent No. 4,510,245 and U.S. Patent No. 4,968,615. Methods for expressing antibodies in plants, including a description of promoters and vectors, as well as transformation of plants is known in the art. See, e.g., United States Patents 6,517,529, herein incorporated by reference. Methods of expressing polypeptides in bacterial cells or fungal cells, e.g., yeast cells, are also well known in the art.

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. Patent 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), the neomycin resistance gene (for G418 selection), and the glutamate synthetase gene.

Nucleic acid molecules encoding anti-MAdCAM antibodies and vectors comprising these nucleic acid molecules can be used for transformation of a suitable mammalian, plant, bacterial or yeast host cell. Antibodies of the invention can be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom.

Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455. The

transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, particle bombardment, encapsulation of the polynucleotide(s) in liposomes, peptide conjugates, dendrimers, and direct microinjection of the DNA into nuclei.

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), including but not limited to Chinese hamster ovary (CHO) cells, NS0 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Non-mammalian cells including but not limited to bacterial, yeast, insect, and plants can also be used to express recombinant antibodies. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The expression methods are selected by determining which system generates the highest expression levels and produce antibodies with constitutive MAdCAM binding properties.

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 sythetase and DHFR gene expression systems are common approaches for enhancing expression under certain conditions. High expressing cell clones can be identified using conventional techniques, such as limited dilution cloning and Microdrop technology. The Glutamine Synthetase system is discussed in whole or part in connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997 and European Patent Application No. 89303964.4.

In connection with the transgenic production in mammals, antibodies can also be produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Pat. Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

The anti-MAdCAM antibodies expressed in cell lines as described above may be purified and/or isolated from the associated cellular material. The antibodies may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. Purification is performed in order to eliminate other cellular components or other contaminants, e.g. other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, column chromatography and others well known in the art. See Ausubel, F., et al., ed. Current Protocols in Molecular Biology, Greene Publishing and Wiley Interscience, New York (1987).

In the present invention, it is possible that the anti-MAdCAM antibodies of the present invention expressed by different cell lines or in transgenic animals will have different glycosylation patterns from each other. However, all of the anti-MAdCAM antibodies encoded by the nucleic acids and amino acids provided herein are considered part of the instant invention, regardless of their glycosylation pattern or modification or deletion thereof.

As used herein, the term "glycosylation" means the pattern of carbohydrate units that are covalently attached to an antibody. When it is said that the anti-MAdCAM antibodies herein have a particular glycosylation pattern, it is meant that the majority of the referenced anti-MAdCAM antibodies have that particular glycosylation pattern. In other aspects, when it is said that the anti-MAdCAM antibodies herein have a particular glycosylation pattern, it is meant that greater than or equal to 50%, 75%, 90%, 95%, 99% or 100% of the referenced anti-MAdCAM antibodies have that particular glycosylation pattern.

The anti-MAdCAM antibodies of the present invention also encompass glycosylation variants thereof (e.g., by insertion of a glycosylation site or deletion of any glycosylation site by deletion, insertion or substitution of suitable amino acid residues). For purposes of the present invention, the anti-MAdCAM antibodies may be glycosylated or non-glycosylated. When the anti-MAdCAM antibodies are glycosylated they may have any possible glycosylation pattern. Moreover, each heavy chain within one antibody may have the same glycosylation pattern or the two heavy chains may have differing glycosylation patterns.

Routes of Administration and Dosages:

The compositions of this invention may be in liquid solutions (e.g., injectable and infusible solutions). 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, intradermal, intraperitoneal, intramuscular, and intrasternally) or by infusion techniques, in the form of sterile injectable liquid or olagenous suspensions. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular, subcutaneous or intradermal injection.

Therapeutic compositions typically are sterile and stable under the conditions of manufacture and storage.

The composition can be formulated as a solution, microemulsion, dispersion, or liposome. Sterile injectable solutions can be prepared by incorporating the anti-MAdCAM antibody in the required amount in an appropriate diluent with one or a combination of ingredients enumerated above, as required, followed by sterilization (e.g., filter sterilization). 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. Such suspensions may be formulated according to the known art using those suitable dispersing of wetting agents and suspending agents or other acceptable agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution

and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, n-3 polyunsaturated fatty acids may find use in the preparation of injectables.

In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin or by formulating the composition into prolonged absorption forms such as, depots, liposomes, polymeric microspheres, polymeric gels, and implants.

Other methods for administration of the antibodies described herein include dermal patches that release the medications directly into a subject's skin. Such patches can contain the antibodies of the present invention in an optionally buffered, liquid solution, dissolved and/or dispersed-in-an-adhesive,-or dispersed in a polymer.

Still other methods for administration of the antibodies described herein include liquid ophthalmological drops for the eyes.

The antibody may be administered once, but more preferably is administered multiple times. For example, the antibody may be administered from once daily to once every six months or longer. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months.

The antibody may also be administered continuously via a minipump. The antibody may be administered once, at least twice or for at least the period of time until the disease is treated, palliated or cured. The antibody typically would be administered as part of a pharmaceutical composition as described supra.

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. In preparing the composition, the therapeutically effective amount of the anti-MAdCAM antibody present in the composition can be determined, for example, by taking into account the desired dose volumes and mode(s) of administration, the nature and severity of the condition to be treated, and the age and size of the subject.

Exemplary, non-limiting dose ranges for administration of the pharmaceutical compositions of the present invention to a subject are from about 0.01 mg/kg to about 200 mg/kg (expressed in terms of milligrams (mg) of anti-MAdCAM antibody administered per kilogram (kg) of subject weight), from about 0.1 mg/kg to about 100 mg/kg, from about 1.0

mg/kg to about 50 mg/kg, from about 5.0 mg/kg to about 20 mg/kg, or about 15 mg/kg. For purposes of the present invention, an average human subject weighs about 70 kg.

Ranges intermediate to any of the concentrations cited herein, e.g., about 6-94 mg/kg, are also intended to be part of this invention. For example, ranges of values using a combination of any of the recited values as upper and/or lower limits are intended to be included.

Dosage regimens can also be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response) by administering several divided doses to a subject 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 predetermined 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 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.

The liquid compositions of the present invention can be prepared as unit dosage forms. For example, a unit dosage per vial may contain from 1 to 1000 milliliters (mls) of different concentrations of an anti-MAdCAM antibody. In other embodiments, a unit dosage per vial may contain about 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml, 15 ml, 20 ml, 30 ml, 40 ml, 50 ml or 100 ml of different concentrations of an anti-MAdCAM antibody. If necessary, these preparations can be adjusted to a desired concentration by adding a sterile diluent to each vial.

Stability Assessment:

The present invention comprises stable liquid pharmaceutical compositions comprising an anti-MAdCAM antibody as described herein and a pharmaceutically acceptable chelating agent. A stable composition is desirable to maintain or resist changes in, for example, product appearance and integrity (including physical or chemical degradation potentially leading to a reduction in biological activity). Various analytical techniques and indicators for measuring protein stability are reported in the literature and a number of these techniques and indicators are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10: 29-90 (1993). In general, the liquid pharmaceutical compositions of the present invention exhibit improved stability when subjected to low storage temperatures over a period of time, and/or when subjected to one or more freeze/thaw cycles.

In one embodiment, the composition when stored at a temperature from about 2°C to about 8°C for at least about 12 months, preferably at least about 18 months and more

preferably at least about 24 months, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time.

In another embodiment, the composition when stored at a temperature from about 25°C to about 30°C for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time.

In another embodiment, the composition when stored at a temperature of about 40°C for at least about 1 month, preferably at least about 2 months, and more preferably at least about 3 months is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time.

As used herein, the term "a freeze/thaw cycle" refers to techniques for using a liquid antibody sample after frozen storage, wherein the temperature of the sample is lowered to a temperature of 0°C or lower in order to freeze the liquid sample, and then subjecting the sample to a temperature which will restore its liquid state for a sufficient period of time to permit use of the sample, followed by and return to frozen storage, preferably at a temperature of 0°C or lower. As used herein, the term "frozen storage" refers to freezing and maintaining a previously liquid antibody sample at a temperature of 0°C or below, and preferably -20°C or lower.

In one embodiment, the composition when subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles, is more stable than an otherwise identical composition lacking the chelating agent that is subjected to the same freeze/thaw conditions.

In another embodiment, the composition satisfies two or more of the following conditions:

(a) the composition when stored at a temperature from about 2°C to about 8°C for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time;

(b) the composition when stored at a temperature from about 25°C to about 30°C for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months, is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time;

(c) the composition when stored at a temperature of about 40°C for at least about 1 month, preferably at least about 2 months, and more preferably at least about 3 months is more stable than an otherwise identical composition lacking the chelating agent that is stored under the same conditions for the same time; or

(d) the composition when subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably

at least 6 freeze/thaw cycles, is more stable than an otherwise identical composition lacking the chelating agent that is subjected to the same freeze/thaw conditions.

In another embodiment, the composition satisfies three or more of the conditions discussed immediately above.

For purposes of the present application, antibody aggregation, antibody fragmentation, and/or composition discoloration, for example, can be used as indicators of the stability of the composition. In general, the liquid pharmaceutical compositions of the present invention exhibit a lower level of at least one of antibody aggregation, antibody fragmentation and composition discoloration when subjected to one or more of the above-described storage or freeze/thaw conditions relative to otherwise identical compositions lacking the chelating agent that are subjected to the same conditions.

Protein aggregation in a liquid pharmaceutical composition can be measured by various methods known in the art. Such methods include gel filtration chromatography to separate proteins on the basis of their molecular weight. A "gel" is a matrix of water and a polymer, such as agarose or polymerized acrylamide. The present invention also encompasses the use of gel filtration HPLC (high performance liquid chromatography). Other recognized methods of measuring aggregation include cation exchange chromatography, which is the general liquid chromatographic technique of ion-exchange chromatography utilizing anion columns. The cations exchanged in the present invention are from the protein molecules. Since multivalent protein aggregates may have some multiple of the net charge of the monomer of the antigen-binding protein, the aggregates can be retained more strongly, and may be separated from the monomer molecules. A preferred cationic exchanger is a polyaspartic acid column. Thus, a monomeric protein can be readily distinguished from an aggregate. However, those of ordinary skill in the art will realize that aggregation assays of the invention are not limited to any particular type of chromatography column, so long as it is capable of separating the two forms of protein molecules.

Protein fragmentation in a liquid pharmaceutical composition can be measured by various methods known in the art. Such methods include, for example, size exclusion chromatography, ultraviolet detection (e.g., at 214 nanometers), SDS-PAGE and/or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS). Protein fragmentation resulting in a charge alteration (e.g., occurring as a result of deamidation) can be evaluated, for example, by ion-exchange chromatography or isoelectric focusing (IEF).

Composition discoloration generally can be measured by visual observation of the composition itself. The present liquid pharmaceutical compositions comprising a chelating agent generally reduce composition discoloration (e.g., pink or yellow) and/or maintain composition clarity (e.g., turbidity, cloudiness and/or particulate formation) relative to otherwise identical compositions that do not contain the chelating agent. For purposes of the present invention, the term "discoloration" refers to both changes in color (e.g., from clear and colorless to pink or yellow) and to changes in clarity (e.g., from clear and colorless to turbid, cloudy and/or having particulates). Composition discoloration generally can be measured using

additional techniques such as by ultraviolet detection at 214 nanometers, detection at other wavelengths such as in the visible/near-UV range and/or by visual comparison against a standard color scale of the compositions with and without the chelating agent. See PhEur 5.0, 2005 Monograph 2.2.2.

In one embodiment, antibody aggregation is determined after the composition is subjected to at least one of the following conditions:

(a) the composition is stored at a temperature from about 2°C to about 8°C for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months;

(b) the composition is stored at a temperature from about 25°C to about 30°C for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months;

(c) the composition is stored at a temperature of about 40°C for at least about 1 month, preferably at least about 2 months, and more preferably at least about 3 months; or

(d) the composition is subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles. Antibody aggregates are then chromatographically separated from the monomers (e.g., using HPLC) and the extent of aggregation determined from the resulting chromatogram. The stable liquid pharmaceutical compositions of the present invention typically have an aggregate peak area on the chromatogram that is less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1.5% of the total peak area on the chromatogram. In one specific example of this technique for measuring aggregation, the composition is stored for 24 weeks at 40°C and chromatographic separation is then conducted using SE-HPLC with ultraviolet detection at 214 nanometers.

In general, the difference between the aggregate chromatogram peak area for a stable liquid pharmaceutical composition of the present invention and the aggregate chromatogram peak area for an otherwise identical composition lacking the chelating agent that is subjected to the same conditions is at least about 2%, at least about 3%, at least about 4%, or at least about 4.5%.

In another embodiment, antibody fragmentation is determined after the composition is subjected to at least one of the following conditions:

(a) the composition is stored at a temperature from about 2°C to about 8°C for at least about 12 months, preferably at least about 18 months and more preferably at least about 24 months;

(b) the composition is stored at a temperature from about 25°C to about 30°C for at least about 3 months, preferably at least 6 months, and more preferably at least about 12 months;

(c) the composition is stored at a temperature of about 40°C for at least about 1 month, preferably at least about 2 months, and more preferably at least about 3 months; or

(d) the composition is subjected to at least 1 freeze/thaw cycle, preferably at least 2 freeze/thaw cycles, more preferably at least 3 freeze/thaw cycles, still more preferably at least 4 freeze/thaw cycles, still more preferably at least 5 freeze/thaw cycles, and still more preferably at least 6 freeze/thaw cycles. Antibody fragments are then electrophoretically separated from the composition (e.g., SDS-PAGE) and the extent of fragmentation determined from the resulting electrophorogram or gel images. The stable liquid pharmaceutical compositions of the present invention typically have a fragment band volume on the SDS-PAGE gel that is less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, or less than about 4.5% of the total band volume on the gel. In one specific example of this technique for measuring fragmentation, the composition is stored for 24 weeks at 40°C and then analyzed using reduced SDS-PAGE (rSDS-PAGE) with band volumes determined by scanning with either a Molecular Dynamics Personal Densitometer PDQC-90 or a Bio-Rad GS800 Imaging Densitometer.

In general, the difference between the fragment band volume for a stable liquid pharmaceutical composition of the present invention and the fragment band volume for an otherwise identical composition lacking the chelating agent that is subjected to the same conditions is at least about 2%, at least about 3%, at least about 4%, or at least about 5%.

Methods of Prevention/Treatment:

Any of the types of antibodies described herein may be used therapeutically. In a preferred embodiment, the anti-MAdCAM antibody is a human antibody. In another preferred embodiment, the MAdCAM is human and the subject is a human subject. In yet another preferred embodiment, the anti-MAdCAM antibody is a human IgG2 antibody. Alternatively, the subject may be a mammal that expresses a MAdCAM protein that the anti-MAdCAM antibody cross-reacts with. The antibody may be administered to a non-human mammal expressing MAdCAM with which the antibody cross-reacts (i.e., a primate) for veterinary purposes or as an animal model of human disease. Such animal models may be useful for evaluating the therapeutic efficacy of antibodies of this invention.

The present invention provides a method for the treatment of an inflammatory disease in a subject, comprising administering to the subject a liquid pharmaceutical composition comprising an anti-MAdCAM antibody; and a chelating agent alone or in combination with other excipients chosen from a buffer, a tonicity agent, or a surfactant, and mixtures thereof. In further embodiments, the aforementioned subject is one that is in need of the prevention or treatment of an inflammatory disease.

In another embodiment, the present invention provides a method for the treatment of an inflammatory disease condition in a subject, comprising administering to the subject a liquid pharmaceutical composition comprising anti-MAdCAM antibody 7.16.6; and pharmaceutically acceptable excipient comprising a chelating agent alone or in combination with other excipients chosen from a buffer, a tonicity agent, or a surfactant, and mixtures thereof.

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, military dermatitis, military 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 desease), chronic pancreatitis, perineural inflammation, colitis (including amoebic colitis, infective colitis, bacterial colitis, Crohn's colitis, ischemic colitis, ulcerative colitis, idiopathic proctocolitis, inflammatory bowel disease, psuodomembranouscolitis), 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, Guilain-Barre syndrome, MS-associated retrovirus), dermatomyositis, diverticulitis, exudative diarrheas, gastritis, granulomatous hepatitis, grenulomatous inflammation, cholecystitis, inulin-dependent diabetes mellitus, liverinflammatory diseases (liver fibrosis primary biliary cirrhosis, hepatitis, sclerosing cholangitis), lung inflammation (idiopathic pulmonary fibrosis, eosinophilic granuloma of the lung, pulmonary histiocytosis X, peribronchiolar inflammation, acute bronchitis), lymphogranuloma venereum, malignant melanoma, mout/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 neuropathy, superficial thrombophlevitits, systemic inflammatory response syndrome, thuroiditis,systemic lupus erythematosus, graft versus host disease, acute burn injury, Behcet's syndrome, Sjogren's syndrome.

In a more preferred embodiment, the anti-MAdCAM antibody is administered to a subject with colitis.

Articles of Manufacture:

In another embodiment of the invention, an article of manufacture is provided comprising a container, which holds the liquid pharmaceutical composition comprising at least one of the monoclonal anti-MAdCAM antibodies of the present invention in a composition comprising a chelating agent alone or in combination with other pharmaceutically acceptable excipients, and optionally provides instructions for its use. Suitable containers include, for

example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as glass or plastic. An exemplary container is a 3-20 cc single use glass vial. Alternatively, for a multidose composition, the container may be a 3-100 cc glass vial. The container holds the composition and the label on, or associated with, the container may indicate directions for use. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use, contraindications, and/or lists of potential side-effects.

The present invention also provides a kit for preparing a liquid composition of a stabilized antibody comprising a first container, comprising monoclonal anti-MAdCAM antibody 7.16.6 in solution, and a second container comprising a sufficient amount of a chelating agent alone or in combination with other excipients in solution to stabilize the antibody.

The following examples describe embodiments of the invention. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims, which follow the examples. In the examples, all percentages are given on a weight basis unless otherwise indicated. The skilled artisan will appreciate that the weight quantities and/or weight-to-volume ratios recited in the examples can be converted to moles and/or molarities using the art-recognized molecular weights of the recited ingredients. Weight quantities exemplified herein (e.g., grams) are for the volumes (e.g., of buffer solutions, antibody composition, etc.) recited. The skilled artisan will appreciate that the weight quantities can be proportionally adjusted when different composition volumes are desired.

EXAMPLE 1:

Generation of anti-MAdCAM producing hybridomas

Antibodies of the invention were prepared in accordance with the present Example. Refer to PCT/US2005/000370.

Primary Immunogen Preparation:

Two immunogens were prepared for immunisation of the XenoMouse™ mice: (i) a MAdCAM-IgG_1 Fc fusion protein and (ii) cell membranes prepared from cells stably transfected with MAdCAM.

(i) MAdCAM-IgG_1 Fc Fusion Protein

Expression vector construction:

An EcoRI/BglII cDNA fragment encoding the mature extracellular, immunoglobulin-like domain of MAdCAM was excised from a pINCY Incyte clone (3279276) and cloned into EcoRI/BamHI sites of the pIG1 vector (Simmons, D. L. (1993) in Cellular Interactions in Development: A Practical Approach, ed. Hartley, D. A. (Oxford Univ. Press, Oxford), pp. 93-127.)) to generate an in frame IgG_1 Fc fusion. The resulting insert was excised with EcoRI/NotI and cloned into pCDNA3.1+ (Invitrogen). The MAdCAM-IgG_1 Fc cDNA in the vector was sequence confirmed. The amino acid sequence of the MAdCAM-IgG_1 Fc fusion protein is shown below:

MAdCAM-IgG_1 Fc Fusion Protein:

MDFGLALLLAGLLGLLLG**QSLQVKPLQVEPPEPVVAVALGASRQLTCRLACADRGASVQWR
GLDTSLGAVQSDTGRSVLTVRNASLSAAGTRVCVGSCGGRTFQHTVQLLVYAFPDQLTVSP
AALVPGDPEVACTAHKVTPVDPNALSFSLLVGGQELEGAQALGPEVQEEEEEPQGDEDVLF
RVTERWRLPPLGTPVPPALYCQATMRLPGLELSHRQAIPVLHSPTSPEPPDTTSPESPDTTS
PESPDTTSQEPPDTTSQEPPDTTSQEPPDTTSPEPPDKTSPEPAPQQGSTHTPRSPGSTRTR
RPE**IQPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKATPPVLDS
DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 5)

Underlined: signal peptide
Bold: MAdCAM extracellular domain

Recombinant Protein Expression/Purification:

CHO-DHFR cells were transfected with pCDNA3.1+ vector containing MAdCAM-IgG_1 Fc fusion protein cDNA and stable clones expressing MAdCAM-IgG_1 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-IgG_1 Fc CHO cells in Iscove's media containing 10% low IgG fetal bovine serum (Gibco), non essential amino acids (Gibco), 2 mM glutamine (Gibco), sodium pyruvate (Gibco), 100 µg/mL G418 and 100 ng/mL methotrexate, and used to generate concentrated media supernatant. The MAdCAM-IgG_1 Fc fusion protein was purified from the harvested supernatant by affinity chromatography. Briefly, supernatant was applied to a HiTrap Protein G Sepharose (5 mL, Pharmacia) column (2 mL/min), washed with 25 mM Tris pH 8, 150 mM NaCl (5 column volumes) and eluted with 100 mM glycine pH 2.5 (1 mL/min), immediately neutralising fractions to pH 7.5 with 1M Tris pH 8. Fractions containing MAdCAM-IgG_1 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-IgG_1 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-IgG_1 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

A SacI/NotI fragment comprising nucleotides 645-1222 of the published MAdCAM 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 MAdCAM-IgG$_1$ Fc, to generate the full length MAdCAM cDNA. A KpnI/NotI fragment containing the MAdCAM 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 $\alpha_4\beta_7^+$ 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 MAdCAM were prepared in the same way, using FlpIn CHO cells (Invitrogen).

MAdCAM-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. MAdCAM-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 $MgCl_2$, 0.015% (w/v) aprotinin, 100 U/mL bacitracin and centrifugation. The final pellet was resuspended in the same buffer, and $50x10^6$ 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:

Eight to ten week old XENOMOUSE™ mice were immunized intraperitoneally or in their hind footpads with either the purified recombinant MAdCAM-IgG$_1$ 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 secreting MAdCAM specific human IgG_{2K} was recovered and sub-cloned.

The following hybridoma producing anti-MAdCAM antibodies designated as follows was deposited at the European Collection of cell Cultures (ECACC), H.P.A. at CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG on 9 September 2003: Hybridoma 7.16.6, Deposit No. 03090909.

Example 2

Antibody Compositions

The following compositions are referred to in the Examples that follow:

Table 2

Composition ID	mAb 7.16.6 mg/ml	Buffer mM; pH	Tonicifier mg/ml	Surfactant mg/ml	Chelating agent Mg/ml	Other Excipients
1	10±1	Acetate, 20mM, pH 5.5				
2	10±1	Acetate, 7mM Citrate, 7mM Phosphate, 7mM, pH 5.5				
3	10±1	EDTA, 20mM pH 5.5				
4	8±1	Sodium acetate, 20mM, pH5.5				NaCl, 140 mM
5	8±1	Sodium acetate, 20	Mannitol, 45 mg/ml			

		mM, pH 5.5				
6	8±1	Sodium acetate, 10 mM, pH 5.5				
7	8±1	Sodium EDTA, 10 mM, pH 5.5				
8	8±1	Sodium EDTA, 10 mM, pH 5.5				NaCl, 140 mM
9	8±1	Sodium EDTA, 10 mM, pH 5.5	Mannitol, 45 mg/ml			
10	8±2	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml		
11	8±2	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
12	8±2	Sodium acetate 20 mM, pH 5.5	NaCl, 8.2 mg/ml	PS80, 0.2 mg/ml		
13	8±2	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$CaCl_2.2H_2O$, 0.3 mg/ml	
14	30±6	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.4 mg/ml		
15	30±6	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
16	30±6	Sodium acetate 20 mM, pH 5.5	NaCl, 8.2 mg/ml	PS80, 0.4 mg/ml		
17	30±6	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.4 mg/ml	$CaCl_2.2H_2O$, 0.3 mg/ml	
18	50±6	Histidine, 10 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	

19	10±2	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
20	10±2	Histidine, 10 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
21	10±2	Histidine, 10 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
22	50±6	Sodium acetate 20 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
23	50±6	Histidine, 10 mM, pH 6.0	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
24	150±6	Histidine, 10 mM, pH 5.5	Mannitol, 45 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
25	50±5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
26	75±5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
27	100±7	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
28	150±10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
29	190±2	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
30	80±10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.02 mg/ml	
31	80±10	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.10 mg/ml	
32	80±10	Histidine,	Trehalos	PS80, 0.4	$Na_2EDTA.2H$	

		10 mM, pH 5.5	e, 90 mg/ml	mg/ml	$_2$0, 0.05 mg/ml	
33	80±10	Histidine, 10 mM, pH 5.5	Trehalos e, 90 mg/ml	PS80, 1.0 mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
34	85±15	Histidine, 10 mM, pH 5.5	Trehalos e, 90 mg/ml	PS80, 0.2 mg/ml	Na_2EDTA.2H $_2$0, 0.10 mg/ml	
35	85±15	Histidine, 10 mM, pH 5.5	Trehalos e, 90 mg/ml	PS80, 0.4 mg/ml	Na_2EDTA.2H $_2$0, 0.10 mg/ml	
36	85±15	Histidine, 10 mM, pH 5.5	Trehalos e, 90 mg/ml	PS80, 1.0 mg/ml	Na_2EDTA.2H $_2$0, 0.10 mg/ml	
37	80±10	Citrate, 5 mM, pH 5.5	Trehalos e, 90 mg/ml	PS80, 0.2 mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
38	80±10	Succinate, 5 mM pH 5.5	Trehalos e, 90 mg/ml	PS80, 0.2 mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
39	80±10	Histidine, 10 mM, pH 5.5	Sucralos e, 85 mg/ml	PS80, 0.2 mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
40	80±10	Histidine, 10 mM, pH 5.5	Sorbitol 45 mg/ml	PS80, 0.2 mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
41	80±10	Histidine, 10 mM, pH 5.5	Xylitol 35 mg/ml	PS80, 0.2 mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
42	80±10	Histidine, 10 mM, pH 5.5	Trehalos e, 90 mg/ml	PEG_{3350}, 10 mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
43	80±10	Histidine, 10 mM, pH 5.5	Trehalos e, 90 mg/ml	NOF PS80 0.2mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
44	80±10	Histidine, 10 mM, pH 5.5	Trehalos e, 90 mg/ml	Poloxamer 407, 1.0 mg/ml	Na_2EDTA.2H $_2$0, 0.05 mg/ml	
45	80±10	Histidine, 10 mM, pH	Trehalos e, 90	Poloxamer 188	Na_2EDTA.2H $_2$0, 0.05	

		5.5	mg/ml	1.0 mg/ml	mg/ml	
46	50±5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
47	50±5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
48	75±15	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
49	75±15	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
50	75±15	Histidine, 10 mM, pH 5.5	Sucrose, 85 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
51	75±5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.2 mg/ml	$Na_2EDTA.2H_20$, 0.05 mg/ml	
52	75±5	Histidine, 10 mM, pH 5.5	Trehalose, 90 mg/ml	PS80, 0.4 mg/ml	$Na_2EDTA.2H_20$, 0.10 mg/ml	

Example 3

A study was conducted to evaluate the effect of several different buffers on anti-MAdCAM antibody 7.16.6 aggregation.

Preparation of Buffer solutions:

The buffer solutions were prepared by first dissolving an amount of the buffer species in water (approximately 90% of target). The pH of each buffer solutions was then adjusted to 5.5 by addition of a sufficient amount of an acid or base solution. After adjustment of pH, an additional amount of water was added to provide a final buffer concentration of 20mM. The buffer concentration of 20 mM was selected to ensure reasonable pH stability at the selected pH of 5.5. The buffer solution was then filtered through a sterilization filter (0.22 micron pore size) into a sterilized receptacle for subsequent use.

Preparation of Antibody compositions:

The antibody compositions were prepared as follows. An antibody bulk solution was obtained as 10.5 mg/ml in 20 mM sodium acetate buffer pH 5.5 + 140 mM sodium chloride. Buffer exchanges of this bulk solution into the composition solutions were carried out by

centrifugation at 4500xg using a molecular weight cut-off membrane (e.g. 30kD). Approximately 8 volume exchanges were made and the final antibody solution was prepared at about 10 mg/ml concentration. Antibody concentrations were determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an extinction coefficient of 1.56 $(mg/ml)^{-1}$ cm^{-1} at 280 nm. The compositions with all ingredients were then sterilized by filtration through sterile 0.22 micron membrane filter. The filtered compositions were then filled into washed and autoclaved vials, which were closed with Daikyo777-1 Flurotec® coated stoppers, crimp sealed and placed in stability chambers.

Specifically, three liquid compositions comprising anti-MAdCAM antibody 7.16.6 and buffered with acetate, EDTA, or a combination of acetate, citrate and phosphate were prepared. The compositions were then stored for 6 weeks at 40°C and aggregation measurements were taken.

Table 3:

Composition ID	7.16.6 mAb mg/mL	Description	% Aggregation	Relative increase in % Aggregation *
1	10 ±1	20mM Acetate pH 5.5	0.8	70
2	10 ±1	Combination, pH 5.5 (acetate 7 mM, citrate 7 mM, phosphate 6 mM)	0.7	60
3	10 ±1	20mM EDTA, pH 5.5	0.5	40

* Relative increase in % Aggregation is calculated by:
[(%aggregation at 6wk/40C) – (%aggregation at initial)}*100] / [% aggregation at initial]

Aggregation Analysis:

The antibody compositions were stored at 40°C. At six weeks, each composition was analyzed for aggregation using size exclusion chromatography (SEC). The size exclusion chromatography was carried out using a TSK gel G3000SWXL-G2000SWXL column, 0.2M sodium phosphate, pH7 mobile phase, a flow rate of 0.7mL/min, and UV detection at 214 nm. Aggregation levels were calculated by integrating the areas under the chromatogram peaks for each composition and reporting the integrated areas under the high molecular weight species peaks as a percentage of total peak area. As is shown in Table 3, the EDTA buffered composition showed the lowest level of aggregation and lowest relative increase in aggregation.

Example 4

A study was conducted to evaluate the effect of buffer concentration and presence/absence of other excipients on anti-MAdCAM antibody 7.16.6 fragmentation.

The compositions shown in Table 4 were prepared by the methods described in Example 2 and evaluated by the methods in Example 3.

Table 4:

Composition ID	7.16.6 mAb mg/mL	Buffer	Excipient	% fragmentation
4	8 ±1	20 mM Sodium acetate, pH 5.5	NaCl, 140 mM	0.9
5	8 ±1	20 mM Sodium acetate, pH 5.5	Mannitol, 45 mg/ml	0.6
6	8 ±1	10 mM Sodium acetate, pH 5.5	-	0.5
7	8 ±1	10 mM Sodium EDTA, pH 5.5	-	0
8	8 ±1	10 mM Sodium EDTA, pH 5.5	NaCl, 140 mM	0
9	8 ±1	10 mM Sodium EDTA, pH 5.5	Mannitol, 45 mg/ml	0

As shown in Table 4, the presence of EDTA in the liquid compositions results in less LMM formation than in compositions containing no EDTA.

Example 5

A study was conducted to assess the effect of EDTA, in liquid anti-MAdCAM antibody compositions, on aggregation and fragmentation.

The buffer solutions were prepared by the methods described in Example 3. The antibody compositions were prepared as follows. An antibody bulk solution was obtained as 9.6 mg/ml in 20 mM sodium acetate buffer pH 5.5. Buffer exchanges of this bulk solution into the composition solutions were carried out by centrifugation at 5000xg using a molecular weight cut-off membrane (e.g. 30 kD). Approximately 8 volume exchanges were made and the final antibody solution was prepared at about 8 mg/ml or about 30 mg/ml protein concentration. Antibody concentrations were determined by Ultraviolet-Visible spectrometry (UV-Vis) method using an extinction coefficient of 1.56 $(mg/ml)^{-1}$ cm^{-1} at 280 nm. A concentrate solution of polysorbate 80 (PS80) (typically 20 mg/ml) was prepared by dilution and dissolution of PS80 by the appropriate buffer. The PS80 concentrate was then added to the antibody solutions to

obtain the final compositions described. The compositions with all ingredients were then sterilized by filtration through sterile 0.22 micron membrane filter. The filtered compositions were then filled into washed, autoclaved vials. The vials were closed with Daikyo 777-1 Flurotec® coated stoppers, crimp sealed, and placed in stability chambers.

The compositions in Table 5 were stored at 40°C for 26 weeks, and evaluated by the SEC method described in Example 3.

Table 5

Composition ID	7.16.6 mAb mg/mL	Buffer (pH 5.5)	Excipient	PS-80, mg/mL	Other excipients	% Aggregate	% fragmentation
10	8±2	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.2	-	6.9	0.6
11	8±2	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.2	$Na_2EDTA.2H_2O$, 0.02 mg/mL	3.7	0
12	8±2	Sodium acetate, 20 mM, pH 5.5	NaCl, 8.2 mg/mL	0.2	-	6.0	0.5
13	8±2	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.2	$CaCl_2.2H_2O$, 0.3 mg/mL	6.8	0.8
14	30±6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	-	7.2	0.2
15	30±6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	$Na_2EDTA.2H_2O$, 0.02 mg/mL	3.7	0
16	30±6	Sodium acetate, 20 mM, pH 5.5	NaCl, 8.2 mg/mL	0.4	-	7.7	0.4
17	30±6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	$CaCl_2.2H_2O$, 0.3 mg/mL	9.8	0.7

The compositions in Table 6 were stored at 25°C for 26 weeks and evaluated by the SEC method described in Example 3.

Table 6

Composition ID	7.16.6 mAb, mg/mL	Buffer	Excipient	PS-80, mg/mL	Other excipients	% Aggregate
14	30±6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	-	1.6
15	30±6	Sodium acetate, 20 mM, pH 5.5	Mannitol, 45 mg/mL	0.4	$Na_2EDTA.2H_2O$, 0.02 mg/mL	0.7
18	50±6	Histidine 10 mM, pH 5.5	Mannitol, 45 mg/mL	0.2	$Na_2EDTA.2H_2O$, 0.02 mg/mL	0.5

This example shows that the presence of EDTA in liquid composition results in less aggregation and less LMM species formation.

Example 6

A study was conducted to compare the effect of acetate and histidine buffers on aggregation and fragmentation of anti-MAdCAM antibody 7.16.6. The compositions shown in Tables 7 and 8 were prepared by the methods described in Example 5. The compositions in Table 7 were stored at 40°C for 26 weeks and analyzed by the SEC methods described in Example 3.

Table 7:

Composition ID	7.16.6 mAb mg/mL	Buffer	pH	Excipient	PS-80, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	% Aggregate	% fragmentation
19	10±2	Sodium acetate, 20 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	3.8	0
20	10±2	Histidine 10 mM	6.0	Mannitol, 45 mg/mL	0.2	0.02	1.2	0
21	10±2	Histidine 10 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	1.5	0
22	50±6	Sodium acetate, 20 mM	5.5	Mannitol, 45 mg/mL	0.4	0.02	5.2	0

Composition ID	7.16.6 mAb mg/mL	Buffer	pH	Excipient	PS-80, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	% Aggregate	% fragmentation
23	50±6	Histidine 10 mM	6.0	Mannitol, 45 mg/mL	0.2	0.02	2.3	0
18	50±6	Histidine 10 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	2.8	0.1

The compositions in Table 8 were stored at 25°C for 52 weeks, and analysed by the SEC methods described in Example 3.

Table 8:

Composition ID	7.16.6 mAb mg/mL	Buffer	pH	Excipient	PS-80, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	% Aggregate	% fragmentation
19	10±2	Sodium acetate, 20 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	0.9	0
20	10±2	Histidine 10 mM	6.0	Mannitol, 45 mg/mL	0.2	0.02	0.4	0
21	10±2	Histidine 10 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	0.3	0
22	50±6	Sodium acetate, 20 mM	5.5	Mannitol, 45 mg/mL	0.4	0.02	1.3	0
23	50±6	Histidine 10 mM	6.0	Mannitol, 45 mg/mL	0.2	0.02	0.8	0
18	50±6	Histidine 10 mM	5.5	Mannitol, 45 mg/mL	0.2	0.02	0.9	0

This example shows that the compositions using histidine as a buffer have less aggregate formation than the compositions using acetate at the same pH.

Example 7

A study was conducted to evaluate the aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions at various antibody concentrations. In this study the compositions in Table 9 were prepared by the methods described in Example 5. The compositions in Table 9

were stored at 5°C, 25°C or 40°C for 26 weeks and then analyzed by the SEC methods described in Example 3.

Table 9

Composition ID	Buffer, mM	pH	mAb 7.16.6, mg/mL	Trehalose.$2H_2O$, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	% Aggregation at 5C	% Aggregation at 25C	% Aggregation at 40C
25	His, 10	5.5	47	90	0.05	0.2	0.3	0.6	2.7
26	His, 10	5.5	75	90	0.05	0.2	0.4	0.7	3.6
27	His, 10	5.5	99	90	0.05	0.2	0.5	0.8	4.4
28	His, 10	5.5	145	90	0.05	0.2	0.7	1.2	6.0
29	His, 10	5.5	183	90	0.05	0.2	0.6	1.3	7.5

As shown in Table 9, the propensity for aggregation increases with increasing concentrations of antibody. However, after storage for 26 weeks, the stabilizing effect of the compositions shown in Table 9 is demonstrated by the accelerated condition data (storage at 25°C and 40°C) that shows relatively low levels of aggregation with high concentration compositions.

Example 8

A study was conducted to evaluate the aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions with various levels of EDTA. The compositions were prepared as described above in example 5, except that the final antibody concentration ws adjusted to 80±10 mg/ml. The compositions in Table 10 were stored for 26 weeks at 5°C or 26°C and then analyzed by SEC as described above.

Table 10

Composition ID	Buffer, mM	pH	Trehalose.$2H_2O$, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	% Aggregation at 5C	% Aggregation at 25C
30	His, 10	5.5	90	0.02	0.2	0.6	0.9
26	His, 10	5.5	90	0.05	0.2	0.4	0.7
31	His, 10	5.5	90	0.10	0.2	0.5	0.8

As shown, there is an improvement at 0.05 mg/ml and 0.10 mg/mL relative to 0.02 mg/mL EDTA, and in each case, aggregation in the presence of EDTA is low after storage at 5°C for 26 weeks.

Example 9

A study was conducted to evaluate stability of compositions of anti-MAdCAM antibody 7.16.6 with various levels of Polysorbate-80. The compositions were prepared as described above, but with the final antibody concentration adjusted to 80± mg/ml. The compositions in Table 11 were stored for 26 weeks at 25°C or 40°C and then analyzed by SEC as described above.

Table 11

Composition ID	Buffer, mM	pH	Trehalose.$2H_2O$, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	% Aggregation at 25C	% Aggregation at 40C
26	His, 10	5.5	90	0.05	0.2	0.7	3.6
32	His, 10	5.5	90	0.05	0.4	0.8	4.2
33	His, 10	5.5	90	0.05	1.0	1.0	4.8

The compositions in Table 12 were subjected to shaking stress applied by orbital shaking at 300rpm for 24 hours at ambient temperature. The compositions were prepared as described above, but with final antibody concentration adjusted to 85±mg/ml

Table 12

Composition ID	Buffer, mM	pH	Trehalose.$2H_2O$, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	Appearance
34	His, 10	5.5	90	0.10	0.2	Presence of few particulates
35	His, 10	5.5	90	0.10	0.4	No particulates
36	His, 10	5.5	90	0.10	1.0	No particulates

Although the above-described storage stability study shows a slight increase in soluble aggregation levels with increasing levels of polysorbate-80, the shaking stress study indicates that a polysorbate-80 level of 0.4 mg/mL provides adequate protection from shaking stress.

Example 10

A study was undertaken to evaluate aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions with various buffers. The compositions were prepared as described above, and adjusted to a final concentration of antibody of 80±10 mg/ml. The compositions in Table 13 were stored at 25°C or 40°C for 26 weeks.

Table 13

Composition ID	Buffer, mM	pH	Trehalose.$2H_2O$, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	% Aggregation at 25C	% Aggregation at 40C
26	His, 10	5.5	90	0.05	0.2	0.7	3.6
37	Citrate, 5	5.5	90	0.05	0.2	1.7	6.0
38	Succinate, 5	5.5	90	0.05	0.2	1.0	4.9

The level of aggregation was lowest in the composition with histidine buffer.

Example 11

A study was undertaken to evaluate aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions with various sugars and polyols. The compositions were prepared as described above, and adjusted to a final antibody concentration of 80±10 mg/ml.. The compositions in Table 14 were stored at 40°C for 26 weeks.

Table 14

Composition ID	Buffer, mM	pH	Sugar/Polyol, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	% Aggregation at 40C
26	His, 10	5.5	Trehalose.$2H_2O$, 90	0.05	0.2	3.6
39	His, 10	5.5	Sucrose, 85	0.05	0.2	4.8
40	His, 10	5.5	Sorbitol, 45	0.05	0.2	4.8
41	His, 10	5.5	Xylitol, 35	0.05	0.2	4.6

The level of aggregation was lower with the composition containing trehalose.

Example 12

A study was undertaken to evaluate antibody aggregation propensity of anti-MAdCAM antibody 7.16.6 in compositions with various surfactants and PEG. The compositions were prepared as described above, and adjusted to a final antibody concentration of 80±10 mg/ml. The final concentration of surfactant of PEG in the antibody compositions was achieved by addition of an appropriate quantity from concentrate stock solutions of surfactant or PEG. The compositions in Table 15 were stored at 40°C for 26 weeks.

Table 15

Composition ID	Buffer, mM	pH	Trehalose. $2H_2O$, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	Surfactant or PEG, mg/mL	% Aggregation at 40C
26	His, 10	5.5	90	0.05	PS80, 0.2	3.6
42	His, 10	5.5	90	0.05	PEG_{3350}, 10	3.9
43	His, 10	5.5	90	0.05	NOF PS80, 0.2	3.9
44	His, 10	5.5	90	0.05	Poloxamer 407, 1.0	3.4
45	His, 10	5.5	90	0.05	Poloxamer 188, 1.0	4.1

Compositions containing PS80 and Poloxamer 407 performed marginally better thanthe other surfactants and amphiphiles.

Example 13

A study was undertaken to evaluate Met256 oxidation in compositions with either trehalose or sucurose. The compositions were prepared as described above, and adjusted to a final antibody concentration of 80±10 mg/ml. The compositions in Table 16 were stored at 5°C or 40°C for 26 weeks. Methionine oxidation was measured by enzymatically digesting the protein using Lysyl endoproteinase and the resulting peptide fragments were separated by reversed-phase HPLC with 214 nm absorbance detection. The peptide fragment containing methionine or its oxidized form was monitored. Percentage oxidation was calculated by peak area of oxidized methionine relative to that of parent methionine.

Table 16

Composition ID	Buffer, mM	pH	Sugar, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	% Met256 oxidation at 5C	% Met256 oxidation at 40C
26	His, 10	5.5	Trehalose.$2H_2O$, 90	0.05	0.2	3.3	7.2
32	His, 10	5.5	Trehalose.$2H_2O$, 90	0.05	0.4	3.2	7.3
39	His, 10	5.5	Sucrose, 85	0.05	0.2	3.2	9.0

The compositions containing trehalose show lower propensity for methionine oxidation relative to those containing sucrose.

Example 14

A study was undertaken to evaluate the chemical stability performance of high antibody concentration compositions. The compositions in Tables 17 and 18 were prepared as described above, and adjusted to a final antibody concentration of 80±10 mg/ml. The compositions in Table 17 were stored at 5°C for 26 weeks. Chemical stability was assessed by

iCE. Measurements were conducted by preparing the protein mix with pI markers, methylcellulose, and pharmalytes to a final protein concentration of approximately 0.22µg/µL. The electrophoresis run was performed with focusing time of 6 min. at 3000 volts and absorbance probe at 280nm. Relative percentage of various charged species was determined by their respective area under the peak.

Table 17

Composition ID	Buffer, mM	pH	Trehalose.$2H_2O$, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	% Major band by iCE (initial)	% Major band by iCE (26 weeks)
26	His, 10	5.5	90	0.05	0.2	66.5	67.9
32	His, 10	5.5	90	0.05	0.4	66.6	67.3
31	His, 10	5.5	90	0.10	0.2	65.7	65.5

The compositions in Table 17 show good chemical stability, as no significant change in the major species as well as in total acidic species or total basic species.

The compositions in Table 18 were stored at 5°C for 26 weeks, and assayed by reduced SDS-PAGE to determine purity. The SDS-PAGE gels were run using NuPAGE 4-12% Bis-Tris gel, and colloidal blue (Coomassie blue) stain. For the reduced gels, reduction was achieved by Nu-PAGE reducing agent. Percent purity in reduced gels was estimated densitometrically by: %purity=(%heavy chain+%light chain).

Table 18

Composition ID	Buffer, mM	pH	Trehalose.$2H_2O$, mg/mL	$Na_2EDTA.2H_2O$, mg/mL	PS80, mg/mL	% Purity by reduced SDS-PAGE (initial)	% Purity by reduced SDS-PAGE (26 weeks)
26	His, 10	5.5	90	0.05	0.2	99.7	98.8
32	His, 10	5.5	90	0.05	0.4	99.7	98.8
31	His, 10	5.5	90	0.10	0.2	99.7	98.8

The compositions in Table 18 show no significant change in purity after storage for 26 weeks at 5°C, indicating good chemical stability.

Example 15

A study was undertaken to evaluate the performance of high concentration compositions against freeze-thaw stress.

The compositions in Table 19 were prepared as described above, and adjusted to a final antibody concentration of 50±10 mg/ml. The compositions in Table 19 were subjected to three freeze-thaw cycles at -70°C/5°C or -20°C/5°C. The studies were conducted in 2ml glass vials with 1ml fill. SE_HPLC measurements were conducted using 0.2M sodium phosphate, pH 7 mobile phase, TSK gel G3000SWXL columns, at a flow rate of 0.7ml/min, probe at 214 nm. Aggregate quantity was determined by summing antibody related peaks that eluted prior to the antibody monomer.

Table 19

Composition ID	Composition Description	Freeze-thaw cycle	Appearance after three freeze-thaw cycles	Change in % soluble aggregation after three freeze-thaw cycles
46	50 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, 0.2 mg/mL polysorbate 80	-70C/5C	Clear; No particulates	0.0
47	50 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, 0.2 mg/mL polysorbate 80	-20C/5C	Clear; No particulates	0.0

The compositions in Table 19 show no increase in aggregation after 3 freeze-thaw cycles at either -70°C/5°C or -20°C/5°C.

The compositions in Table 20 were prepared as described above, and adjusted to a final antibody concentration of 75±15 mg/ml. The compositions in Table 20 were subjected to four freeze-thaw cycles at -20°C/5°C. These freeze-thaw studies were conducted in 10ml glass vials with 10ml fill. SEC measurements were conducted as indicated above in this example.

Table 20

Composition ID	Composition Description	Freeze-thaw cycle	Appearance after four freeze-thaw cycles	Change in % soluble aggregation after four freeze-thaw cycles
48	75 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, 0.4 mg/mL polysorbate 80	-20C/5C	Clear; No particulates	0.0
49	75 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, 0.2 mg/mL polysorbate 80	-20C/5C	Clear; No particulates	0.0
50	75 mg/mL mAb 7.16.6, 10 mM histidine, pH 5.5, 85 mg/mL sucrose, 0.05 mg/mL disodium EDTA dihydrate, 0.4 mg/mL polysorbate 80	-20C/5C	Clear; No particulates	0.0

The compositions in Table 20 show no increase in aggregation after four freeze-thaw cycles at -20°C/5°C.

Example 16

A study was undertaken to assess the stability of a high concentration composition during frozen storage. The composition in Table 21 was prepared as described above, and final antibody concentration was adjusted to about 75 mg/ml. The composition in Table 21 was stored at -20°C for 13 weeks, and aggregation assessed as described in Example 15.

Table 21

Composition ID	Buffer, mM	pH	Trehalose.2 H_2O, mg/mL	$Na_2EDTA.2$ H_2O, mg/mL	PS80, mg/mL	% Increase in aggregation at -20C
51	His, 10	5.5	90	0.05	0.2	0.0

The high concentration (75mg/ml antibody) composition shows no increase in aggregation after 13 weeks of storage at -20°C.

Example 17

A study was conducted to assess the viscosity of a high concentration composition. The composition in Table 22 was prepared as described above, and final antibody concentration

was adjusted to about 75 mg/ml. Viscosity measurements were conducted by applying an average shear rate of 300^{s-1} to the composition placed on a rheometer plate.

Composition 52:	mAb 7.16.6, mg/mL	Viscosity at 5C, cP
10 mM histidine, pH 5.5, 90 mg/mL trehalose dihydrate, 0.10 mg/mL disodium EDTA dihydrate, 0.4 mg/mL polysorbate 80	75	5.7

The composition shows a viscosity suitable for subcutaneous administration.

SEQUENCES
Signal Sequence: underlined

SEQ ID NO. 1
7.16.6 Heavy Chain Nucleotide Sequence

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 151    TATGGTATCA ACTGGGTGCG ACAGGCCCCT GGACAAGGGC TTGAGTGGAT
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 301    GACCTGAGGA GCCTGAGATC TGACGACACG GCCGTGTATT ACTGTGCGAG
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 401    GCCAAGGGAC CACGGTCACC GTCTCCTCAG CCTCCACCAA GGGCCCATCG
 451    GTCTTCCCCC TGGCGCCCTG CTCCAGGAGC ACCTCCGAGA GCACAGCGGC
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 851    TGGTGGTGGA CGTGAGCCAC GAAGACCCCG AGGTCCAGTT CAACTGGTAC
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SEQ ID NO. 2
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 151    VFPLAPCSRS TSESTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL
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 251    PCPAPPVAGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVQFNWY
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SEQ ID NO. 3
7.16.6 Kappa Light Chain Nucleotide Sequence

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601  AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA
651  CGCCTGCGAA GTCACCCATC AGGGCCTGAG CTCGCCCGTC ACAAAGAGCT
701  TCAACAGGGG AGAGTGTTAG TGA
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SEQ ID NO. 4
7.16.6 Kappa Light Chain Protein Sequence

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101  SRVEAEDVGI YYCMQNIQLP WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL
151  KSGTASVVCL LNNFYPREAK VQWKVDNALQ SGNSQESVTE QDSKDSTYSL
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SEQ ID NO:5
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WHAT IS CLAIMED IS:

1. A composition comprising:

at least one chelating agent; and .

at least one antibody comprising:

an amino acid sequence that is at least 99% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2; and

an amino acid sequence that is at least 99% identical to a light chain amino acid sequence shown in SEQ ID NO: 4;

wherein the antibody binds to human MAdCAM.

2. The composition according to claim 1, wherein the composition is a liquid composition and the antibody is a human IgG2 antibody and the antibody does not comprise a signal sequence.

3. The composition according to claim 1, wherein the antibody comprises a heavy chain amino acid sequence comprising SEQ ID NO: 2 and a light chain amino acid sequence comprising SEQ ID NO: 4.

4. The composition according to claim 1, wherein the composition comprises at least one chelating agent that is EDTA.

5. The composition according to claim 1, wherein the composition further comprises a buffer.

6. The composition according to claim 1, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises histidine.

7. The composition according to claim 1, wherein the composition further comprises a buffer and a surfactant

8. The composition according to claim 1, wherein the composition further comprises a buffer, a surfactant, and a tonicity agent.

9. The composition according to claim 1, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises a buffer, a surfactant, and a tonicity agent.

10. The composition according to claim 1, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises histidine, a surfactant, and a tonicity agent.

11. The composition according to claim 1, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises histidine, polysorbate 80, and a tonicity agent.

12. The composition according to claim 1, wherein the composition comprises at least one chelating agent that is EDTA, and further comprises histidine, polysorbate 80, and trehalose.

13. The composition according to claim 1, wherein the composition comprises:
from 1 mg/ml to 200 mg/ml of antibody;
from 0.01 millimolar to 5.0 millimolar of a chelating agent; and
from 1 mM to 100 mM of histidine.

14. The composition according to claim 1, wherein the composition comprises:
from 1 mg/ml to 200 mg/ml of antibody;
from 0.01 millimolar to 5.0 millimolar of EDTA; and
from 1 mM io 100 mM of histidine.

15. The composition according to claim 1, wherein the composition comprises:
from 1 mg/ml to 200 mg/ml of antibody;
from 0.01 millimolar to 5.0 millimolar of a chelating agent;
from 1 mM to 100 mM of a buffer;
from 0.005 millimolar to 10 millimolar of a surfactant; and
from 100 millimolar to 400 millimolar of a tonicity agent.

16. The composition according to claim 1, wherein the composition comprises:
from 1 mg/ml to 200 mg/ml of antibody;
from 0.01 millimolar to 5.0 millimolar of EDTA;

from 1 mM to 100 mM of histidine;
from 0.005 millimolar to 10 millimolar of polysorbate 80; and
from 100 millimolar to 400 millimolar of a tonicity agent.

17. The composition according to claim 1, wherein the composition comprises:
from 1 mg/ml to 200 mg/ml of antibody;
from 0.01 millimolar to 5.0 millimolar of EDTA;
from 1 mM to 100 mM of histidine;
from 0.005 millimolar to 10 millimolar of polysorbate 80; and
from 100 millimolar to 400 millimolar of trehalose.

18. The composition according to claim 1, wherein the composition comprises:
from 0.1 mg/ml to 100 mg/ml of antibody;
from 0.001 mg/ml to 1.0 mg/ml of EDTA;
from 1 mM to 50 mM of histidine;
from 0.01 mg/ml to 10 mg/ml of polysorbate 80; and
from 10 mg/ml to 100 mg/ml of trehalose.

19. A liquid pharmaceutical composition comprising:
at least one antibody comprising an amino acid sequence that is at least 99% identical to a heavy chain amino acid sequence shown in SEQ ID NO: 2, and further comprising an amino acid sequence that is at least 99% identical to a light chain amino acid sequence shown in SEQ ID NO: 4, wherein the antibody binds to human MAdCAM;

and a pharmaceutically acceptable excipient, wherein the composition contains a concentration of antibody that is at least 50 mg/ml.

20. A process for preparing a liquid pharmaceutical composition comprising mixing at least one anti-MAdCAM antibody having the heavy chain amino acid sequence of SEQ ID NO:2 and light chain amino acid sequence of SEQ ID NO:4, in solution, with at least one chelating agent.

SEQUENCE LISTING

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tga 723

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<213> Homo sapiens

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

Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser
        35                  40                  45

Leu Leu His Thr Asp Gly Thr Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
    50                  55                  60

Pro Gly Gln Pro Pro Gln Leu Leu Ile Tyr Glu Val Ser Asn Arg Phe
65                  70                  75                  80

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
                85                  90                  95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Ile Tyr Tyr
            100                 105                 110

Cys Met Gln Asn Ile Gln Leu Pro Trp Thr Phe Gly Gln Gly Thr Lys
        115                 120                 125

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

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                 150                 155                 160

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

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
            180                 185                 190

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

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
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Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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Met Asp Phe Gly Leu Ala Leu Leu Leu Ala Gly Leu Leu Gly Leu Leu
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Leu Gly Gln Ser Leu Gln Val Lys Pro Leu Gln Val Glu Pro Pro Glu
20 25 30

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

Leu Ala Cys Ala Asp Arg Gly Ala Ser Val Gln Trp Arg Gly Leu Asp
50 55 60

Thr Ser Leu Gly Ala Val Gln Ser Asp Thr Gly Arg Ser Val Leu Thr
65 70 75 80

Val Arg Asn Ala Ser Leu Ser Ala Ala Gly Thr Arg Val Cys Val Gly
85 90 95

Ser Cys Gly Gly Arg Thr Phe Gln His Thr Val Gln Leu Leu Val Tyr
100 105 110

Ala Phe Pro Asp Gln Leu Thr Val Ser Pro Ala Ala Leu Val Pro Gly
115 120 125

Asp Pro Glu Val Ala Cys Thr Ala His Lys Val Thr Pro Val Asp Pro
130 135 140

Asn Ala Leu Ser Phe Ser Leu Leu Val Gly Gly Gln Glu Leu Glu Gly
145 150 155 160

Ala Gln Ala Leu Gly Pro Glu Val Gln Glu Glu Glu Glu Glu Pro Gln
165 170 175

Gly Asp Glu Asp Val Leu Phe Arg Val Thr Glu Arg Trp Arg Leu Pro
180 185 190

Pro Leu Gly Thr Pro Val Pro Pro Ala Leu Tyr Cys Gln Ala Thr Met
195 200 205

Arg Leu Pro Gly Leu Glu Leu Ser His Arg Gln Ala Ile Pro Val Leu
210 215 220

His Ser Pro Thr Ser Pro Glu Pro Pro Asp Thr Thr Ser Pro Glu Ser
225 230 235 240

Pro Asp Thr Thr Ser Pro Glu Ser Pro Asp Thr Thr Ser Gln Glu Pro
245 250 255

Pro Asp Thr Thr Ser Gln Glu Pro Pro Asp Thr Thr Ser Gln Glu Pro
260 265 270

Pro Asp Thr Thr Ser Pro Glu Pro Pro Asp Lys Thr Ser Pro Glu Pro
275 280 285

Ala Pro Gln Gln Gly Ser Thr His Thr Pro Arg Ser Pro Gly Ser Thr
290 295 300

Arg Thr Arg Arg Pro Glu Ile Gln Pro Lys Ser Cys Asp Lys Thr His
305 310 315 320

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
325 330 335

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
340 345 350

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
355 360 365

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
370 375 380

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
385 390 395 400

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
405 410 415

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
420 425 430

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
435 440 445

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
450 455 460

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
465 470 475 480

Gly Gln Pro Glu Asn Asn Tyr Lys Ala Thr Pro Pro Val Leu Asp Ser
485 490 495

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
500 505 510

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
515 520 525

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
530 535 540