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(54) Title: HUMANIZED ANTIBODIES TARGETING THE EC1 DOMAIN OF CADHERIN-11 AND RELATED COMPOSITIONS AND METHODS

(57) Abstract: The present invention relates to humanized antibodies that specifically bind an EC1 domain of a mammalian Cadherin-11 protein and compositions (e.g., pharmaceutical compositions) comprising such antibodies. The invention also relates to methods for treating Cadherin-11 -mediated disorders in a mammalian subject by administering a therapeutically effective amount of a humanized antibody of the invention. Cadherin-11 -mediated disorders suitable for treatment by the methods of the invention include inflammatory disorders (e.g., inflammatory joint disorders, such as rheumatoid arthritis), fibrosis and cancer.

HUMANIZED ANTIBODIES TARGETING THE EC1 DOMAIN OF CADHERIN-11 AND RELATED COMPOSITIONS AND METHODS

RELATED APPLICATION(S)

This application claims the benefit of U.S. Provisional Application No. 61/364,698, filed on July 15, 2010. The entire teachings of the above application(s) are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Patients with advanced chronic joint inflammation suffer from severe joint deterioration including bone and cartilage destruction, resulting in long-term pain, deformity, loss of joint function, reduced mobility and shortened life expectancy. Joint inflammation is associated with an increased number of cells and inflammatory substances in the joint, which cause irritation, wearing down of cartilage and swelling of the joint lining. Several different autoimmune disorders are known to trigger inappropriate or misdirected inflammation in a joint, resulting in chronic inflammation in the joints of individuals who suffer from these disorders. Common inflammatory joint disorders include rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome and ankylosing spondylitis.

Rheumatoid arthritis (RA) is the most common form of inflammatory arthritis and is estimated to affect approximately 1 percent of the U.S. population, or about 2.1 million Americans. RA is a chronic disease that is characterized by inflammation of the lining, or synovium, of the joints, and can lead to significant bone and cartilage damage over time. RA is more common in women than in men and as many as 3% of women may develop rheumatoid arthritis in their lifetime. Currently, the cause of RA is unknown.

RA can lead to long-term joint damage, resulting in chronic pain, loss of function and disability. In addition, recent research indicates that people with RA, particularly those whose disease is not well controlled, may have a higher risk for heart disease, stroke and the development of fibrosis, particularly lung fibrosis.

Lung fibrosis, including idiopathic pulmonary fibrosis and radiation-induced fibrosis, as well as fibrosis caused by smoking, chemical exposure, scleroderma, sarcoidosis, therapeutic radiation, RA, or lupus, afflicts 5,000,000 patients worldwide, and over 500,000 individuals in the U.S. The 5-year mortality from idiopathic pulmonary fibrosis is approximately 80%, accounting for 40,000 deaths per year in the U.S. alone. There currently is no approved therapy for idiopathic pulmonary fibrosis in the U.S. or Europe.

Thus, fibrosis and chronic joint inflammation caused by RA and other inflammatory conditions are international health burdens. There is a need to develop new agents for the prevention and treatment of these and other disorders.

SUMMARY OF THE INVENTION

The present invention relates, in one embodiment, to a humanized antibody that specifically binds an EC1 domain of a mammalian Cadherin-11 protein, comprising an antibody variable heavy chain region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67 and SEQ ID NO:69. In a particular embodiment, the humanized antibody antagonizes the mammalian Cadherin-11 protein. In a further embodiment, the humanized antibody comprises an antibody variable heavy chain region comprising SEQ ID NO:69.

In another embodiment, the invention relates to a humanized antibody that specifically binds an EC1 domain of a mammalian Cadherin-11 protein, comprising an antibody variable heavy chain region comprising SEQ ID NO:69 and an antibody variable light chain region comprising SEQ ID NO:71. In a particular embodiment, the humanized antibody antagonizes the mammalian Cadherin-11 protein.

In an additional embodiment, the invention provides a method of treating a Cadherin-11-mediated disorder (*e.g.*, an inflammatory joint disorder) in a mammalian subject (*e.g.*, a human) in need thereof. The method comprises administering to the subject a therapeutically effective amount of a humanized antibody that specifically binds an EC1 domain of a mammalian Cadherin-11 protein and antagonizes the mammalian Cadherin-11 protein, thereby resulting in a desired therapeutic effect in the subject. In a particular embodiment, the humanized antibody comprises an antibody variable heavy chain region comprising SEQ ID NO:69. In a preferred embodiment, the Cadherin-11-mediated disorder is rheumatoid arthritis.

In yet another embodiment, the invention relates to a pharmaceutical composition comprising a humanized antibody that specifically binds an EC1 domain of a mammalian Cadherin-11 protein and a pharmaceutically-acceptable carrier. In a particular embodiment, the humanized antibody comprises an antibody variable heavy chain region comprising SEQ ID NO:69. In a further embodiment, the pharmaceutical composition further comprises a second agent, such as a disease-modifying anti-rheumatic drug or an anti-inflammatory agent.

The present invention provides new agents and therapies having improved efficacy for the treatment of inflammation and other conditions mediated by Cadherin-11 in humans.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIG. 1A is a Western blot showing detection of a Cadherin-11-EC1-5-Fc fusion protein using anti-Cad-11 antibodies 23C6, 13C2 and 27F3 (see solid arrows). These antibodies did not recognize the Cadherin-11-EC1-Fc and Cadherin-11-EC1/2-Fc fusion proteins that were also present on the membrane (see open arrows for positions of the Cadherin-11-EC1-Fc and Cadherin-11-EC1/2-Fc proteins on the blot).

FIG. 1B is a graph depicting the binding of public Cadherin-11 antibodies 13C2, 23C6 and 5F82 to human Cad-11-EC1-5-Fc fusion protein, but not the Cad-11-EC1-Fc fusion protein, as determined by ELISA. In contrast, the EC1 antibody H1M1 binds both Cad-11-EC1-Fc fusion protein and the Cad-11-EC1-5-Fc fusion protein.

FIG. 2 is an amino acid sequence alignment of the first 34 amino acids of the EC1 domains of human Cad-11 (SEQ ID NO:3), MN-Cad (SEQ ID NO:4), and Cad-8 (SEQ ID NO:5) that are involved in cadherin binding. Donor sequences containing residues that extend into the pocket of a cadherin counter-receptor are indicated by underlining of the left half of sequence and residues of the pocket sequence are indicated by the underlining of the right half of sequence in SEQ ID NO:3.

FIG. 3 is a graph depicting the binding of a Cadherin-11-binding Fab to a human Cad-11 EC1 domain peptide as well as the Cad-11-EC1-Fc fusion protein, but not the Cad-8 or MN-Cad EC1 domain peptides, as determined by ELISA. Clone 7 demonstrated significant binding to the Cad-11 EC1 domain peptide and fusion protein, but not the MN-Cad or Cad-8 EC1 domain peptides.

FIG. 4 is a graph depicting data from an *in vitro* Cad-11 cell aggregation assay. Cad-11 antagonists added to the media, such as a Fab made from the anti-Cad-11 antibody 13C2, or varying concentrations of an anti-Cadherin-11 EC1 Fab directed to the first 35 amino acids of the EC1 domain of Cadherin-11 (designated EC1 Fab clone 7), block Cad-11 mediated 431-D-11 cell aggregation. The anti-Cadherin-11 EC1 Fab (clone 7) inhibited aggregation of A-431-D-11 epidermoid carcinoma cells at all concentrations tested in a range of 0.3 μg/ml to 10 μg/ml. In contrast, the Fab made from the 13C2 anti-Cadherin-11 antibody only inhibited cell aggregation at a concentration of 10 μg/ml.

FIG. 5 is a graph depicting data from a second *in vitro* Cad-11 cell aggregation assay. Percent aggregation of 431-D-11 cells is shown at 40 min. after addition of either SME media (designated control), varying concentrations of a fusion protein comprising the EC1 domain of Cad-11 fused to the human IgG2 hinge, CH2 and CH3 domains (designated Cad-11-EC1-Fc), varying concentrations of an anti-Cadherin-11 EC1 Fab directed to the first 35 amino acids of the EC1 domain of Cadherin-11 (designated Cad-11 EC1 Fab) or varying concentrations of a control anti-green fluorescent protein (anti-GFP) Fab (designated GFP fAb). The anti-Cadherin-11 EC1 Fab (clone 7) inhibited aggregation of Cad-11 expressing 431-D-11 cells at concentrations of 3 μg/ml, 1 μg/ml and 0.1 μg/ml. The EC1-Fc fusion protein inhibited aggregation of 431-D-11 cells at concentrations of 3 μg/ml.

In contrast, the anti-GFP Fab failed to inhibit cell aggregation significantly at any of the test concentrations.

FIG. 6 is a graph depicting inhibition of Cad-11 mediated cell aggregation by various anti-Cadherin-11 EC1 Fabs that have binding specificity for Cad-11 alone (EC1 fAb clone 7 and clone 4), Cad-11 and Cad-8 (EC1 fAb clone 6), or Cad-11 and MN-Cad (EC1 fAb clone 5), using an *in vitro* cell aggregation assay. All Fabs tested inhibited 431-D-11 cell aggregation relative to the control (D-11 SME; left bar).

FIG. 7 is a graph depicting inhibition of Cad-11 mediated cell aggregation by anti-Cad-11 Fabs that have binding specificity for Cad-11 alone (EC1 fAb clone 7), or Cad-11 and MN-Cad (EC1 fAb clone 8), using an *in vitro* cell aggregation assay. The specificity of the Fabs tested is shown in parentheses next to each Fab designation. Both cadherin-specific Fabs inhibited cell aggregation (middle and right bars) relative to a control Fab that was specific for GFP (left bar).

FIG. 8 shows the nucleotide (DNA) sequence (SEQ ID NO:6) of the human Cad-11-EC1-hIgG2-Fc1 fusion protein (Cad-11-EC1-Fc). The sequence of the human Cadherin-11 extracellular domain is shown in italics, the BglII site is underlined, and the sequence encoding the human IgG_2-Fc1 region is shown in bold lettering.

FIG. 9 shows the amino acid sequence (SEQ ID NO:7) of the human Cad-11-EC1-hIgG2-Fc1 fusion protein (Cad-11-EC1-Fc). The sequence of the human Cadherin-11 extracellular domain is shown in italics, the sequence encoded by the BglII site is underlined, and the sequence of the human IgG_2-Fc1 region is shown in bold lettering.

FIG. 10 is an image of an SDS polyacrylamide gel that has been stained with Coomassie Blue, which shows the predominant intense bands corresponding to the monomeric form of the purified Cad-11-EC1-$hIgG_2$-Fc1 (middle lane) and Cad-11-EC1/2-$hIgG_2$-Fc1 (right lane) fusion proteins, respectively, following purification from cell culture medium using a protein A column. Molecular weight standards are shown in the left lane.

FIG. 11 is a Western blot showing the detection of human Cad-11-EC1-$hIgG_2$-Fc1 (middle lane) and Cad-11-EC1/2-$hIgG_2$-Fc1 (right lane) fusion proteins using an anti-human IgG antibody conjugated to horse radish peroxidase (HRP). The predominant observed band in each lane corresponds to the locations of the monomeric forms of the fusion proteins. The locations of the dimeric forms of the fusion proteins are also visible (see less intense higher molecular weight bands), due to incomplete reducing conditions. Molecular weight standards are shown in the left lane.

FIG. 12 is a graph depicting that a Cad-11-EC1-Fc fusion protein and a mouse anti-Cad-11 antibody, 13C2, inhibit the invasion of Cad-11 expressing human fibroblast-like synoviocytes into a matrigel plug at the indicated concentrations compared to untreated cells, labeled *Invasion*. Data is pooled from two independent experiments.

FIGS. 13A and B are graphs depicting data from two *in vitro* Cad-11 cell aggregation assays. Percent aggregation of Cad-11 expressing 431-D-11 cells is shown at 40 min. after addition of either SME media (designated control) or a Cad-11 fusion protein. FIG. 13A shows the inhibition of aggregation in the presence of varying concentrations of a fusion protein comprising the 5 extracellular domains of Cad-11 fused to the human IgG2 hinge, CH2 and CH3 domains (designated Cad-11-EC1-5-Fc). FIG. 13B shows the inhibition of aggregation of varying concentrations of a fusion protein comprising either the N-terminal extracellular domain (EC1 domain) of Cad-11 fused to the human IgG2 hinge, CH2 and CH3 domains (designated Cad-11-EC1-Fc) or Cad-11-EC1-5-Fc.

FIGS. 14A-C show the human Cadherin-11 cDNA sequence (SEQ ID NO:1; see Genbank Accession No. NM001797).

FIG. 15 shows the human Cadherin-11 protein sequence (SEQ ID NO:2; see Genbank Accession No. NP001788).

FIG. 16 is a graph depicting the level of binding of antibodies in media from peptide 4 hybridomas (HL), or control hybridoma media (Media), to proteins containing the EC1-2 domains of Cad-11, Cad-8 or MN-Cad, as determined by ELISA.

FIGS. 17A-C are representative graphs depicting the intensity of cell staining (MFI; mean fluorescence intensity) as a measure of binding of H14 antibody to Cad-11-expressing 431-D-11 cells.

FIGS. 17D-F are representative graphs depicting the absence of 431-D cell staining (MFI; mean fluorescence intensity) relative to FIGS. 17A-C, indicating a lack of binding of H14 antibody to the Cad-11 negative cells.

FIGS. 17G-I are representative graphs depicting the intensity of cell staining (MFI; mean fluorescence intensity) as a measure of binding of H1M1 antibody to Cad-11-expressing 431-D-11 cells.

FIG. 18A is a graph depicting the binding of H14 antibody to Cad-11-expressing cells, and the absence of H14 binding to Cad-11 negative control cells, at varying concentrations of antibody, as measured by the intensity of cell staining (MFI; mean fluorescence intensity).

FIG. 18B is a graph depicting the binding of H1M1 antibody to Cad-11-expressing cells, and the absence of binding of H1M1 to Cad-11 negative control cells, at varying concentrations of antibody, as measured by the intensity of cell staining (MFI; mean fluorescence intensity).

FIG. 19A is a graph depicting the degree of binding of the H14 anti-Cad-11 antibody to Cad-11 and Cad-8 EC1 domain peptides at various antibody concentrations, as determined by ELISA.

FIG. 19B is a graph depicting the absence of binding of the H14 anti-Cad-11 antibody to Cad7, MNCad, Cad9, Cad18, Cad20 or Cad24 EC1 domain peptides at various antibody concentrations, as determined by ELISA.

FIG. 20 is a graph depicting the binding of the H1M1 anti-Cad-11 antibody to Cad-11, Cad-8, Cad-7, MN-Cad, Cad-9, Cad-18, Cad-20 and Cad-24 EC1 domain peptides at varying antibody concentrations, as determined by ELISA.

FIG. 21A is a graph depicting the degree of binding of the H1M1 anti-Cad-11 antibody to various Cad-11 EC1 domain peptide immunogens (PEP1, PEP2, PEP3 and PEP4), as well as the Cad-11 EC1 domain fusion protein (EFL) and human IgG control (Fc block), as determined by ELISA.

FIG. 21B is a graph depicting the degree of binding of the H14 anti-Cad-11 antibody to various Cad-11 EC1 domain peptide immunogens (PEP1, PEP2, PEP3 and PEP4), as well as the Cad-11 EC1 domain fusion protein (EFL) and human IgG control (Fc block), as determined by ELISA.

FIG. 22 is a schematic diagram depicting the sequence of the first 37 amino acids of the EC1 domain of human Cadherin-11 and the portions of this sequence encompassed by each of Peptides 1-4. Amino acid residues shared by Peptides 2 and 4 that are upstream of Peptide 3 are highlighted in the boxed region. Amino acids directly involved in Cad-11 to Cad-11 binding are underlined.

FIG. 23A is a photograph showing a large mass of aggregated Cad-11-expressing cells that were treated with a control isotype antibody.

FIG. 23B is a photograph showing small clumps of H1M1-treated Cad-11-expressing cells that did not progress to form the large masses observed in FIG. 23A.

FIG. 23C is a photograph showing that untreated parental Cad-11 negative cells remain as groups of single or double cells.

FIG. 24A is a photograph depicting a culture of Cad-11 expressing cells with large masses of aggregated cells.

FIG. 24B is a photograph depicting a culture of Cad-11 expressing cells with predominantly single cells with small and infrequent cell clusters relative to those shown in FIG. 24A following treatment with the H14 Cad-11 EC1 domain antibody.

FIG. 25 is a graph depicting inhibition of arthritis-associated joint swelling in mice treated with increasing dosages of H1M1 anti-Cad-11 antibody relative to untreated control mice.

FIG. 26 is a graph depicting inhibition of arthritis-associated joint swelling in mice treated with 0.3 mg of either H14 or H1M1 anti-Cad-11 antibodies every second day relative to untreated control mice.

FIG. 27 is a graph showing that treatment with 0.3 mg of either H1M1 or H14 antibody delayed the development of arthritis in a mouse model compared to an untreated control.

FIG. 28 is a graph depicting the degree of binding of antibody-containing media from peptide 3 hybridomas (HL), or control hybridoma media (Media), to the EC1-2 domains of Cad-11, Cad-8, and MN-Cadherin, or a Cad-11 EC1-Fc fusion protein.

FIG. 29 is a graph depicting the degree of binding of anti-Cad-11 antibodies from the peptide 3 hybridomas to cells expressing human Cad-11 protein (see arrow) and non-Cad-11-expressing control cells that expressed Neos.

FIG. 30A is a photograph depicting fibroblast-like synoviocytes (FLS) incubated with media. Cell aggregates and cells that associate through cell processes are visible.

FIG. 30B is a photograph depicting FLS incubated with 30 μg/ml control antibody. Cell aggregates and cells that associate through cell processes are visible.

FIG. 30C is a photograph depicting FLS incubated with 30 μg/ml H1M1 antibody. Few cell aggregates and cell processes are visible.

FIG. 31 is a graph depicting inhibition of FLS invasion into matrigel-coated membrane following contact with H1M1 antibodies relative to controls, including other Cad-11 antibodies that bind outside the EC1 region.

FIG. 32 is a graph depicting inhibition of joint swelling in a KBN mouse model of arthritis following administration of H1M1 antibodies relative to control.

FIG. 33 depicts the H1M1 variable heavy chain nucleotide and deduced amino acid sequences. CDR definitions and protein sequence numbering are according to Kabat. CDR nucleotide and protein sequences are shown in gray.

FIG. 34 depicts the H1M1 variable light chain nucleotide and deduced amino acid sequences. CDR definitions and protein sequence numbering are according to Kabat. CDR nucleotide and protein sequences are shown in gray.

FIGS. 35A-H depict the nucleotide and amino acid sequences of the 5 VH chains (A-E) (SEQ ID Nos.:60-69) and 3 Vκ chains (F-H) (SEQ ID Nos.:70-75) used in the design of H1M1/SYN0012 humanized anti-Cad-11 antibody variants. CDR definitions and protein sequence numbering are according to Kabat. CDR nucleotide and protein sequences are highlighted in red.

FIG. 36 is a vector diagram illustrating the pANTVK and pANTVhG4 vectors. Both pANTVK and pANTVhG4 vectors contain genomic DNA fragments incorporating introns and poly A sequences. Expression of both chains is driven by a CMV promoter. The DHFR mini gene on the heavy chain vector is used for selection.

FIG. 37 depicts a Coomassie Blue-stained SDS-PAGE gel of protein A-purified antibodies. (a) Chimeric anti-Cad-11 EC1 IgG4 (SYN0014); (b) Humanized anti-Cad-11 antibody variants (SDP011-SDP151). Size marker is pre-stained protein standard Fermentas PageRuler (Cat. No. SM1811). Note: SDP111 was not run.

FIGS. 38A-C are graphs depicting the results of an anti-Cad-11 competition ELISA. A dilution series of humanized anti-Cad-11 EC1 antibodies (SDP011 to SDP151) were tested against a fixed concentration of biotinylated murine SYN0012 antibody for binding to a recombinant human Cad-11 fusion protein. Binding of biotinylated antibody decreases with increasing amounts of test and control antibodies. A: SDP011 to SDP051; B: SDP061 to SDP101; C: SDP111 to SDP151.

FIGS. 39A-O are graphs depicting titration of humanized anti-Cad-11 antibodies in a cadherin cross reactivity assay with peptides encompassing the Cad-11 peptide immunogen (red line in each curve) and the corresponding homologous sequences from Cadherins-7, -8, -9, -11, -18, -20, and -24. The peptide coating concentration was 500 ng/ml. A: SDP011; B: SDP021; C: SDP031; D: SDP041; E: SDP051; F: SDP061; G: SDP071; H: SDP081; I: SDP091; J: SDP101; K: SDP111; L: SDP121; M: SDP131; N: SDP141; and O: SDP151.

FIGS. 40A-D depict amino acid sequences of the light and heavy chain variable region domains of the humanized anti-Cad-11 antibodies (A) SDP031 (SEQ ID Nos.:71,65), (B) SDP051 (SEQ ID Nos.:71,69), (C) SDP061 (SEQ ID Nos.:73,61), (D), SDP071 (SEQ ID Nos.:73,63).

FIG. 41A is a graph depicting titration of SDP051 in a cadherin cross reactivity assay with peptides encompassing the Cad-11 peptide immunogen (red) or the corresponding homologous sequences from Cadherins-7, -8, -9, -11, -18, -20, and -24. The peptide coating concentration was 500 ng/ml.

FIG. 41B is a graph depicting titration of SDP051 in a cadherin cross reactivity assay with peptides encompassing the Cad-11 peptide immunogen (red) or the corresponding homologous sequences from Cadherins-8 and -18. The peptide coating concentration was 50 ng/ml.

FIG. 42 is a sensogram plot for SDP051 binding to recombinant human Cad-11 (rhCad-11).

FIG. 43 is a graph depicting antibody concentration dependent binding of humanized anti-Cad-11 EC1 antibodies SDP051 and SDP071 to Cad-11 expressing 431D-11 cells as measured by FACS (Mean Fluorescence Intensity (MFI)). Also shown is the absence of binding to the Cad-11 non-expressing 431D cells.

FIGS. 44A-C are graphs depicting the specificity of SDP051 antibody for Cad-11 using an assay that measures the effect of a peptide encompassing the Cad-11 antigen on the ability of SDP051 antibody to bind to Cad-11 expressing cells relative to peptides encompassing related Cad-20 or Cad-24 (FIG. 44A), Cad-7 or Cad-18 (FIG. 44B), or Cad-8 or Cad-9 (FIG. 44C) antigens, on the ability of SDP051 antibody to bind to Cad-11 expressing cells, as measured by FACS (Mean Fluorescence Intensity (MFI)).

FIGS. 45A and B are graphs depicting the immunogenicity of (A) the murine/human chimeric anti-Cad-11 EC1 antibody SYN0014 and (B) the humanized anti-Cad-11 EC1 antibody SDP051 as determined by an immunogenicity time course T cell assay using PBMC from 25 donors. Results for each triplicate sample were averaged and normalized by conversion to Stimulation Index (SI). The SI for each time point with each donor is shown. The cut-off for determining positive responses with an SI ≥ 2.0 is highlighted by the red line and significant responses ($p<0.05$ in a student's t-test) are indicated (*).

FIG. 46 is a graph showing that SDP-051 at a dose of 0.3 μg/ml inhibits FLS invasion by 50% compared to a 3 μg/ml dose of isotype control antibody (AC1-P).

FIG. 47 is a graph showing that SDP-051 at a dose of 0.3 μg/ml inhibits MMP expression compared to a 3 μg/ml dose of isotype control antibody (AC1-P).

FIG. 48 is a graph illustrating a 47% reduction in joint swelling in SDP051 treated mice relative to mice treated with AC1-P control antibody.

FIG. 49 is a graph depicting serum levels of SDP051 antibody following administration to mice (KBN arthritis model).

FIG. 50 is a graph depicting changes in ankle thickness in mice treated with different doses of SDP051 antibody relative to mice treated with AC3-1P control antibody (murine KBN arthritis model; KBN024 Study).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the terms "Cadherin-11," "Cad-11," and "OB-Cadherin" refer to a naturally occurring or endogenous Cadherin-11 (*e.g.*, mammalian, for example human) protein, and to proteins having an amino acid sequence that is the same as that of naturally occurring or endogenous Cadherin-11 protein (*e.g.*, recombinant proteins, synthetic proteins). Accordingly, the terms "Cadherin-11," "Cad-11," and "OB-Cadherin," which are used interchangeably herein, include polymorphic or allelic variants and other isoforms of a Cadherin-11 protein (*e.g.*, mammalian, human) produced by, *e.g.*, alternative splicing or other cellular processes, that occur naturally in mammals (*e.g.*, humans, non-human primates). Preferably, the Cadherin-11 protein is a human protein that has the amino acid sequence of SEQ ID NO:2 (See, Genbank Accession No. NP001788 and FIG. 15).

As defined herein, a "Cadherin-11 antagonist" is an agent (*e.g.*, antibody, fusion protein, peptide, peptidomimetic, small molecule, nucleic acid) that specifically binds an EC1 domain of a Cadherin-11 protein and inhibits (*e.g.*, reduces, prevents) one or more Cadherin-11-mediated activities in a cell. Cadherin-11-mediated activities include, but are not limited to, binding of a Cadherin-11 protein to one or more other Cadherin-11 proteins in a homotypic fashion, aggregation of cells that express Cadherin-11, induction of enzyme (*e.g.*, collagenase, serine proteases, MMP1, MMP3, MMP13) expression or activity, and induction of cytokines (*e.g.*, inflammatory cytokines) or growth factors (*e.g.*, IL-6, IL-8 or RANKL or TRANCE), migration of Cadherin-11-expressing cells and destruction of cartilage by Cadherin-11-expressing cells. In one embodiment, the Cadherin-11 antagonist can inhibit the binding of a Cadherin-11 protein to one or more other Cadherin-11 proteins by, for example, blocking the interaction between the donor sequences in the EC1 domain of a Cad-11 protein (*e.g.*, a Cad-11 protein expressed on the surface of a cell) with the pocket sequence in the EC1 domain of one or more other Cad-11 proteins (*e.g.*, one or more Cad-11 proteins expressed on the surface of another cell).

As used herein, a Cadherin-11 antagonist that "specifically binds" an EC1 domain of a Cadherin-11 protein refers to a Cadherin-11 antagonist that binds (*e.g.*, under physiological conditions) an EC1 domain of a Cadherin-11 protein with an affinity (*e.g.*, a binding affinity) that is at least about 5 fold, preferably at least about 10 fold, greater than the affinity with which the Cadherin-11 antagonist binds an EC1 domain of another cadherin protein (*e.g.*, MN-Cadherin, Cadherin-8). In a particular embodiment, the Cadherin-11 antagonist that specifically binds an EC1 domain of a Cadherin-11 protein binds an epitope present in SEQ ID NO:3, the N-terminal portion of the EC1 domain of human Cadherin-11, with an affinity that is at least about 5 fold, preferably at least about 10 fold, greater than the affinity with which the Cadherin-11 antagonist binds an epitope present in SEQ ID NO:4, the N-terminal portion of the EC1 domain of human MN-Cadherin, and the affinity with which the Cadherin-11 antagonist binds an epitope present in SEQ ID NO:5, the N-terminal portion of the EC1 domain of human Cadherin-8.

As used herein, the term "antibody" is intended to encompass both whole antibodies and antibody fragments (*e.g.*, antigen-binding fragments of antibodies, for example, Fv, Fc, Fd, Fab, Fab', F(ab'), and dAb fragments). "Antibody" refers to both polyclonal and monoclonal antibodies and includes naturally-occurring and engineered antibodies. Thus, the term "antibody" includes, for example, human, chimeric, humanized, primatized, veneered, single chain, and domain antibodies (dAbs). (See *e.g.*, Harlow *et al.* , *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988).

The term "epitope" refers to a unit of structure conventionally bound by an immunoglobulin V_H/V_L pair. An epitope defines the minimum binding site for an antibody, and thus represent the target of specificity of an antibody.

The term "fusion protein" refers to a naturally occurring, synthetic, semi-synthetic or recombinant single protein molecule that comprises all or a portion of two or more heterologous polypeptides

The term "polypeptide" refers to a polymer of amino acids, and not to a specific length; thus, peptides, oligopeptides and proteins are included within the definition of a polypeptide.

As used herein, the term "peptide" refers to a compound consisting of from about 2 to about 100 amino acid residues wherein the amino group of one amino acid is linked to the carboxyl group of another amino acid by a peptide bond. Such peptides are typically less than about 100 amino acid residues in length and preferably are about 10, about 20, about 30, about 40 or about 50 residues.

As used herein, the term "peptidomimetic" refers to molecules which are not peptides or proteins, but which mimic aspects of their structures. Peptidomimetic antagonists can be prepared by conventional chemical methods (see *e.g.*, Damewood J.R. "Peptide Mimetic Design with the Aid of Computational Chemistry" in *Reviews in Computational Biology*, 2007, Vol. 9, pp.1-80, John Wiley and Sons, Inc., New York, 1996; Kazmierski W.K., "*Methods of Molecular Medicine: Peptidomimetic Protocols,*" Humana Press, New Jersey, 1999).

As defined herein, "therapy" is the administration of a particular therapeutic or prophalytic agent to a subject (*e.g.*, a mammal, a human), which results in a desired therapeutic or prophylactic benefit to the subject.

As defined herein a "treatment regimen" is a regimen in which one or more therapeutic or prophalytic agents are administered to a mammalian subject at a particular dose (*e.g.*, level, amount, quantity) and on a particular schedule or at particular intervals (*e.g.*, minutes, days, weeks, months).

As defined herein, a "therapeutically effective amount" is an amount sufficient to achieve the desired therapeutic or prophylactic effect under the conditions of administration, such as, but not limited to, an amount sufficient to inhibit (*i.e.*, reduce, prevent) inflammation in a joint (*e.g.*, by inhibiting the aggregation of cells, for example synoviocytes, that express Cadherin-11) or the formation, growth or metastasis of a tumor. The effectiveness of a therapy (*e.g.*, the reduction of inflammation in a joint and/or prevention of inflammation in a joint) can be determined by suitable methods (*e.g.*, imaging methods, such as MRI, NMR, CT).

Cadherins

Cadherins belong to a large family of Ca^{2+}-dependent adhesion molecules that mediate cell adhesion by binding to other cadherins in a homotypic manner (MJ

Wheelock and KR Johnson, *Ann. Rev. Cell Dev. Biol. 19*: 207-235 (2003). Classical cadherins are single-pass transmembrane proteins that contain five extracellular cadherin (EC) domains, each approximately 110 amino acids in length, a transmembrane region and a conserved cytoplasmic domain. Cadherins are divided into either type I or type II cadherins based on the degree of homology between the EC domains. Type II cadherins include human Cadherins-5, -6, -8, -11, and -12, and MN-cadherin. The relative importance of the role of each of the extracellular domains in mediating inter-cellular binding is unclear.

Cadherin-11 activity in synoviocytes

Cadherin-11 mediates synoviocyte to synoviocyte binding in the synovial lining of articulated joints (Valencia *et al.*, *J. Exp. Med. 200*(12):1673-1679 (2004); Kiener and Brenner, *Arthritis Res Ther. 7*(2):49-54 (2005)). A fusion protein that comprised all five extracellular cadherin domains of human Cadherin-11, fused to the hinge-CH2-CH3 domain of human IgG_2, inhibited synoviocyte lining formation *in vitro* (Kiener *et al.*, *Am. J. Pathol. 168* (2006)). In addition, antagonistic anti-Cadherin-11 antibodies and a fusion protein that comprised EC1-5 of murine Cadherin-11, fused to the hinge-CH_2-CH_3 domains of murine IgG2a, inhibited inflammation and joint swelling in murine models of rheumatoid arthritis (Lee *et al.*, *Science 315*:1006-1010 (2007)).

Cadherin-11 antagonists

A Cadherin-11 antagonist of the invention can be any agent that specifically binds an EC1 domain of a Cadherin-11 protein and inhibits (*e.g.*, reduces, prevents) one or more Cadherin-11-mediated activities in a cell. Cadherin-11-mediated activities include, but are not limited to, aggregation of cells that express Cadherin-11 on the cell surface, and expression or secretion of factors such as, for example, collagenase, serine proteases, MMP1, MMP3, IL-6, IL-8 or RANKL/TRANCE. The agent can be an antibody, a fusion protein, a peptide, a peptidomimetic, a small molecule, or a nucleic acid, among others.

Cadherin-11 antibodies

As described herein, antibodies that bind an epitope within an N-terminal portion of the EC1 domain of human Cadherin-11 that comprises the donor sequences and cadherin-binding pocket of Cad-11 (*e.g.*, SEQ ID NO:3), block Cadherin-11 activity *in vitro* more effectively than antibodies that bind to epitopes in other regions of this protein (See Examples 1 and 2).

Accordingly, in one embodiment, the invention provides an antibody or antigen-binding fragment thereof that binds (*e.g.*, specifically binds) an epitope that is present in the N-terminal portion of the EC1 domain of a Cadherin-11 protein that comprises the donor sequences and cadherin-binding pocket of Cad-11. The term "antibody" is intended to encompass all types of polyclonal and monoclonal antibodies (*e.g.*, human, chimeric, humanized, primatized, veneered, single chain, domain antibodies (dAbs)) and antigen-binding fragments of antibodies (*e.g.*, Fv, Fc, Fd, Fab, Fab', F(ab'), dAb). (See *e.g.*, Harlow *et al.* , *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988). In a particular embodiment, the Cad-11 EC1 domain-specific antibody is a human antibody or humanized antibody. Cad-11 EC1 domain-specific antibodies can also be directly or indirectly linked to a cytotoxic agent.

Other antibodies or antibody fragments that specifically bind to an N-terminal portion of the EC1 domain of a Cad-11 protein and inhibit the activity of the Cad-11 protein can also be produced, constructed, engineered and/or isolated by conventional methods or other suitable techniques. For example, antibodies which are specific for the EC1 domain of a Cadherin-11 protein can be raised against an appropriate immunogen, such as a recombinant mammalian (*e.g.*, human) Cadherin-11 EC1 domain peptide (*e.g.*, SEQ ID NO:3) or a portion thereof (including synthetic molecules, *e.g.*, synthetic peptides). A variety of methods have been described (see *e.g.*, Kohler *et al.* , *Nature, 256:* 495-497 (1975) and *Eur. J. Immunol. 6:* 511-519 (1976); Milstein *et al.* , *Nature 266: 550-552 (1977);* Koprowski *et al.* , U.S. Patent No. 4,172,124; Harlow, E. and D. Lane, 1988, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory: Cold Spring Harbor, NY); *Current Protocols In Molecular Biology*, Vol. 2 (Supplement 27, Summer '94), Ausubel, F.M. *et al.* , Eds., (John Wiley & Sons: New York, NY), Chapter 11, (1991)). Antibodies can also be raised by immunizing a suitable host (*e.g.*, mouse) with cells that express the EC1 domain of Cadherin-11 (*e.g.*, cancer cells/cell lines) or cells engineered to express the EC1 domain of Cadherin-11 (*e.g.*, transfected cells). (See *e.g.*, Chuntharapai *et al.* , *J. Immunol.*, 152:1783-1789 (1994); Chuntharapai *et al.* U.S. Patent No. 5,440, 021). For the production of monoclonal antibodies, a hybridoma can be produced by fusing a suitable immortal cell line (*e.g.*, a myeloma cell line such as SP2/0 or P3X63Ag8.653) with antibody producing cells. The antibody producing cells can be obtained from the peripheral blood, or preferably, the spleen or lymph nodes, of humans or other suitable animals immunized with the antigen of interest. The fused cells (hybridomas) can be isolated using selective culture conditions, and cloned by limited dilution. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (*e.g.*, ELISA).

Antibody fragments can be produced by enzymatic cleavage or by recombinant techniques. For example, papain or pepsin cleavage can generate Fab or $F(ab')_2$ fragments, respectively. Other proteases with the requisite substrate specificity can also be used to generate Fab or $F(ab')_2$ fragments. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons has been introduced upstream of the natural stop site. For example, a chimeric gene encoding a $F(ab')_2$ heavy chain portion can be designed to include DNA sequences encoding the CH_1 domain and hinge region of the heavy chain. Single chain antibodies, and human, chimeric, humanized or primatized (CDR-grafted), or veneered antibodies, as well as chimeric, CDR-grafted or veneered single chain antibodies, comprising portions derived from different species, and the like are also encompassed by the present invention and the term "antibody". The various portions of these antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, *e.g.*, Cabilly *et al.* , U.S. Patent No. 4,816,567; Cabilly *et al.* , European Patent No. 0,125,023 B1; Boss *et al.* , U.S. Patent No. 4,816,397; Boss *et al.* , European Patent

No. 0,120,694 B1; Neuberger, M.S. *et al.* , WO 86/01533; Neuberger, M.S. *et al.*, European Patent No. 0,194,276 B1; Winter, U.S. Patent No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Queen *et al.* , European Patent No. 0 451 216 B1; and Padlan, E.A. *et al.* , EP 0 519 596 A1. See also, Newman, R. *et al.* , *BioTechnology, 10:* 1455-1460 (1992), regarding primatized antibody, and Ladner *et al.*, U.S. Patent No. 4,946,778 and Bird, R.E. *et al.* , *Science, 242:* 423-426 (1988)) regarding single chain antibodies.

Humanized antibodies can be produced using synthetic or recombinant DNA technology using standard methods or other suitable techniques. Nucleic acid (*e.g.*, cDNA) sequences coding for humanized variable regions can also be constructed using PCR mutagenesis methods to alter DNA sequences encoding a human or humanized chain, such as a DNA template from a previously humanized variable region (see *e.g.*, Kamman, M., *et al.* , *Nucl. Acids Res., 17*: 5404 (1989)); Sato, K., *et al.* , *Cancer Research, 53*: 851-856 (1993); Daugherty, B.L. *et al.* , *Nucleic Acids Res., 19(9):* 2471-2476 (1991); and Lewis, A.P. and J.S. Crowe, *Gene, 101*: 297-302 (1991)). Using these or other suitable methods, variants can also be readily produced. In one embodiment, cloned variable regions (*e.g.*, dAbs) can be mutated, and sequences encoding variants with the desired specificity can be selected (*e.g.*, from a phage library; see *e.g.*, Krebber *et al.* , U.S. 5,514,548; Hoogenboom *et al.* , WO 93/06213, published April 1, 1993).

Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, for example, methods which select a recombinant antibody or antibody-binding fragment (*e.g.*, dAbs) from a library (*e.g.*, a phage display library), or which rely upon immunization of transgenic animals (*e.g.*, mice). Transgenic animals capable of producing a repertoire of human antibodies are well-known in the art (*e.g.*, Xenomouse® (Abgenix, Fremont, CA)) and can be produced using suitable methods (see *e.g.*, Jakobovits *et al.* , *Proc. Natl. Acad. Sci. USA, 90*: 2551-2555 (1993); Jakobovits *et al.* , *Nature, 362*: 255-258 (1993); Lonberg *et al.* , U.S. Patent No. 5,545,806; Surani *et al.* , U.S. Patent No. 5,545,807; Lonberg *et al.* , WO 97/13852).

The invention encompasses, in one embodiment, a Cad-11 antibody that binds to an epitope that is present in the first about 37 amino acids of the EC1 domain of human Cad-11 (SEQ ID NO: 13). In a particular embodiment, the invention relates to a Cad-11 antibody that binds to an epitope that is present in SEQ ID NO:10. In a further embodiment, the invention relates to a Cad-11 antibody that binds to an epitope that comprises SEQ ID NO:11. In another embodiment, the invention relates to a Cad-11 antibody that binds to an epitope that is present in SEQ ID NO:12.

In one embodiment, the invention relates to a Cad-11 antibody produced by hybridoma H1M1 (ATCC Patent Deposit Designation PTA-9699), having been deposited on January 8, 2009 at the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, Virginia 20108, United States of America. In another embodiment, the invention provides a Cad-11 antibody produced by hybridoma H14 (ATCC Patent Deposit Designation PTA-9701), having been deposited on January 9, 2009 at the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, Virginia 20108, United States of America.

The invention also encompasses antibodies that specifically compete with a Cad-11 antibody produced by hybridoma H1M1 and/or a Cad-11 antibody produced by hybridoma H14 for binding to a human Cad-11 protein or an EC1-domain containing portion thereof (*e.g.*, SEQ ID NO:3, 10, 12, 13). In a particular embodiment, an antibody that specifically competes with a Cad-11 antibody produced by hybridoma H1M1 and/or hybridoma H14 blocks (*e.g.*, inhibits, diminishes, prevents) the binding of a Cad-11 antibody produced by hybridoma H1M1 and/or hybridoma H14 to a human Cad-11 protein or EC1-domain containing portion thereof (*e.g.*, SEQ ID NO:3, 10, 12, 13).

In addition, the invention encompasses antibodies having a binding affinity for a human Cad-11 protein or EC1-domain containing portion thereof (*e.g.*, SEQ ID NO:3, 10, 12, 13) that is at least as great as the binding affinity of a Cad-11 antibody produced by hybridoma H1M1 and/or a Cad-11 antibody produced by hybridoma H14 for a human Cad-11 protein or EC1-domain containing portion thereof.

Cadherin-11 fusion proteins

In addition, immunoglobulin fusion proteins that contain only the EC1 domain of human Cad-11 (*e.g.*, the EC1 domain of human Cad-11 fused to a portion of human IgG) inhibited Cad-11 activity *in vitro* more effectively than a fusion protein that included a larger portion of the EC region of Cad-11, which contained all 5 EC domains.

Cadherin-11 antagonists also encompass chimeric, or fusion, proteins that comprise at least about the N-terminal 35 amino acids of the EC1 domain of human Cad-11 (SEQ ID NO:2) operatively linked to all or a portion of a heterologous protein. "Operatively linked" indicates that the portion of the Cad-11 EC1 domain and the heterologous protein are fused in-frame. The heterologous protein can be fused to the N-terminus or C-terminus of the protein. For example, the fusion protein can be a GST-fusion protein in which the protein sequences are fused to the C-terminus of a GST sequence. Other types of fusion proteins include, but are not limited to, enzymatic fusion proteins, for example, β-galactosidase fusion proteins, yeast two-hybrid GAL fusion proteins, poly-His fusions, FLAG-tagged fusion proteins, GFP fusion proteins, and immunoglobulin (Ig) fusion proteins. Such fusion protein can facilitate purification (*e.g.*, of a recombinant fusion protein). In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of a protein can be increased by using a heterologous signal sequence. Therefore, in another embodiment, the fusion protein contains a heterologous signal sequence at its N-terminus.

EP-A-O 464 533 discloses fusion proteins comprising various portions of immunoglobulin constant regions. The Fc is useful in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (see, for example, EP-A 0232 262). In drug discovery, for example, human proteins have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists (Bennett *et al.*, *Journal of Molecular Recognition 8*:52-58 (1995); Johanson *et al.*, *J. Biol. Chem., 270(16)*:9459-9471 (1995)). Thus, this invention also encompasses soluble fusion proteins containing a protein Cad-11 antagonist of the invention and various portions of the constant regions of heavy and/or light chains of immunoglobulins of various subclasses (*e.g.*, IgG, IgM, IgA, IgE). Advantages of immunoglobulin fusion proteins of the present invention include one or more of the following: (1) increased avidity for multivalent ligands due to the resulting bivalency of dimeric fusion proteins, (2) longer serum half-life, (3) the ability to activate effector cells via the Fc domain, (4) ease of purification (for example, by protein A chromatography), (5) affinity for Cad-11 and (6) the ability to block Cad-11 mediated activity.

Accordingly, in particular embodiments, the Cad-11 antagonist is a fusion protein that comprises a portion of the extracellular region of a Cadherin-11 protein that includes an N-terminal portion of the EC1 domain (amino acids 54-90 of SEQ ID NO:2), operatively linked to all, or a portion of, a mammalian immunoglobulin protein. In a particular embodiment, the immunoglobulin fusion proteins of the invention do not comprise a portion of the extracellular region of Cadherin-11 that includes all five EC domains that are contained within amino acids 1-609 of SEQ ID NO:2. In certain embodiments, the portion of the human Cadherin-11 extracellular region can include, for example, amino acids 1-160, amino acids 1-259 or amino acids 1-269 of SEQ ID NO:2. In a particular embodiment, the fusion protein lacks the leader and pro-region of human Cadherin-11 (amino acids 1-53 of SEQ ID NO:2) and uses a heterogologous leader sequence. The immunoglobulin portion can be from any vertebrate source, such as murine, but preferably, is a human immunoglobulin protein. In one embodiment, the mammalian immunoglobulin protein is a human IgG_2 protein or a portion thereof, such as the hinge-CH_2-CH_3 portion of human IgG_2.

A chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for different protein sequences (*e.g.*, a Cad-11 EC1 domain peptide and a mammalian immunoglobulin) are ligated together in-frame in accordance with conventional techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of nucleic acid fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive nucleic acid fragments that can subsequently be annealed and re-amplified to generate a chimeric nucleic acid sequence (see Ausubel *et al.*, Current Protocols in Molecular Biology, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST moiety, an Fc moiety). A nucleic acid molecule encoding protein Cad-11 antagonist can be cloned into such an expression vector that the fusion moiety (*e.g.*, immunoglobulin) is linked in-frame to the protein.

The immunoglobulin fusion proteins of the invention can be provided as monomers, dimers, tetramers or other multimers (*e.g.*, polymers). For example, variable domains of the immunoglobulin portion of the fusion protein may be linked together to form multivalent ligands by, for example, provision of a hinge region at the C-terminus of each V domain and disulphide bonding between cysteines in the hinge regions; or provision of heavy chains each with a cysteine at the C-terminus of the domain, the cysteines being disulphide bonded together; or production of V-CH & V-CL to produce a Fab format; or use of peptide linkers (for example Gly_4Ser linkers) to produce dimers, trimers and further multimers. For example, such ligands can be linked to an antibody Fc region comprising one or both of C_H2 and C_H3 domains, and optionally a hinge region. For example, vectors encoding ligands linked as a single nucleotide sequence to an Fc region may be used to prepare such ligands (*e.g.*, by expression).

The immunoglobulin fusion proteins of the invention can be conjugated to other moieties including, but not limited to, multimers of polyethelene glycol (PEG) or its derivatives (*e.g.*, poly methyl ethylene glycol), radionuclides, cytotoxic agents and drugs, and subsequently used for *in vivo* therapy. Examples of radionuclides include ^{212}Bi, ^{131}I, ^{186}Re, and ^{90}Y, among others. The radionuclides exert their cytotoxic effect by locally irradiating the cells, leading to various intracellular lesions, as is known in the art of radiotherapy. Cytotoxic drugs that can be conjugated to the fusion proteins include, but are not limited to, daunorubicin, doxorubicin, methotrexate, and Mitomycin C. Cytotoxic drugs interfere with critical cellular processes including DNA, RNA, and protein synthesis. For a fuller exposition of these classes of drugs, which are known in the art, and their mechanisms of action, see Goodman, A.G., *et al., Goodman and Gilman's The Pharmacological Basis of Therapeutics,* 8th Ed., Macmillan Publishing Col, 1990. Katzung, ed., Basic and Clinical Pharmacology, Fifth Edition, p 768-769, 808-809, 896, Appleton and Lange, Norwalk, Conn.

As used herein, the term “immunoglobulin fusion protein” includes fragments of the immunoglobulin fusion proteins of the invention. Such fragments are intended to be within the scope of this invention. For example, once the molecules are isolated, they can be cleaved with protease to generate fragments that remain capable of binding the EC1 domain of human Cad-11.

Peptide antagonists

The Cadherin-11 antagonist of the invention can also be a peptide that binds to the EC1 domain of a Cadherin-11 protein. The peptide can comprise any suitable L-and/or D-amino acid, for example, common α-amino acids (*e.g.*, alanine, glycine, valine), non-α-amino acids (*e.g.*, β-alanine, 4-aminobutyric acid, 6-aminocaproic acid, sarcosine, statine), and unusual amino acids (*e.g.*, citrulline, homocitruline, homoserine, norleucine, norvaline, ornithine). The amino, carboxyl and/or other functional groups on a peptide can be free (*e.g.*, unmodified) or protected with a suitable protecting group. Suitable protecting groups for amino and carboxyl groups, and methods for adding or removing protecting groups are known in the art and are disclosed in, for example, Green and Wuts, *"Protecting Groups in Organic Synthesis"*, John Wiley and Sons, 1991. The functional groups of a peptide can also be derivatized (*e.g.*, alkylated) using art-known methods.

The peptide Cad-11 antagonist can comprise one or more modifications (*e.g.*, amino acid linkers, acylation, acetylation, amidation, methylation, terminal modifiers (*e.g.*, cyclizing modifications)), if desired. The peptide can also contain chemical modifications (*e.g.*, N-methyl-α-amino group substitution). In addition, the peptide antagonist can be an analog of a known and/or naturally-occurring peptide, for example, a peptide analog having conservative amino acid residue substitution(s). These modifications can improve various properties of the peptide (*e.g.*, solubility, binding), including its Cadherin-11 antagonist activity.

Cad-11 antagonists that are peptides can be linear, branched or cyclic, *e.g.*, a peptide having a heteroatom ring structure that includes several amide bonds. In a particular embodiment, the peptide is a cyclic peptide. Such peptides can be produced by one of skill in the art using standard techniques. For example, a peptide can be derived or removed from a native protein by enzymatic or chemical cleavage, or can be synthesized by suitable methods, for example, solid phase peptide synthesis (*e.g.*, Merrifield-type synthesis) (see, *e.g.*, Bodanszky *et al.* *"Peptide*

Synthesis," John Wiley & Sons, Second Edition, 1976). Peptides that are Cadherin-11 antagonists can also be produced, for example, using recombinant DNA methodologies or other suitable methods (see, *e.g.*, Sambrook J. and Russell D.W., *Molecular Cloning: A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).

Peptides can be synthesized and assembled into libraries comprising a few to many discrete molecular species. Such libraries can be prepared using methods of combinatorial chemistry, and can be screened using any suitable method to determine if the library comprises peptides with a desired biological activity. Such peptide antagonists can then be isolated using suitable methods.

Peptidomimetic antagonists

Cadherin-11 antagonists can also be peptidomimetics. For example, polysaccharides can be prepared that have the same functional groups as peptides. Peptidomimetics can be designed, for example, by establishing the three dimensional structure of a peptide agent in the environment in which it is bound or will bind to a target molecule. The peptidomimetic comprises at least two components, the binding moiety or moieties and the backbone or supporting structure.

The binding moieties are the chemical atoms or groups which will react or form a complex (*e.g.*, through hydrophobic or ionic interactions) with a target molecule, for example, with amino acids in the EC1 domain of Cad-11. For example, the binding moieties in a peptidomimetic can be the same as those in a peptide or protein antagonist. The binding moieties can be an atom or chemical group which reacts with the receptor in the same or similar manner as the binding moiety in the peptide antagonist. For example, computational chemistry can be used to design peptide mimetics of the donor sequences of the EC1 domain of a Cadherin-11 protein, for instance, which can bind to the pocket sequence in the EC1 domain of Cad-11 proteins. Examples of binding moieties suitable for use in designing a peptidomimetic for a basic amino acid in a peptide include nitrogen containing groups, such as amines, ammoniums, guanidines and amides or phosphoniums. Examples of binding moieties suitable for use in designing a peptidomimetic for an acidic amino acid include, for example, carboxyl, lower alkyl carboxylic acid ester, sulfonic acid, a lower alkyl sulfonic acid ester or a phosphorous acid or ester thereof.

The supporting structure is the chemical entity that, when bound to the binding moiety or moieties, provides the three dimensional configuration of the peptidomimetic. The supporting structure can be organic or inorganic. Examples of organic supporting structures include polysaccharides, polymers or oligomers of organic synthetic polymers (such as, polyvinyl alcohol or polylactide). It is preferred that the supporting structure possess substantially the same size and dimensions as the peptide backbone or supporting structure. This can be determined by calculating or measuring the size of the atoms and bonds of the peptide and peptidomimetic. In one embodiment, the nitrogen of the peptide bond can be substituted with oxygen or sulfur, for example, forming a polyester backbone. In another embodiment, the carbonyl can be substituted with a sulfonyl group or sulfinyl group, thereby forming a polyamide (*e.g.*, a polysulfonamide). Reverse amides of the peptide can be made (*e.g.*, substituting one or more-CONH-groups for a-NHCO-group). In yet another embodiment, the peptide backbone can be substituted with a polysilane backbone.

These compounds can be manufactured by known methods. For example, a polyester peptidomimetic can be prepared by substituting a hydroxyl group for the corresponding α-amino group on amino acids, thereby preparing a hydroxyacid and sequentially esterifying the hydroxyacids, optionally blocking the basic and acidic side chains to minimize side reactions. Determining an appropriate chemical synthesis route can generally be readily identified upon determining the chemical structure.

Peptidomimetics can be synthesized and assembled into libraries comprising a few to many discrete molecular species. Such libraries can be prepared using well-known methods of combinatorial chemistry, and can be screened to determine if the library comprises one or more peptidomimetics which have the desired activity. Such peptidomimetic antagonists can then be isolated by suitable methods.

Small molecule antagonists

Cadherin-11 antagonists can also be small molecules. Examples of small molecules include organic compounds, organometallic compounds, inorganic compounds, and salts of organic, organometallic or inorganic compounds. Atoms in a small molecule are typically linked together via covalent and/or ionic bonds. The arrangement of atoms in a small organic molecule may represent a chain (*e.g.* a carbon-carbon chain or a carbon-heteroatom chain), or may represent a ring containing carbon atoms, *e.g.* benzene or a policyclic system, or a combination of carbon and heteroatoms, *i.e.*, heterocycles such as a pyrimidine or quinazoline. Although small molecules can have any molecular weight, they generally include molecules that are less than about 5,000 daltons. For example, such small molecules can be less than about 1000 daltons and, preferably, are less than about 750 daltons or, more preferably, are less than about 500 daltons. Small molecules and other non-peptidic Cadherin-11 antagonists can be found in nature (*e.g.*, identified, isolated, purified) and/or produced synthetically (*e.g.*, by traditional organic synthesis, bio-mediated synthesis, or a combination thereof). See *e.g.* Ganesan, Drug Discov. Today 7(1): 47-55 (January 2002); Lou, Drug Discov. Today, 6(24): 1288-1294 (December 2001). Examples of naturally occurring small molecules include, but are not limited to, hormones, neurotransmitters, nucleotides, amino acids, sugars, lipids, and their derivatives.

A small molecule Cadherin-11 antagonist according to the present invention, and physiologically acceptable salts thereof, can inhibit the homotypic binding of a Cadherin-11 protein (*e.g.*, by directly competing with a donor sequence in the EC1 domain of a Cad-11 protein for binding to the binding pocket of another Cadherin-11, by directly competing with the binding pocket in the EC1 domain of a Cad-11 protein for binding to a donor sequence of another Cadherin-11).

Nucleic acid antagonists

Cad-11 antagonists of the invention can also be nucleic acid molecules (*e.g.*, oligonucleotides) that bind to the EC1 domain of a human Cadherin-11. Suitable nucleic acid Cad-11 antagonists include aptamers, which are capable of binding to a particular molecule of interest (*e.g.*, the EC1 domain of human Cadherin-11) with high affinity and specificity through interactions other than classic Watson-Crick base pairing (Tuerk and Gold, Science 249:505 (1990); Ellington and Szostak, Nature 346:818 (1990)).

Aptamers, like peptides generated by phage display or monoclonal antibodies (MAbs), are capable of specifically binding to selected targets and, through binding, block their targets' ability to function. Created by an *in vitro* selection process from pools of random sequence oligonucleotides, aptamers have been generated for over 100 proteins including growth factors, transcription factors, enzymes, immunoglobulins, and receptors. A typical aptamer is 10-15 kDa in size (30-45 nucleotides), binds its target with sub-nanomolar affinity, and discriminates against closely related targets (*e.g.*, will typically not bind other proteins from the same gene family). A series of structural studies have shown that aptamers are capable of using the same types of binding interactions (hydrogen bonding, electrostatic complementarity, hydrophobic contacts, steric exclusion, etc.) that drive affinity and specificity in antibody-antigen complexes.

An aptamer that binds to a target of interest (*e.g.*, an EC1 domain of a human Cad-11 protein) can be generated and identified using a standard process known as "Systematic Evolution of Ligands by Exponential Enrichment" (SELEX), described in, *e.g.*, U.S. Pat. Nos. 5,475,096 and U.S. Pat. No. 5,270,163.

Identification of Cadherin-11 antagonists

Agents having Cadherin-11 binding specificity, including small molecules, can be identified in a screen, for example, a high-throughput screen of chemical compounds and/or libraries (*e.g.*, chemical, peptide, nucleic acid libraries).

Antibodies that specifically bind the EC1 domain of human Cadherin-11 can be identified, for example, by screening commercially available combinatorial antibody libraries (Dyax Corp., MorphoSys AG). Suitable combinatorial antibody libraries and standard methods of screening these libraries are described in Hoet *et al.*, *Nature Biotechnology 23*(3):344-348 (2005)and Rauchenberger *et al.*, *J. Biol. Chem. 278*(40):38194-38205 (2003), the contents of which are incorporated herein by reference. Such libraries or collections of molecules can also be prepared using well-known chemical methods.

Alternatively murine antibodies that specifically bind the EC1 domain of human Cadherin-11 can be identified, for example, by immunizing mice with EC1 protein domains or EC1 peptides along with an adjuvant to break tolerance to the antigen. These antibodies can be screened for the desired specificity and activity and then humanized using known techniques to create suitable agents for the treatment of human disease.

Compounds or small molecules can be identified from numerous available libraries of chemical compounds from, for example, the Chemical Repository of the National Cancer Institute and the Molecular Libraries Small Molecules Repository (PubChem), as well as libraries of the Institute of Chemistry and Cell Biology at Harvard University and other libraries that are available from commercial sources (*e.g.*, Chembridge, Peakdale, CEREP, MayBridge, Bionet). Such libraries or collections of molecules can also be prepared using well-known chemical methods, such as well-known methods of combinatorial chemistry. The libraries can be screened to identify compounds that bind and inhibit Cadherin-11.

Identified compounds can serve as lead compounds for further diversification using well-known methods of medicinal chemistry. For example, a collection of compounds that are structural variants of the lead can be prepared and screened for Cadherin-11 binding and/or inhibitory activity. This can result in the development of a structure activity relationship that links the structure of the compounds to biological activity. Compounds that have suitable binding and inhibitory activity can be developed further for *in vivo* use.

Agents that bind Cadherin-11 can be evaluated further for Cadherin-11 antagonist activity. For example, a composition comprising a Cadherin-11 protein can be used in a screen or binding assay to detect and/or identify agents that bind and antagonize the Cadherin-11 protein. Compositions suitable for use include, for example, cells that naturally express a Cadherin-11 protein (*e.g.*, a synoviocyte), extracts of such cells, and recombinant Cadherin-11 protein.

An agent that binds a Cadherin-11 protein can be identified in a competitive binding assay, for example, in which the ability of a test agent to inhibit the binding of Cadherin-11 to a reference agent is assessed. The reference agent can be a full-length Cad-11 protein or a portion thereof that comprises the EC1 domain. The reference agent can be labeled with a suitable label (*e.g.*, radioisotope, epitope label, affinity label (*e.g.*, biotin and avidin or streptavadin), spin label, enzyme, fluorescent group, chemiluminescent group, dye, metal (*e.g.*, gold, silver), magnetic bead) and the amount of labeled reference agent required to saturate the Cadherin-11 protein in the assay can be determined. The specificity of the formation of the complex between the Cadherin-11 protein and the test agent can be determined using a suitable control (*e.g.*, unlabeled agent, label alone).

The capacity of a test agent to inhibit formation of a complex between the reference agent and a Cadherin-11 protein can be determined as the concentration of test agent required for 50% inhibition (IC_{50} value) of specific binding of labeled reference agent. Specific binding is preferably defined as the total binding (*e.g.*, total label in complex) minus the non-specific binding. Non-specific binding is preferably defined as the amount of label still detected in complexes formed in the presence of excess unlabeled reference agent. Reference agents suitable for use in the method include molecules and compounds which specifically bind to Cadherin-11, *e.g.*, an antibody that binds Cadherin-11.

An agent that antagonizes a Cadherin-11 protein can be identified by screening for agents that have an ability to antagonize (reduce, prevent, inhibit) one or more activities of Cadherin-11, such as, for example, a binding activity (*e.g.*, homotypic Cad-11 binding). Such activities can be assessed using an appropriate *in vitro* or *in vivo* assay. Exemplary assays for Cadherin-11 activity have been described previously (Patel, SD, *et al.*, *Cell 124*: 1255–1268 (2006); Lee *et al.*, *Science 315*:1006-1010 (2007)).

Once a Cadherin-11 antagonist is identified, the ability of the Cadherin-11 antagonist to interfere with (*e.g.*, reduce, inhibit, prevent) one or more biological functions or properties associated with Cadherin-11 activity in a cell can be assessed, for example, using a cell-based assay designed to measure a particular biological function or property associated with Cadherin-11. Biological functions and properties that are known to be associated with Cadherin-11 expression and/or activity include, but are not limited to, cell adhesion, cell migration, cell invasion, cell sorting, cell condensation, cell rearrangement, maintenance of tissue integrity and architecture, contact inhibition of cell proliferation and malignant transformation of cancer (*e.g.*, tumor) cells (Kiener and Brenner, *Arthritis Res Ther.* 7(2):49-54 (2005)). In addition Cad-11 antagonists are shown herein to inhibit production of active MMPs by synoviocytes. Suitable assays for assessing one or more biological functions of cadherins are known to those of skill in the art (see, *e.g.*, Patel, SD, *et al.*, *Cell 124*:_1255–1268 (2006)) and include, for example, the cell aggregation assay described herein (see Exemplification, Materials and Methods section).

Methods of Therapy

Without wishing to be bound by any one theory, it is believed that the first about 35 amino acids (*e.g.*, about 33 to about 37 amino acids) of the EC1 domain of Cad-11 are required for homotypic cadherin binding and that agents that specifically bind to this region of Cad-11 can effectively inhibit binding between Cad-11 molecules. Accordingly, such agents are useful in the treatment and prevention of disorders (*e.g.*, inflammatory disorders, fibrosis, cancer) associated with Cad-11 expression or activity in cells (*e.g.*, synoviocytes). Thus, one aspect of the present invention relates to a method for treating a Cadherin-11-mediated disorder in a mammalian subject comprising administering to the subject a therapeutically effective amount of a Cadherin-11 antagonist that binds a human Cadherin-11 EC1 domain peptide (SEQ ID NO:3).

As used herein, a "Cadherin-11-mediated disorder" refers to a disease, disorder or condition that involves, or is mediated by (*e.g.*, caused by), cells that express Cadherin-11 protein. Cadherin-11-mediated disorders that can be treated by the methods of the invention include, but are not limited to, inflammatory disorders (*e.g.*, inflammatory joint disorders), fibrosis disorders (*e.g.*, dermal fibrosis, pulmonary fibrosis) and cancer.

Using the methods of the invention, a Cadherin-11-mediated disorder in a mammal (*e.g.*, a human) can be treated by administering a Cadherin-11 antagonist of the invention (*e.g.*, antibodies, fusion proteins, small molecules, nucleic acids, peptides, peptidomimetics) in an amount that is sufficient to provide a therapeutic benefit, for example, by inhibiting the aggregation of cells, or inhibiting the migration of cells, or inhibiting expression of active proteases or inflammatory molecules by cells, that express Cadherin-11 (*e.g.*, synoviocytes, fibroblasts, tumor cells).

Accordingly, one aspect of the invention relates to a method for treating an inflammatory disorder in a mammalian subject comprising administering to the subject a therapeutically effective amount of a Cadherin-11 antagonist of the invention. Inflammatory disorders are generally characterized by expression of pro-inflammatory molecules (*e.g.*, inflammatory cytokines and growth factors) and activation of immune cells (*e.g.*, macrophages, monocytes, lymphocytes, plasma cells, leukocytes, fibroblasts, neutrophils, T cells). Inflammatory disorders include, for example, inflammatory joint disorders, inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis), psoriasis, dermatitis (*e.g.*, eczema), renal amyloidosis, glomerular nephritis, vasculitis, pelvic inflammatory disease, chronic prostatitis, Graves opthalmopathy. In a particular embodiment, the inflammatory disorder is an autoimmune disorder.

In a particular embodiment, the invention relates to a method for treating an inflammatory joint disorder in a mammalian subject comprising administering to the subject a therapeutically effective amount of a Cadherin-11 antagonist of the invention. The inflammatory joint disorder can be any disorder that is associated with or characterized by Cadherin-11 expression in cells of an articulated joint (*e.g.*, synoviocytes). Examples of inflammatory joint disorders that can be treated by the present invention include, but are not limited to, rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, juvenile chronic arthritis, chronic Lyme disease and joint arthritis associated with systemic lupus erythrematosus. In a particular embodiment, the inflammatory joint disorder is rheumatoid arthritis.

In another aspect, the invention relates to a method for treating fibrosis in a mammalian subject comprising administering to the subject a therapeutically effective amount of a Cadherin-11 antagonist of the invention. As used herein, the term "fibrosis" refers to the formation or development of excess fibrous connective tissue in an organ or tissue as a reparative or reactive process. Examples of fibrosis include, but are not limited to vascular fibrosis (*e.g.*, vascular fibrosis associated with pulmonary hypertension), kidney fibrosis, liver fibrosis (*e.g.*, cirrhosis), skin/dermal fibrosis (*e.g.*, scleroderma), lung/pulmonary fibrosis (*e.g.*, idiopathic pulmonary fibrosis), myelofibrosis, endomyocardial fibrosis, fibrosis of the joint, fibrosis of the mesothelium, fibrosis of the eyes, fibrosis of the gut and interstitial fibrosis. In a particular embodiment, the fibrosis is pulmonary or lung fibrosis. In another embodiment, the fibrosis is dermal or skin fibrosis.

Without wishing to be bound by any particular theory, it is believed that certain types of tumor cells express Cadherin-11, which can promote tumor metastasis. Accordingly, a Cad-11 antagonist of the invention may be used to inhibit tumor formation, growth and/or metastasis in a subject. Thus, in another aspect, the invention relates to a method for treating a cancer (*e.g.*, a cancer characterized by cells that express Cad-11) in a mammalian subject comprising administering to the subject a therapeutically effective amount of a Cadherin-11 antagonist of the invention. Cancers involving cells that express Cad-11 protein can include, for example, leukemia (*e.g.*, AML, CLL, pro-lymphocytic leukemia), lung cancer (*e.g.*, small cell and non-small cell lung carcinoma), esophageal cancer, gastric cancer, colorectal cancer, brain cancer (*e.g.*, astrocytoma, glioma, glioblastoma, medulloblastoma, meningioma, neuroblastoma), bladder cancer, breast cancer, cervical cancer, epithelial cancer, nasopharyngeal cancer (*e.g.*, oral or laryngeal squamous cell carcinoma), lymphoma (*e.g.*, follicular lymphoma), uterine cancer (*e.g.*, malignant fibrous histiocytoma), hepatic cancer (*e.g.*, hepatocellular carcinoma), head-and-neck cancer (*e.g.*, head-and-neck squamous cell carcinoma), renal cancer, male germ cell tumors, malignant mesothelioma, myelodysplastic syndrome, ovarian cancer, pancreatic or biliary cancer, prostate cancer, thyroid cancer (*e.g.*, sporadic follicular thyroid tumors), and urothelial cancer.

In one aspect, a therapeutically effective amount of a Cadherin-11 antagonist is administered to a patient in need thereof. The amount of the Cadherin-11 antagonist to be administered (*e.g.*, a therapeutically effective amount) can be determined by a clinician using the guidance provided herein and other methods known in the art and is dependent on several factors including, for example, the particular agent chosen, the subject's age, sensitivity, tolerance to drugs and overall well-being. For example, suitable dosages for Cad-11 antagonists that are antibodies can be from about 0.01 mg/kg to about 300 mg/kg body weight per treatment and preferably from about 0.01 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 1 mg/kg to about 10 mg/kg body weight per treatment. Suitable dosages for a small molecule Cad-11 antagonist can be from about 0.001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.01 mg/kg to about 1 mg/kg body weight per treatment. Suitable dosages for Cadherin-11 antagonists that are proteins or peptides (linear, cyclic, mimetic), will result in a plasma concentration of the peptide from about 0.1 μg/mL to about 200 μg/mL. Determining the dosage for a particular agent, patient and cancer is well within the abilities of one skilled in the art. Preferably, the dosage does not cause, or produces minimal, adverse side effects (*e.g.*, immunogenic response, nausea, dizziness, gastric upset, hyperviscosity syndromes, congestive heart failure, stroke, pulmonary edema).

A therapeutically effective amount of a Cadherin-11 antagonist can be administered alone, or in combination with one or more other therapeutic agents (*e.g.*, anti-inflammatory agents, chemotherapeutic agents). Suitable anti-inflammatory agents that are useful for treating inflammatory joint disorders, particularly RA, which can be administered in combination with Cad-11 antagonists of the invention, include, but are not limited to, (i) non-steroidal anti-inflammatory drugs (NSAIDs; *e.g.*, detoprofen, diclofenac, diflunisal, etodolac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenameate, mefenamic acid, meloxicam, nabumeone, naproxen sodium, oxaprozin, piroxicam, sulindac, tolmetin, celecoxib, rofecoxib, aspirin, choline salicylate, salsalte, and sodium and magnesium salicylate); (ii) steroids (*e.g.*, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone); (iii) DMARDs, *i.e.*, disease modifying antirheumatic drugs (*e.g.*, cyclosporine, azathioprine, methotrexate, leflunomide, cyclophosphamide, hydroxychloroquine, sulfasalazine, D-penicillamine, minocycline, and gold); or (iv) recombinant proteins (*e.g.*, ENBREL® (etanercept, a soluble TNF receptor), REMICADE® (infliximab, a chimeric monoclonal anti-TNF antibody), ORENCIA® (abatabacept, a soluble

CTLA4 receptor) , ACTEMRA® (Tocilizumab, a monoclonal antibody to the IL-6 receptor), and RITUXAN® (rituximab, a monoclonal antibody to CD20).

Thus, a Cadherin-11 antagonist can be administered as part of a combination therapy (*e.g.*, with one or more other therapeutic agents). The Cad-11 antagonist can be administered before, after or concurrently with one or more other therapeutic agents. In some embodiments, the Cadherin-11 antagonist and other therapeutic agent can be co-administered simultaneously (*e.g.*, concurrently) as either separate formulations or as a joint formulation. Alternatively, the agents can be administered sequentially, as separate compositions, within an appropriate time frame as determined by the skilled clinician (*e.g.*, a time sufficient to allow an overlap of the pharmaceutical effects of the therapies). The Cadherin-11 antagonist and one or more other therapeutic agents can be administered in a single dose or in multiple doses, in an order and on a schedule suitable to achieve a desired therapeutic effect (*e.g.*, a reduction in and/or inhibition of joint inflammation). Suitable dosages and regimens of administration can be determined by a clinician and are dependent on the agent(s) chosen, pharmaceutical formulation and route of administration, various patient factors and other considerations.

The effectiveness of a therapy (*e.g.*, the reduction or elimination of joint inflammation and/or the prevention or inhibition of joint inflammation) can be determined by any suitable method (*e.g.*, imaging (MRI, NMR)).

According to the methods of the invention, a therapeutically effective amount of a Cad-11 antagonist is administered to a mammalian subject to treat an inflammatory joint disorder. The term "mammalian subject" is defined herein to include mammals such as primates (*e.g.*, humans) cows, sheep, goats, horses, dogs cats, rabbits, guinea pigs, rats, mice or other bovine, ovine, equine, canine feline, rodent and murine species.

Agents that are Cad-11 antagonists can be administered to a mammalian subject by a variety of routes. For example, the agent can be administered by any suitable parenteral or nonparenteral route, including, for example, topically (*e.g.*, cream, ointment), or nasally (*e.g.*, solution, suspension). Parenteral administration can include, for example, intraarticular, intramuscular, intravenous, intraventricular, intraarterial, intrathecal, subcutaneous, or intraperitoneal administration. The agent can also be administered orally (*e.g.*, in capsules, suspensions, tablets or dietary), transdermally, intradermally, topically, by inhalation (*e.g.*, intrabronchial, intranasal, oral inhalation or intranasal drops), transmucosally or rectally. Administration can be local or systemic as appropriate, and more than one route can be used concurrently, if desired. Localized administration of a Cad-11 antagonist can be achieved by intraarticular injection (*e.g.*, direct injection of the agent into a joint). The preferred mode of administration can vary depending upon the particular agent chosen. However, systemic intravenous or subcutaneous administration is generally preferred for antibodies.

Delivery can also be by injection into the brain or body cavity of a patient or by use of a timed release or sustained release matrix delivery systems, or by onsite delivery using micelles, gels and liposomes. Nebulizing devices, powder inhalers, and aerosolized solutions are representative of methods that may be used to administer such preparations to the respiratory tract. Delivery can be *in vitro*, *in vivo*, or *ex vivo*.

Agents that are proteins (*e.g.*, fusion protein) can be administered via *in vivo* expression of recombinant protein. *In vivo* expression can be accomplished by somatic cell expression according to suitable methods (see, *e.g.*, U.S. Patent No. 5,399,346). Further, a nucleic acid encoding the protein can also be incorporated into retroviral, adenoviral or other suitable vectors (preferably, a replication deficient infectious vector) for delivery, or can be introduced into a transfected or transformed host cell capable of expressing the protein for delivery. In the latter embodiment, the cells can be implanted (alone or in a barrier device), injected or otherwise introduced in an amount effective to express the protein in a therapeutically effective amount.

Nucleic acid-based Cadherin-11 antagonists (*e.g.*, aptamers) can be introduced into a mammalian subject of interest in a number of ways. For instance, nucleic acids may be expressed endogenously from expression vectors or PCR products in host cells or packaged into synthetic or engineered compositions (*e.g.*, liposomes, polymers, nanoparticles) that can then be introduced directly into the bloodstream of a mammalian subject (by, *e.g.*, injection, infusion). Anti-Cadherin-11 nucleic acids or nucleic acid expression vectors (*e.g.*, retroviral, adenoviral, adeno-associated and herpes simplex viral vectors, engineered vectors, non-viral-mediated vectors) can also be introduced into a mammalian subject directly using established gene therapy strategies and protocols (see *e.g.*, Tochilin V.P. *Annu Rev Biomed Eng* 8:343-375, 2006; Recombinant DNA and Gene Transfer, Office of Biotechnology Activities, National Institutes of Health Guidelines).

Agents that are Cadherin-11 antagonists (*e.g.*, small molecules) can be administered to a mammalian subject as part of a pharmaceutical or physiological composition, for example, as part of a pharmaceutical composition comprising a Cadherin-11 antagonist and a pharmaceutically acceptable carrier. Formulations or compositions comprising a Cadherin-11 antagonist or compositions comprising a Cadherin-11 antagonist and one or more other therapeutic agents (*e.g.*, an anti-inflammatory agent) will vary according to the route of administration selected (*e.g.*, solution, emulsion or capsule). Suitable pharmaceutical carriers can contain inert ingredients which do not interact with the Cadherin-11 antagonist. Standard pharmaceutical formulation techniques can be employed, such as those described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. Suitable pharmaceutical carriers for parenteral administration include, for example, sterile water, physiological saline, bacteriostatic saline (saline containing about 0.9% mg/ml benzyl alcohol), phosphate-buffered saline, Hank's solution, Ringer's lactate and the like. Formulations can also include small amounts of substances that enhance the effectiveness of the active ingredient (*e.g.*, emulsifying agents, solubilizing agents, pH buffering agents, wetting agents). Methods of encapsulation compositions (such as in a coating of hard gelatin or cyclodextran) are known in the art. For inhalation, the agent can be solubilized and loaded into a suitable dispenser for administration (*e.g.*, an atomizer or nebulizer or pressurized aerosol dispenser).

The pharmaceutical agent can be administered as a neutral compound or as a salt or ester. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic or tartaric acids, and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc. Salts of compounds containing an amine or other basic group can be obtained, for example, by reacting with a suitable organic or inorganic acid, such as hydrogen chloride, hydrogen bromide, acetic acid, perchloric acid and the like. Compounds with a quaternary ammonium group also contain a counteranion such as chloride, bromide, iodide, acetate, perchlorate and the like. Salts of compounds containing a carboxylic acid or other acidic functional group can be prepared by reacting with a suitable base, for example, a hydroxide base. Salts of acidic functional groups contain a countercation such as sodium or potassium.

The present invention will now be illustrated by the following Examples, which are not intended to be limiting in any way.

Exemplification

Example 1: Identification of Fabs having binding specificity for an epitope within the N-terminal 35 amino acids of the EC1 domain of human Cadherin-11.

Materials and Methods

Western blotting

Proteins were separated by SDS-PAGE and transferred to a nitrocellulose (NC) membrane using standard methods. Briefly NC membrane was rinsed with tris buffered-saline-tween (TBST) (8.8 g/L of NaCl, 0.2g/L of KCl, 3g/L of Tris base, 500ul/L of Tween-20, pH to 7.4). The membrane was blocked with 4% BSA dissolved in TBST for hour at 22°C. The NC membrane was rinsed 3X for 5 min each with TBST. Mouse anti-human Cad-11 antibody was diluted to 0.5 μg/ml in TBST and the NC was incubated for 1 hour at 22°C. The NC membrane was rinsed 3X for 5 minutes each in TBST. Goat anti-mouse Ig antibody conjugated with horse radish peroxidase (HRP) was diluted to 1 μg/ml in TBST and the NC membrane was incubated in secondary solution for a minimum time of 1 hour @ room temperature (RT) at 22°C. The NC membrane was rinsed 3X for 5 min each in TBST. Signal was developed using standard HRP method.

ELISA

The antigen (either 5 µg/ml or 50 µg of Cad-11-EC-1-Fc or 5 µg/ml of Cadherin peptide) was diluted in a buffer and used to coat the plates overnight at 4°C. The plates were washed and then blocked with 1.5 % BSA, 5% low fat milk powder in PBS Dilution buffer: 1.5 % BSA, 2.5% low fat milk powder, 0.1% Tween-20 in PBS. The plates were then incubated with bacterial lysate containing the anti-Cad-11 human fAbs or purified anti-Cad-11 human fAbs for 1 hr. After washing, the secondary antibody (Cy5-conjugated a-hu-Fab diluted 1/100) was applied for 25 min. The plates were then washed and the resulting fluorescence read.

Results

Three sets of previously reported Cadherin-11-specific antibodies were tested for an ability to bind to the EC1 domain of human Cadherin-11. These antibodies included antibodies that were raised against a mouse Cadherin-11-Fc fusion protein immunogen in Cadherin-11 knock-out or deficient mice (Lee *et al.*, *Science 315*:1006-1010 (2007)), antibodies that were raised in Cadherin-11 wild type mice against a human Cadherin-11-Fc fusion protein immunogen that had been produced in CHO cells (Valencia *et al.*, *J. Exp. Med. 200*(12):1673-1679 (2004)), and antibodies that were raised in Cadherin-11 wild type mice against a bacterially-produced protein containing the EC1-3 domains of human Cadherin-11. These antibodies were tested by western analysis for an ability to bind fusion proteins that contained only the EC1 domain of human Cad-11(Cadherin-11-EC1-Fc), the EC1 and EC2 domains of Cad-11 (Cad-11-EC1/2-Fc) or all 5 EC domains of Cad-11 (Cadherin-11-EC1-5-Fc). None of the antibodies tested recognized the EC1-Fc or the EC1-2-Fc fusion proteins on a Western blot (FIG. 1A). However, antibodies from each of the three sets tested recognized the human Cad-11-Fc fusion protein that included extracellular domains 1 through 5 (FIG. 1B). These results indicate that the tested antibodies did not bind to the EC1 or EC2 domains of human Cad-11, but recognized epitopes elsewhere in the extracellular region of this protein.

The available published anti-Cad-11 antibodies that bind Cad-11 expressing cells, 13C2, 23C6, 5F82 (Lifespan Science) and 283416 (R&D Systems), as well as the Cad-11 EC1-binding antibody H1M1, and the control antibody, MOPC, were tested by ELISA for the ability to bind fusion proteins that contained only the EC1 domain of human Cad-11 (Cadherin-11-EC1-Fc) or all 5 EC domains of Cad-11 (Cadherin-11-EC1-5-Fc). None of the available published anti-Cad-11 antibodies tested recognized the EC1-Fc (FIG. 1B, open bars) (data for 283416 is not shown here). However, the Cad-11 EC1 binding H1M1 antibody bound both the Cadherin-11-EC1-Fc and Cadherin-11-EC1-5-Fc (FIG. 1B closed bar). The control MOPC antibody bound neither fusion protein. These results indicate that the available published anti-Cad-11 antibodies do not bind to the EC1 domain of human Cad-11, but recognized epitopes elsewhere in the extracellular region of this protein.

To create an antibody specific for an epitope within the N-terminal 35 amino acids of the EC1 domain of human Cadherin-11, a phage display library (MorphoSys AG) encoding human Fabs was screened. Candidate Fabs were identified using two selection criteria - a positive selection for binding to a peptide that included the first 35 amino acids of the human Cadherin-11 EC1 domain, and a negative selection for binding to corresponding peptides from the EC1 domains of two closely related and highly homologous cadherins, Cadherin-8 and MN-Cadherin (FIG. 2). ELISA was used to assess binding.

Two screens were conducted. In the first screen, 96 Fab clones that bound the Cadherin-11 EC1 peptide were identified by ELISA. Seven (7) candidate Fabs bound the Cad-11 EC-1 peptide; however, only two of these bound to both EC-1 peptide and the EC1-2-Fc fusion protein. One of these two Fabs also bound to MN-Cad peptide. Accordingly, only one of the seven Fab clones specifically bound the EC1-Fc fusion protein, but did not bind to both MN-Cad and Cadherin-8 EC1 domain peptides. In a second screen, similar results were observed, as only 1 of 96 Fabs (clone F9) showing specificity for the Cadherin-11 EC1 peptide and EC1-2-Fc fusion protein, failed to bind MN-Cad and Cadherin-8 EC1 domain peptides (FIG. 3). The majority of the Cad-11 EC1 domain-binding Fabs tested showed cross reactivity with the MN-Cad peptide, which contains an EEY CAR sequence that overlaps with the EEY CAR sequence of Cad-11.

Example 2: A Fab that binds the EC1 domain of Cadherin-11 inhibits Cad-11 mediated cell aggregation in an *in vitro* assay

Materials and Methods

In vitro Cadherin-11 aggregation assay

431-D cells grow in suspension and do not normally express any cadherins and do not aggregate. 431-D-11 cells have been genetically modified to express Cad-11. When 431-D-11 cells are incubated in media alone and they begin to aggregate over 40 min and the clumps of cells settle to the bottom of well and the remaining non-aggregated cells in suspension can be measured and the percentage of aggregated 431-D-11 calculated. For the aggregation assay, 431-D-11 cells (D-11 cells) were grown to sub-confluence in a flask and then were removed from the flask using 0.05% Trypsin plus 0.53 mM EDTA. Approximately 2 x 10^6 431-D-11 cells were added to 2 ml of SME media (Dulbecco's Modified Eagle's Medium-high glucose, 0.1M Hepes pH 7.4 and 5U/ml DNAse) and were preincubated for 15 min on ice, either in the absence or presence of a test agent (*e.g.*, antibody, Fab, fusion protein). After pre-incubation with the test agent, the cells were transferred to a round bottom well on a 24-well plate and incubated at 37°C while rotating at 130 rpm on a rotary shaker. As cells aggregate they sink to the bottom of the well. At 0 min and 40 min, 200 µl from the middle of the sample were removed from the well and mixed with 25 µl of 8% glutaraldehyde to fix the cells. 200 µl of the fixed sample of cells were added to 9.8 ml of Coulter Counter isotonic saline solution and counted using a Coulter Counter set at the 8 µm to 24 µm threshold. 3 cell counts per sample were recorded. The percentage of cell decrease or aggregated cells at 40 min. compared to the percentage at the 0 min. time point was calculated.

Results

A candidate Fab (clone F9) having binding specificity for an epitope within the N-terminal 35 amino acids of the Cadherin-11 EC1 domain, which does not bind the EC1 domains of MN-Cad or Cad-8, was tested for an ability to inhibit Cad-11 mediated cell aggregation using an *in vitro* Cadherin-11 cell aggregation assay. The candidate Fab significantly inhibited Cadherin-11 mediated aggregation of cells at concentrations of 1 µg/ml or lower (FIGS. 4 and 5). In contrast, a Fab made from the 13C2 antibody that binds to an epitope in the extracellular region of Cad-11 outside the EC1/2 domains inhibited Cadherin-11 aggregation only at a concentration of 10 μg/ml, suggesting that the F9 Fab inhibits Cad-11 activity more effectively at lower concentrations than antibodies which bind to other portions of the extracellular domain of Cad-11.

Cad-11 mediated cell aggregation was also inhibited by various anti-Cadherin-11-EC1 domain Fabs that were specific for either Cad-11 alone, Cad-11 and Cad-8, Cad-11 and MN-Cad, or Cad-11, Cad-8 and MN-Cad (FIGS. 6 and 7). All cadherin-EC1-domain specific Fabs that were tested inhibited cell aggregation *in vitro* relative to the control samples (*e.g.*, SME medium (FIG. 6), a Fab specific for GFP (FIG. 7).

Example 3: Generation of Cadherin-11/Immunoglobulin Fusion Proteins Containing the EC1 domain of human Cadherin-11.

The Cadherin-11 EC1 region was prepared from a vector encoding the full length human Cadherin-11 cDNA (human Cad-11 cloned into the Not1 and Kpn-1 sites of the Invitrogen pCEP4® vector) using polymerase chain reaction (PCR) performed under standard conditions using the following oligonucleotide primers to introduce EcoR1 and BglII sites (see underlined sequences in forward and reverse primers, respectively) into the amplified product:

Forward Primer:
tttttttt<u>gaattc</u>atgaaggagaactactgtttacaagc (SEQ ID NO:8)
EcoR1

Reverse Primer:
tttttttt<u>agatct</u>ctggaccttgacaatgaattccgacgg (SEQ ID NO:9)
BglII

The amplified product was digested with restriction enzymes EcoR1 and BglII, and the digestion product was isolated and ligated into the pFUSE-hIgG2e1-Fc1 vector (InvivoGen) using the corresponding EcoR1 and BglII sites. TOP10 competent bacteria (Invitrogen) were transformed as described by the manufacturer with the ligation product and bacteria with the Cadherin-11-EC1-Fc plasmid were selected with zeomycin. Cadherin-11-EC1-Fc plasmid was isolated, sequenced and then used to transiently transfect 293F cells. Conditioned media was collected and the Cadherin-11-EC1-Fc fusion protein (SEQ ID NO:9) was purified using tangential flow filtration followed by isolation on a 50/50 mix protein A/protein G column equilibrated in 20 mM HEPES pH 7.4, 137mM NaCl, 3mM KCl and 1mMCaCl2. The purified Cadherin-11-EC1-Fc fusion protein was eluted from the column using 0.1 M Glycine (pH 3) and 1 mM CaCl2 and into tubes containing 200 µl of 1M Tris pH 7.4, and 1mM $CaCl_2$. The eluates with the Cad-11 fusion protein were then dialyzed against 20 mM Hepes (pH 7.4), 137 mM NaCl, 3 mM KCl and 1 mM $CaCl_2$. The size of the protein was confirmed by SDS PAGE (FIG. 10) and identity was confirmed by Western analysis using an antibody that recognizes the human Fc region (FIG. 11) and N-terminal sequencing (not shown). Cad-11-EC1-2-Fc was produced using techniques and conditions similar those described above.

Example 4: A Cad-11-EC1-Fc immunoglobulin fusion protein inhibits Cad-11 mediated cell aggregation in an *in vitro* assay

Materials and methods

Cell invasion/migration into a matrigel plug

Fibroblast-like synoviocye (FLS) migratory activity was assessed in Matrigel ECM-coated transwells in FLS media (Dulbecco's Modified Eagle's Medium-high glucose [Sigma #D7777], 10% Fetal Bovine Serum [Benchmark #100-106], 1% Pencillin-Streptomycin [Gibco 315140-122], 1% L-Glutamine [Gibco #25030], 0.5% Gentamicin [Gibco #15710-064]. Human FLS cell suspensions in FLS medium containing $1x10^4$ cells were added to the control insert or matrigel coated insert set in the well of a 24-well plate containing 0.750 mL of FLS medium. The plates were then incubated in a humidified tissue culture incubator at 37°C, 5% CO_2 atmosphere for 22 hours.

To calculate the number of cells that migrated, non-invading cells were removed from the upper surface of the membrane of control inserts by wiping with a cotton swab. A second wipe using a cotton swap wetted with FLS medium is repeated. Control inserts were then fixed and stained using a differential staining kit [Fisher #122-911]. Inserts are allowed to dry and cells are counted in 4 quadrants of the control insert using a microscope with a 10x objective. Triplicate inserts are counted and the totals averaged.

To calculate the number of cells that invaded the matrigel inserts, non-invading cells were gently removed from the surface of the matrigel insert by wiping with a cotton swab. A second wipe using a cotton swap wetted with FLS medium is repeated. Inserts were then fixed and stained using a differential staining kit [Fisher #122-911]. Inserts are allowed to dry and cells are counted in 4 quadrants of the control insert using a microscope with a 10x objective. Triplicate inserts are counted and the totals averaged.

Results

Cad-11-EC1-Fc significantly inhibited cell aggregation at a concentration of 3 μg/ml, while the full length Cad-11-EC1-5-Fc protein containing all 5 EC domains of human Cad-11 inhibited Cad-11 mediated aggregation at a concentration of 100 μg/ml (FIG. 13). These data show that the Cad-11-EC1-Fc immunoglobulin fusion protein effectively inhibits Cad-11 mediated cell aggregation in an *in vitro* assay.

In addition, the ability of the Cad-11-EC1-Fc immunoglobulin fusion protein to inhibit the invasion of human fibroblast like synoviocytes (FLS) into a matrigel plug was tested *in vitro*. Invasion of the FLS into matrigel is a complex process that involves the expression of MMP1, MMP-3, MMP-13, serine proteases, and other proteins by the FLS to degrade the matrigel as well as the migration of the FLS into matrigel. In a separate assay we saw no inhibition of migration of FLS through a normal fiber insert. This suggests the impact of the EC-Fc or 13C2 mAb is to inhibit the degradation of the matrigel (a surrogate for joint cartilage). Both the Cad-11-EC1-Fc and murine anti-Cad-11 mAb 13C2 inhibited FLS invasion into a matrigel plug in two independent experiments.

Example 5: Generation of antibodies against an EC1 domain peptide of human Cadherin-11

Materials and Methods

Balb/c mice were immunized bi-weekly in the foot pad nine times over a one month period with 0.01 mg of a peptide corresponding to the first 33 amino acids of the human Cad-11 EC1 domain (GWVWN QFFVI EEYTG PDPVL VGRLH SDIDS GDG (SEQ ID NO:10)), covalently linked to BSA. This peptide is referred to herein as Peptide 4. Spleens from the immunized mice were harvested and fused with a murine fusion partner, P3X63-Ag8.653, to create antibody-producing hybridomas. The hybridomas were expanded and subcloned at either 10, 3 or 0.5 cells/well, and the anti-Cad-11 antibody-containing media from the hybridomas were screened in an ELISA for the ability to specifically bind Cad-11 EC1-2 domain-containing protein produced in bacteria. Anti-Cad-11 antibody-containing media from these Peptide 4 hybridomas were screened concurrently for the absence of binding to proteins encompassing the EC1-2 domains of human Cad-8 and MN-Cadherin. 96-well EIA plates were coated overnight at 4°C with 0.05 ml of 0.0 to 0.3 mg/ml of each of the EC1-2 Cad proteins and then washed several times with saline buffer. Plates were then blocked with 0.25 ml of casein-PBS buffer and washed several times with saline buffer. Hybridoma media containing the anti-Cad-11 antibody were incubated neat in each well for 1 hr at 22°C and then washed twice with PBS-Tween (0.05%). 100 µl of a 1/1000 dilution of a goat anti-mouse IgG secondary antibody were added to each well, incubated for 30 min at 22°C, and then washed twice with PBS-Tween (0.05%). 100 µl/well of room temperature TMB (3, 3', 5, 5'-tetramethylbenzidine) reagent was added to each well and color was allowed to develop for 5 min at 22°C. The reaction was stopped with 100 µl of room temperature 2N sulfuric acid and the plate was read at 450 nm on a Wallac 1420 microplate reader.

The specificity of H1M1 and H14 anti-Cad-11 antibodies was tested further using an ELISA against the first 33 amino acids of the EC1 domains of Cad-11, Cad-7, Cad-8, Cad-20, Cad-24, Cad-9, Cad-18, and MN-Cad. Peptides corresponding to the region of Cad-7, Cad-8, Cad-20, Cad-24, Cad-9, Cad-18, MN-

Cad that overlapped with the G1-G33 region of the Cad-11 EC1 domain were synthesized and conjugated to biotin. 100 μl of a 30 ng/ml solution of each of these peptides in PBS-Tween (0.05%) were incubated in each well of a 96-well Netravidin plate for 2-3 hrs at 4°C and then washed twice with PBS-Tween (0.05%). Various concentrations of the anti-Cad-11 antibody were incubated in each well for 1 hr at 22°C and then washed twice with PBS-Tween (0.05%). 100 μl of a 1/1000 dilution of a goat anti-mouse IgG secondary antibody were added to each well, incubated for 30 min at 22°C, and then washed twice with PBS-Tween (0.05%). 100 μl/well of room temperature TMB (3, 3', 5, 5'-tetramethylbenzidine) reagent was added to each well and color was allowed to develop for 5 min at 22°C. The reaction was stopped with 100 μl of room temperature 2N sulfuric acid and the plate was read at 450 nm on a Wallac 1420 microplate reader.

Media from wells containing positive anti-Cad-11 antibody hybridomas were tested for the ability to bind to Cad-11 expressing cells. Frozen Cad-11-expressing 431D cells were thawed and washed twice in Hanks Balanced Saline Solution (HBSS) containing Ca^{2+} (0.137 M NaCl, 5.4 mM, KCl 0.25, mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 4.2 mM NaHCO) and then resuspended at 10^6 cells/ml in HBSS containing Ca^{2+}. 10^5 cells/well were stained with either a 50% or 16% anti-Cad-11 antibody media for 45 min on ice, washed twice in HBSS containing Ca^{2+}, stained with a secondary goat anti-mouse IgG antibody conjugated with phytoerytherin (Jackson ImmunoResearch, West Grove, PA) a concentration of 1% for 45 min on ice and then washed again twice in HBSS containing Ca^{2+}. Cells were then resuspended in 400 μl of HBSS containing Ca^{2+} and 1% formaldehyde and subsequently analyzed on a FACScalibur (Becton Dickenson, Franklin Lakes, NJ) for PE positive cells.

Results

Anti-Cad-11 antibody-containing media from the Peptide 4 hybridomas bound to the Cad-11 EC1-2 protein (FIG. 16, HL vs Cad-11), but not proteins containing the EC1-2 domains of Cad-8 and MN-Cad (FIG. 16, HL vs Cad8 and HL vs MNCad, respectively). Control hybridoma media did not bind any of the cadherin proteins tested (FIG. 16, Media vs Cad-11, Media vs Cad8, and Media vs

MNCad). These data demonstrate the presence of Cad-11 specific antibodies to Peptide 4 in the hybridomas.

Two Peptide 4 hybridomas, referred to herein as H1M1 and H14, bound to cells expressing human Cad-11 protein (FIGS. 17A-C and 17G-I), but not to non-Cad-11 control 431-D cells (FIGS. 17D-17F). The hybridoma cell line referred to as H1M1 has the A.T.C.C. Patent Deposit Designation PTA-9699, having been deposited on January 8, 2009. The hybridoma cell line referred to as H14 has the A.T.C.C. ATCC Patent Deposit Designation PTA-9701, having been deposited on January 9, 2009. These hybridomas contain anti-Cad-11 antibodies that recognize both Peptide 4 and Cad-11-expressing cells *in vitro*. The binding of these antibodies to Cad-11-expressing cells was shown to titrate with the amount of Peptide 4 antibody that was used, as shown in the plots of the titration of H1M1 (FIG. 18A) and H14 (FIG. 18B) versus the intensity of cell staining from the mean fluorescence intensity (MFI).

The H1M1 and H14 Peptide 4 anti-Cad-11 antibodies demonstrated >100-fold higher binding to Cad-11 than to any of the other cadherins tested, which included Cad-7, Cad-8, Cad-20, Cad-24, Cad-9, Cad-18, and MN-Cad. In most cases, no binding of H1M1 and H14 anti-Cad-11 antibodies to the other cadherins was observed. The anti-Cad-11 antibody H14 showed strong binding to Cad-11 (FIG. 19A), with 468-fold lower binding to Cad-8 (FIG. 19A), and virtually no binding to Cad-7, MN-Cad, Cad-9, Cad-18, Cad-20 or Cad-24 (FIG. 19B). Similarly, the anti-Cad-11 antibody H1M1 showed strong binding to Cad-11 (FIG. 20), with 1500-fold lower binding to Cad-8, and substantially no binding to Cad-7, MN-Cad, Cad-9, Cad-18, Cad-20 or Cad-24 (FIG. 20).

Example 6: The anti-Cad-11 EC1 domain antibodies H1M1 and H14 bind epitopes in the Cad-11 EC1 domain that include the amino acid sequence GPDP

Materials and Methods

To determine the epitope within the Cad-11 EC1 domain that the Peptide 4 Cad-11 EC1 antibodies H1M1 and H14 bind, four different peptides spanning the first 37 amino acids of the EC1 region (see FIG. 22) were immobilized in an ELISA format and the ability of the H1M1 and H14 antibodies to bind each of the four peptides was determined. 96-well Reactibind plates were coated overnight at 4°C with 0.3 ng/well of Peptide 1 (amino acids G1-P18 of the Cad-11 EC1 domain), 0.3 ng/well of Peptide 2 (amino acids G15-N34 of the Cad-11 EC1 domain), 0.3 ng/well of Peptide 3 (amino acids V19-Y37 of the Cad-11 EC1 domain), 0.3 ng/well of the immunogen Peptide 4 (amino acids G1-G33 of the Cad-11 EC1 domain), 20 ng of a fusion protein including the entire EC1 domain (EFL), or 20 ng of control human Ig (Fc-block). The wells were washed twice with PBS-Tween (0.05%), blocked with casein in dH_20 for 3 hrs at 22°C and then washed again twice with PBS-Tween (0.05%). Various dilutions of the different Peptide 4 Cad-11 EC1 domain antibodies were transferred to the peptide- or protein-coated wells, incubated for 45 min at 22°C and then washed twice with PBS-Tween (0.05%). 100 µl of a 1/1000 dilution of goat anti-mouse IgG secondary antibody (Jackson ImmunoResearch, West Grove, PA) were added to each well, incubated for 30 min at 22°C and then washed twice with PBS-Tween (0.05%). 100 µl/well of room temperature TMB reagent was added to each well and color was allowed to develop for 5 min at 22°C. The reaction was stopped with 100 µl of room temperature 2 N sulfuric acid and the plate was read at a wavelength of 450 nm on a Wallac 1420 microplate reader.

Results

The Peptide 4 anti-Cad-11 antibodies H1M1 at 1:11 (FIG. 21A) and H14 at 1:23 (FIG. 21B) both bound the Peptide 4 (PEP4) immunogen, as well as the EC1 domain fusion protein (EFL), in the ELISA as indicated by elevated OD450 plate readings relative to the control. Neither of these antibodies bound to the human IgG control (Fc block). In addition, both antibodies bound Peptide 2 (PEP2), but not Peptide 1 (PEP1) or Peptide 3 (PEP3), in the ELISA (FIGS. 21A and 21B).

These results suggest that the anti-Cad-11 EC1 domain antibodies H1M1 and H14 bind a common epitope in Peptides 2 and 4 that is not present in the overlapping Peptide 3. Amino acids shared by Peptides 2 and 4 that are upstream of Peptide 3 are highlighted in the boxed region shown in FIG. 22. These four amino acids, GPDP (SEQ ID NO:11), beginning at G15 of the Cad-11 EC1 domain, are likely part of the epitope recognized by the H1M1 and H14 antibodies.

Example 7: The anti-Cad-11 EC1 domain antibodies H1M1 and H14 inhibit aggregation of Cad-11-expressing cells *in vitro*

Materials and Methods

To assess the ability of the Cad-11 antibodies to inhibit Cad-11 mediated cell aggregation, 30 µg/ml of the H1M1 Peptide 4 antibody was cultured with 75,000 Cad-11 expressing A-431-D epidermoid carcinoma cells in 0.5 ml of DMEM-high glucose, 20mM Hepes pH 7.4, 10% FCS and 10U/ml DNAse in a 24-well round bottom polypropylene plate. The 24-well plates were placed on a rotating platform at approximately 60 rpm and incubated with 5% CO_2 overnight at 37°C. The next day, cell aggregation was assessed after photographing the plates at 100x (for H1M1 experiment) or 40x (for H14 experiment) magnification.

Results

In the presence of a control isotype antibody (30 µg/ml), the Cad-11-expressing cells formed large masses (FIG. 23A), while the parental Cad-11 negative cells remain as single or double cell groups (FIG. 23C). The H1M1-treated Cad-11 cells remained as small clumps of cells (FIG. 23B) that did not progress to form the large masses obtained using the control antibody.

Using the same assay, the anti-Cad-11 antibody H14 was also shown to inhibit Cad-11-mediated aggregation. While the parental Cad-11-expressing cells formed large clusters of aggregated cells (FIG. 24A), the H14 antibody (FIG. 24B) inhibited aggregation at a concentration of 30 µg/ml, as cell clusters were small and infrequent. These results indicate that the anti-Cad-11 antibodies H1M1 and H14 inhibit Cad-11-mediated cell aggregation *in vitro*.

Example 8: The anti-Cad-11 EC1 domain antibodies, H1M1 and H14, inhibit arthritis-associated joint swelling *in vivo* in a murine model of rheumatoid arthritis

Materials and Methods

Study 1 – Six-week-old male C57/Bl6 mice were injected with 150 μl of KBN sera on day 0 and day 2. KBN sera-treated mice received either saline injections (FIG. 25, unfilled triangles) or were treated with different doses of the H1M1 anti-Cad-11 EC1 antibody. Treatment regimens included dosing on day 0 with 0.5 mg of antibody/mouse and every second day (q2d) thereafter with 0.1 mg of antibody/mouse (0.5 mg+0.1 mg) (FIG. 25, filled triangles); dosing on day 0 with 0.5 mg of antibody/mouse (0.5 mg) (FIG. 25, diamonds); dosing every second day (q2d) with 0.1 mg of antibody/mouse (0.1 mg+0.1 mg) (FIG. 25, squares); or dosing every second day (q2d) with 0.3 mg of antibody/mouse (0.3 mg+0.3 mg) (FIG. 25, circles). The control group consisted of 5 mice and the treatment group consisted of 7 mice. Arthritis-associated joint swelling was determined by caliper measurements taken every second day.

Study 2 – Six-week-old male C57/Bl6 mice were injected with 150 μl of KBN sera on day 0 and day 2, and then were treated with either saline every second day (q2d) (FIG. 26, triangles), or one of the anti-Cad-11 antibodies, H1M1 (FIG. 26, squares) or H14 (FIG. 26, circles), at 0.3mg/dose q2d. The control group consisted of 5 mice and the treatment group consisted of 7 mice. Arthritis-associated joint swelling was determined by caliper measurements taken every second day.

Results

Study 1 – The H1M1 anti-Cad-11 antibody inhibited joint swelling relative to the control mice. The greatest inhibition of arthritis-associated joint swelling was observed by dosing KBN-treated mice with 0.3 mg of H1M1 antibody every second day (FIG. 25, circles).

Study 2 - Both of the anti-Cad-11 antibodies inhibited joint swelling relative to the control. In this study, the H14 antibody significantly delayed the onset of arthritis compared to the control animals (FIG. 27). All mice in the control group developed arthritis by day 3, while the H14-treated mice required 6 days before all of the animals developed arthritis.

These studies indicate that antibodies against the EC1 domain of human Cad-11 can inhibit the development and severity of arthritis *in vivo*.

Example 9: Generation of antibodies against another EC1 domain peptide of human Cadherin-11

Materials and Methods

Balb/c mice were immunized bi-weekly in the foot pad nine times over a 1 month period with 0.01 mg of peptide V19-Y37 (VL VGRLH SDIDS GDGNI KY (SEQ ID NO:12)), corresponding to 19 amino acids of the human Cad-11 EC1 domain, covalently linked to BSA. This peptide is referred to herein as Peptide 3. Spleens from the immunized mice were harvested and fused with a murine fusion partner P3X63-Ag8.653, to create antibody-producing hybridomas. These hybridomas were expanded and the anti-Cad-11 antibody-containing media from the hybridomas were screened for the ability to bind to a protein corresponding to the EC1-2 domain of Cad-11, which was produced in bacteria. The anti-Cad-11 antibody-containing media from these Peptide 3 hybridomas were screened concurrently for the absence of binding to proteins encompassing the EC1-2 domains of Cad-8 and MN-Cadherin. 96-well EIA plates were coated overnight at 4°C with 0.05 ml of 0 to 300 mg/ml of one of each of the EC1-2 Cad proteins, or CHO cell produced EC1-Fc fusion protein, and then washed several times with saline buffer. Plates were then blocked using 0.25 ml of casein-PBS buffer and subsequently washed several times with saline buffer. Hybridoma media containing the Peptide 3 anti-Cad-11 antibodies were incubated neat in each well for 1 hr at 22°C and then washed twice with PBS-Tween (0.05%). 100 μl of a 1/1000 dilution of a goat anti-mouse IgG secondary antibody were added to each well, incubated for 30 min at 22°C, and then washed twice with PBS-Tween (0.05%). 100 μl/well of room temperature TMB (3, 3', 5, 5'-tetramethylbenzidine) reagent was added to each well and color was allowed to develop for 5 min at 22°C. The reaction was stopped with 100 μl of room temperature 2N sulfuric acid and the plate was read at 450 nm on a Wallac 1420 microplate reader.

Media from the Peptide 3 hybridomas were also tested for the ability to bind to human Cad-11 protein expressed on cells. Frozen Cad-11-expressing 431D cells were thawed and washed twice in HBSS with Ca^{2+} and then resuspended at 10^6 cells/ml in HBSS containing Ca^{2+}. 10^5 cells/well were stained with either a 50% or

16% anti-Cad-11 antibody media for 45 min on ice, washed twice in HBSS containing Ca^{2+}, and then stained with a secondary goat anti-mouse IgG antibody conjugated with phytoerytherin at a concentration of 1% for 45 on ice and then washed again twice in HBSS containing Ca^{2+}. Cells were then resuspended in 400 µl of HBSS containing Ca^{2+} and 1% formaldehyde and subsequently analyzed on a FACScalibur for PE positive cells.

Results

Anti-Cad-11 antibody-containing media from the Peptide 3 hybridomas bound to the Cad-11 EC1-2 protein and the EC1-Fc fusion protein (FIG. 28, HL vs Cad-11 and HL vs Cad-11-EC1, respectively), but did not bind proteins containing the EC1-2 domains of Cad-8 and MN-Cad (FIG. 28, HL vs Cad8 and HL vs MNCad, respectively). Control hybridoma media did not bind any of the cadherin proteins (FIG. 28, Media vs Cad-11, Media vs Cad8, and Media vs MNCad).

Anti-Cad-11 antibodies from the Peptide 3 hybridomas also bound to cells expressing human Cad-11 protein (FIG. 29, see arrow), but not to non-Cad-11-expressing control cells that expressed Neos. This result confirmed the presence of anti-Cad-11 antibodies in the hybridomas that recognize both Peptide 3 and Cad-11-expressing cells *in vitro.*

Example 10: The anti-Cad-11 EC1 domain-specific antibody, H1M1, prevents the aggregation of human primary fibroblast like synoviocytes in vitro.

Materials and Methods

Aggregation Assay

In this assay, human fibroblast like synoviocytes (FLS) lines were cultured in FLS media (DMEM, 10% FCS, 1% Penicillin-Streptomycin, 1% L-Glutamine, 0.05% Gentamycin, 1% HEPES) until 90-100% confluent. The day of the assay, the media was removed from the FLS and the cells were removed from the flask using 0.05% Trypsin-EDTA, washed with MM media (DMEM, 10% FCS, 1% Penicillin-Streptomycin, 1% L-Glutamine, 0.05% Gentamycin, 1% HEPES) and then washed twice with HBS with calcium (0.137 M NaCl, 5.4 mM, KCl 0.25, mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 4.2 mM NaHCO).

Trypsonized FLS were resuspended in MM media to $4x10^4$ cells/ml, and $2x10^4$ cells/well were placed in a 24-well dish containing either media alone, isotype control antibody or various anti-Cad-11 antibodies at 30 µg/ml. The plate was incubated for 24 hrs in a 5% CO_2 incubator and the following day the wells were examined with a light microscope and photographed.

FLS Invasion Assay

To test the ability of the H1M1 antibody to inhibit FLS invasion into matrigel, which consists of a variety of extracellular matrix proteins, an invasion assay using a matrigel split well system was employed. This in vitro model of FLS biology mimics the ability of FLS to degrade and bore into human cartilage in articulated joints. In this assay, human FLS lines were cultured in FLS MM media until 90-100% confluent. The day before the assay was begun, the FLS were serum starved for 24 hrs in FLS media without serum. On the day of the assay, the media was removed from the serum-starved FLS and the cells were removed from the flask using 0.05% Trypsin-EDTA, washed with MM media and then washed 2x with HBS with calcium. Trypsonized FLS were resuspended in MM media to $4x10^5$ cells/ml. Prepared matrigel-coated inserts were placed in a 24-well plate containing the MM media with FCS, which acts as a mitogen for the FLS. On the other side of the chamber, 50 µl of MM media containing either no antibody or twice the working concentration of treatment antibody, to which 50 µl of $4x10^5$ cells/ml cell suspension was added, was introduced into each well. The chambers with the FLS and antibodies were incubated for 18 hrs in a 37 °C incubator.

After 24 hrs, the inserts with cells were fixed in methanol, the FLS stuck to the outside of the matrigel coated membrane were removed using a cotton swab, and the membranes were dried and then stained with propidium iodide (PI) for 30 min in the dark at room temperature. The PI stain was removed and the inserts were washed with D-glucose (1 mg/ml) and then dried in the dark. The membranes were excised and imaged on slides using a fluorescent microscope and the number of FLS that migrated into the membrane were quantified.

Results

FLS incubated with either media or control antibody formed cell aggregates and a broad network of cells that associate through cell processes (FIGS. 30A and B). In contrast, cells treated with the anti-Cad-11 antibody, H1M1, formed few cell aggregates and few connections (FIG. 30C).

In a series of FLS invasion assays with different primary human FLS, 30 μg/ml of H1M1 antibody consistently inhibited the invasion of FLS into the membrane. Specifically, H1M1 at a dose of 30 μg/ml inhibited FLS invasion by 80% compared to control antibody. This activity was greater than that observed with other Cad-11 antibodies that bound outside the EC1 region, including the 13C2 antibody (FIG. 31).

Example 11: H1M1 antibodies inhibit joint swelling in a KBN arthritis mouse model

Materials and methods

The anti-Cad-11 antibody, H1M1, was tested in a KBN model of arthritis where disease was induced by the administration of 2 doses of 75 μl KBN sera delivered on days 0 and 2. Groups of 7 male C57BL/6 mice were administered 10 mg/kg of H1M1 intraperitoneally every second day starting on day 0 and joint swelling in the mice was monitored daily. Joint swelling, or ankle thickness, was measured at the malleoli with the ankle in a fully flexed position, using spring-loaded dial calipers (Long Island Indicator Service, Hauppauge, NY).

Results

The H1M1 antibody suppressed joint swelling by 53% ($p<0.001$) compared to the control group (FIG. 32).

Example 12: Sequencing of H1M1 Variable Domains and Complementarity Determining Regions

Materials and Methods

RNA was extracted from 3 clones of H1M1 producing hybridoma cells (H1, H17 and H27). RT-PCR was performed using degenerate primer pools for murine signal sequences with constant region primers for each of IgGVH, IgMVH, IgκVL and IGλVL. Heavy chain variable (V)-region RNA was amplified using a set of six degenerate primer pools (HA to HF) and light chain V-region mRNA was amplified using a set of seven degenerate primer pools for the κ cluster (κA to κG) and one degenerate primer for the λ cluster (see Table 1).

For all RNA samples, amplification products from the heavy chain were obtained from the RNA reverse transcribed with IgGVH reverse transcription primer combined with heavy chain primer pools B and E. Amplification products were obtained from the IgκVL reverse transcription primer and with κ light chain primer pools B, C and G. The PCR products from each of the above pools were purified and cloned, and at least four clones for each product were sequenced.

For the H1M1-producing clones H1, H17 and H27, single functional heavy and light chain V-region sequences were identified for each sample and the three antibodies were found to be identical (Table 2). The heavy and light chain V-region sequences, and their CDR sequences, are shown in FIGS. 33 and 34, respectively.

The heavy and light chain V-regions from the three H1M1 hybridoma clones show good homology to their closest human germline sequences (64% and 82%, respectively) and the individual framework sequences have close homologues in the human germline database (Table 2). This high degree of homology reduces the likelihood that extensive engineering will be needed to produce a successful humanized antibody.

Table 1. Primers Used for Sequencing of H1M1 Variable Domains and Complementarity Determining Regions

Name	Bases	Degeneracy	Amino Acid Position	Sequence	SEQ ID NO:
MuIgVH5'-A	33	512	-20 to -13	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT	14
MuIgVH5'-B	34	64	-20 to -13	GGGAATTCATGRAATGSASCTGGGTYWTYCTCTT	15
MuIgVH5'-C	39	-	-20 to -11	ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTCCT	16
	36	48	-20 to -12	ACTAGTCGACATGGCTGTCYTRGBGCTGYTCYTCTG	17
	39	24	-20 to -11	ACTAGTCGACATGGVTTGGSTGTGGAMCTTGCYATTCCT	18
MuIgVH5'-D	36	8	-20 to -12	ACTAGTCGACATGAAATGCAGCTGGRTYATSTTCTT	19
	36	32	-20 to -12	ACTAGTCGACATGGRCAGRCTTACWTYYTCATTCCT	20
	36	-	-20 to -12	ACTAGTCGACATGATGGTGTTAAGTCTTCTGTACCT	21
MuIgVH5'-E	36	8	-20 to -12	ACTAGTCGACATGGGATGGAGCTRTATCATSYTCTT	22
	33	24	-20 to -13	ACTAGTCGACATGAAGWTGTGGBTRAACTGGRT	23
	35	64	-20 to -13	ACTAGTCGACATGGRATGGASCKKIRTCTTTMTCT	24
MuIgVH5'-F	35	32	-20 to -13	ACTAGTCGACATGAACTTYGGGYTSAGMTTGRTTT	25
	35	-	-20 to -13	ACTAGTCGACATGTACTTGGGACTGAGCTGTGTAT	26
	33	-	-20 to -13	ACTAGTCGACATGAGAGTGCTGATTCTTTTGTG	27
	38	-	-20 to -12	ACTAGTCGACATGGATTTTGGGCTGATTTTTTTTATTG	28
MuIgMVH3'-1	32	-	125 to 118	CCCAAGCTTACGAGGGGGAAGACATTTGGGAA	29
uIgGVH3'-2	35	32	126 to 119	CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	30
MuIgκVL5'-A	32	32	-20 to -13	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT	31
MuIgκVL5'-B	33	-	-20 to -13	GGGAATTCATGGAGACAGACACACTCCTGCTAT	32
MuIgκVL5'-C	39	8	-20 to -11	ACTAGTCGACATGGAGWCAGACACACTSCTGYTATGGGT	33
MuIgκVL5'-D	42	16	-20 to -10	ACTAGTCGACATGAGGRCCCCTGCTCAGWTTYTTGGIWTCTT	34
	41	128	-24 to -14	ACTAGTCGACATGGGCWTCAAGATGRAGTCACAKWYYCWGG	35
MuIgκVL5'-E	39	4	-20 to -11	ACTAGTCGACATGAGTGTGCYCACTCAGGTCCTGGSGTT	36
	41	32	-15 to -5	ACTAGTCGACATGTGGGGAYCGKTTTYAMMCTTTTCAATTG	37
	38	-	-20 to -11	ACTAGTCGACATGGAAGCCCCAGCTCAGCTTCTCTTCC	38

MuIgκVL5'-F	36	32	-20 to -12	ACTAGTCGACATGAGIMMKTCIMTTCAITTCYTGGG	39
	36	96	-20 to -12	ACTAGTCGACATGAKGTHCYCIGCTCAGYTYCTIRG	40
	35	8	-20 to -12	ACTAGTCGACATGGTRTCCWCASCTCAGTTCCTTG	41
	37	-	-16 to -8	ACTAGTCGACATGTATATATGTTTGTTGTCTATTTCT	42
MuIgκVL5'-G	39	-	-19 to -10	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT	43
	39	8	-22 to -13	ACTAGTCGACATGGATTTWCARGTGCAGATTWTCAGCTT	44
	37	12	-15 to -7	ACTAGTCGACATGGTYCTYATVTCCTTGCTGTTCTGG	45
	37	24	-15 to -7	ACTAGTCGACATGGTYCTYATVTTRCTGCTGCTATGG	46
MuIgκVL3'-1	30	-	122 to 116	CCCAAGCTTACTGGATGGTGGGAAGATGGA	47
MuIgλVL5'-A	33	128	-20 to -13	GGGAATTCATGGCCTGGAYTYCWCTYWTMYTCT	48
MuIgλVL3'-1	32	32	125 to 118	CCCAAGCTTAGCTCYTCWGWGGAIGGYGGRAA	49

* Amino acid position of the primer relative to the start codon of the Ig variable region coding sequence.

Mouse 5' primers A-B and the 3' primers contain 500 pmol of each primer at a concentration of 10 pmol/μl. Mouse 5' leader primers C-F (heavy chain) and D-G (light chain) contain an equimolar mixture (100 pmol of each primer at a concentration of 5 pmol/μl) of the indicated sequences.

Table 2: Sequence analysis of H1M1 hybridoma clones H1, H17 and H27

	H Chain	L Chain
CDR 1 Length	5aa	16aa
CDR 2 Length	17aa	7aa
CDR 3 Length	10aa	9aa
Closest Human Germline[b]	IGHV1-46*01 (64%)	IGKV2-29*02 (82%)
Closest Human FW1[b]	IGHV7-4-1*01 (80%)	IGKV2-30*01 (78%)
Closest Human FW2[b]	IGHV3-73*01 (64%)	IGKV2-40*01 (93%)
Closest Human FW3[b]	IGHV1-69*02 (69%)	IGKV2-40*01 (94%)
Closest Human J[b]	IGHJ6 (91%)	IGKJ2 or IGKJ4 (90%)

[a] CDR definitions and sequence numbering according to Kabat

[b] Germline ID(s) indicated followed by % homology

Example 13: Generation of Humanized Anti-Cad-11 Antibodies

Design of Humanized Anti-Cad-11 Antibody Variable Region Sequences

Structural models of the antibody variable (V) regions of the murine H1M1 monoclonal antibody described herein, also referred to as SYN0012 antibody, were produced using the Swiss protein database and analyzed in order to identify important "constraining" amino acids in the V regions that were predicted to be essential for the binding properties of the SYN0012/H1M1 antibody. Residues contained within the CDRs (using both Kabat and Chothia definitions) together with a number of framework residues were considered to be important. Both the VH and Vκ sequences of the SYN0012/H1M1 antibody contain typical framework residues and the CDR 1, 2 and 3 motifs are comparable to many murine antibodies.

From the above analysis, it was considered that composite human sequences of the SYN0012/H1M1 antibody could be created with a wide latitude of alternatives outside of CDRs but with only a narrow selection of possible alternative residues within the CDR sequences. Preliminary analysis indicated that corresponding sequence segments from several human antibodies could be combined to create

CDRs similar or identical to those in the murine SYN0012/H1M1 antibody sequences. For regions outside of and flanking the CDRs, a wide selection of human sequence segments were identified as possible components of humanized anti-Cad-11 antibody V regions.

Design of Variants

Based upon the above analysis, a large preliminary set of sequence segments that could be used to create SYN0012 humanized anti-Cad-11 antibody variants were selected and analyzed for *in silico* analysis of peptide binding to human MHC class II alleles (Perry *et al* 2009), and using the TCEDTM (T Cell Epitope Database of Antitope LLC, Cambridge UK) of known antibody sequence-related T cell epitopes (Bryson C, Jones TD and Baker MP. Prediction of immunogenicity of therapeutic proteins: validity of computational tools. *Biodrugs* 2010, 24(1): 1-8). Sequence segments that were identified as significant non-human germline binders to human MHC class II or that scored significant hits against the TCEDTM were discarded. This resulted in a reduced set of segments, and combinations of these were again analyzed, as above, to ensure that the junctions between segments did not contain potential T cell epitopes. Selected segments were then combined to produce heavy and light chain V region sequences for synthesis. For SYN0012 humanized anti-Cad-11 antibody variants, 5 VH chains (VH1-VH5; SEQ ID Nos:61, 63, 65, 67, 69) and three Vκ chains (Vκ1- Vκ3; SEQ ID Nos:71, 73, 75) were designed with sequences as detailed in FIGS. 35A-35H.

Construction of Composite Human Antibody Variants

All variant composite human antibody VH and Vκ region genes for SYN0012/H1M1 were synthesized using a series of overlapping oligonucleotides that were annealed, ligated and PCR amplified to give full length synthetic V regions. The assembled VH variants were cloned using Mlu I and Hind III sites, and the assembled Vκ variants were cloned using Bss HII and Bam H1 restriction sites directly into the pANT expression vector system (Antitope, Cambridge UK) for IgG4 (S241P) VH chains and Vκ chains (FIG. 36). All constructs were confirmed by sequencing.

Construction, Expression and Purification of Antibodies

All 15 combinations of the composite humanized IgG4(S241P) VH and Vκ chains were stably transfected into NS0 cells via electroporation and selected using 200nM methotrexate (Sigma Cat. No. M8407, Sigma Aldrich, UK). Methotrexate resistant colonies for each construct were tested for IgG expression levels using an IgG4 ELISA, and the best expressing lines were selected, expanded and frozen in liquid nitrogen. Cells expressing each of the 15 humanized antibody variants were successfully generated.

The fifteen humanized IgG4 (S241P) composite variants of SYN0012, named SDP011 (Vκ1/VH1), SDP021 (Vκ1/VH2), SDP031 (Vκ1/VH3), SDP041 (Vκ1/VH4), SDP051 (Vκ1/VH5), SDP061 (Vκ2/VH1), SDP071 (Vκ2/VH2), SDP081 (Vκ2/VH3), SDP091 (Vκ2/VH4), SDP101 (Vκ2/VH5), SDP111 (Vκ3/VH1), SDP121 (Vκ3/VH2), SDP131 (Vκ3/VH3), SDP141 (Vκ3/VH4), and SDP151 (Vκ3/VH5), were purified from NS0 cell culture supernatants on a Protein A sepharose column (GE Healthcare Cat. No. 110034-93) and antibody was quantified by OD280nm using an extinction coefficient (Ec(0.1%) = 1.51) based on the predicted amino acid sequence. It was noted during the purification of murine IgG1 anti-Cad-11 EC1 antibody (SYN0011) that the antibody precipitated as a flocculate at a pH of approximately 7.5; therefore all antibodies were neutralized post-acid elution to a pH of approximately 6.5 and buffer exchanged into 10 mM sodium acetate buffer pH 5.5 (1.4 mM acetic acid, 8.6 mM sodium acetate). Each humanized anti-Cad-11 antibody variant was purified and analyzed by reducing SDS-PAGE. Samples were loaded on a NuPage 4-12% Bis-Tris gel (Invitrogen Cat. No. NP0322BOX) and run at 200 V for 30 min. Bands corresponding to the predicted sizes of the VH and Vκ chains were observed with no evidence of any contaminating proteins (FIG. 37).

Example 14: Characterization of Binding of Humanized Anti-Cad-11 Antibodies to Cad-11 Proteins

Materials and Methods

Binding of Purified Antibodies to Recombinant Human Cad-11 EC1

The binding of the purified humanized composite variant antibodies (SDP011 to SDP151) to a recombinant Cad-11 EC1 human IgG1 fusion protein was assessed in a competition binding ELISA. A dilution series from 5 μg/ml to 0.0022 μg/ml of the humanized or control (SYN0012/H1M1) antibodies was premixed with a constant concentration of biotinylated murine anti-Cad-11 antibody, SYN0012/H1M1 (0.1 μg/ml, final concentration), before incubating for 1 hour at room temperature on a Nunc Immuno MaxiSorp 96 well flat bottom microtitre plate (Fisher Cat. No. DIS-971-030J) pre-coated with 0.25 μg/ml recombinant human Cad-11 EC1 (R&D Systems Cat. No. 1790-CA-050) diluted in PBS. The binding of the biotinylated mAb was detected with streptavidin-HRP (Sigma Cat. No. S5512) and tetramethylbenzidine (TMB) substrate (Invitrogen Cat. No. 00-2023).

Cadherin-Peptide Cross Reactivity ELISA

To assess which of the humanized anti-Cad-11 antibodies showed the greatest specificity for Cad-11, the humanized antibodies were tested in an ELISA format for ability binding to 37-mer peptides linked to BSA that encompassed the Cad-11 immunogen or the corresponding sequences of the 6 most closely related sequences to the Cad-11 immunogen, taken from Cadherins-7, -8, -9, -18, -20, and -24.

Reactibind plates (ThermoScientific) were coated with 500ng/ml of the BSA coupled cadherin 37-mer peptide (NE peptide) corresponding to Cadherins-7, -8, -9, -11, -18, -20, and -24 in 0.05% Tween (EMD, product code 9480) in PBS (pH 7.2) (Gibco(Invitrogen), #20012) at 100 μl volume/well for overnight at 4°C. The peptide containing solution was removed from the well and the plate blocked with a 2% bovine serum albumin (BSA) in PBS, 200 μl volume/well, for 2h at 22°C. The plate was then washed twice with 200 μl/well of PBS-Tween 0.05% at 22°C and blotted dry. A dilution series from 5 μg/ml to 8 ng/ml in a volume of 100μl/well of each the humanized anti-Cad-11 antibodies were incubated in the petide coated wells and incubated at 22°C for 1hr. Antibody solution was removed from the wells and the plate washed twice with 200 μl/well of 0.05% Tween-PBS at 22°C and blotted dry. The goat-anti mouse IgG (Pierce # 31432) was added to each well to plate at a 1:1000 dilution, 100 μl/well and incubate at 22°C for 30 min. The secondary antibody solution was removed and the plate washed twice with 200 μl/well of 0.05% Tween-PBS at 22°C and blotted dry. The reaction was developed by adding 100μl/well tetramethylbenzidine (TMB) reagent IgG (Pierce # 34022, 1 step Turbo TMB-ELISA) to plate and developed at 22°C for 5 min. The reaction was stopped using 100 μl RT 2N Sulfuric acid and the plate was read at 450nm on a microplate reader (Wallac 1420).

Binding to Cad-11 in Surface Plasmon Resonance (SPR)

To determine the affinity of SDP051 for recombinant his-tagged human Cad-11, rhCad-11 (R&D Systems) was immobilized to the chip at a single concentration, high resolution kinetics were determined for SDP051, and a 2-state reaction binding model was successfully fitted to the kinetic data.

Kinetic data were obtained at a flow rate of 40 μL/min to minimize any potential mass transfer effects. An analyte concentration series ranging from 12.5 nM to 0.195 nM was prepared by serial dilution. Two repeats of the blank (no mAb) and the 3.125 nM (first and last cycle) concentration of the analyte were programmed into the kinetic runs in order to check the stability of both the surface and analyte over the kinetic cycles. The SDP051 was injected over the Fcs for 360 seconds, long enough to observe the curvature in association phase.

Cad-11 Cell Binding Assay

Frozen Cad-11 expressing 431D cells (K. Johnson, U. Nebraska, Omaha) or the non-Cad-11 expressing parental 431D cells (K. Johnson, U. Nebraska, Omaha) were thawed, washed and resuspended at $2x10^6$ cells/ml and 50μl added to each well. Cells were then stained on ice for 45 minutes with increasing concentrations of the humanized SDP051 or SDP071 antibodies from 1ng/ml to 30μg/ml. After staining the cells were washed twice with Hanks balanced salt solution (HBSS) with 1% fetal bovine solution (FBS), resuspended and stained with mouse anti-human IgG4-PE (100x dilution) on ice for 45' protected from light. After staining the cells with the secondary agent, cells were washed twice with HBSS with 1% FBS, resuspended in 200 μl of 2% formaldehyde in HBSS with 1% FBS and then analyzed with a BD FACSCalibur.

Cadherin-Peptide Cross Reactivity FACS Assay

Frozen Cad-11 expressing 431D cells or the non-Cad-11 expressing parental 431D cells were thawed, washed and resuspended at $2x10^6$ cells/ml and 100,000 cells added to each well. Cells were the stained on ice for 45 minutes with increasing concentration of either the 37-mer Cad-11 peptide or Cad-7, Cad-8, Cad-9, Cad-18, Cad-20, or Cad-24 peptides with 0.5µg/ml SDP051. After staining the cells were washed twice with Hanks balanced salt solution (HBSS) containing 1% fetal bovine solution (FBS), resuspended and then stained with a 100µl of goat anti-mouse IgG-PE (100x dilution) on ice for 30' protected from light. After staining the cells with the secondary agent, cells were washed twice with HBSS with 1% FBS, resuspended and fixed in 200 µl of 2% formaldehyde in HBSS with 1% FBS and then analyzed with a BD FACSCalibur.

Results

All of the humanized anti-Cad-11 antibodies efficiently competed with SYN0012 for binding to the Cad-11 fusion protein (FIGS. 38A to C). This indicated that they bound the same epitope as SYN0012 or an overlapping epitope on Cad-11 and that they bound at least as well or better than SYN0012 to recombinant human Cad-11. The curves were used to calculate IC50 values for each antibody and these were normalized to the IC50 of SYN0012, which was included on each ELISA plate so that comparisons between plates could be made (Table 3). The expression levels of each cell line and the calculated isoelectric point (pI) of each antibody are also shown (Table 3).

SDP051, a Vκ1/VH5 antibody, bound Cad-11 with the greatest specificity, demonstrating over 100-fold greater binding to the Cad-11 antigen than the related antigens from the other cadherins (FIGS. 39A-O). The greatest cross reactivity was seen against Cad-8. SDP031, SDP061 and SDP071 also showed significant specificity for Cad-11, demonstrating over 20-fold greater binding to the Cad-11 antigen than the related antigens from the other cadherins. The light and heavy chain amino acid sequences for SDP031, SDP051, SDP061 and SDP071 are shown in FIGS. 40A-40D.

Table 3: Characterization of composite human anti-Cad-11 antibodies.

Synovex Number	V Region IDs	IC50 µg/ml	PI	Expression Levels mg/L
SDP011	VK1/VH1	0.91	7.64	20
SDP021	VK1/VH2	0.67	7.78	15
SDP031	VK1/VH3	0.84	7.78	12
SDP041	VK1/VH4	0.65	7.78	13
SDP051	VK1/VH5	0.99	7.78	15
SDP061	VK2/VH1	0.47	7.64	12
SDP071	VK2/VH2	0.33	7.78	10
SDP081	VK2/VH3	0.42	7.78	12
SDP091	VK2/VH4	0.57	7.78	8
SDP101	VK2/VH5	0.53	7.78	10
SDP111	VK3/VH1	0.68	7.64	6
SDP121	VK3/VH2	0.52	7.78	20
SDP131	VK3/VH3	0.55	7.78	15
SDP141	VK3/VH4	0.61	7.78	8
SDP151	VK3/VH5	0.72	7.78	34

The relative IC50 was calculated by dividing the value for the test antibody by that of SYN0012. Antibody expression levels given are for static cultures in T flasks. PI are calculated.

In the initial screen of the humanized anti-Cad-11 antibodies against the Cad-11 antigen and Cad-7, -8, -9, -11, -18, -20, -24 antigens at a coat of 500ng/ml (FIG.41A), SDP051 demonstrated far greater binding to Cad-11 than any other cadherin. The greatest degree of cross reactivity was seen with the Cad-8 antigen. This assay was repeated with the Cad-11, Cad-8 and Cad-18 antigens with a lower antigen coat concentration of 50ng/ml and a wider dilution series of the SDP051 antibody (FIG. 41B). In this assay, SDP051 had an EC50 of 6.8 ng/ml for Cad-11 and an EC50 of 1421 ng/ml of Cad-8, a binding differential of over 200-fold. An EC50 for Cad-18 could not be established due to the low level of binding observed. These results confirmed that SDP051 was highly specific for Cad-11. A BLAST search with the sequence of the Cad-11 EC1 domain indicated that the closest matches were among the Cad-7, -8, -9, -11, -18, -20, and -24 antigens. A BLAST search with the elucidated hexapeptide SDP051 binding epitope in the Cad-11 EC1 domain indicated that no other known human protein possessed this sequence.

SDP051 had sub-nanomolar affinity for the rhCad-11 protein with a equilibrium dissociation constant (KD) of 0.37 nM (Table 4).

Table 4: Physical Properties of SDP051.

mAb	SDP051
ka1 (1/Ms)	6.13×10^{5}
kd1 (1/s)	0.004301
ka2 (1/s)	0.01160
kd2 (1/s)	6.618×10^{-4}
KD (M)	3.787×10^{-10}
Chi^2	0.194

Characterization and control experiments for the chip surface suggested that the rhCad-11 immobilized at the single density was suitable to determine kinetic values for the anti-Cad-11 antibody interactions.

For the kinetic analysis, a 2 state reaction model was used (Equation 1, FIG. 42) based on the linked reaction control results, which indicated that another time dependent event was occurring once the antigen had bound to the antibody. As rhCad-11 is homodimeric and therefore contained two identical binding epitopes, it is likely that individual mAb molecules become bound sequentially through a first binding site and then both binding sites.

Equation 1: Two State Reaction $$A + B \underset{kd1}{\overset{ka1}{\rightleftarrows}} AB \underset{kd2}{\overset{ka2}{\rightleftarrows}} AB^{*}$$

In cell binding assays, SDP051 and SDP071 demonstrated dose dependent binding to Cad-11 expressing cells and no binding to the Cad-11 negative parental cell line (FIG. 43).

An alternative way to determine the specificity of SDP051 for Cad-11 is to measure the ability of different cadherins to block binding of SDP051 to Cad-11 expressing cells. This was performed for each of Cadherins-7, -8, -9, -18, -20, -24 or –MN. Only the Cad-11 peptide significantly blocked SDP051 binding to Cad-11^{+} cells. Results of the analysis are shown in FIGS. 44A-C.

Example 15: SDP051, a Humanized Anti-Cad-11 EC1 Antibody, Exhibits Low Immunogenicity in Assays Using Human Cells

Materials and Methods

Purification of Antibodies

SDP051 was purified from 5L cultures of antibody expressing cells grown to saturation. Supernatants were separated from cells and debris, adjusted to pH 7.4, filter sterilized and run through 5 ml Hi-Trap Mab Select Sure protein A affinity columns (GE Healthcare, Amersham, UK), which had previously been sanitized with 0.5M NaOH, at a flow rate of 5 ml/min. The column was washed with 100ml PBS pH 7.4. Antibody was eluted in 5 ml fractions with 0.1M sodium citrate pH 3.0 and each fraction immediately neutralized with 0.25 ml 1M Tris-HCl. The protein content of each fraction was monitored by UV absorption at 280 nm and protein containing fractions were pooled, buffer exchanged into 10 mM sodium acetate pH 5.5 and concentrated. The antibody was further purified by size exclusion chromatography using a 16/60 Sephacryl S200 column (GE Healthcare, Amersham, UK). The major peak fractions were collected, pooled, filter sterilized and tested for endotoxin levels using an Endosafe®-PTSTM (Charles River, Margate, UK). The purified antibody was stored at +4°C. Final concentrations were determined by UV absorption using calculated molar extinction coefficients, where A280 1.0 = 1.51 mg/ml. Each antibody was then diluted to 100 μg/ml in AIMV culture medium (Invitrogen, Paisley, UK).

Immunogenicity Assay

PBMC were isolated from healthy donor buffy coats (from blood drawn within 24 hours) obtained from the National Blood Transfusion Service (Addenbrooke's Hospital, Cambridge, UK). PBMC were isolated from buffy coats by Lymphoprep® (Axis-Shield, Dundee, UK) and density centrifugation, and CD8+ T cells were depleted using CD8+ RosetteSep® (StemCell Technologies Inc., London, UK).

Donors were characterized by identifying HLA-DR haplotypes using a Biotest SSP-PCR based tissue-typing kit (Biotest, Landsteinerstraße, Denmark) and by determining T cell responses to a 'reproducibility' control antigen (KLH: Pierce, Cramlington, UK). PBMC were then frozen and stored in liquid nitrogen until required.

PBMCs from each donor were thawed, counted and assessed for viability. Cells were revived in room temperature AIMV culture medium (Invitrogen, Paisley, UK) and resuspended in AIMV to 4-6x10^6 PBMC/ml. For each donor, bulk cultures were established in which a total of 1ml proliferation cell stock was added a 24 well plate. A total of 1ml of each diluted test sample was added to the PBMC to give a final concentration of 50μg/ml per sample. For each donor, a positive control (cells incubated with 100μg/ml KLH) and a negative control (cells incubated with culture media only) were also included. For the first 4 donors, an additional control was included to test for modulation of T cell responses by the test samples, where test sample and KLH were both added to the PBMC. Comparison of these samples with KLH alone can be used to assess the effects of the test samples on proliferation.

Cultures were incubated for a total of 8 days at 37°C with 5% CO_2. On days 5, 6, 7 and 8, the cells in each well were gently resuspended and 3 x 100μl aliquots were transferred to individual wells of a round bottomed 96 well plate. The cultures were pulsed with 1μCi [^{3}H]-Thymidine (Perkin Elmer®, Waltham, Massachusetts, USA) in 100μl AIMV culture medium and incubated for a further 18 hours before harvesting onto filter mats (Perkin Elmer®, Waltham, Massachusetts, USA) using a TomTec® Mach III cell harvester. Cpm for each well were determined by Meltilex™ (Perkin Elmer®, Waltham, Massachusetts, USA) scintillation counting on a Microplate Beta Counter in paralux, low background counting mode.

Cell Proliferation Assays

For proliferation assays, an empirical threshold of a SI equal to or greater than 2 (SI≥2.0) has been previously established whereby samples inducing proliferative responses above this threshold are deemed positive (where included, borderline SIs ≥1.90 are highlighted). Extensive assay development and previous studies have shown that this is the minimum signal to noise threshold allowing maximum sensitivity without detecting large numbers of false positive responses. For proliferation data sets (n=3), positive responses were defined by statistical and empirical thresholds:

1. Significance ($p<0.05$) of the response by comparing cpm of test wells against medium control wells using unpaired two sample student's t-test.
2. SI equal to or greater than 2 (SI≥2.0).

Results

To further assess potential immunogenicity, the humanized anti-Cad-11 EC1 antibody SDP051 was tested for its potential to induce the proliferation of CD8 depleted PBMC (CD4 T cells) from 25 different human T cell donors in an 8 day assay. While the chimeric SYN0014 antibody, which is a mouse-human IgG4 Fc chimeric version of the parental murine H1M1/SYN0012 antibody, induced proliferation in 28% of donors, the SDP051 antibody did not induce the proliferation of any T cell donor. These results support the earlier *in silico* finding that SDP051 has a low immunogenicity profile.

The results from the immunogenicity time course proliferation assay with the control chimeric SYN0014 antibody and humanized SDP051 antibody are shown in FIGS. 45A and B, respectively. The mouse-human chimeric SYN0014 antibody stimulated responses in 7 of the 25 donors (28% of donors) with one of the donor responses being borderline (1.98 for donor 16) but still significantly different from background (p<0.05, FIG. 45A). The humanized antibody SDP051 did not stimulate any responses in any of the donors (0 of the 25 or 0% of donors) indicating its low potential immunogenicity.

Example 16: SDP051, a Humanized Anti-Cad-11 EC1 Antibody, Inhibits Synoviocyte Invasion and MMP3 Expression

Materials and Methods

FLS Invasion Assay

Primary human FLS lines were cultured in FLS MM media (DMEM, 10% FCS, 1% Penicillin-Streptomycin, 1% L-Glutamine, 0.05% Gentamycin, 1%

HEPES) until 90-100% confluent. The day before the assay wa to begin, the FLS were serum starved for 24h in FLS media without serum. The day of the assay the media was removed from the serum-starved FLS and the cells were removed from flask using 0.05% Trypsin-EDTA and then washed 2x with HBS with calcium. Trypsinized FLS were resuspended in MM media to $4x10^5$ cells/ml. Prepared matrigel coated inserts were placed in the 24-well plate containing the MM media with 5% fetal calf serum (FCS) which acts as a chemoattractant for the FLS. On the other side of the chamber was placed 50μl of MM media containing either 6μg/ml or 0.6μg/ml of SDP051 (twice the working concentration) or 6μg/ml of the control AC1-P antibody, to which was then added 50 μl of $4x10^5$ cells/ml cell suspension to each well. The chambers with the FLS and antibodies were incubated for 18h in a 37C incubator

After 24hrs, the inserts with cells are fixed in 100% methanol for 30' at -20°C and the non-invading FLS stuck to the interior of the matrigel coated membrane were removed using a cotton swab. The membranes were dried and then stained with propidium iodide (PI) for 30min in the dark at room temperature. The PI stain was removed and the inserts were washed with D-glucose (1mg/ml) and then dried in the dark. The membranes were excised and imaged on slides using a fluorescent microscope and the number of FLS that have migrated through the membrane were quantified.

Results

Cad-11 is a critical cadherin required for FLS biology. As a result, Cad-11 antagonists are expected to inhibit the ability of human primary FLS to degrade and invade into a layer of matrigel, separating two wells. Matrigel consists of a variety of extracellular matrix proteins. This *in vitro* model of FLS biology replicates aspects of FLS biology including their ability to degrade and bore into cartilage.

SDP051 significantly inhibitED FLS invasion into matrigel. 0.3 μg/ml of SDP051 antibody was sufficient to inhibit 50% the invasion of primary human FLS into the matrigel membrane compared to the isotype control antibody (FIG. 46).

FLS express MMP3, which is thought to be one of the enzymes involved in the degradation of cartilage *in vivo* and matrigel in the *in vitro* invasion assay.

Supernatants from the invasion assay were harvested prior to fixation of the cells and frozen at -80° C. These supernatants were thawed and tested for the presence of MMP3 in the culture media. MMP3 levels were significantly increased in the well containing control antibody (AC1-P)-treated FLS that were stimulated to migrate with FCS (FIG. 47). MMP3 levels were significantly reduced in the SDP051 treated FLS stimulated with FCS. Levels were reduced about 50% compared to cells treated with control antibody.

Example 17: SDP051, a Humanized Anti-Cad-11 EC1 Antibody, Inhibits Joint Inflammation in an Animal Model of Arthritis

To test the SDP051 antibody in an animal model of arthritis, the K/BxN serum transfer model of arthritis developed by D. Mathis and C. Benoist was chosen (Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, *et al.* From systemic T cell self-reactivity to organ- specific autoimmune disease via immunoglobulins. *Immunity* 1999;10:451–61). This model presents extensive synovitis and therefore was a good test for the anti-inflammatory and joint protective properties of the SDP051 antibody.

Six to eight week-old C57Bl/6 mice were injected intraperitonealy (IP) with 75μl KBN sera (Jackson Labs, Bar Harbor, ME) both on Day 0 and Day 2. Mice were dosed IP with either 10 mg/kg of SDP051 or control (AC1-P) on Days -1, 0, 1, 2, 4 and 6. Joint swelling, or ankle thickness, was measured at the malleoli of both hind ankles of each mouse holding the ankle in a fully flexed position on Days 0, 2, 4, 6, 8, and 10 of the study, using spring-loaded dial calipers (Mitutoyo thickness gage model 7308, Long Island Indicator Service, Hauppauge, NY). The differential between the joint thickness on day 0 and the day of measurement was determined. Changes in ankle thickness over the course of the study are graphed in Figure 48. Differences in the ankle thickness scores were significant on days 6, 8 and 10. On Day 10, ankle swelling in the SDP051 group was 47% lower than swelling in the control group.

On Day 10, serum was collected from a terminal bleed of each study mouse. Serum was frozen on dry ice and stored at -80°C. Serum levels of the SDP051 were determined by testing sera in an ELISA using a Cad-11 peptide as a capture reagent, and then detecting SDP051 with an anti-human IgG. SDP051 could be readily detected in the sera of mice (FIG. 49).

Ankles were harvested at Day 10 and the histopathology was assessed. The severity of cartilage erosion, bone erosion and inflammation (cellular infiltrate) were assessed on a score of 0-5 by a qualified technician blinded to the study groups. Results of histopathology at Day 10 are summarized in Table 5. SDP051-treated animals demonstrated a 35% reduction in cartilage erosion scores, a 27% reduction in bone erosion scores and a 25% reduction in inflammation scores.

Table 5. Histopathology Results at Day 10 (KBN013)

Antibody	Ankle Histopath. Score: Cartilage Erosion	Ankle Histopath. Score: Bone Erosion	Ankle Histopath. Score: Inflammation
AC1-P	3.1 (± 0.3)	3.7 (+/-0.3)	4.4 (± 0.3)
SDP051	2.0 (± 0.4)*	2.7 (± 0.4)	3.3 (± 0.5)

* = $p<0.05$; ± = Standard error of the mean

Example 18: SDP051 Dose response in KBN Arthritis model

Mice were dosed with KBN sera on days 0 and 2. Mice were dosed IP with 10, 3, or 1 mg/kg of SDP051 or control AC3-1-P on Days 0, 2, 4 and 6. Changes in ankle thickness are shown in Figure 50. A trend to reduction in ankle thickness was seen in mice treated with 3 or 10 mg/kg SDP051. The reduction of ankle swelling in the 10 mg/kg group was significant on Days 6, 8 and 10. On Day 10, ankle swelling in this group was 44% lower than swelling in the control group. Over a series of studies, 10mg/kg of SDP051 significantly reduced ankle swelling. At 3mg/kg there was a trend for reduced ankle swelling.

Example 19. SDP051 Reduces Cytokines Implicated in Pathology of Rheumatoid Arthritis

Mice were dosed by tail vein IV with 10mg/kg of SDP051 or AC3-1-P control antibody on days 0, 1, 2, 4 and 6. On Day 6, the SDP051 group demonstrated 33% lower ankle joint swelling than the control group (Table 6). Cytokine analysis was performed on ankles harvested on day 7 from these mice as follows: Ankle joints were harvested, the skin and fur were removed and the joints were snap frozen in liquid nitrogen. Joints were then solubilized in homogenization buffer (Affymetrix tissue homogenization buffer (part# PC6002), supplemented with 2 mM PMSF (Sigma part# P7626) and 1X protease inhibitor cocktail (Sigma part #P8340), 0.1M indomethacin) and centrifuged, and the resulting supernatants were assayed for the levels of various cytokines and chemokines in multiplex assays (OceanRidge Biosciences).

The SDP051 treatment group demonstrated a 29% to 51% reduction in levels of the osteoclast differentiation factor, soluble Receptor Activator of Nuclear Factor Kappa-B Ligand (sRANKL), the osteoclastic/lymphoid chemokine, Macrophage Inflammatory Protein-1 (MIP1α), the moonocyte chemokine Monocyte Chemotactic Protein-1 (MCP1), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), and the leukocyte chemokine, Regulated upon Activation, Normal T-cell Expressed and Secreted (RANTES) (Table 6). Little to no effect was seen in levels of IL-6 or TNFα. A modest increase was seen in Vascular Endothelial Growth Factor (VEGF) while IL-23 could not be detected in the test groups.

The proposed mechanism of action of SDP051 is that the antibody binds Cad-11 on the fibroblast-like synoviocytes in the mouse joint resulting in reduced expression of pro-inflammatory cytokines and chemokines. Indeed, in the SDP051 treated mice, there was a reduction in several leukocyte chemokines that are suggested to be involved in FLS biology, including MCP-1, RANTES and MIP1α, in the joints of patients with RA (Garcia-Vicuna R, Gomez-Gaviro M, Dominguez-Luis M, Pec M, Gonzalez-Alvaro M, Alvaro-Gracia M and Diaz-Gonzalez F, 2004 Arth & Rheum, 50, pp3866-3877) (Table 6). In addition, sRANKL is an important mediator of osteoblast development and RANKL expression is induced on FLS by various inflammatory cytokines (Hashizume M, Hayakawa N and Mihara M, 2008, Rheumatology 47 pp.1635-1640). SDP051-treated mice demonstrated a reduction in sRANKL levels in their joints (Table 6). While these effects were modest, the local-region effects of these changes could be amplified.

Table 6. Day 7 Cytokine Results from KBN018 Study

Test Group	Ankle Thickness Change (1/100mm)	GM-CSF	IL-23	IL-6	MCP-1	MIP1 alpha	RANTES	sRANKL	TNF alpha	VEGF
AC3-1-P	39.29 (± 8.3)	4.9	0.0	26.1	188.0	99.5	20.5	327.7	2.2	17.3
SDP051	26.07 (± 8.6)	3.4	0.0	24.0	126.9	58.5	14.5	154.0	2.2	20.1
Naïve	n/a	2.3	5.4	1.7	21.8	10.7	11.1	17.2	0.1	15.5

The relevant teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A humanized antibody that specifically binds an EC1 domain of a mammalian Cadherin-11 protein and antagonizes said mammalian Cadherin-11 protein, comprising an antibody variable heavy chain region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67 and SEQ ID NO:69.

2. The humanized antibody of Claim 1, wherein the antibody inhibits one or more Cadherin-11-mediated activities selected from the group consisting of binding of the mammalian Cadherin-11 protein to one or more other Cadherin-11 proteins, aggregation of cells that express the mammalian Cadherin-11 protein, induction of enzyme expression, induction of cytokine expression, induction of growth factor expression, migration of cells expressing the mammalian Cadherin-11 protein and destruction of cartilage.

3. The humanized antibody of Claim 1, wherein the antibody variable heavy chain region comprises SEQ ID NO:69.

4. The humanized antibody of Claim 1, wherein the antibody comprises an antibody variable light chain region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:71, SEQ ID NO:73, and SEQ ID NO:75.

5. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:61 and the antibody variable light chain region comprises SEQ ID NO:71.

6. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:61 and the antibody variable light chain region comprises SEQ ID NO:73.

7. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:61 and the antibody variable light chain region comprises SEQ ID NO:75.

8. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:63 and the antibody variable light chain region comprises SEQ ID NO:71.

9. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:63 and antibody variable light chain region comprises SEQ ID NO:73.

10. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:63 and the antibody variable light chain region comprises SEQ ID NO:75.

11. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:65 and the antibody variable light chain region comprises SEQ ID NO:71.

12. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:65 and the antibody variable light chain region comprises SEQ ID NO:73.

13. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:65 and the antibody variable light chain region comprises SEQ ID NO:75.

14. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:67 and the antibody variable light chain region comprises SEQ ID NO:71.

15. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:67 and the antibody variable light chain region comprises SEQ ID NO:73.

16. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:67 and the antibody variable light chain region comprises SEQ ID NO:75.

17. The humanized antibody of Claim 4, wherein the antibody variable light chain region comprises SEQ ID NO:71.

18. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:69 and the antibody variable light chain region comprises SEQ ID NO:73.

19. The humanized antibody of Claim 4, wherein the antibody variable heavy chain region comprises SEQ ID NO:69 and the antibody variable light chain region comprises SEQ ID NO:75.

20. The humanized antibody of Claim 1, wherein the antibody binds an epitope that is present in SEQ ID NO:3.

21. The humanized antibody of Claim 1, wherein the antibody binds an epitope that is present in SEQ ID NO:10.

22. The humanized antibody of Claim 1, wherein the antibody binds an epitope that comprises SEQ ID NO:11.

23. The humanized antibody of Claim 1, wherein the antibody is a whole antibody.

24. The humanized antibody of Claim 1, wherein the antibody is an antibody fragment.

25. The humanized antibody of Claim 24, wherein the antibody fragment is selected from the group consisting of an Fab, an Fab', an $F(ab')_2$ and an scFv.

26. A humanized antibody that specifically binds an EC1 domain of a mammalian Cadherin-11 protein and antagonizes said mammalian Cadherin-

11 protein, comprising an antibody variable heavy chain region comprising SEQ ID NO:69 and an antibody variable light chain region comprising SEQ ID NO:71.

27. The humanized antibody of Claim 26, wherein the antibody inhibits one or more Cadherin-11-mediated activities selected from the group consisting of binding of the mammalian Cadherin-11 protein to one or more other Cadherin-11 proteins, aggregation of cells that express the mammalian Cadherin-11 protein, induction of enzyme expression, induction of cytokine expression, induction of growth factor expression, migration of cells expressing the mammalian Cadherin-11 protein and destruction of cartilage.

28. The humanized antibody of Claim 26, wherein the antibody is a whole antibody.

29. The humanized antibody of Claim 26, wherein the antibody is an antibody fragment.

30. The humanized antibody of Claim 29, wherein the antibody fragment is selected from the group consisting of an Fab, an Fab', an $F(ab')_2$ and an scFv.

31. A pharmaceutical composition comprising a humanized antibody that specifically binds an EC1 domain of a mammalian Cadherin-11 protein and antagonizes said mammalian Cadherin-11 protein, and a pharmaceutically-acceptable carrier, wherein the antibody comprises an antibody variable heavy chain region comprising SEQ ID NO:69 and antagonizes said mammalian Cadherin-11 protein.

32. The pharmaceutical composition of Claim 31, wherein the antibody comprises an antibody variable light chain region comprising SEQ ID NO:71.

33. The pharmaceutical composition of Claim 31, wherein the antibody inhibits aggregation of cells that express said mammalian Cadherin-11.

34. The pharmaceutical composition of Claim 31, wherein the antibody is a whole antibody.

35. The pharmaceutical composition of Claim 31, wherein the antibody is an antibody fragment.

36. The pharmaceutical composition of Claim 31, further comprising a disease-modifying anti-rheumatic drug.

37. The pharmaceutical composition of Claim 36, wherein the disease-modifying anti-rheumatic drug is methotrexate.

38. The pharmaceutical composition of Claim 31, further comprising an anti-inflammatory agent.

39. The pharmaceutical composition of Claim 38, wherein the anti-inflammatory agent is an NSAID or a steroid.

40. An isolated nucleic acid encoding the humanized antibody of Claim 1.

41. The isolated nucleic acid of Claim 40, wherein said nucleic acid is present in a vector.

42. An isolated cell expressing the humanized antibody of Claim 1.

43. A method of treating a Cadherin-11-mediated disorder in a mammalian subject in need thereof, comprising administering to the subject a therapeutically effective amount of a humanized antibody that specifically binds an EC1 domain of a mammalian Cadherin-11 protein, wherein the antibody comprises an antibody variable heavy chain region comprising SEQ ID NO:69 and antagonizes said mammalian Cadherin-11 protein.

44. The method of Claim 43, wherein aggregation of cells that express said mammalian Cadherin-11 protein is inhibited in one or more joints of said subject.

45. The method of Claim 43, wherein the antibody comprises an antibody variable light chain region comprising SEQ ID NO:71.

46. The method of Claim 43, wherein the antibody is a whole antibody.

47. The method of Claim 43, wherein the antibody is an antibody fragment.

48. The method of Claim 43, wherein the Cadherin-11-mediated disorder is selected from the group consisting of an inflammatory disorder, a fibrosis, and a cancer.

49. The method of Claim 43, wherein the Cadherin-11-mediated disorder is an inflammatory joint disorder selected from the group consisting of rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, ankylosing spondylitis, juvenile chronic arthritis, chronic Lyme disease and joint arthritis associated with systemic lupus erythrematosus.

50. The method of Claim 43, wherein the mammalian subject is a human.

51. The method of Claim 43, wherein the antibody is administered systemically.

52. The method of Claim 43, wherein the antibody is administered intravenously.

53. The method of Claim 43, wherein the antibody is administered by direct injection into a joint.

54. The method of Claim 43, wherein the antibody inhibits migration, adhesion, invasion into cartilage, or intercellular signaling of cells that express said mammalian Cadherin-11 protein in one or more joints of said subject.

55. The method of Claim 43, wherein the antibody inhibits induction of expression or activity of an enzyme selected from the group consisting of a collagenase, a serine protease, and a matrix metalloproteinase in cells that express said mammalian Cadherin-11 protein in one or more joints of said subject.

56. The method of Claim 43, wherein the antibody inhibits induction of expression or activity of a cytokine or growth factor selected from the group consisting of a IL-6, IL-8, RANKL and TRANCE in cells that express said mammalian Cadherin-11 protein in one or more joints of said subject.

57. The method of Claim 43, wherein the antibody is administered in combination with at least one agent selected from the group consisting of a disease-modifying anti-rheumatic drug and an anti-inflammatory agent.

58. Use of a humanized antibody for the treatment of a Cadherin-11-mediated disorder in a mammalian subject in need thereof, wherein the antibody specifically binds an EC1 domain of a mammalian Cadherin-11 protein and comprises an antibody variable heavy chain region comprising SEQ ID NO:69.

59. The use of Claim 58, wherein the Cadherin-11-mediated disorder is an inflammatory disorder.

60. The use of Claim 59, wherein the inflammatory disorder is an inflammatory joint disorder.

61. The use of Claim 60, wherein the inflammatory joint disorder is rheumatoid arthritis.

62. The use of Claim 58, wherein the Cadherin-11-mediated disorder is fibrosis.

63. The use of Claim 58, wherein the Cadherin-11-mediated disorder is cancer.

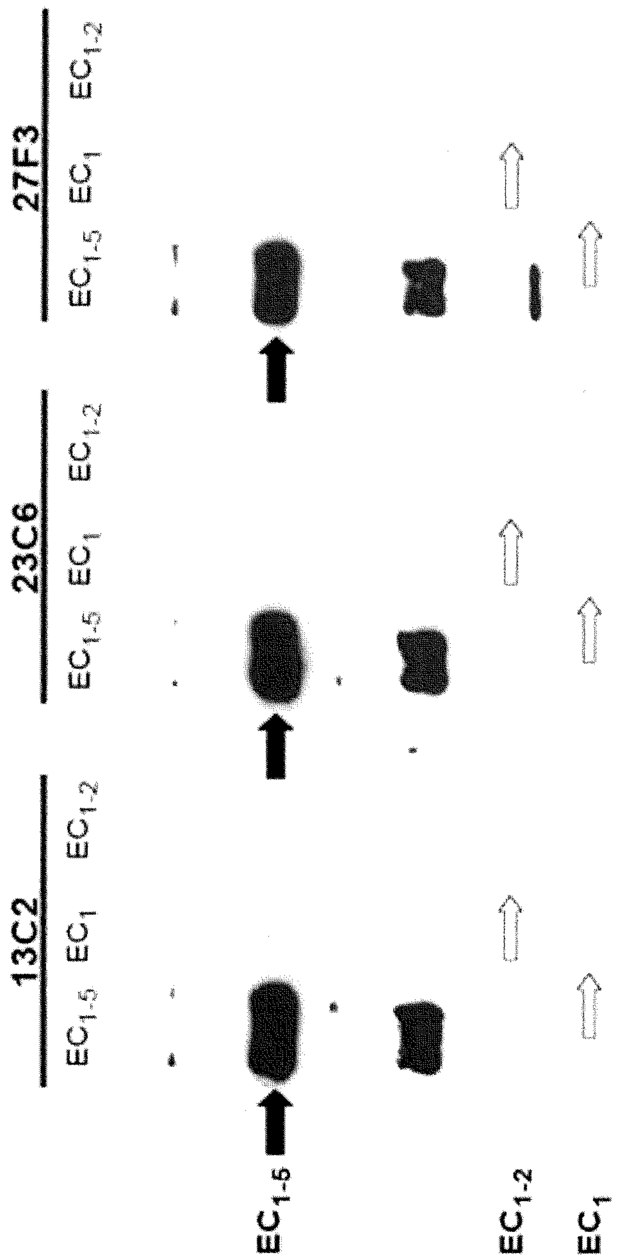

13C2
23C6
27F3
EC1-5 EC1 EC1-2
EC1-5 EC1 EC1-2
EC1-5 EC1 EC1-2
EC1-5
EC1-2
EC1

FIG. 1A

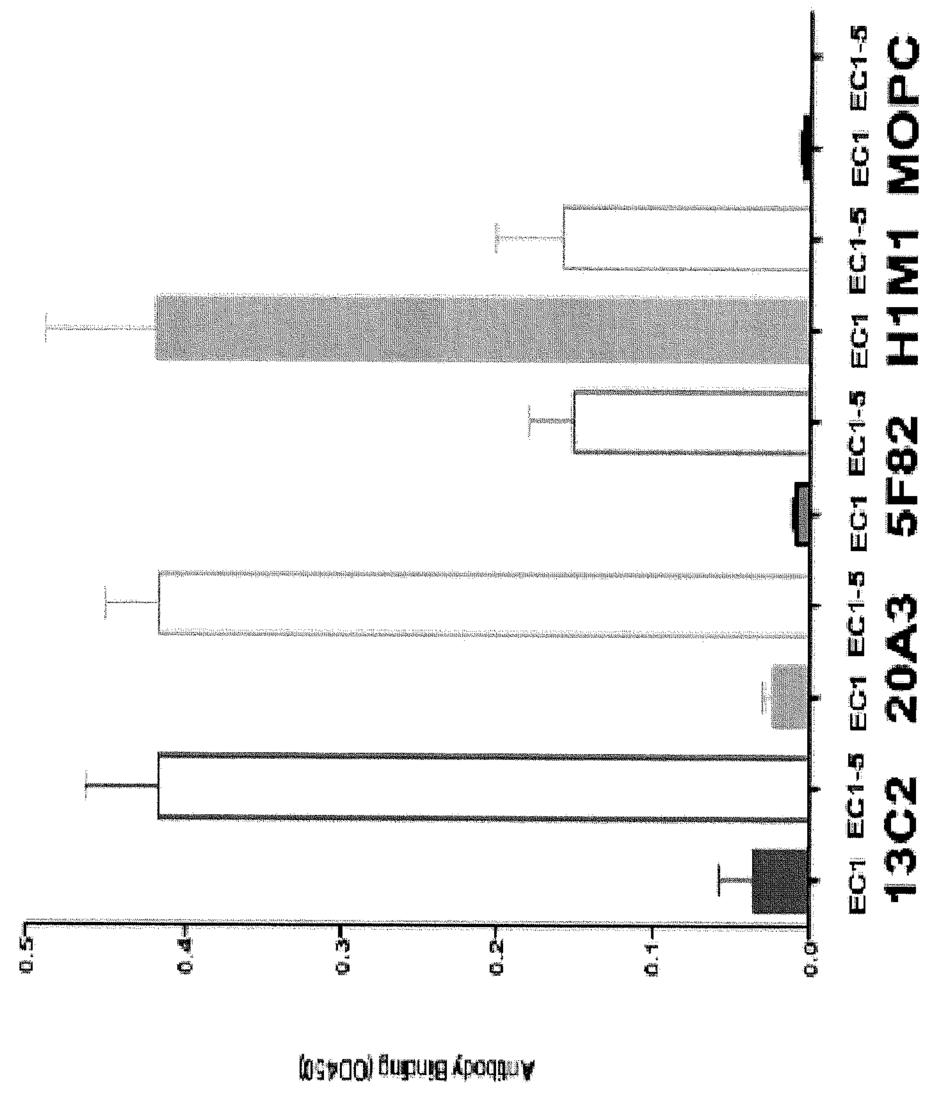

Antibody Binding (OD450)
0.0
0.1
0.2
0.3
0.4
0.5
EC1 EC1-5
13C2
EC1 EC1-5
20A3
EC1 EC1-5
5F82
EC1 EC1-5
H1M1
EC1 EC1-5
MOPC

FIG. 1B

Human Cad-11	GWVWN	QFFVI	EEYTG	PDPVL	VGRLH	SDIDS	GDGN	(SEQ ID NO:3)
Human Cad-8	GWVWN	QMFVL	EEFSG	PEPIL	VGRLH	TDLDP	GSKK	(SEQ ID NO:4)
Human MN-Cad	SWVWN	QFFVL	EEYTG	TDPLY	VGKLH	SDMDR	GDGS	(SEQ ID NO:5)

FIG. 2

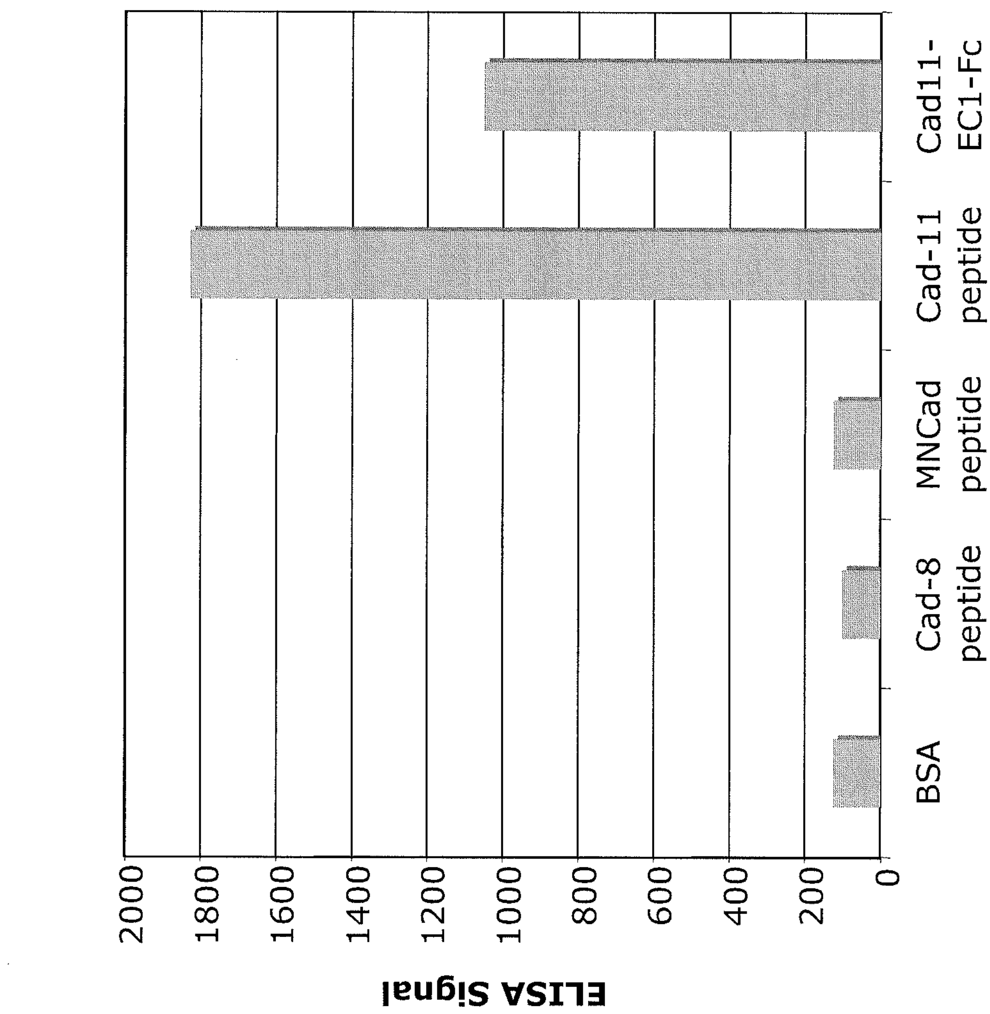
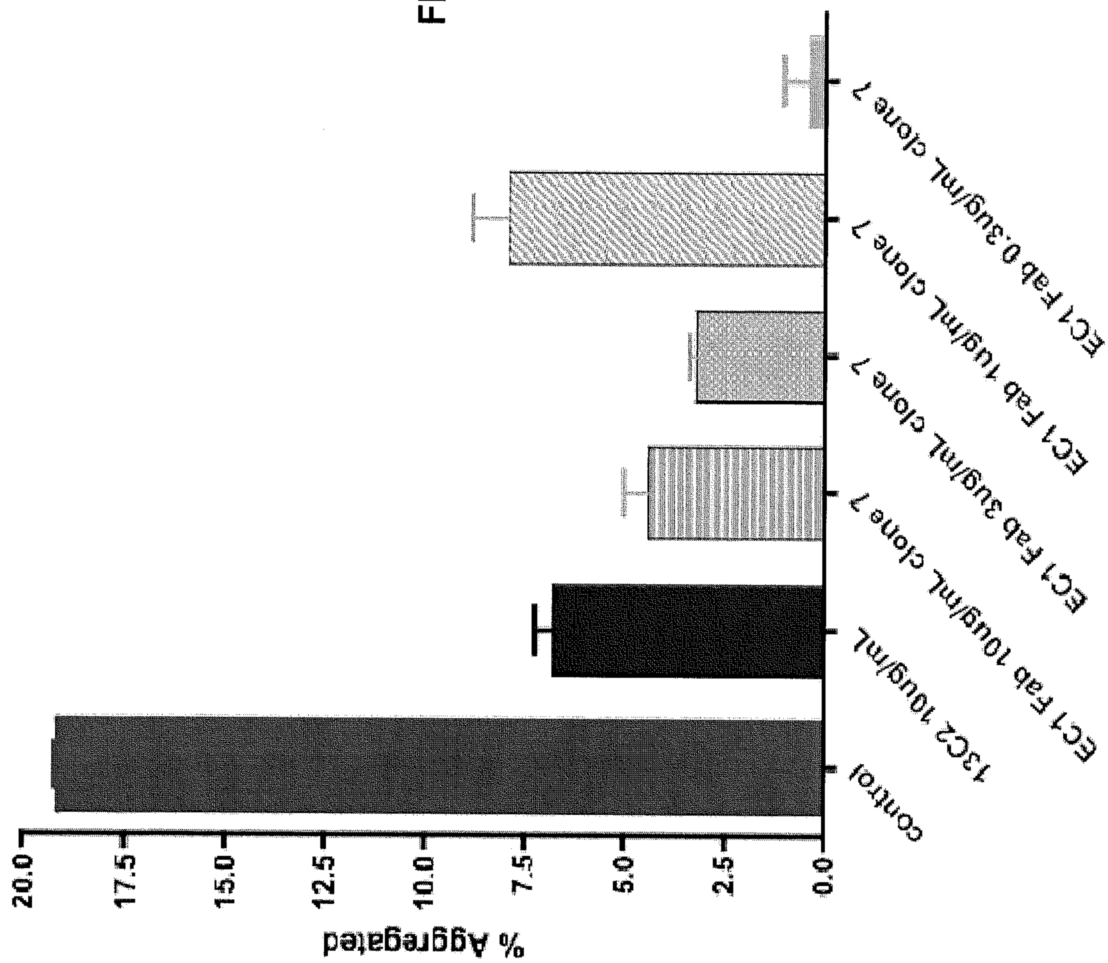

ELISA Signal
2000
1800
1600
1400
1200
1000
800
600
400
200
0
BSA
Cad-8 peptide
MNCad peptide
Cad-11 peptide
Cad11-EC1-Fc

FIG. 3

FIG. 4


% Aggregated
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
control
13C2 10ug/mL
EC1 Fab 10ug/mL clone 7
EC1 Fab 3ug/mL clone 7
EC1 Fab 1ug/mL clone 7
EC1 Fab 0.3ug/mL clone 7

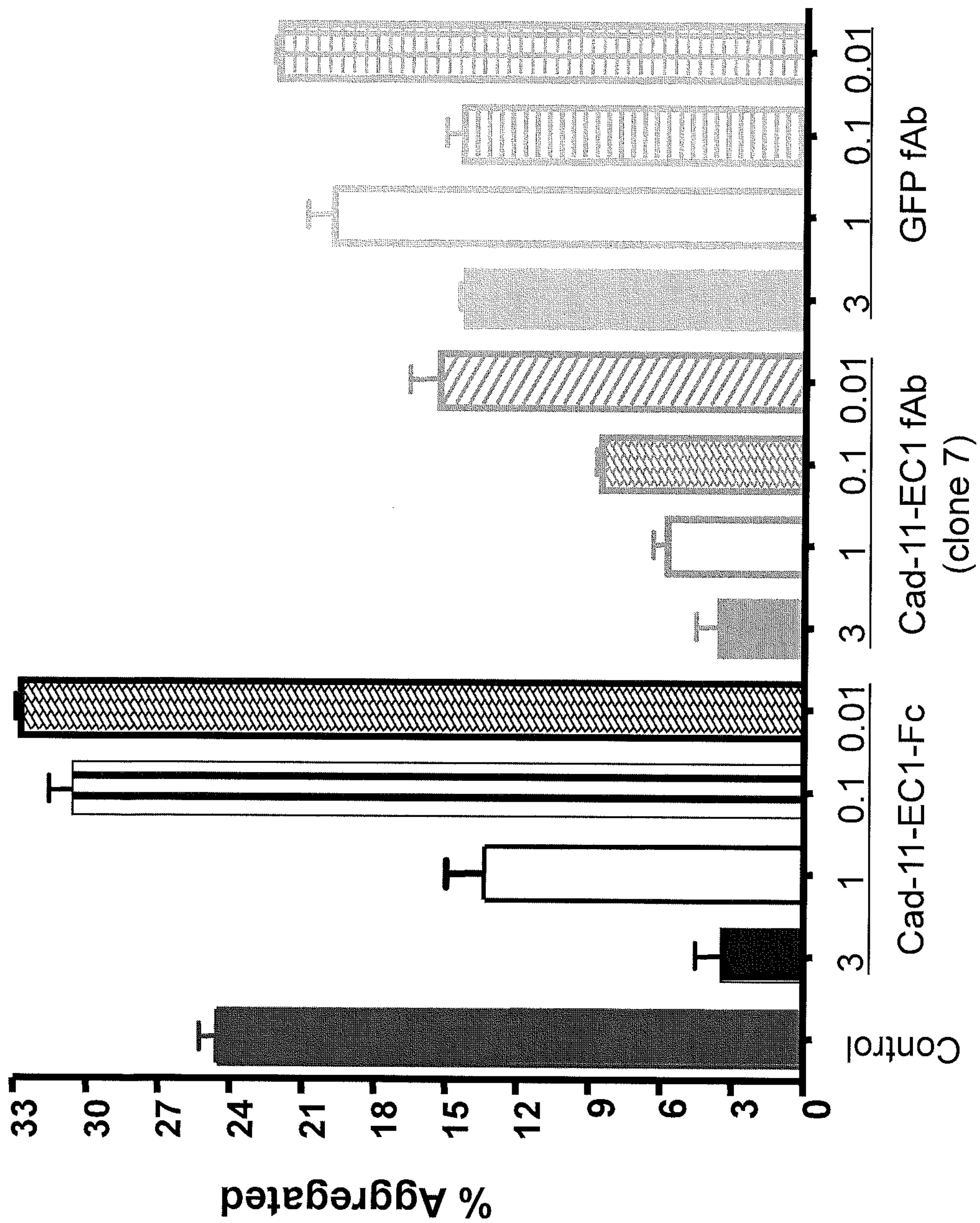

% Aggregated
0
3
6
9
12
15
18
21
24
27
30
33
Control
3
1
0.1
0.01
Cad-11-EC1-Fc
3
1
0.1
0.01
Cad-11-EC1 fAb
(clone 7)
3
1
0.1
0.01
GFP fAb

FIG. 5

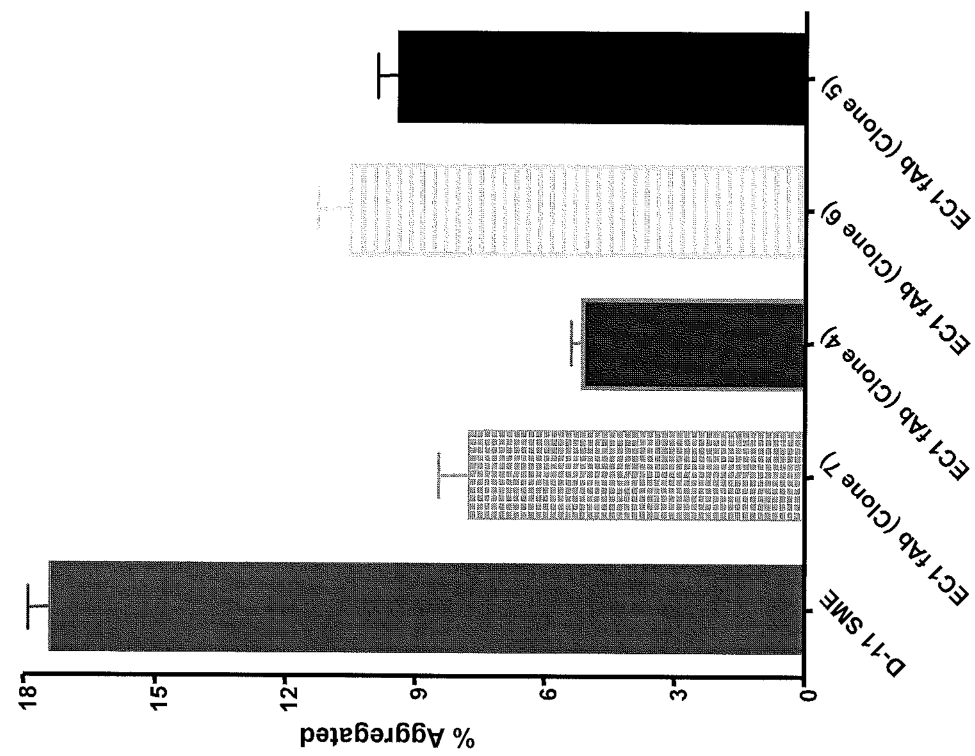

% Aggregated
0
3
6
9
12
15
18
D-11 SME
EC1 fAb (Clone 7)
EC1 fAb (Clone 4)
EC1 fAb (Clone 6)
EC1 fAb (Clone 5)

FIG. 6

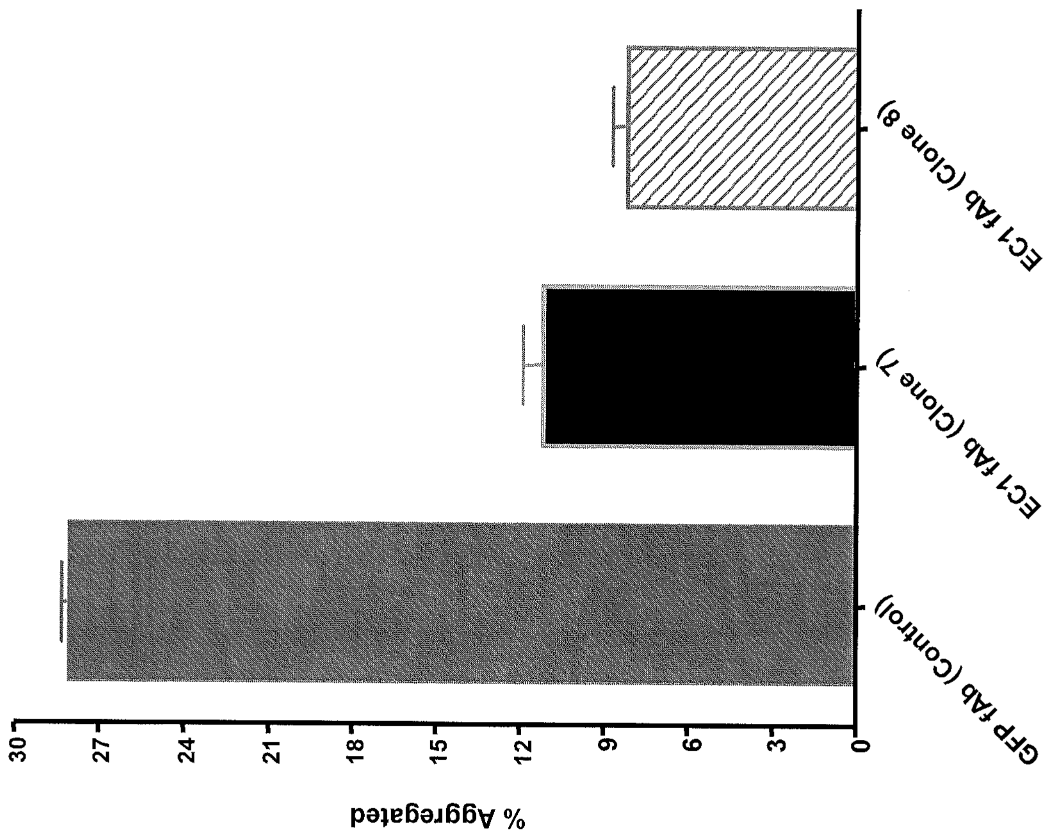

% Aggregated
0
3
6
9
12
15
18
21
24
27
30
GFP fAb (Control)
EC1 fAb (Clone 7)
EC1 fAb (Clone 8)

FIG. 7

ATGAAGGAGAACTACTGTTTACAAGCCGCCCTGGTGTGCCTGGGCATGCTGTGCCACAGCCATGCCTTTGC
CCCAGAGCGGCGGGGGCACCTGCGGCCCTCCTTCCATGGGCACCATGAGAAGGGCAAGGAGGGGCAGGT
GCTACAGCGCTCCAAGCGTGGCTGGGTCTGGAACCAGTTCTTCGTGATAGAGGAGTACACCGGGCCTGAC
CCCGTGCTTGTGGGCAGGCTTCATTCAGATATTGACTCTGGTGATGGGAACATTAAATACATTCTCTCAGGGG
AAGGAGCTGGAACCATTTTTGTGATTGATGACAAATCAGGGAACATTCATGCCACCAAGACGTTGGATCGAG
AAGAGAGAGCCCAGTACACGTTGATGGCTCAGGCGGTGGACAGGGACACCAATCGGCCACTGGAGCCACC
*GTCGGAATTCATTGTCAAGGTCCAG*AGATCT**GTGGAGTGCCCACCTTGCCCAGCACCACCTGTGGCAGGA**
CCTTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTGATGATCTCCAGAACCCCTGAGGTCACGT
GCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCATGGAG
GTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTC
ACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCC
AGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC
CCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAG
CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACACCTCCCATGCT
GGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAA
CGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCT
CCGGGTAAATGAGTGCCACGGCTAGCTGG (SEQ ID NO:6)

FIG. 8

MKENYCLQAALVCLGMLCHSHAFAPERRGHLRPSFHGHHEKGKEGQVLQRSKR
GWVWNQFFVIEEYTGPDPVLVGRLHSDIDSGDGNIKYILSGEGAGTIFVIDDKSGNI
HATKTLDREERAQYTLMAQAVDRDTNRPLEPPSEFIVKVQ
RS**VECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFN**
WYVDGMEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLP
APIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGKVPRLA (SEQ ID NO:7)

FIG. 9

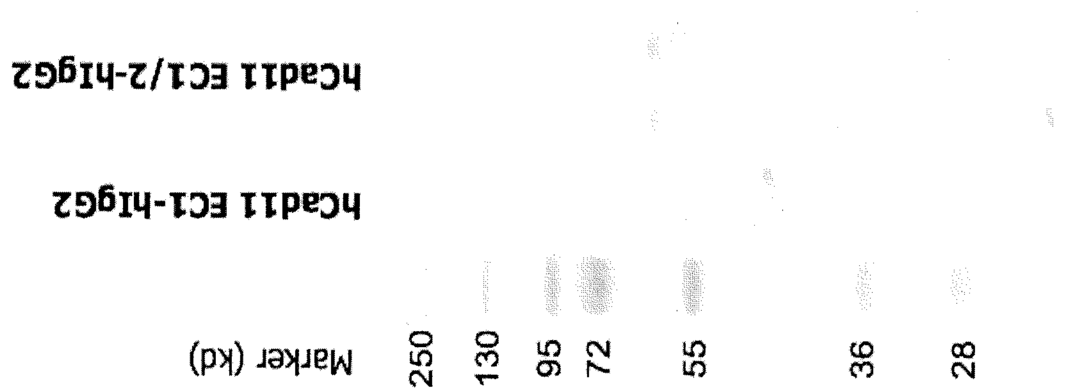

hCad11 EC1/2-hIgG2
hCad11 EC1-hIgG2
Marker (kd)
250
130
95
72
55
36
28

FIG. 10

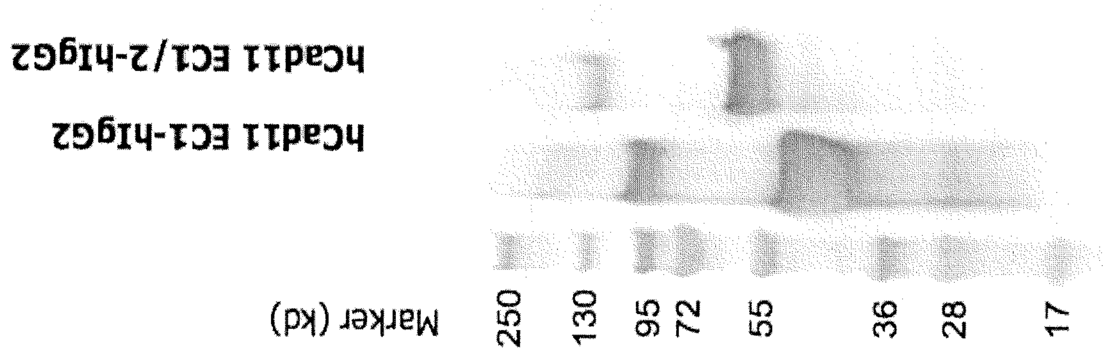

hCad11 EC1/2-hIgG2
hCad11 EC1-hIgG2
Marker (kd)
250
130
95
72
55
36
28
17

FIG. 11

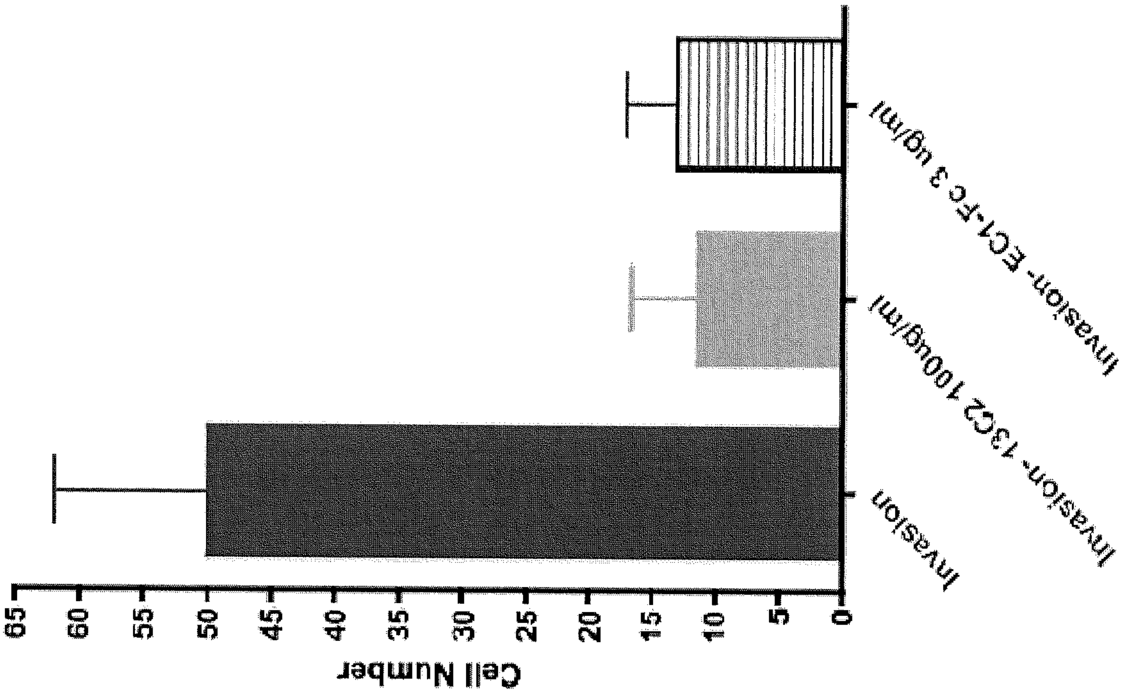

Cell Number
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Invasion
Invasion- 13C2 100ug/ml
Invasion- EC1-Fc 3 ug/ml

FIG. 12

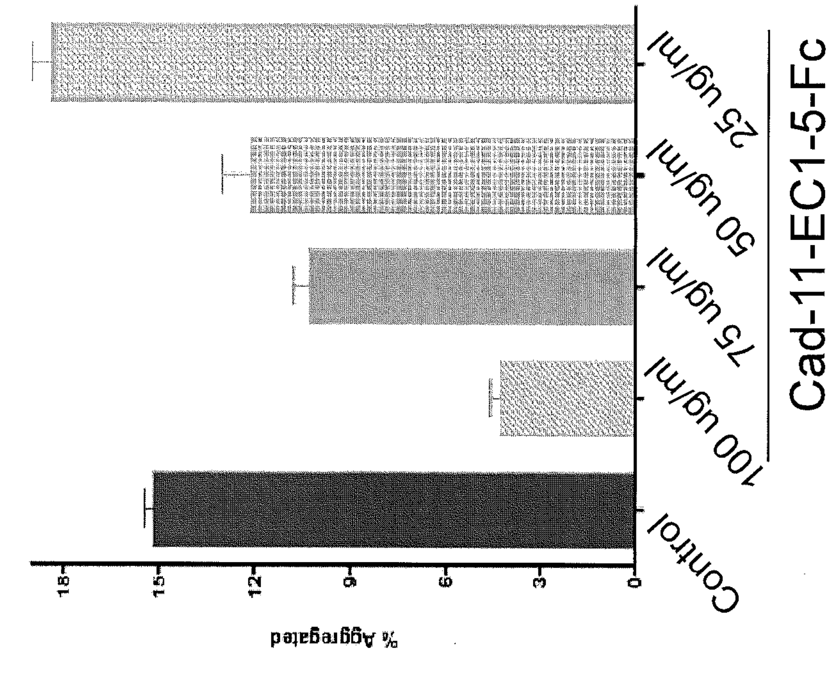


FIG. 13A

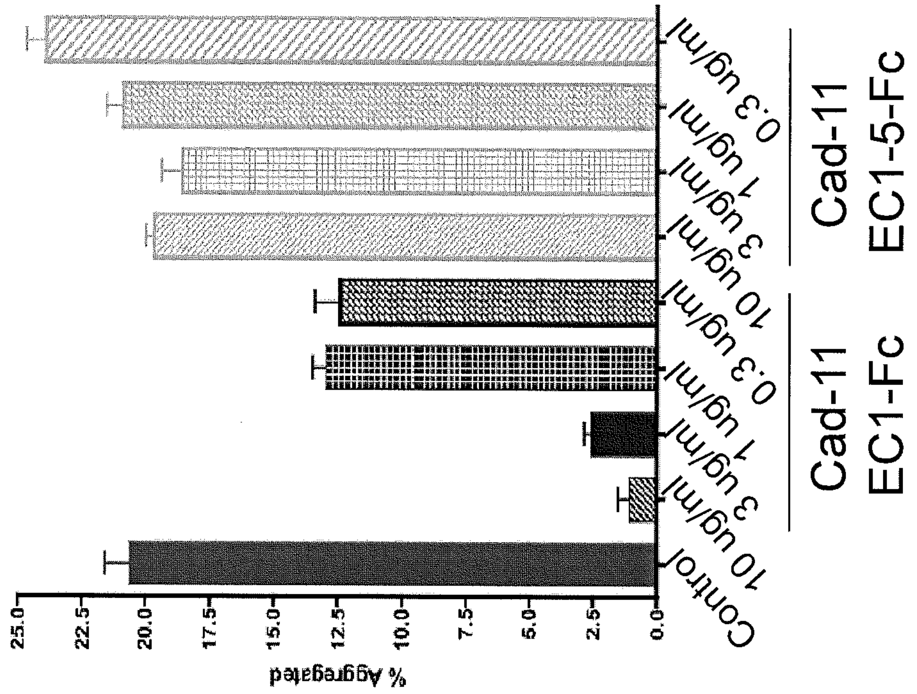


FIG. 13B

1 agatgccgcg ggggccgctc gcagccgccg ctgacttgtg aatgggaccg ggactggggc

61 cgggactgac accgcagcgc ttgccctgcg ccagggactg gcggctcgga ggttgcgtcc

121 accctcaagg gccccagaaa tcactgtgtt ttcagctcag cggccctgtg acattccttc

181 gtgttgtcat ttgttgagtg accaatcaga tgggtggagt gtgttacaga aattggcagc

241 aagtatccaa tgggtgaaga agaagctaac tggggacgtg ggcagccctg acgtgatgag

301 ctcaaccagc agagacattc catcccaaga gaggtctgcg tgacgcgtcc gggaggccac

361 cctcagcaag accaccgtac agttggtgga aggggtgaca gctgcattct cctgtgccta

421 ccacgtaacc aaaaatgaag gagaactact gtttacaagc cgccctggtg tgcctgggca

481 tgctgtgcca cagccatgcc tttgccccag agcggcgggg gcacctgcgg ccctccttcc

541 atgggcacca tgagaagggc aaggaggggc aggtgctaca gcgctccaag cgtggctggg

601 tctggaacca gttcttcgtg atagaggagt acaccgggcc tgaccccgtg cttgtgggca

661 ggcttcattc agatattgac tctggtgatg ggaacattaa atacattctc tcaggggaag

721 gagctggaac catttttgtg attgatgaca aatcagggaa cattcatgcc accaagacgt

781 tggatcgaga agagagagcc cagtacacgt tgatggctca ggcggtggac agggacacca

841 atcggccact ggagccaccg tcggaattca ttgtcaaggt ccaggacatt aatgacaacc

901 ctccggagtt cctgcacgag acctatcatg ccaacgtgcc tgagaggtcc aatgtgggaa

961 cgtcagtaat ccaggtgaca gcttcagatg cagatgaccc cacttatgga aatagcgcca

1021 agttagtgta cagtatcctc gaaggacaac cctatttttc ggtggaagca cagacaggta

1081 tcatcagaac agccctaccc aacatggaca gggaggccaa ggaggagtac cacgtggtga

1141 tccaggccaa ggacatgggt ggacatatgg gcggactctc agggacaacc aaagtgacga

1201 tcacactgac cgatgtcaat gacaacccac caaagtttcc gcagagcgta taccagatgt

1261 ctgtgtcaga agcagccgtc cctggggagg aagtaggaag agtgaaagct aaagatccag

1321 acattggaga aaatggctta gtcacataca atattgttga tggagatggt atggaatcgt

1381 ttgaaatcac aacggactat gaaacacagg agggggtgat aaagctgaaa aagcctgtag

1441 attttgaaac caaaagagcc tatagcttga aggtagaggc agccaacgtg cacatcgacc

1501 cgaagtttat cagcaatggc cctttcaagg acactgtgac cgtcaagatc tcagtagaag

1561 atgctgatga gccccctatg ttcttggccc caagttacat ccacgaagtc caagaaaatg

1621 cagctgctgg caccgtggtt gggagagtgc atgccaaaga ccctgatgct gccaacagcc

FIG. 14A

1681 cgataaggta ttccatcgat cgtcacactg acctcgacag atttttcact attaatccag
1741 aggatggttt tattaaaact acaaaacctc tggatagaga ggaaacagcc tggctcaaca
1801 tcactgtctt tgcagcagaa atccacaatc ggcatcagga agccaaagtc ccagtggcca
1861 ttagggtcct tgatgtcaac gataatgctc ccaagtttgc tgccccttat gaaggtttca
1921 tctgtgagag tgatcagacc aagccacttt ccaaccagcc aattgttaca attagtgcag
1981 atgacaagga tgacacggcc aatggaccaa gatttatctt cagcctaccc cctgaaatca
2041 ttcacaatcc aaatttcaca gtcagagaca accgagataa cacagcaggc gtgtacgccc
2101 ggcgtggagg gttcagtcgg cagaagcagg acttgtacct tctgcccata gtgatcagcg
2161 atggcggcat cccgcccatg agtagcacca acaccctcac catcaaagtc tgcgggtgcg
2221 acgtgaacgg ggcactgctc tcctgcaacg cagaggccta cattctgaac gccggcctga
2281 gcacaggcgc cctgatcgcc atcctcgcct gcatcgtcat tctcctggtc attgtagtat
2341 tgtttgtgac cctgagaagg caaaagaaag aaccactcat tgtctttgag gaagaagatg
2401 tccgtgagaa catcattact tatgatgatg aagggggtgg ggaagaagac acagaagcct
2461 ttgatattgc caccctccag aatcctgatg gtatcaatgg atttatcccc cgcaaagaca
2521 tcaaacctga gtatcagtac atgcctagac ctgggctccg gccagcgccc aacagcgtgg
2581 atgtcgatga cttcatcaac acgagaatac aggaggcaga caatgacccc acggctcctc
2641 cttatgactc cattcaaatc tacggttatg aaggcagggg ctcagtggcc gggtccctga
2701 gctccctaga gtcggccacc acagattcag acttggacta tgattatcta cagaactggg
2761 gacctcgttt taagaaacta gcagatttgt atggttccaa agacactttt gatgacgatt
2821 cttaacaata acgatacaaa tttggcctta agaactgtgt ctggcgttct caagaatcta
2881 gaagatgtgt aaacaggtat ttttttaaat caaggaaagg ctcatttaaa acaggcaaag
2941 ttttacagag aggatacatt taataaaact gcgaggacat caaagtggta aatactgtga
3001 aataccttttt ctcacaaaaa ggcaaatatt gaagttgttt atcaacttcg ctagaaaaaa
3061 aaaacacttg gcatacaaaa tatttaagtg aaggagaagt ctaacgctga actgacaatg
3121 aagggaaatt gtttatgtgt tatgaacatc caagtctttc ttcttttttta agttgtcaaa
3181 gaagcttcca caaaattaga aaggacaaca gttctgagct gtaatttcgc cttaaactct
3241 ggacactcta tatgtagtgc atttttaaac ttgaaatata taatattcag ccagcttaaa
3301 cccatacaat gtatgtacaa tacaatgtac aattatgtct cttgagcatc aatcttgtta
3361 ctgctgattc ttgtaaatct ttttgcttct actttcatct taaactaata cgtgccagat

FIG. 14B

3421 ataactgtct tgtttcagtg agagacgccc tatttctatg tcatttttaa tgtatctatt

3481 tgtacaattt taaagttctt attttagtat acgtataaat atcagtattc tgacatgtaa

3541 gaaaatgtta cggcatcaca cttatatttt atgaacattg tactgttgct ttaatatgag

3601 cttcaatata agaagcaatc tttgaaataa aaaaagattt ttttttaaaa aaaa (SEQ ID NO:1)

FIG. 14C

1 mkenyclqaa lvclgmlchs hafaperrgh lrpsfhghhe kgkegqvlqr skrgwvwnqf
61 fvieeytgpd pvlvgrlhsd idsgdgniky ilsgegagti fviddksgni hatktldree
121 raqytlmaqa vdrdtnrple ppsefivkvq dindnppefl hetyhanvpe rsnvgtsviq
181 vtasdaddpt ygnsaklvys ilegqpyfsv eaqtgiirta lpnmdreake eyhvviqakd
241 mgghmgglsg ttkvtitltd vndnppkfpq svyqmsvsea avpgeevgrv kakdpdigen
301 glvtynivdg dgmesfeitt dyetqegvik lkkpvdfetk rayslkveaa nvhidpkfis
361 ngpfkdtvtv kisvedadep pmflapsyih evqenaaagt vvgrvhakdp daanspirys
421 idrhtdldrf ftinpedgfi kttkpldree tawlnitvfa aeihnrhqea kvpvairvld
481 vndnapkfaa pyegficesd qtkplsnqpi vtisaddkdd tangprfifs lppeiihnpn
541 ftvrdnrdnt agvyarrggf srqkqdlyll pivisdggip pmsstntlti kvcgcdvnga
601 llscnaeayi lnaglstgal iailacivil lvivvlfvtl rrqkkepliv feeedvreni
661 ityddeggge edteafdiat lqnpdgingf iprkdikpey qymprpglrp apnsvdvddf
721 intriqeadn dptappydsi qiygyegrgs vagslssles attdsdldyd ylqnwgprfk
781 kladlygskd tfddds (SEQ ID NO: 2)

FIG. 15

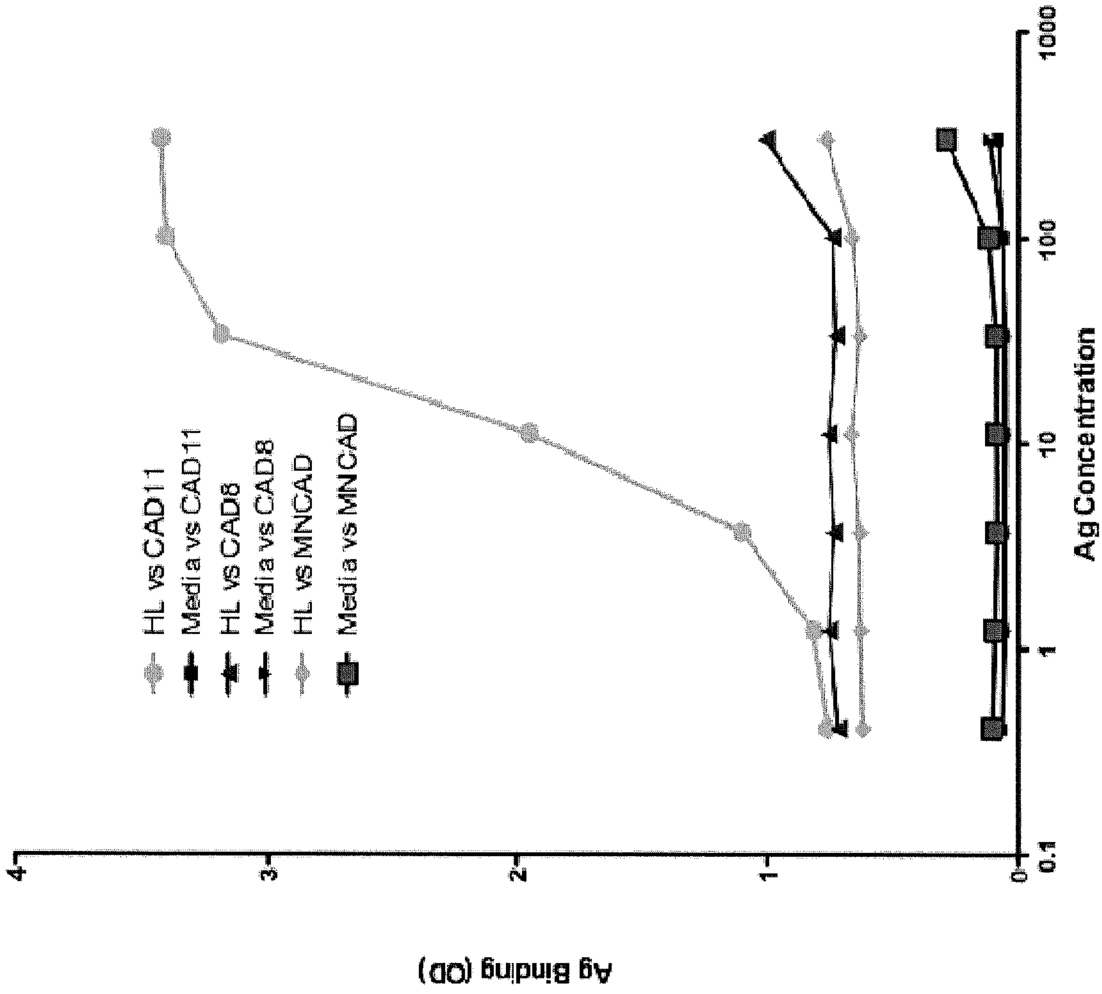

HL vs CAD11
Media vs CAD11
HL vs CAD8
Media vs CAD8
HL vs MNCAD
Media vs MNCAD
Ag Binding (OD)
0
1
2
3
4
0.1
1
10
100
1000
Ag Concentration

FIG. 16

H14

Cad11+ cells

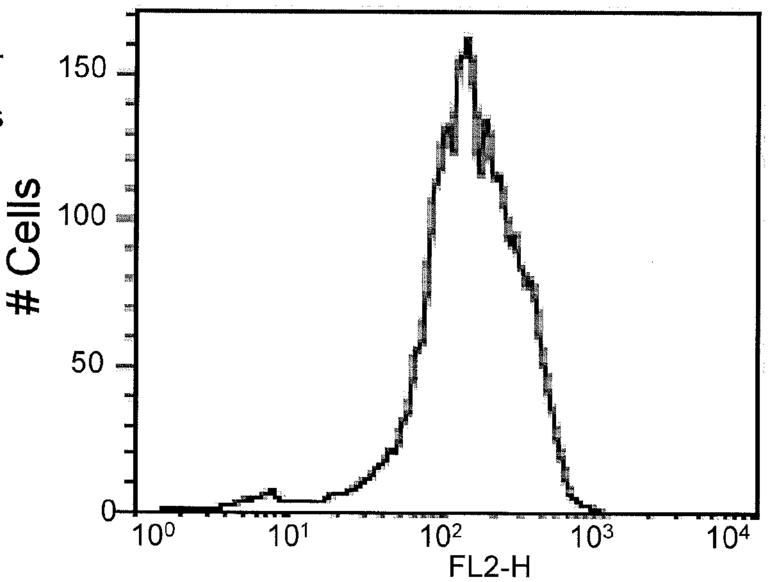


FIG. 17A

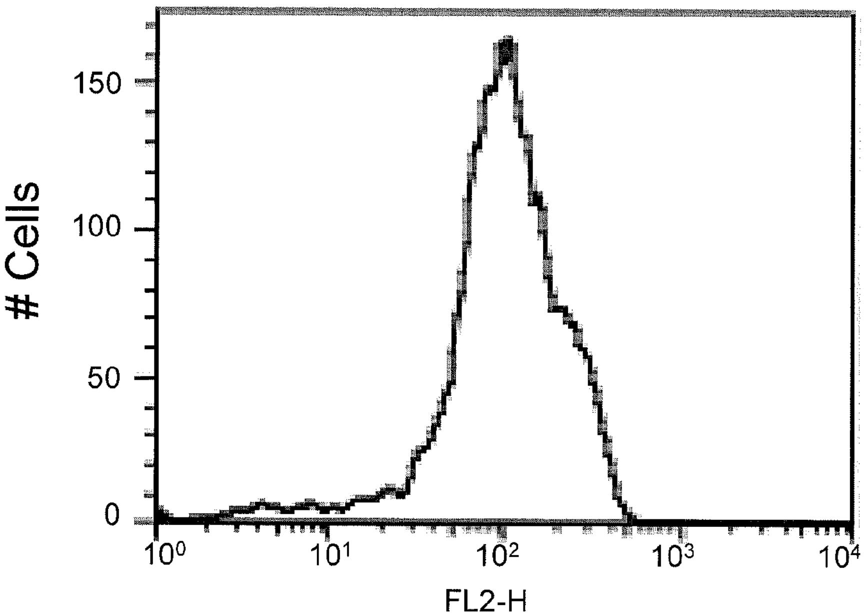


FIG. 17B

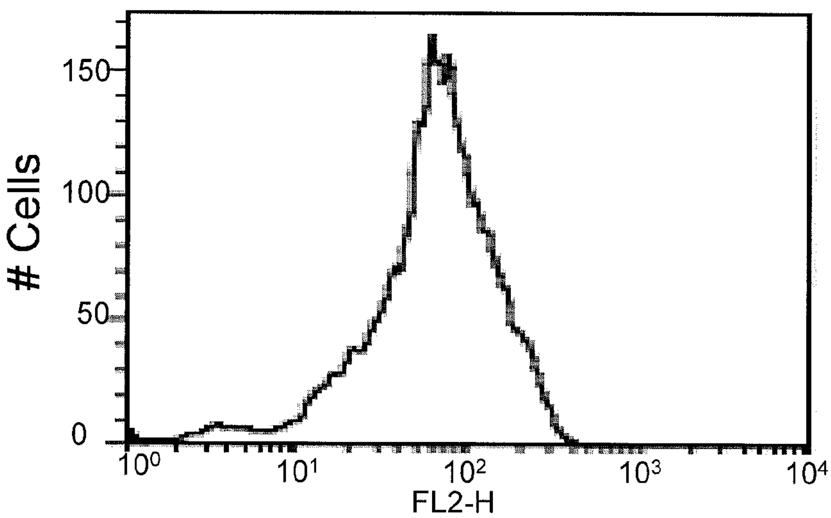


FIG. 17C

H14

Cad11⁻ cells

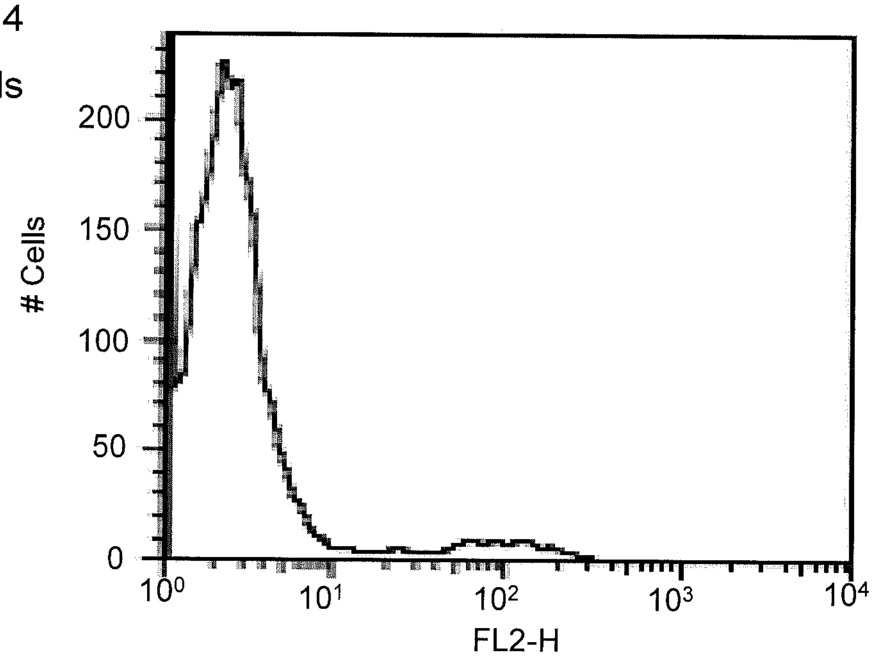


FIG. 17D

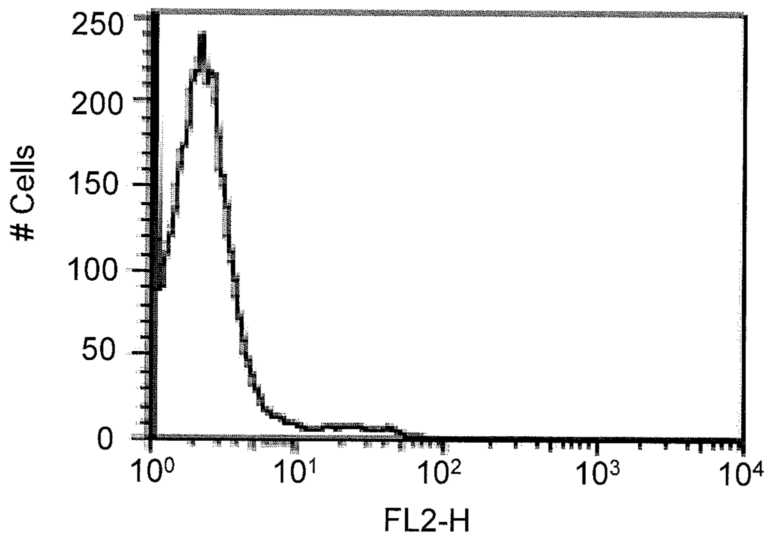


FIG. 17E

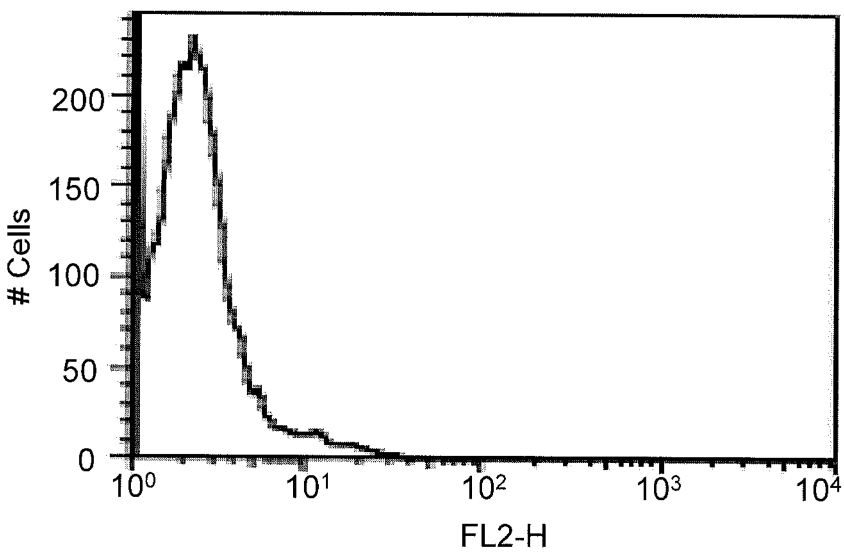


FIG. 17F

H1M1

CAD11+ cells

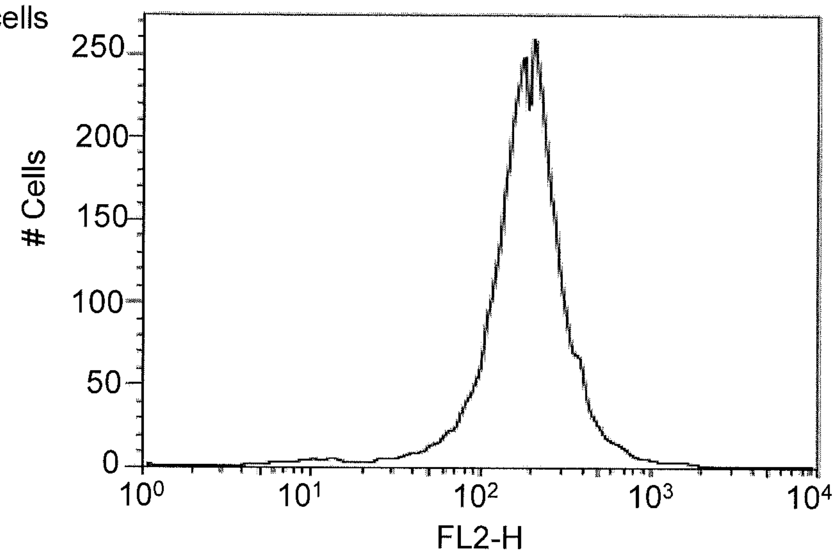


FIG. 17G

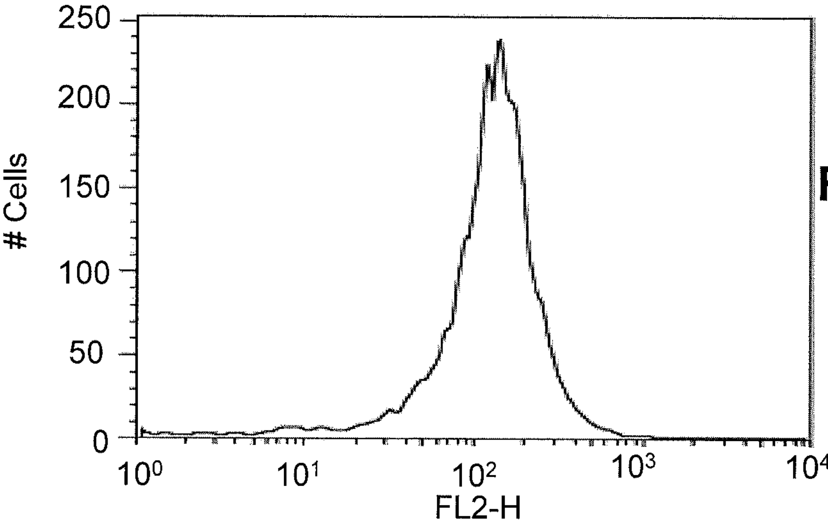


FIG. 17H

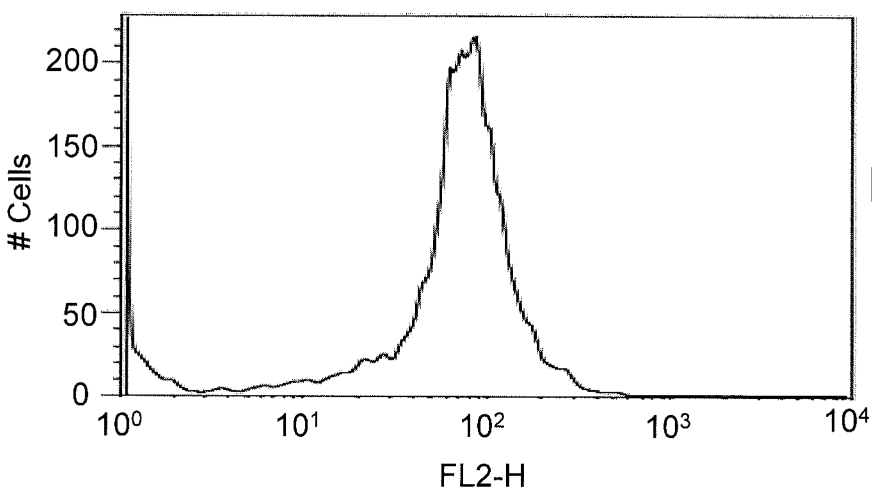


FIG. 17I

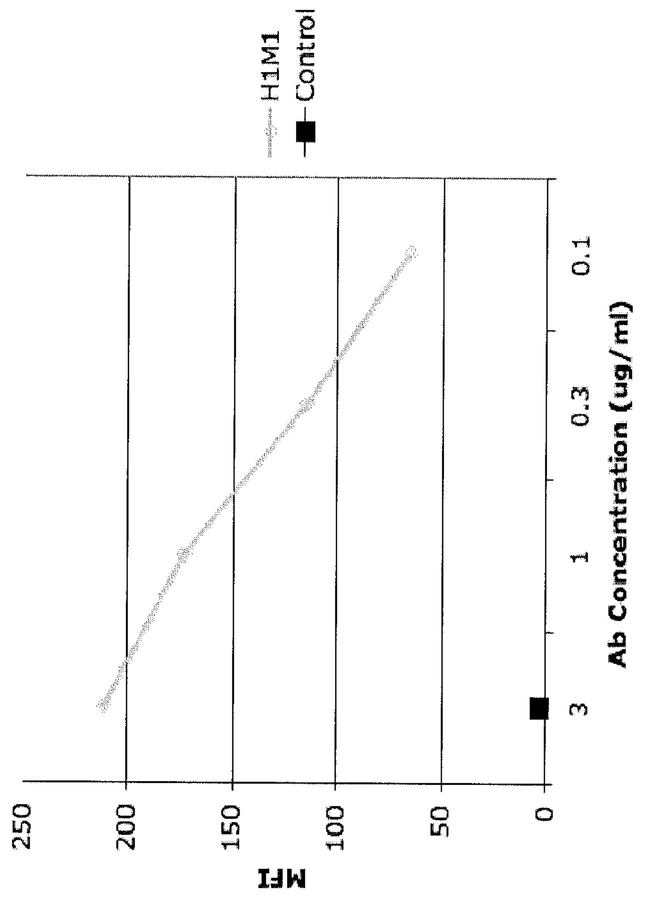


FIG. 18B

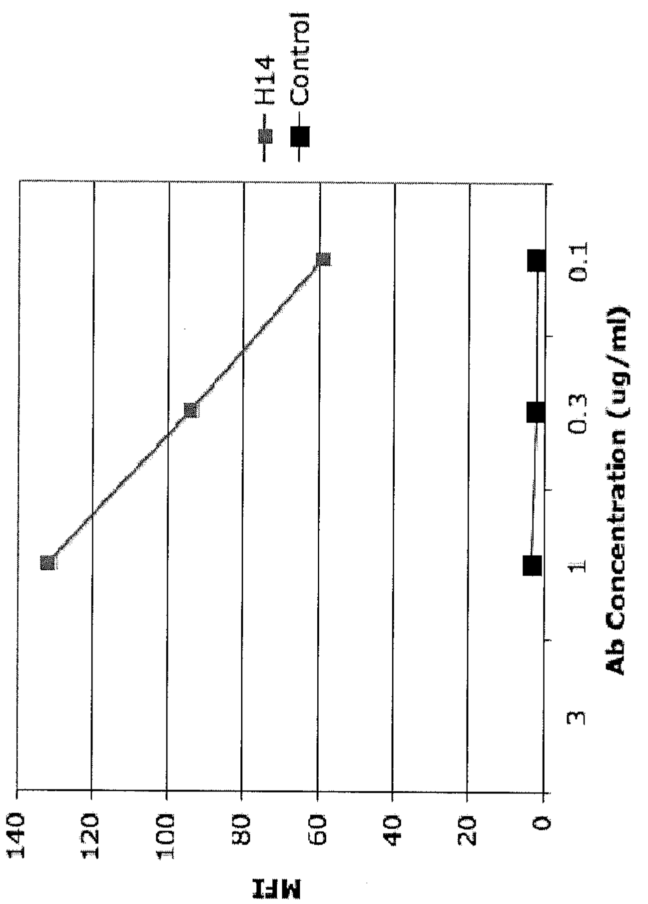


FIG. 18A

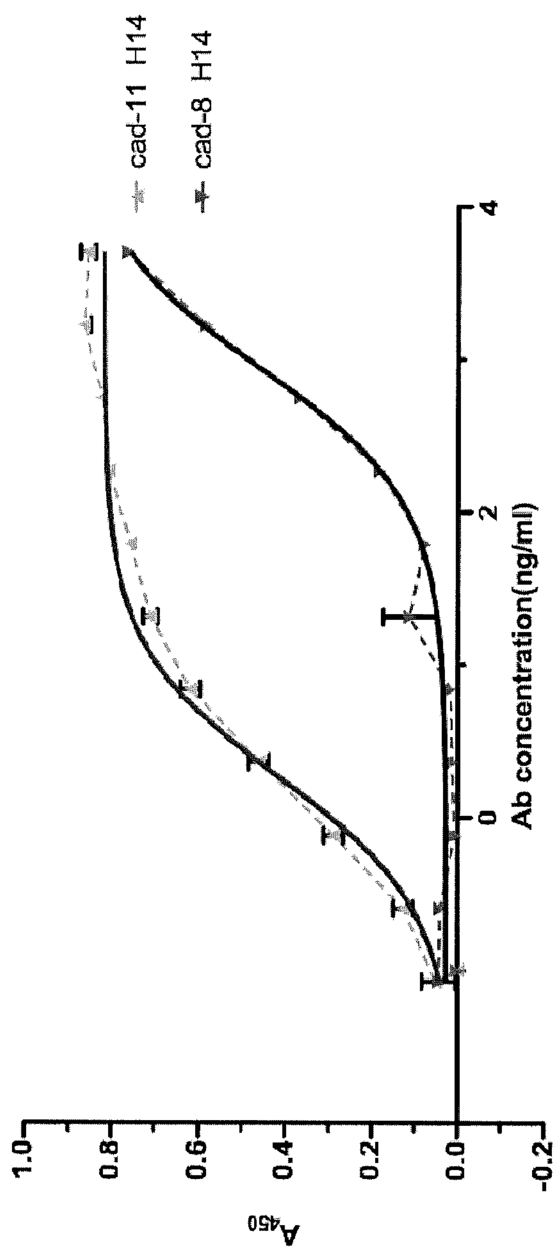


FIG. 19A

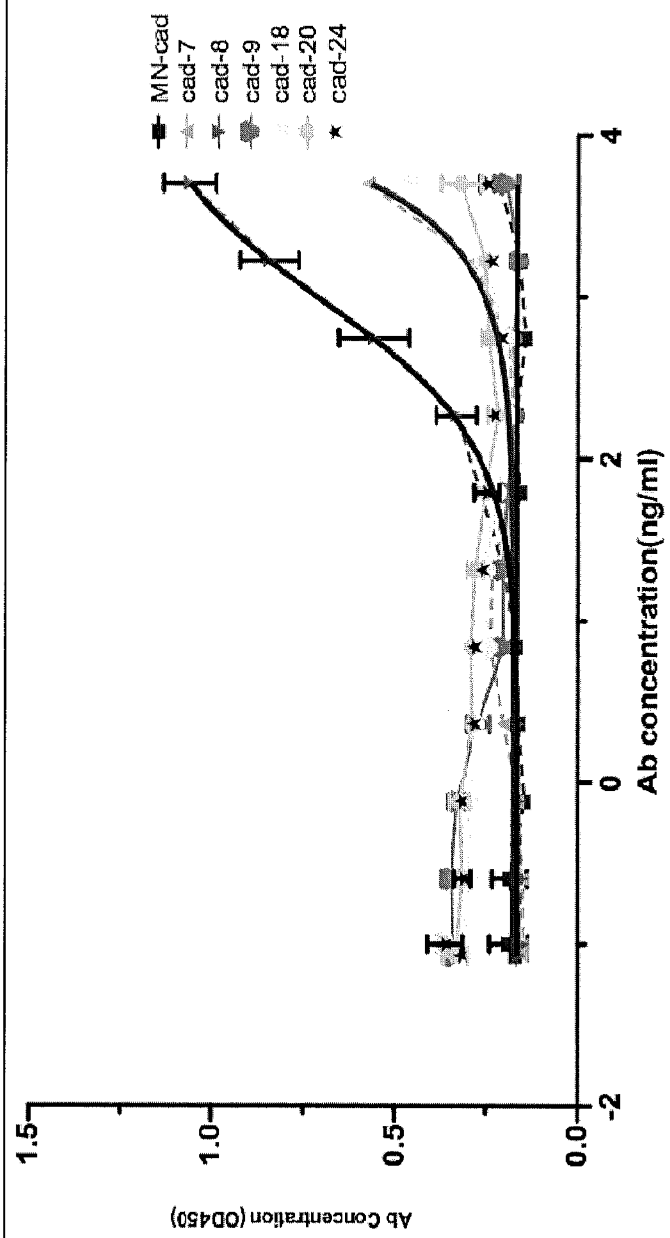


FIG. 19B

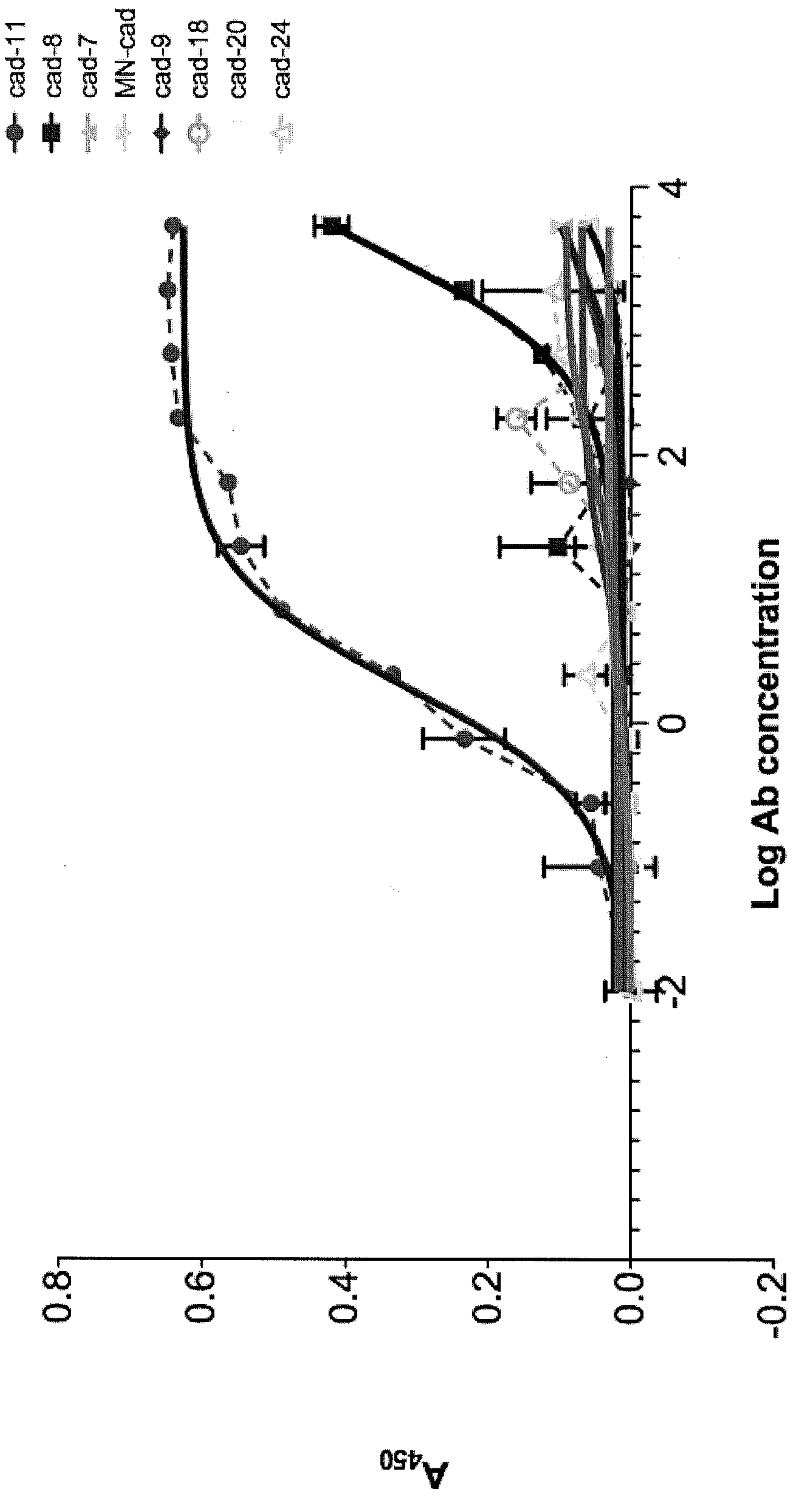

cad-11
cad-8
cad-7
MN-cad
cad-9
cad-18
cad-20
cad-24
0.8
0.6
0.4
0.2
0.0
-0.2
-2
0
2
4
Log Ab concentration
A_{450}

FIG. 20

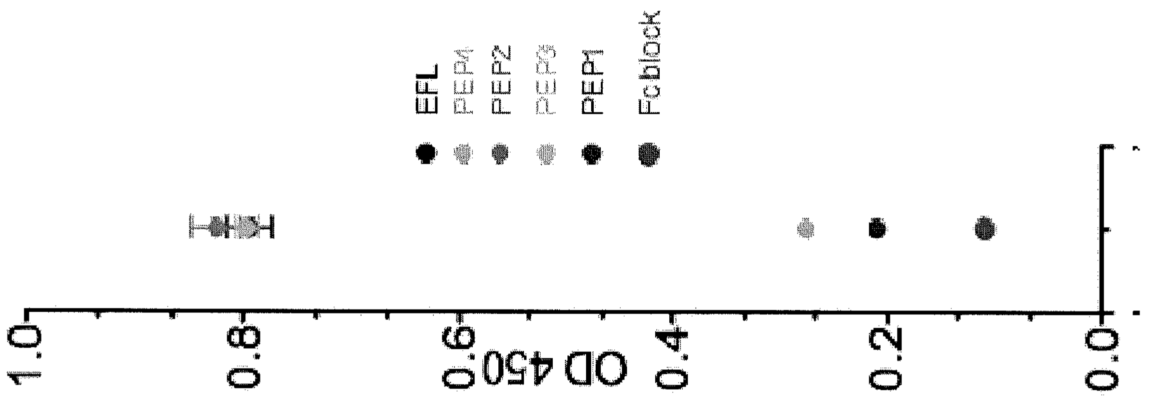


FIG. 21A

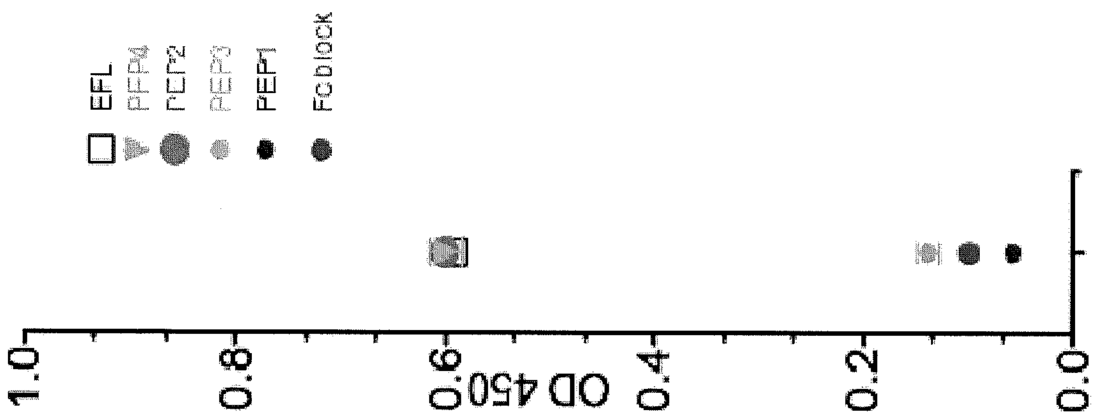


FIG. 21B

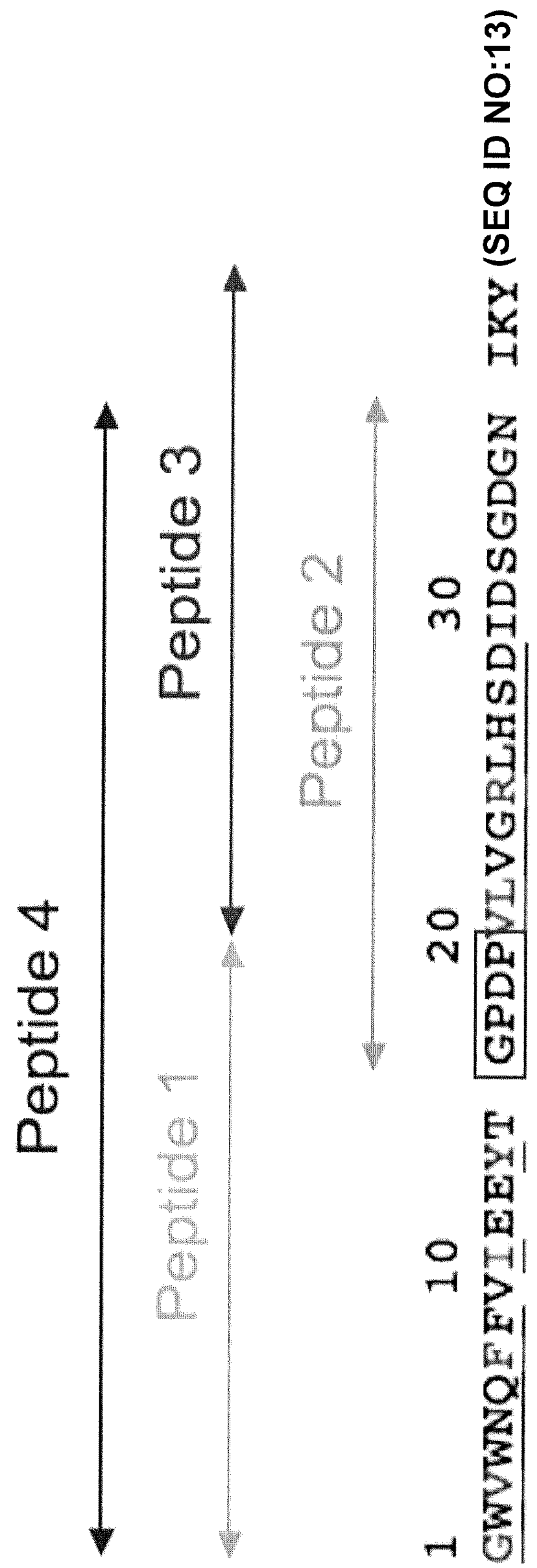

Peptide 4
Peptide 1
Peptide 3
Peptide 2
1
10
20
30
GWVWNQFFVIEEYT GPDPVLVGRLHSDIDSGDGN IKY (SEQ ID NO:13)

FIG. 22

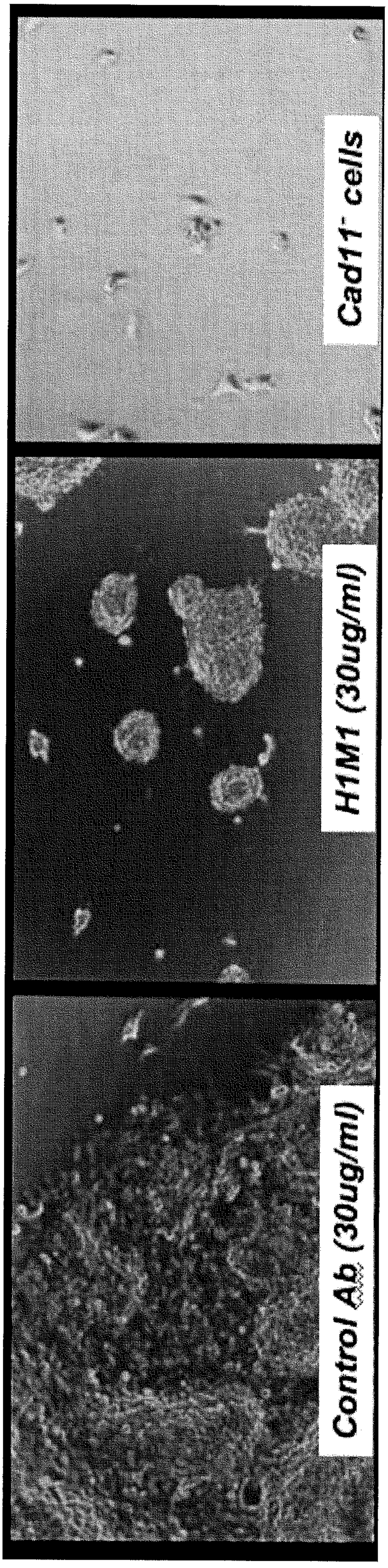
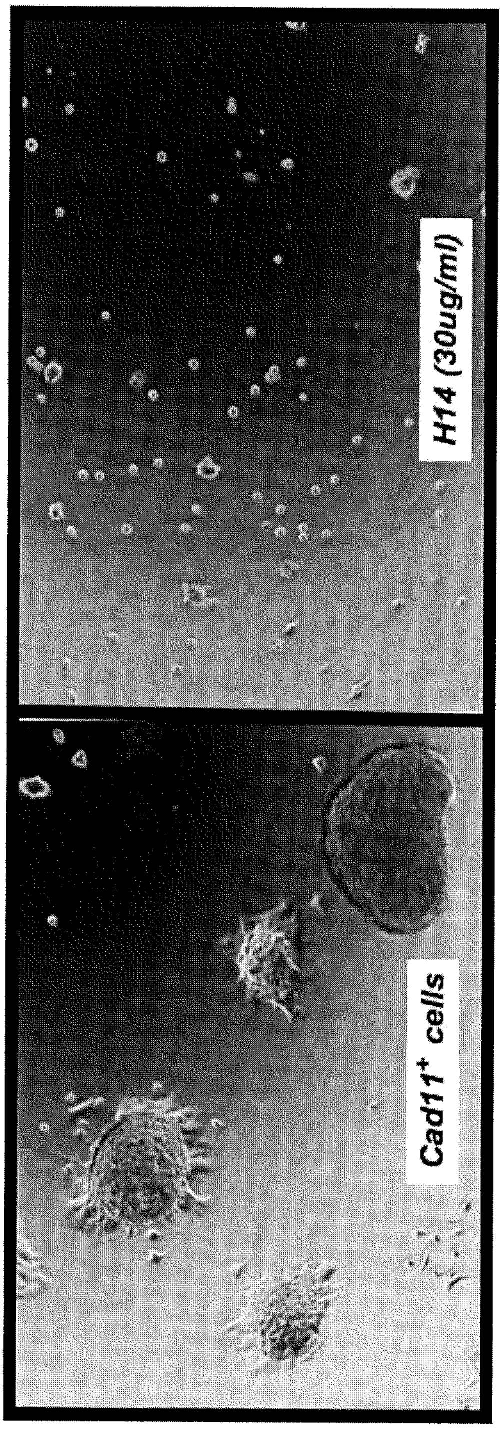


FIG. 23A

FIG. 23B

FIG. 23C



40x

FIG. 24A

FIG. 24B

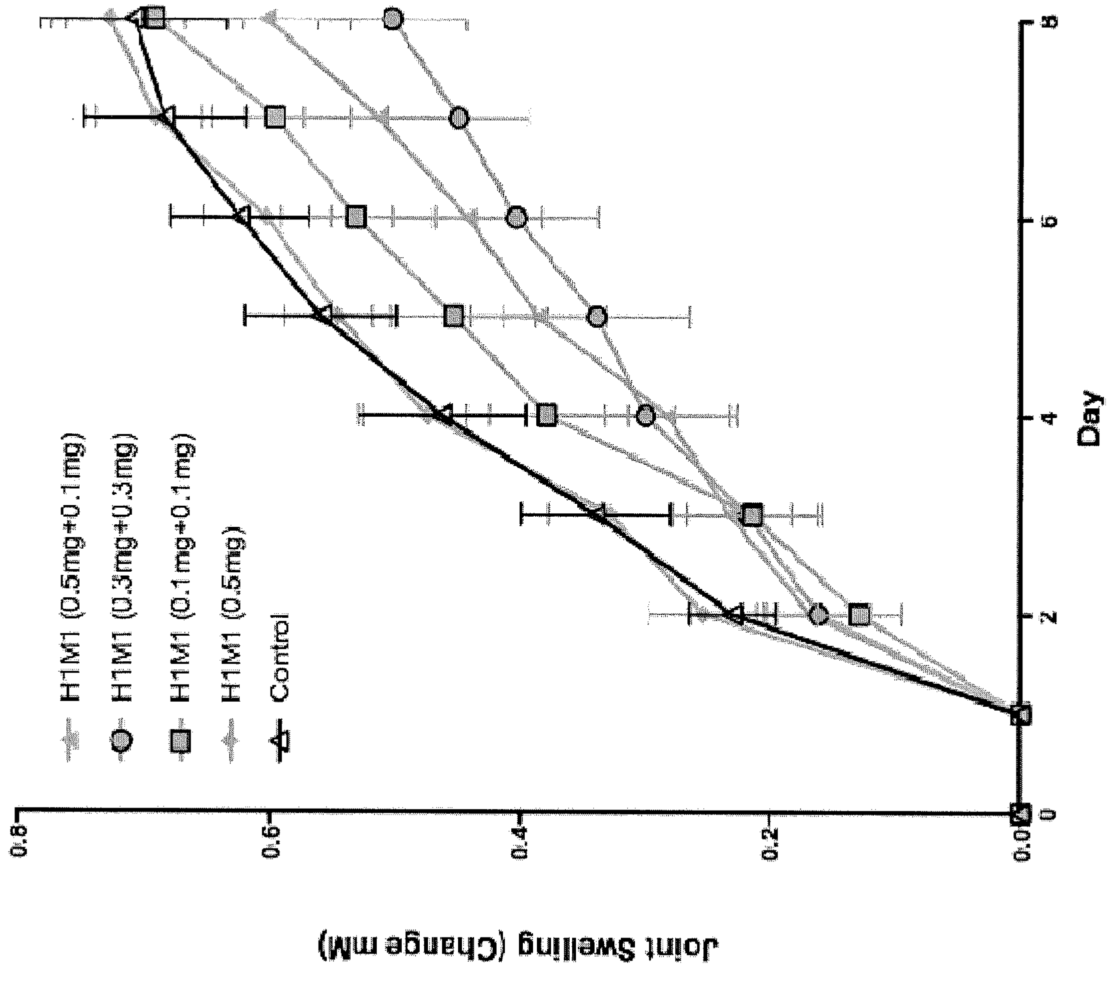

H1M1 (0.5mg+0.1mg)
H1M1 (0.3mg+0.3mg)
H1M1 (0.1mg+0.1mg)
H1M1 (0.5mg)
Control
Joint Swelling (Change mM)
0.0
0.2
0.4
0.6
0.8
0
2
4
6
8
Day

FIG. 25

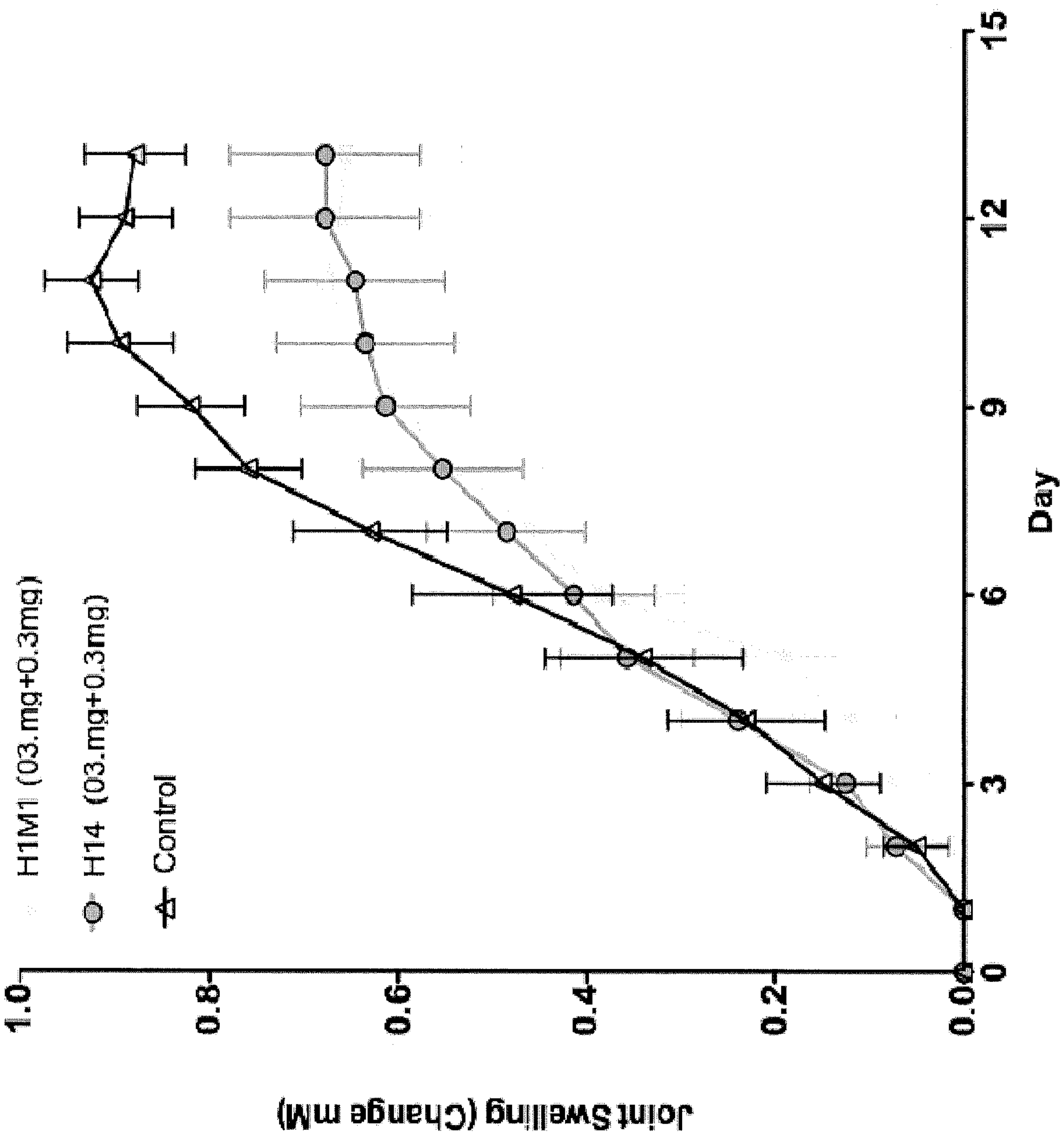

H1M1 (03.mg+0.3mg)
H14 (03.mg+0.3mg)
Control
Joint Swelling (Change mM)
1.0
0.8
0.6
0.4
0.2
0.0
0
3
6
9
12
15
Day

FIG. 26

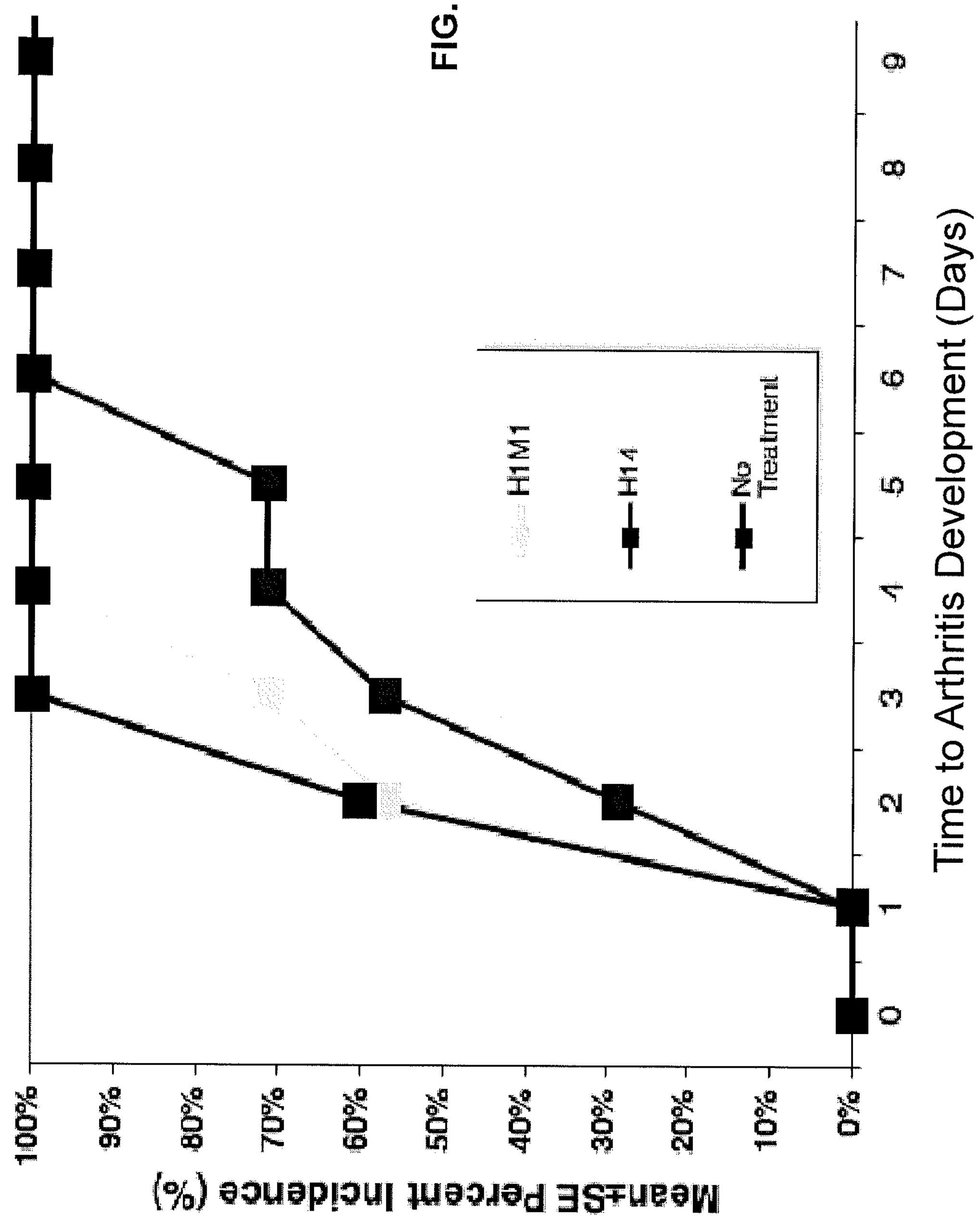
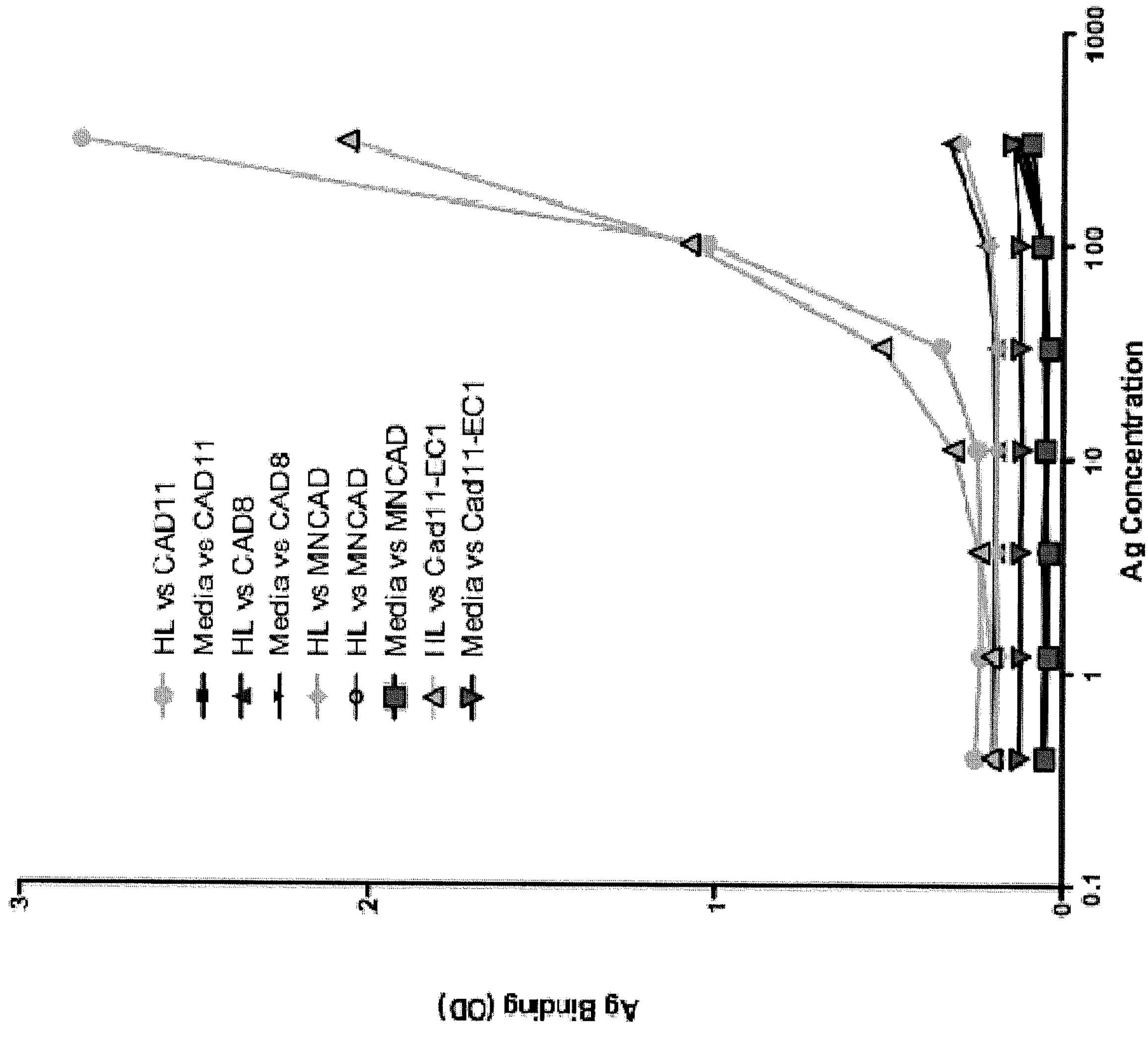

Mean±SE Percent Incidence (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
9
Time to Arthritis Development (Days)
H1M1
H14
No Treatment

FIG. 27

FIG. 28


HL vs CAD11
Media vs CAD11
HL vs CAD8
Media vs CAD8
HL vs MNCAD
HL vs MNCAD
Media vs MNCAD
HL vs Cad11-EC1
Media vs Cad11-EC1
Ag Binding (OD)
0
1
2
3
Ag Concentration
0.1
1
10
100
1000

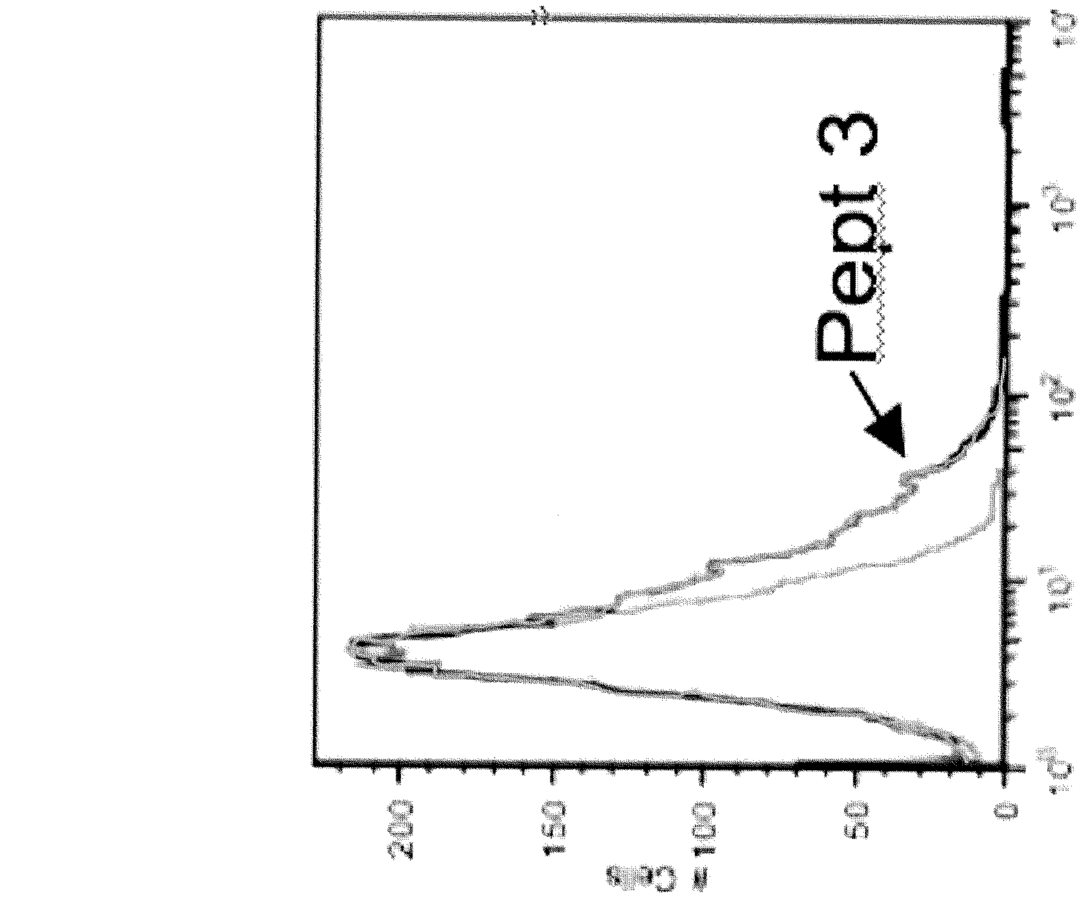


FIG. 29

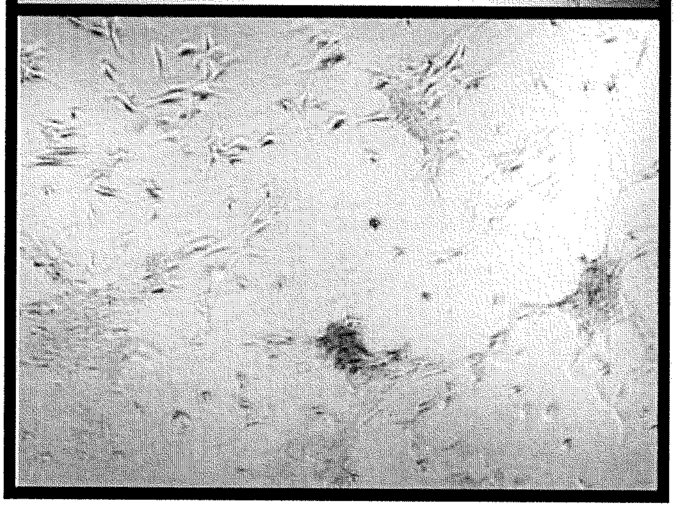


FIG. 30A

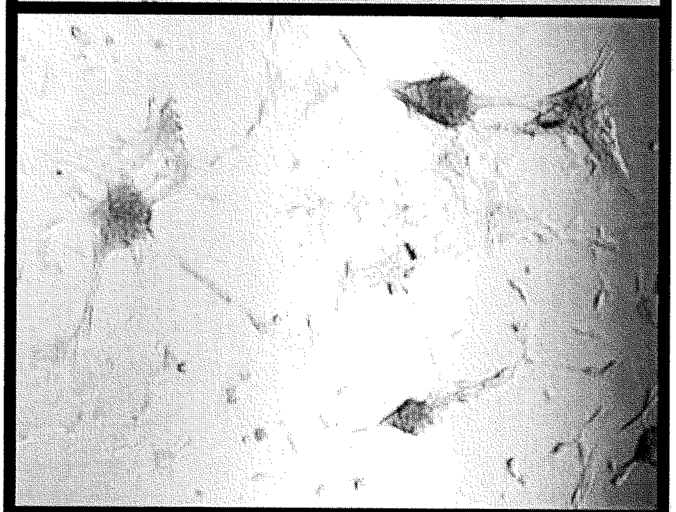


FIG. 30B

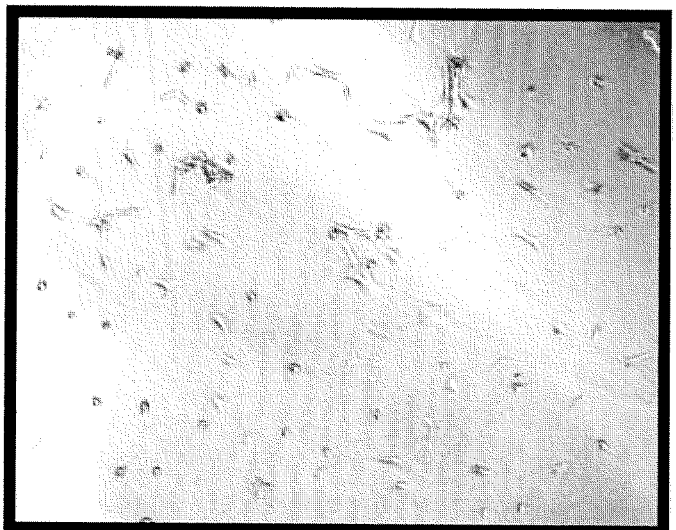


FIG. 30C

FIG. 31

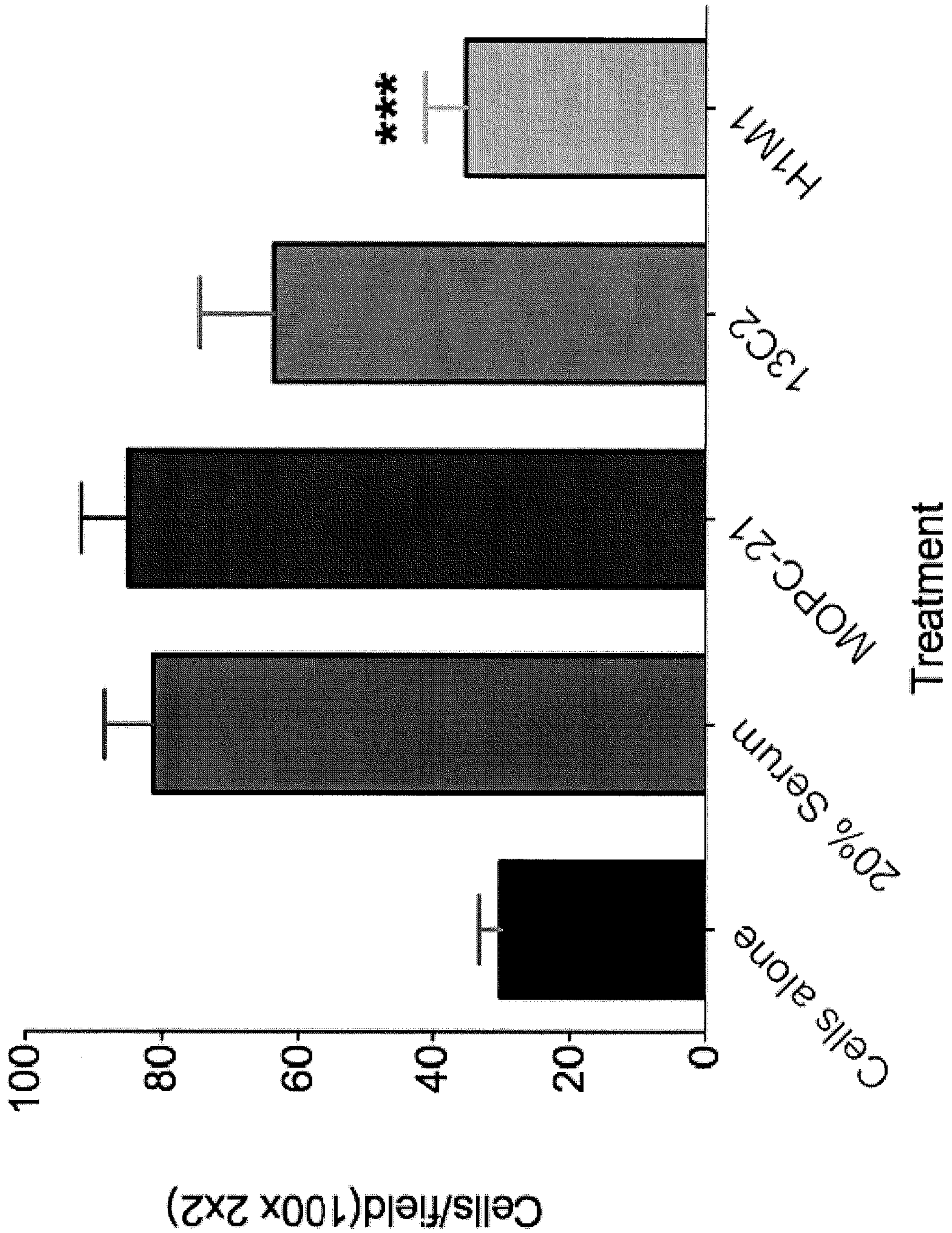
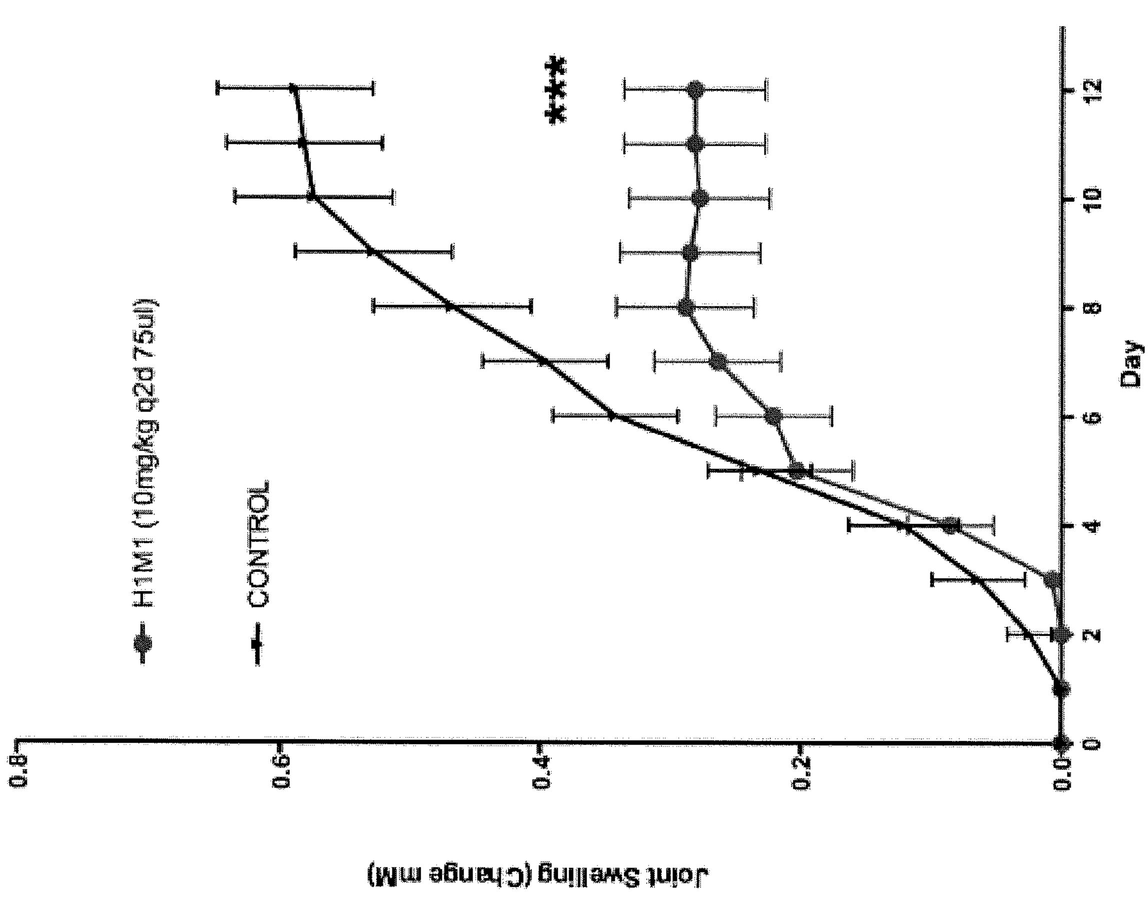

Cells/field(100x 2x2)
100
80
60
40
20
0
Cells alone
20% Serum
MOPC-21
13C2
H1M1
***
Treatment

FIG. 32


H1M1 (10mg/kg q2d 75ul)
CONTROL
***
Joint Swelling (Change mM)
Day
0.0
0.2
0.4
0.6
0.8
0
2
4
6
8
10
12

```
        10        20        30        40        50        60        70        80        90       100
GAGGTTCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGTTACTCATTTACTGGCTACTTTA
 E  V  Q  L  Q  Q  S  G  P  E  L  V  K  P  G  A  S  V  K  I  S  C  K  A  S  G  Y  S  F  T  G  Y  F
                            10                            20                            30

       110       120       130       140       150       160       170       180       190       200
TGAACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGACGTATTAATCCTTACACTGGTGATACTTTCTACAACCAGAAGTTCAAGGGCAA
M  N  W  V  K  Q  S  H  G  K  S  L  E  W  I  G  R  I  N  P  Y  T  G  D  T  F  Y  N  Q  K  F  K  G  K
                  40                            50    52 52a                     60

       210       220       230       240       250       260       270       280       290       300
GGCCACATTGACTGTTGACAAATCCTCTAGCACAGCCCACATGGAGCTCCTGAGCCTGTCATCTGAAGACTCTGCAGTCTATTATTGTGGACGACTCGGT
  A  T  L  T  V  D  K  S  S  S  T  A  H  M  E  L  L  S  L  S  S  E  D  S  A  V  Y  Y  C  G  R  L  G
        70                                    80    82 82a b  c                        90

       310       320       330       340       350
AGTAGGTACTGGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTC  (SEQ ID NO:50)
S  R  Y  W  Y  F  D  V  W  G  A  G  T  T  V  T  V  S  S    (SEQ ID NO:51)
        100 a  b                                110
```

CDR1: GYFMN (SEQ ID NO:52)
CDR2: RINPYTGDTFYNQKFKG (SEQ ID NO:53)
CDR3: LGSRYWYFDV (SEQ ID NO:54)

FIG. 33

```
         10         20         30         40         50         60         70         80         90        100
GATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTATACATAGTAATG
 D  V  L  M  T  Q  T  P  L  S  L  P  V  S  L  G  D  Q  A  S  I  S  C  R  S  S  Q  S  I  I  H  S  N
                            10                            20                         27 27a b  c  d  e

        110        120        130        140        150        160        170        180        190        200
GAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTT
G  N  T  Y  L  E  W  Y  L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S  N  R  F  S  G  V  P  D  R  F
   30                            40                            50                            60

        210        220        230        240        250        260        270        280        290        300
CACTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCT
  T  G  S  G  S  G  T  D  F  T  L  K  I  S  R  V  E  A  E  D  L  G  V  Y  Y  C  F  Q  G  S  H  V  P
                          70                            80                            90

        310        320        330
TGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAA   (SEQ ID NO:55)
 W  T  F  G  G  G  T  K  L  E  I  K    (SEQ ID NO:56)
          100                106 106a
```

CDR1: RSSQSIIHSNGNTYLE (SEQ ID NO:57)
CDR2: KVSNRFS (SEQ ID NO:58)
CDR3: FQGSHVPWT (SEQ ID NO:59)

FIG. 34

Anti-CAD11 EC1 VH1 Heavy Chain Sequences

```
        10        20        30        40        50        60        70        80        90       100
GAGGTTCAGCTGGTGCAGTCTGGACCTGAGCTGAAGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGTTACTCATTTACTGGCTACTTTA
 E  V  Q  L  V  Q  S  G  P  E  L  K  K  P  G  A  S  V  K  I  S  C  K  A  S  G  Y  S  F  T  G  Y  F
                            10                            20                            30

       110       120       130       140       150       160       170       180       190       200
TGAACTGGGTGAAGCAGGCCCATGGACAGGGCCTTGAGTGGATTGGACGTATTAATCCTTACACTGGTGATACTTTCTACAACCAGAAGTTCAAGGGCAG
M  N  W  V  K  Q  A  H  G  Q  G  L  E  W  I  G  R  I  N  P  Y  T  G  D  T  F  Y  N  Q  K  F  K  G  R
                  40                            50    52 a                    60

       210       220       230       240       250       260       270       280       290       300
GGCCACATTGACTGTTGACAAATCCTCTAGCACAGCCTACATGGAGCTCGTGAGCCTGTCATCTGAAGACTCTGCAGTCTATTATTGTGGACGACTCGGT
  A  T  L  T  V  D  K  S  S  S  T  A  Y  M  E  L  V  S  L  S  S  E  D  S  A  V  Y  Y  C  G  R  L  G
           70                            80    82 a  b  c                       90

       310       320       330       340       350
AGTAGGTACTGGTACTTCGATGTCTGGGGCCAGGGGACCACGGTCACCGTCTCCTCA  (SEQ ID NO:60)
 S  R  Y  W  Y  F  D  V  W  G  Q  G  T  T  V  T  V  S  S   (SEQ ID NO:61)
       100 a  b                         110
```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red

FIG. 35A

Anti-CAD11 EC1 VH2 Heavy Chain Sequences

```
        10        20        30        40        50        60        70        80        90       100
GAGGTTCAGCTGGTGCAGTCTGGAGCCGAGGTGAAGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGTTACTCATTTACTGGCTACTTTA
 E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  I  S  C  K  A  S  G  Y  S  F  T  G  Y  F
                            10                                     20                                30

       110       120       130       140       150       160       170       180       190       200
TGAACTGGGTGAAGCAGGCCCCTGGACAGGGCCTTGAGTGGATTGGACGTATTAATCCTTACACTGGTGATACTTTCTACAACCAGAAGTTCAAGGGCAG
M  N  W  V  K  Q  A  P  G  Q  G  L  E  W  I  G  R  I  N  P  Y  T  G  D  T  F  Y  N  Q  K  F  K  G  R
                 40                                50    52  a                             60

       210       220       230       240       250       260       270       280       290       300
GGCCACATTGACTGTTGACAAATCCACCAGCACAGCCTACATGGAGCTCTCCAGCCTGAGATCTGAAGACACCGCAGTCTATTATTGTGGACGACTCGGT
  A  T  L  T  V  D  K  S  T  S  T  A  Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  G  R  L  G
           70                                  80    82  a  b  c                          90

       310       320       330       340       350
AGTAGGTACTGGTACTTCGATGTCTGGGGCCAGGGGACCACGGTCACCGTCTCCTCA (SEQ ID NO:62)
 S  R  Y  W  Y  F  D  V  W  G  Q  G  T  T  V  T  V  S  S  (SEQ ID NO:63)
       100  a  b                          110
```

CDR definitions and protein sequence numbering according to Kabat. CDR nucleotide and protein sequences are highlighted in red

FIG. 35B

Anti-CAD11 EC1 VH3 Heavy Chain Sequences

```
         10        20        30        40        50        60        70        80        90       100
GAGGTTCAGCTGGTGCAGTCTGGAGCCGAGGTGAAGAAGCCTGGGGCTTCAGTGAAGATATCCTGCAAGGCTTCTGGTTACTCATTTACTGGCTACTTTA
 E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  I  S  C  K  A  S  G  Y  S  F  T  G  Y  F
                            10                            20                            30

        110       120       130       140       150       160       170       180       190       200
TGAACTGGGTGAGGCAGGCCCCTGGACAGGGCCTTGAGTGGATTGGACGTATTAATCCTTACACTGGTGATACTTTCTACAACCAGAAGTTCAAGGGCAG
 M  N  W  V  R  Q  A  P  G  Q  G  L  E  W  I  G  R  I  N  P  Y  T  G  D  T  F  Y  N  Q  K  F  K  G  R
                40                            50    52 a                    60

        210       220       230       240       250       260       270       280       290       300
GGCCACATTGACTGTTGACAAATCCACCAGCACAGCCTACATGGAGCTCTCCAGCCTGAGATCTGAAGACACCGCAGTCTATTATTGTGGACGACTCGGT
  A  T  L  T  V  D  K  S  T  S  T  A  Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  G  R  L  G
           70                         80    82 a  b  c                    90

        310       320       330       340       350
AGTAGGTACTGGTACTTCGATGTCTGGGGCCAGGGGACCACGGTCACCGTCTCCTCA (SEQ ID NO:64)
 S  R  Y  W  Y  F  D  V  W  G  Q  G  T  T  V  T  V  S  S  (SEQ ID NO:65)
      100 a  b                             110
```

FIG. 35C

Anti-CAD11 EC1 VH4 Heavy Chain Sequences

```
         10        20        30        40        50        60        70        80        90       100
GAGGTTCAGCTGGTGCAGTCTGGAGCCGAGGTGAAGAAGCCTGGGGCTTCAGTGAAGGTGTCCTGCAAGGCTTCTGGTTACTCATTTACTGGCTACTTTA
 E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  V  S  C  K  A  S  G  Y  S  F  T  G  Y  F
                            10                                     20                                     30

        110       120       130       140       150       160       170       180       190       200
TGAACTGGGTGAGGCAGGCCCCTGGACAGGGCCTTGAGTGGATTGGACGTATTAATCCTTACACTGGTGATACTTTCTACAACCAGAAGTTCAAGGGCAG
M  N  W  V  R  Q  A  P  G  Q  G  L  E  W  I  G  R  I  N  P  Y  T  G  D  T  F  Y  N  Q  K  F  K  G  R
                  40                                     50    52 a                             60

        210       220       230       240       250       260       270       280       290       300
GGCCACAATCACTGTTGACAAATCCACCAGCACAGCCTACATGGAGCTCTCCAGCCTGAGATCTGAAGACACCGCAGTCTATTATTGTGGACGACTCGGT
  A  T  I  T  V  D  K  S  T  S  T  A  Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  G  R  L  G
           70                                      80    82 a  b  c                            90

        310       320       330       340       350
AGTAGGTACTGGTACTTCGATGTCTGGGGCCAGGGGACCACGGTCACCGTCTCCTCA(SEQ ID NO:66)
 S  R  Y  W  Y  F  D  V  W  G  Q  G  T  T  V  T  V  S  S (SEQ ID NO:67)
       100 a  b                                110
```

FIG. 35D

Anti-CAD11 EC1 VH5 Heavy Chain Sequences

```
        10        20        30        40        50        60        70        80        90       100
GAGGTTCAGCTGGTGCAGTCTGGAGCCGAGGTGAAGAAGCCTGGGGCTTCAGTGAAGGTGTCCTGCAAGGCTTCTGGTTACTCATTTACTGGCTACTTTA
 E  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  A  S  V  K  V  S  C  K  A  S  G  Y  S  F  T  G  Y  F
                            10                            20                            30

       110       120       130       140       150       160       170       180       190       200
TGAACTGGGTGAGGCAGGCCCCTGGACAGGGCCTTGAGTGGATTGGACGTATTAATCCTTACACTGGTGATACTTTCTACAACCAGAAGTTCAAGGGCAG
M  N  W  V  R  Q  A  P  G  Q  G  L  E  W  I  G  R  I  N  P  Y  T  G  D  T  F  Y  N  Q  K  F  K  G  R
                  40                            50    52  a                       60

       210       220       230       240       250       260       270       280       290       300
GGTGACAATCACTGTTGACAAATCCACCAGCACAGCCTACATGGAGCTCTCCAGCCTGAGATCTGAAGACACCGCAGTCTATTATTGTGGACGACTCGGT
  V  T  I  T  V  D  K  S  T  S  T  A  Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  G  R  L  G
        70                                  80    82  a  b  c                       90

       310       320       330       340       350
AGTAGGTACTGGTACTTCGATGTCTGGGGCCAGGGGACCACGGTCACCGTCTCCTCA (SEQ ID NO:68)
 S  R  Y  W  Y  F  D  V  W  G  Q  G  T  T  V  T  V  S  S  (SEQ ID NO:69)
        100 a  b                          110
```

FIG. 35E

Anti-CAD11 EC1 VK1 Light Chain Sequences

```
        10        20        30        40        50        60        70        80        90       100
GATGTTTTGATGACCCAAACTCCACTCTCCTCCCCTGTCACCCTTGGACAGCCAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTATACATAGTAATG
 D  V  L  M  T  Q  T  P  L  S  S  P  V  T  L  G  Q  P  A  S  I  S  C  R  S  S  Q  S  I  I  H  S  N
                            10                            20                      27 a  b  c  d  e

       110       120       130       140       150       160       170       180       190       200
GAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCACAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTT
G  N  T  Y  L  E  W  Y  L  Q  K  P  G  Q  S  P  Q  L  L  I  Y  K  V  S  N  R  F  S  G  V  P  D  R  F
  30                            40                            50                            60

       210       220       230       240       250       260       270       280       290       300
CACTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCT
  T  G  S  G  S  G  T  D  F  T  L  K  I  S  R  V  E  A  E  D  V  G  V  Y  Y  C  F  Q  G  S  H  V  P
                          70                            80                            90

       310       320       330
TGGACGTTCGGTCAGGGCACCAAGCTGGAAATCAAA(SEQ ID NO:70)
 W  T  F  G  Q  G  T  K  L  E  I  K (SEQ ID NO:71)
          100                106  a
```

FIG. 35F

Anti-CAD11 EC1 VK2 Light Chain Sequences

```
         10        20        30        40        50        60        70        80        90       100
GATGTTTTGATGACCCAAACTCCACTCTCCTCCCCTGTCACCCTTGGACAGCCAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTATACATAGTAATG
 D  V  L  M  T  Q  T  P  L  S  S  P  V  T  L  G  Q  P  A  S  I  S  C  R  S  S  Q  S  I  I  H  S  N
                            10                                      20                    27 a  b  c  d  e

        110       120       130       140       150       160       170       180       190       200
GAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCACAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTT
G  N  T  Y  L  E  W  Y  L  Q  K  P  G  Q  S  P  Q  L  L  I  Y  K  V  S  N  R  F  S  G  V  P  D  R  F
 30                          40                            50                            60

        210       220       230       240       250       260       270       280       290       300
CAGCGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCT
  S  G  S  G  S  G  T  D  F  T  L  K  I  S  R  V  E  A  E  D  V  G  V  Y  Y  C  F  Q  G  S  H  V  P
                        70                            80                            90

        310       320       330
TGGACGTTCGGTCAGGGCACCAAGCTGGAAATCAAA (SEQ ID NO:72)
 W  T  F  G  Q  G  T  K  L  E  I  K  (SEQ ID NO:73)
         100            106  a
```

FIG. 35G

Anti-CAD11 EC1 VK3 Light Chain Sequences

```
       10        20        30        40        50        60        70        80        90       100
GATGTTGTGATGACCCAAACTCCACTCTCCTCCCCTGTCACCCTTGGACAGCCAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTATACATAGTAATG
 D  V  V  M  T  Q  T  P  L  S  S  P  V  T  L  G  Q  P  A  S  I  S  C  R  S  S  Q  S  I  I  H  S  N
                         10                            20                       27 a  b  c  d  e

      110       120       130       140       150       160       170       100       190       200
GAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCACAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTT
G  N  T  Y  L  E  W  Y  L  Q  K  P  G  Q  S  P  Q  L  L  I  Y  K  V  S  N  R  F  S  G  V  P  D  R  F
 30                          40                            50                            60

      210       220       230       240       250       260       270       200       290       300
CAGCGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTGGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCT
  S  G  S  G  S  G  T  D  F  T  L  K  I  S  R  V  E  A  E  D  V  G  V  Y  Y  C  F  Q  G  S  H  V  P
                       70                            80                            90

      310       320       330
TGGACGTTCGGTCAGGGCACCAAGCTGGAAATCAAA (SEQ ID NO:74)
 W  T  F  G  Q  G  T  K  L  E  I  K  (SEQ ID NO:75)
          100               106  a
```

FIG. 35H

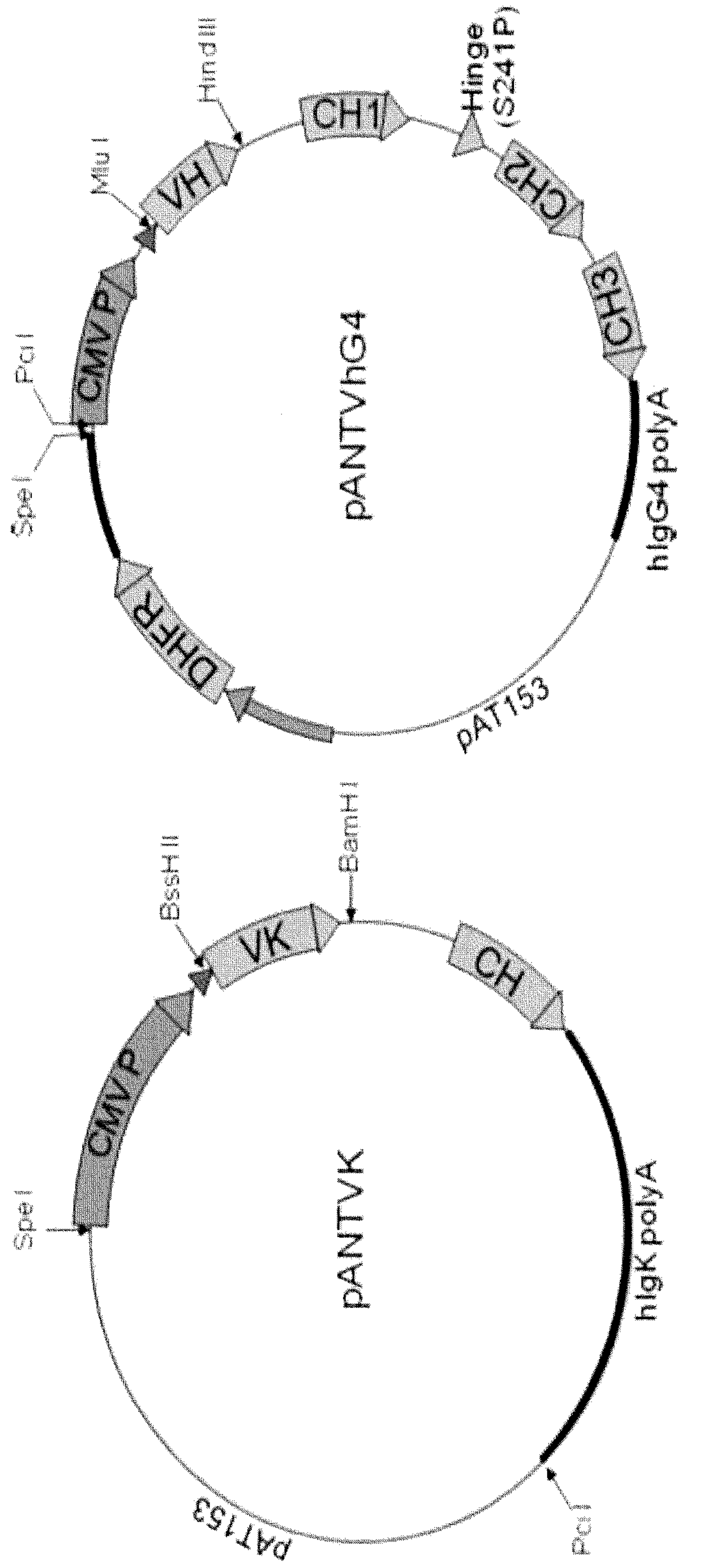

pANTVhG4
Spe I
Pci I
CMV P
Mlu I
VH
Hind III
CH1
Hinge
(S241P)
CH2
CH3
hIgG4 polyA
pAT153
DHFR
pANTVK
Spe I
CMV P
BssH II
VK
BamHI
CH
hIgK polyA
Pci I
pAT153

FIG. 36

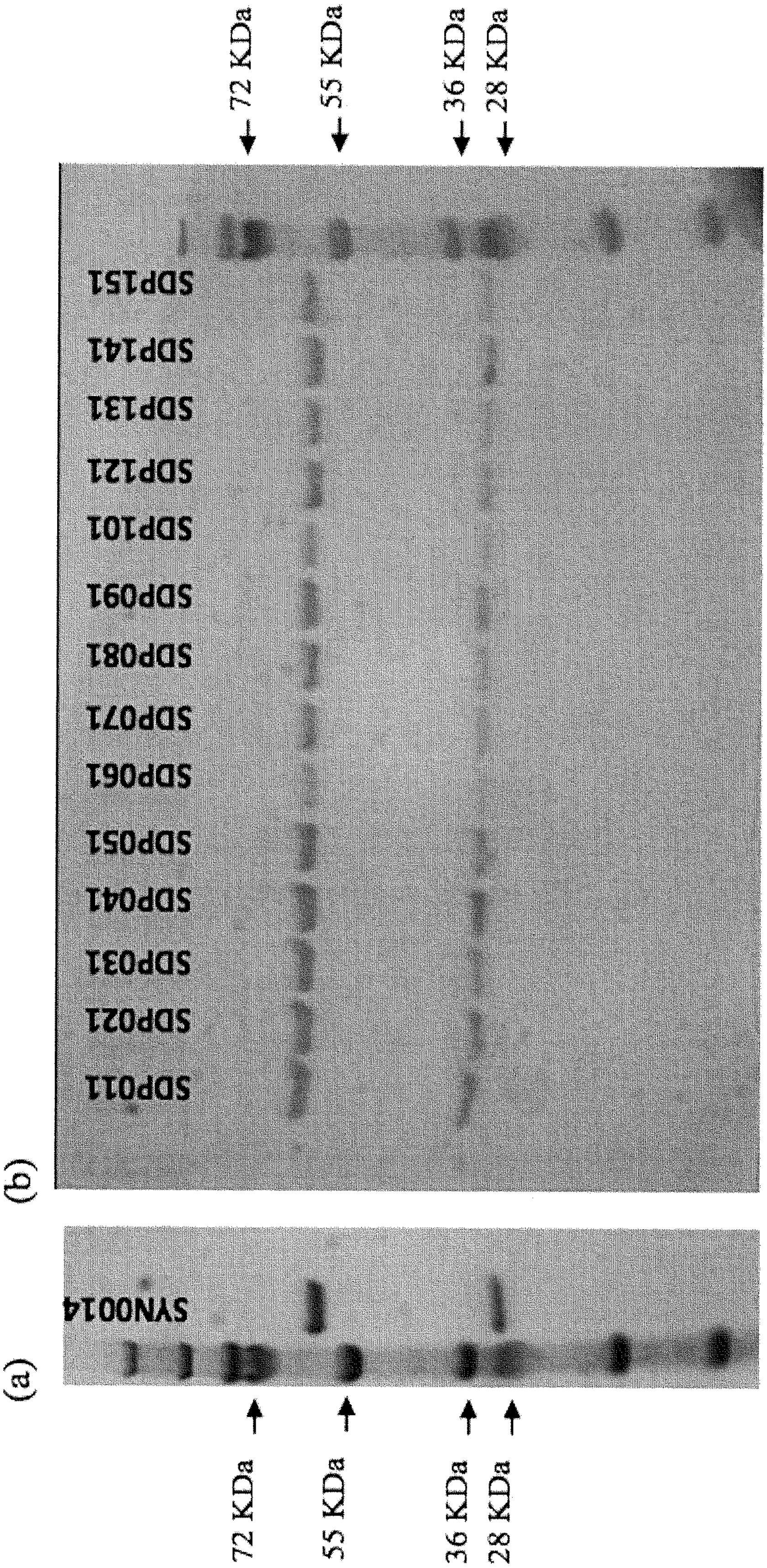

(a)
(b)
72 KDa
55 KDa
36 KDa
28 KDa
SYN0014
SDP011
SDP021
SDP031
SDP041
SDP051
SDP061
SDP071
SDP081
SDP091
SDP101
SDP121
SDP131
SDP141
SDP151

FIG. 37

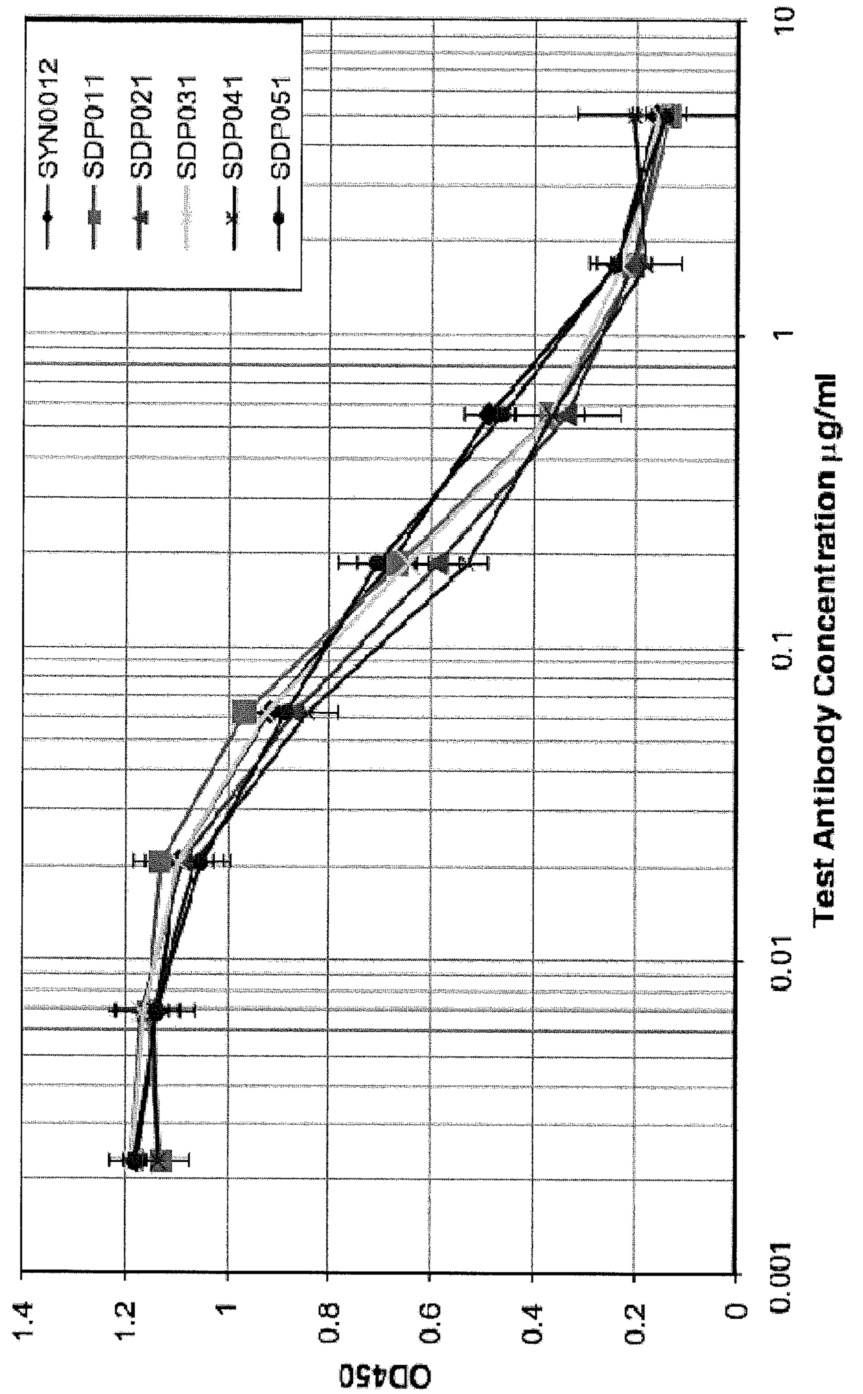

SYN0012
SDP011
SDP021
SDP031
SDP041
SDP051
OD450
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.001
0.01
0.1
1
10
Test Antibody Concentration μg/ml

FIG. 38A

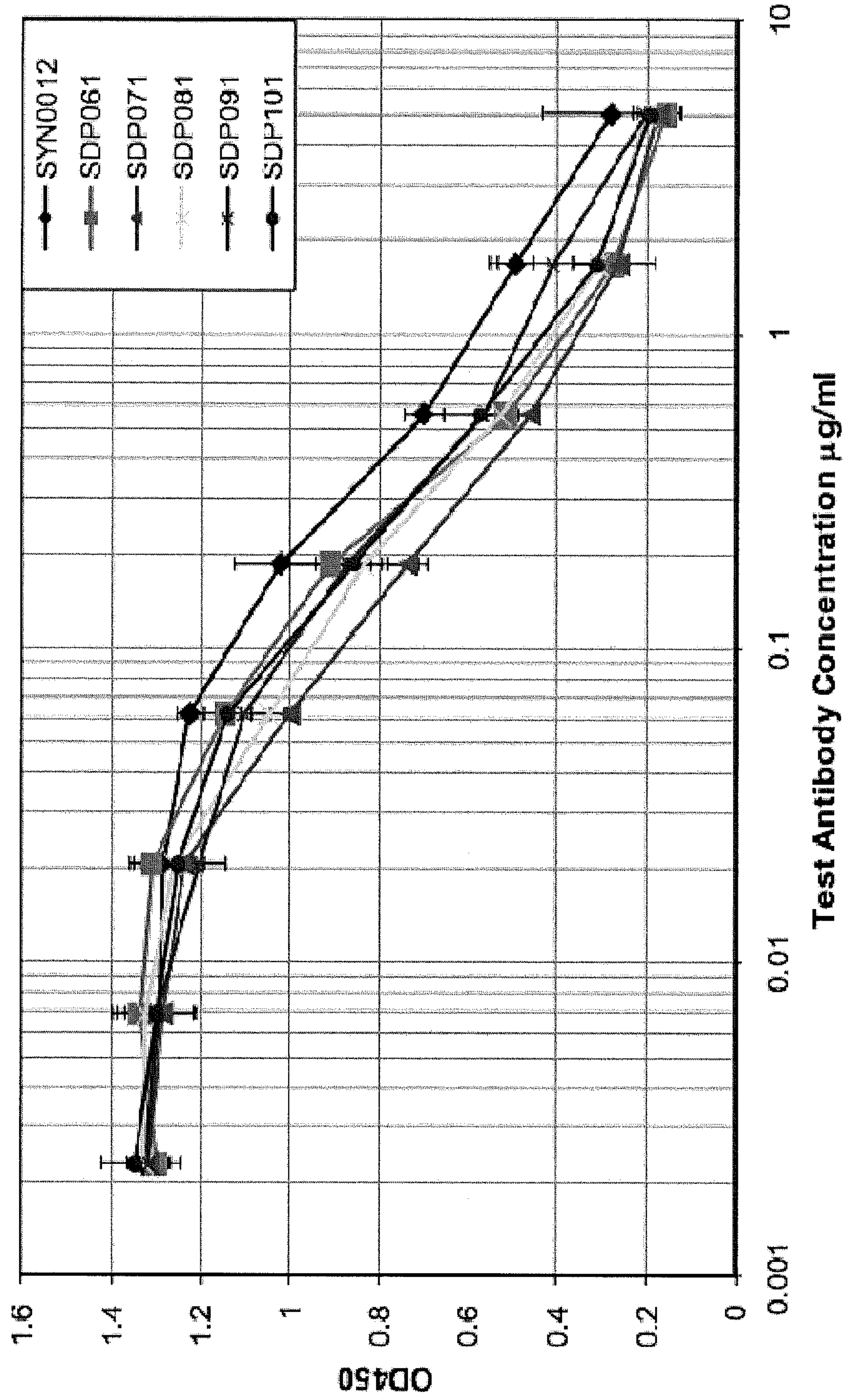

SYN0012
SDP061
SDP071
SDP081
SDP091
SDP101
OD450
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.001
0.01
0.1
1
10
Test Antibody Concentration µg/ml

FIG. 38B

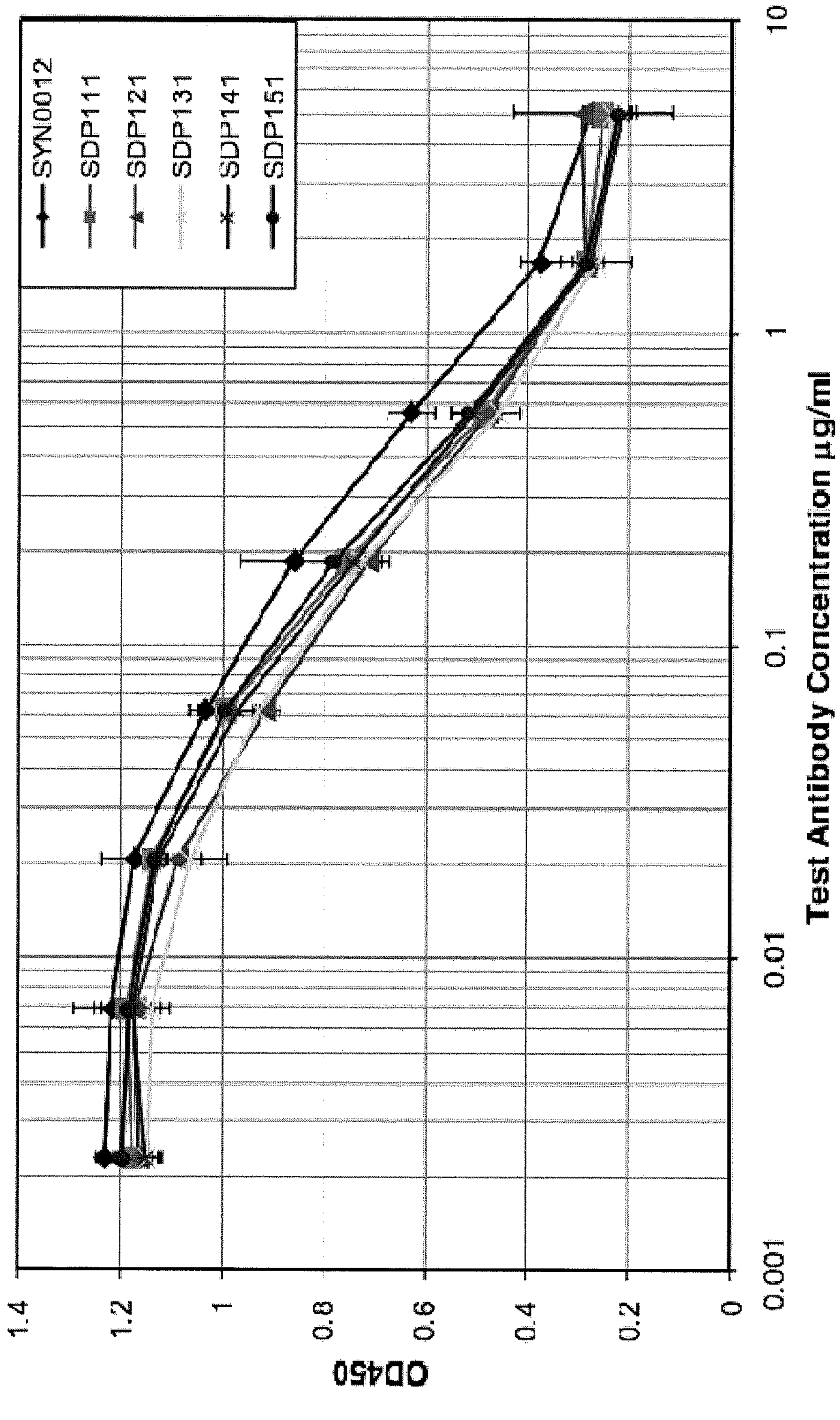

SYN0012
SDP111
SDP121
SDP131
SDP141
SDP151
OD450
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.001
0.01
0.1
1
10
Test Antibody Concentration µg/ml

FIG. 38C

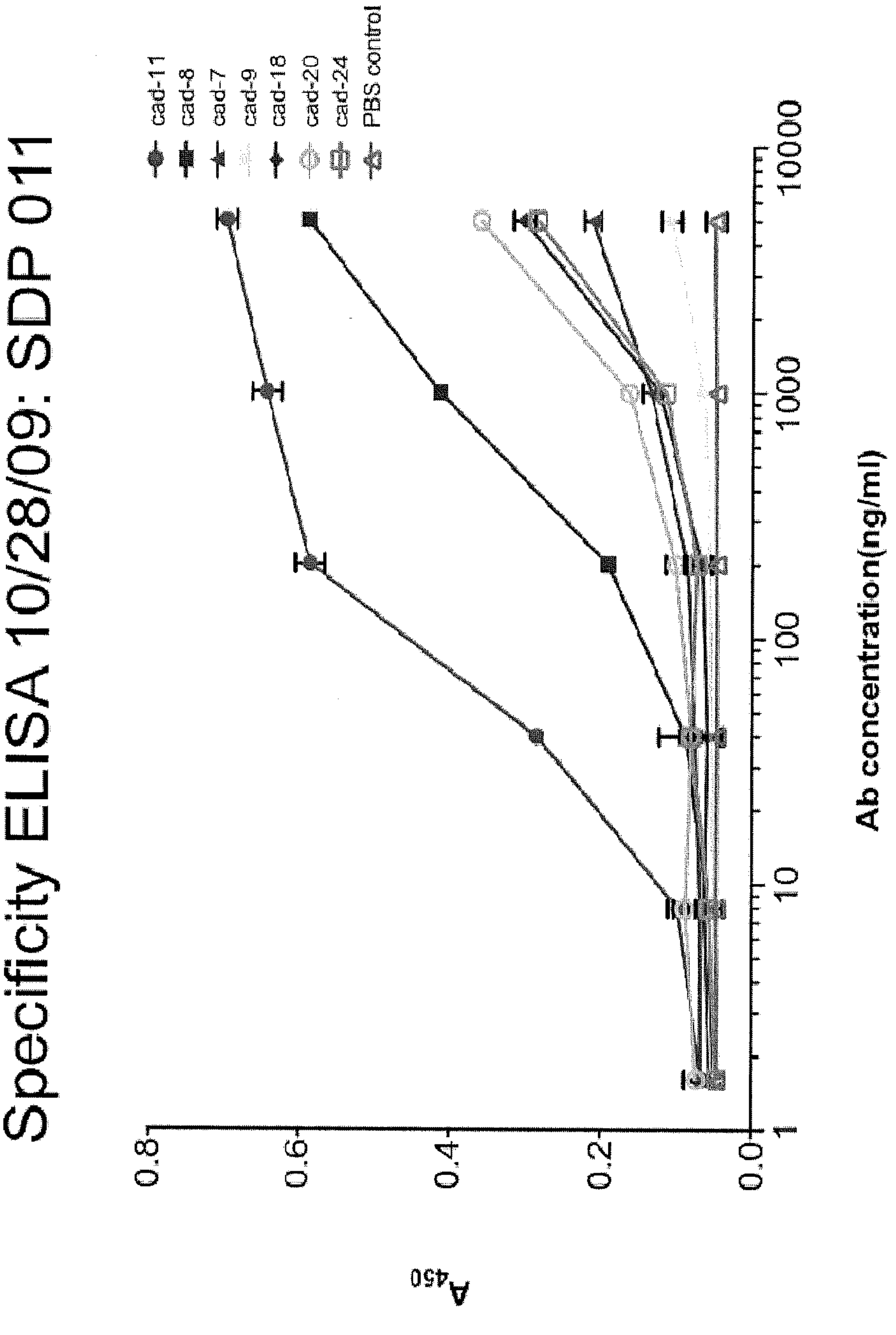

Specificity ELISA 10/28/09: SDP 011
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
A_{450}
0.0
0.2
0.4
0.6
0.8
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39A

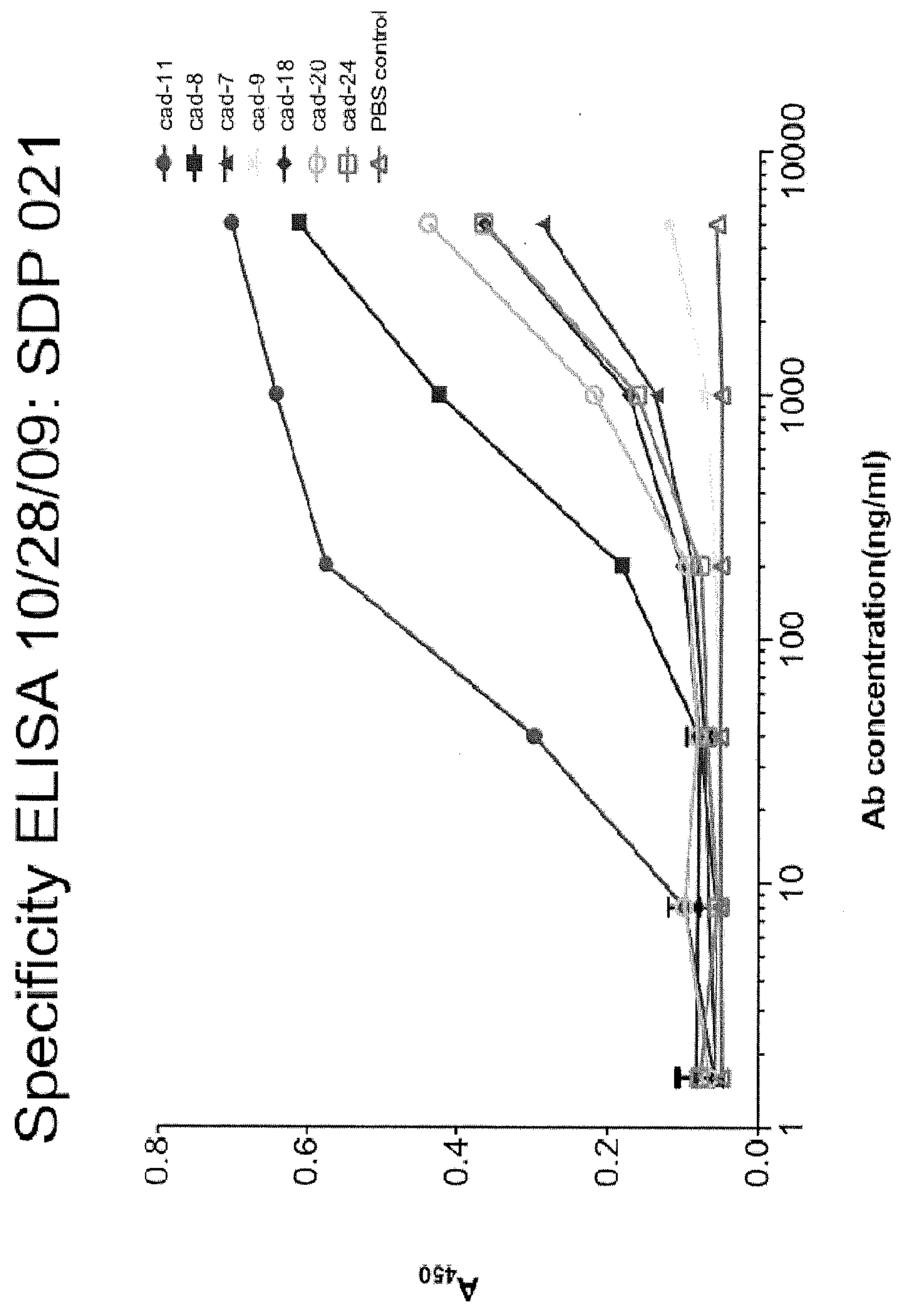

Specificity ELISA 10/28/09: SDP 021
A450
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control

FIG. 39B

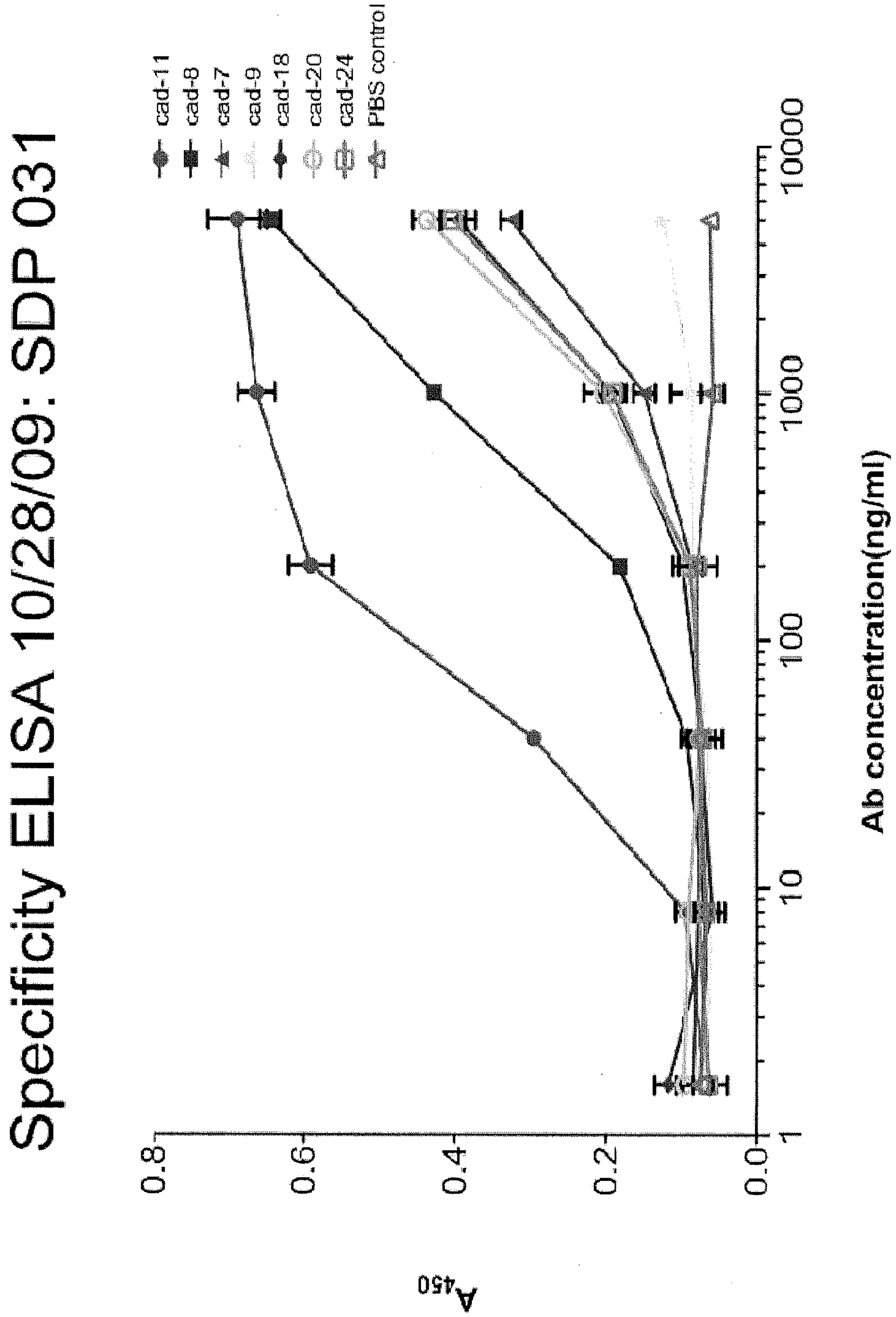

Specificity ELISA 10/28/09: SDP 031
A450
0.0
0.2
0.4
0.6
0.8
1
10
100
1000
10000
Ab concentration(ng/ml)
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control

FIG. 39C

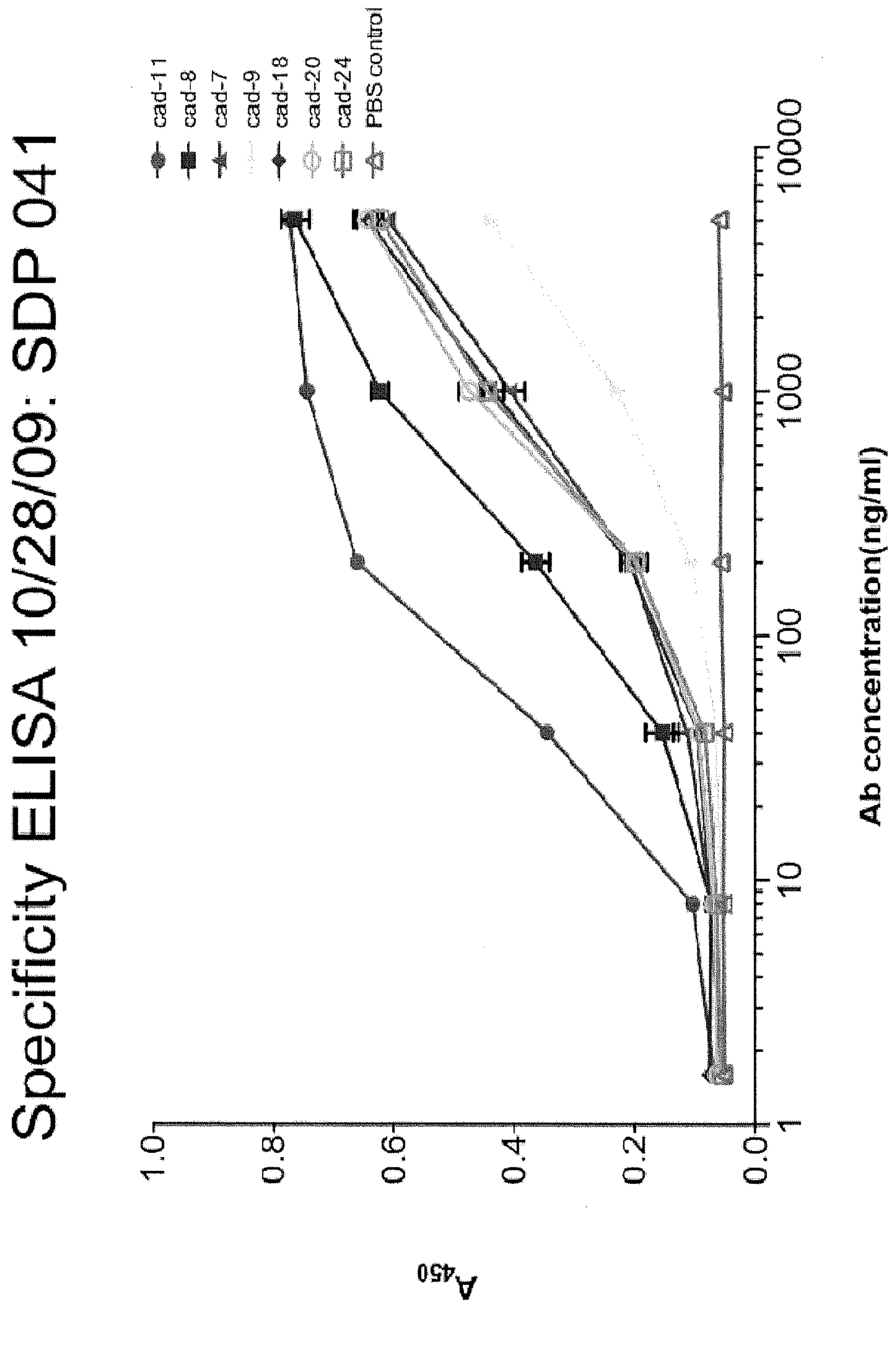

Specificity ELISA 10/28/09: SDP 041
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
1.0
0.8
0.6
0.4
0.2
0.0
A_{450}
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39D

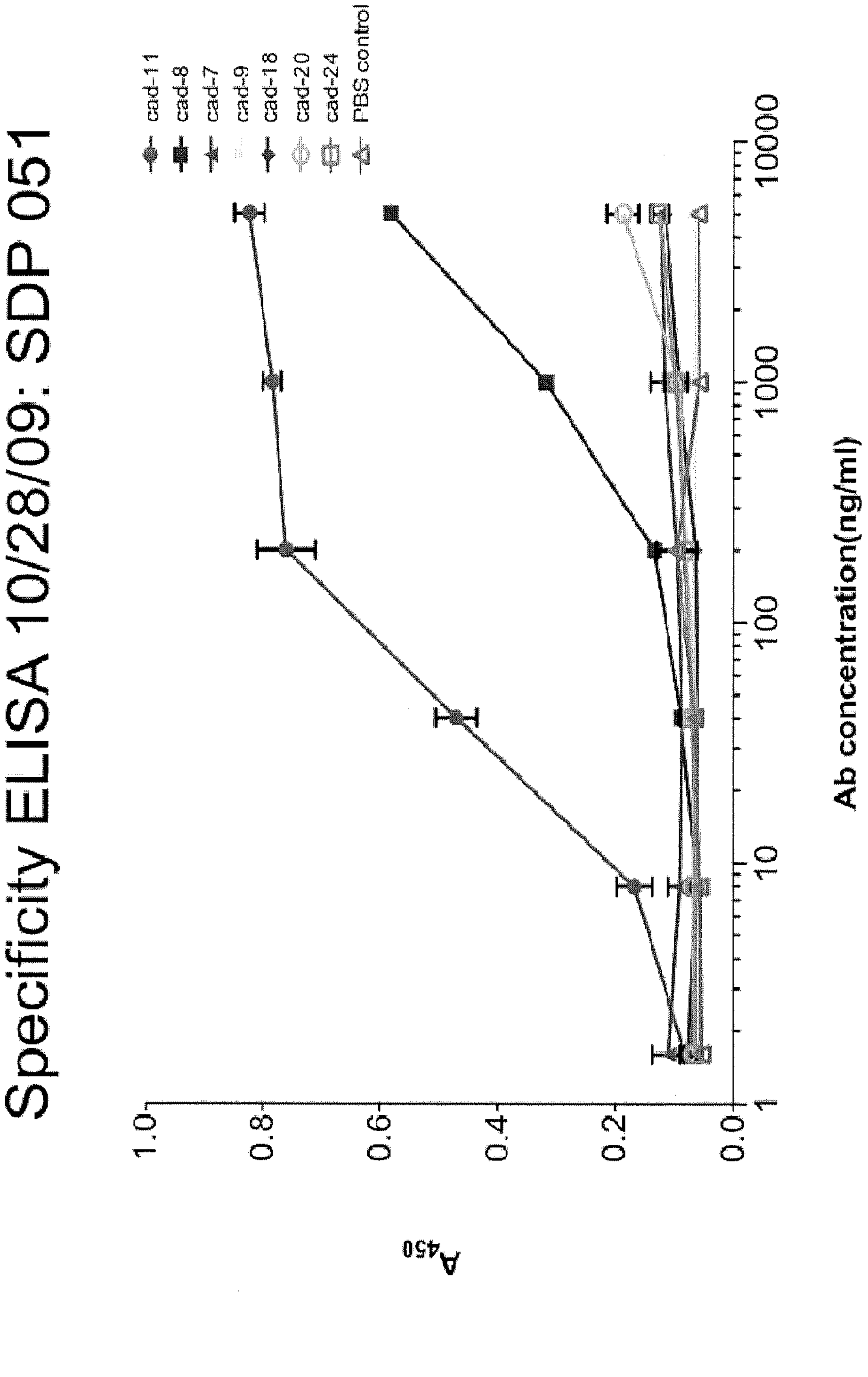

Specificity ELISA 10/28/09: SDP 051
A_{450}
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control

FIG. 39E

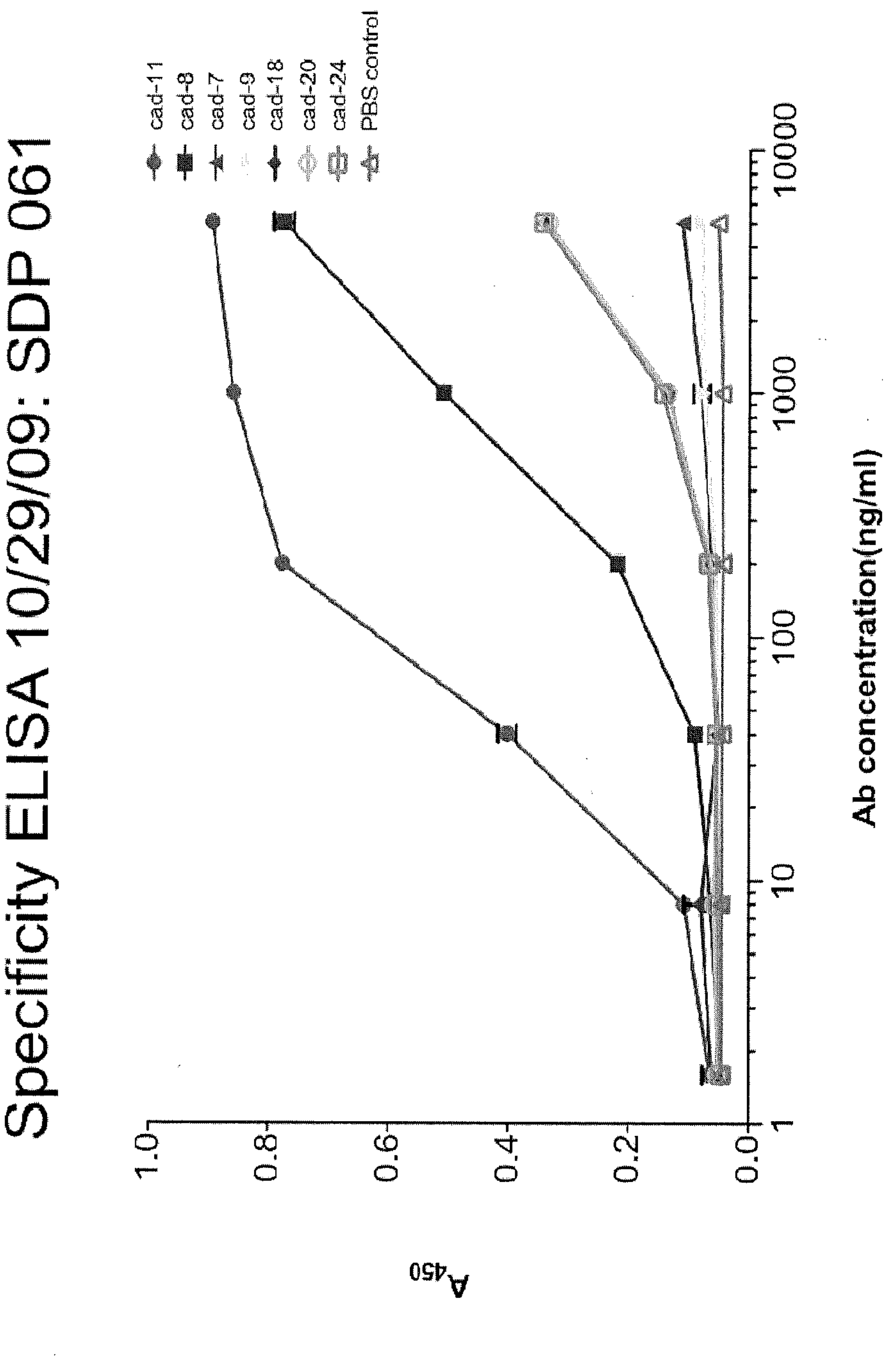

Specificity ELISA 10/29/09: SDP 061
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
A_{450}
0.0
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39F

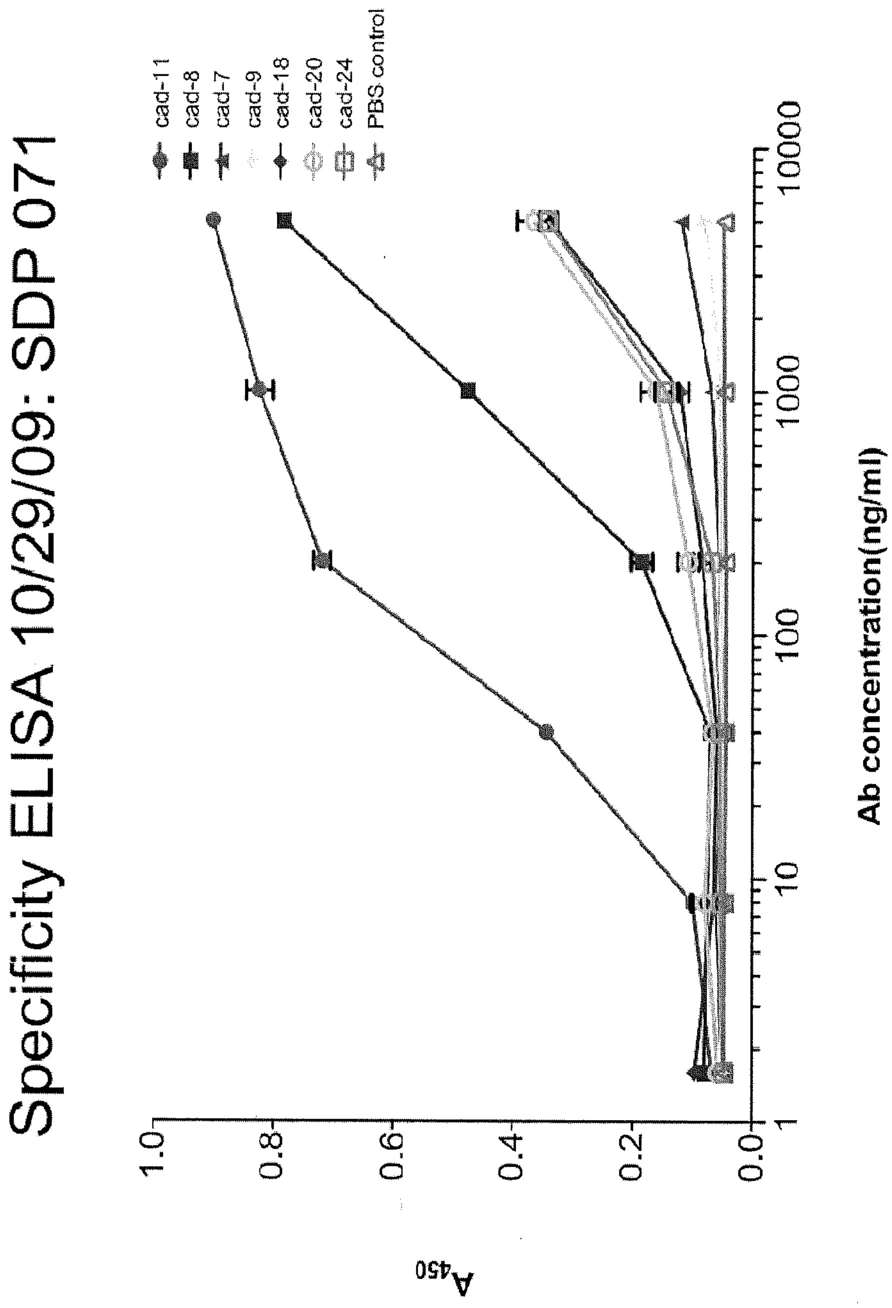
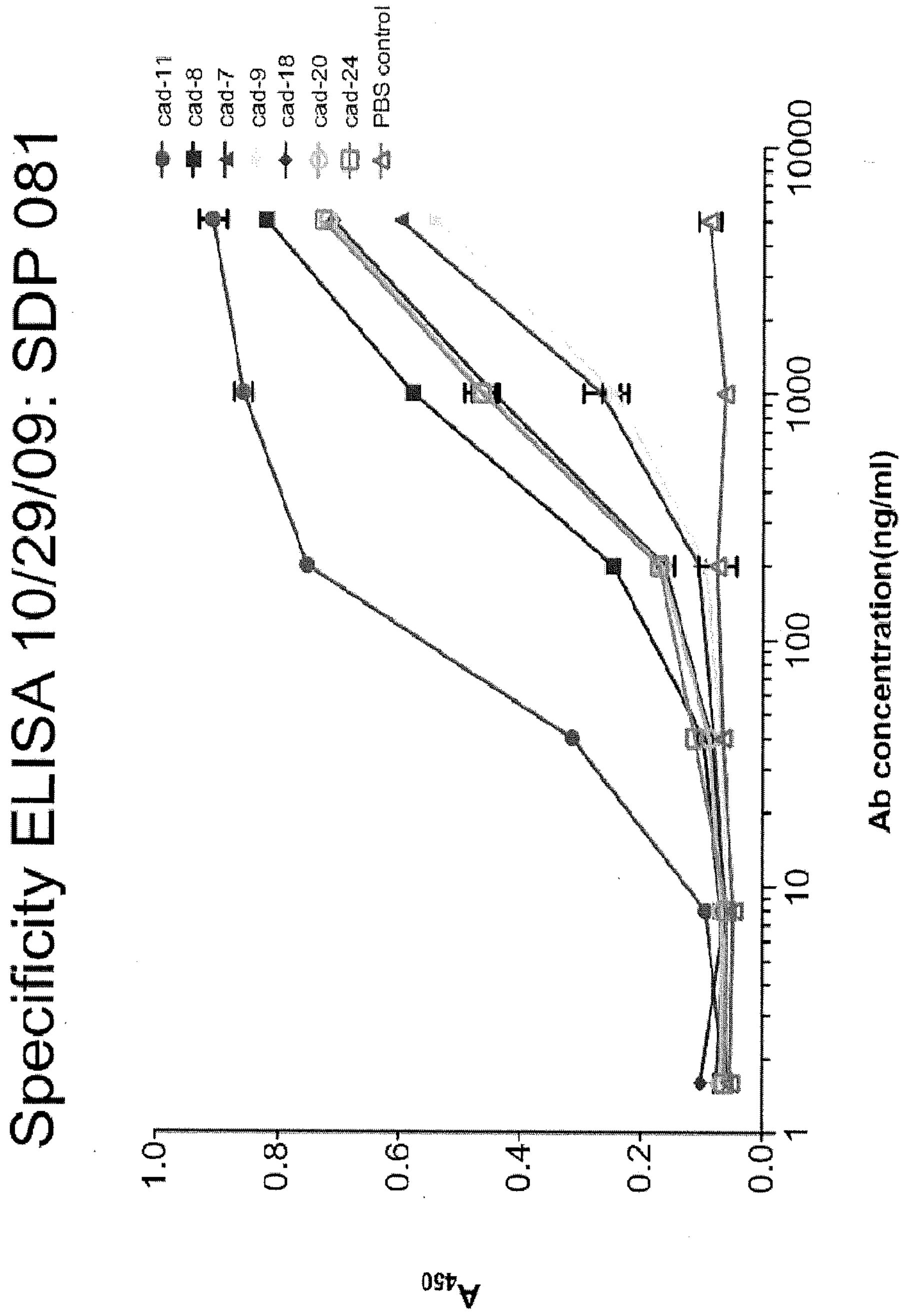

Specificity ELISA 10/29/09: SDP 071
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
A450
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39G



Specificity ELISA 10/29/09: SDP 081
A450
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control

FIG. 39H

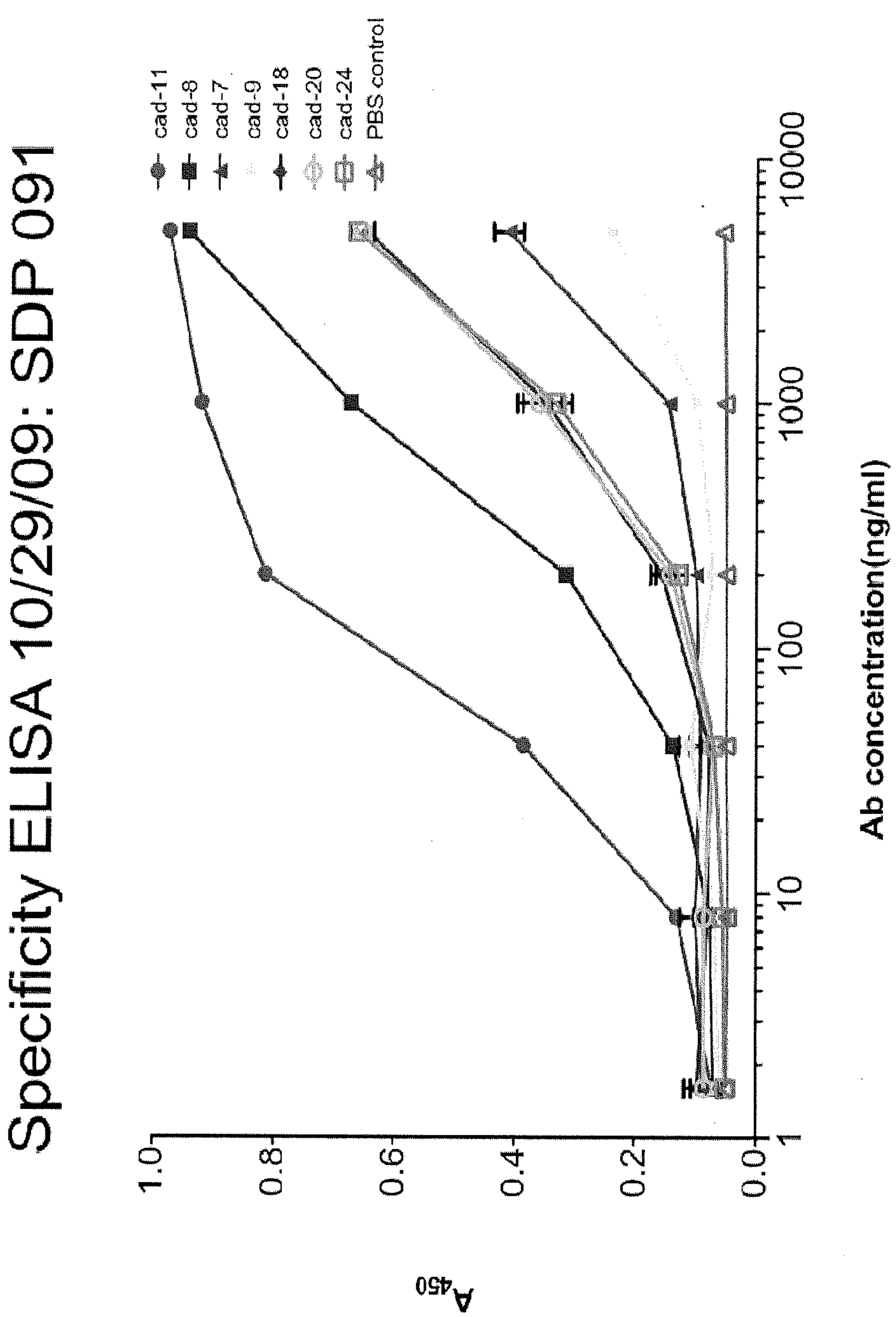

Specificity ELISA 10/29/09: SDP 091
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
A_{450}
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39I

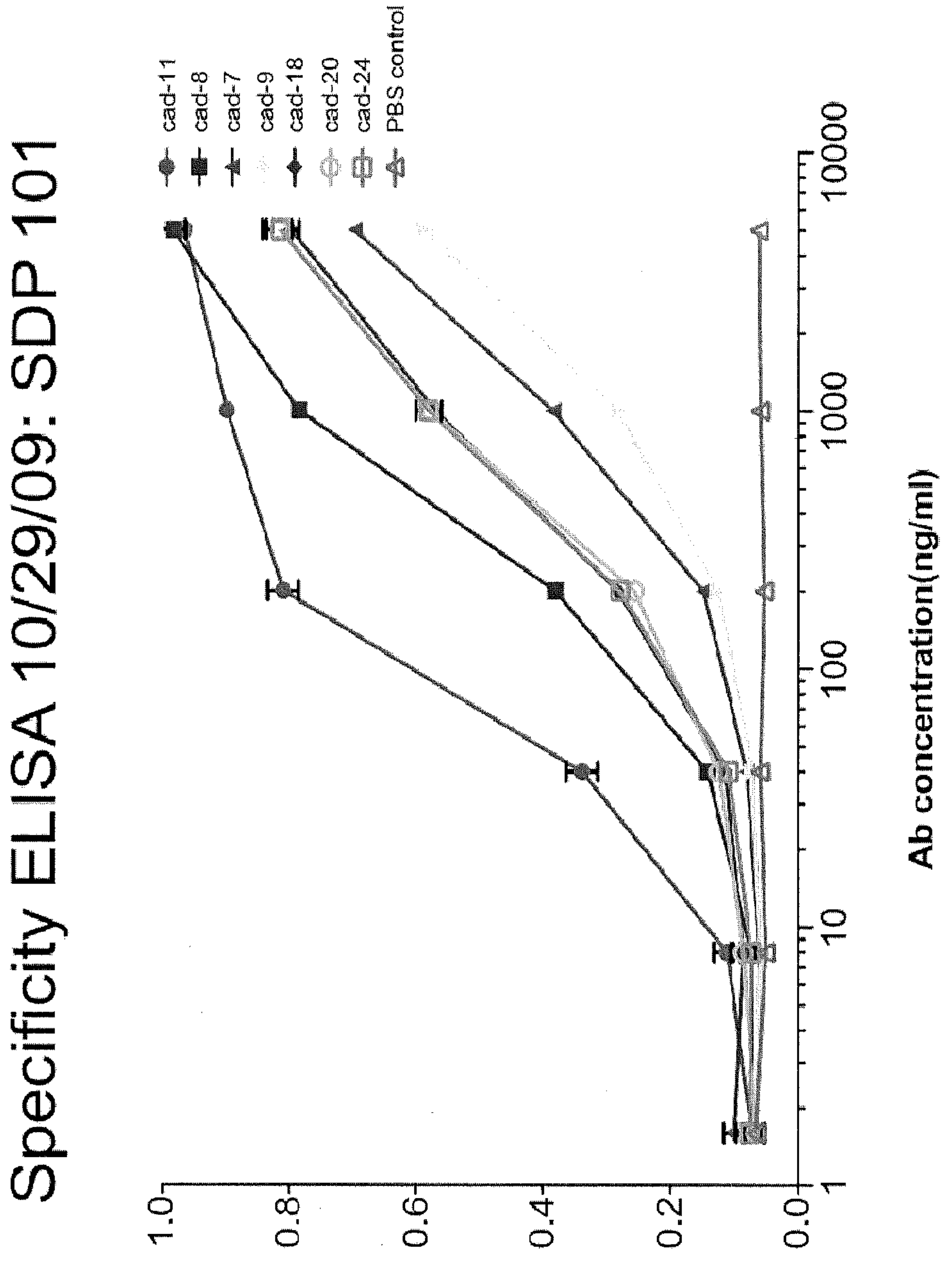

Specificity ELISA 10/29/09: SDP 101
cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
A450
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39J

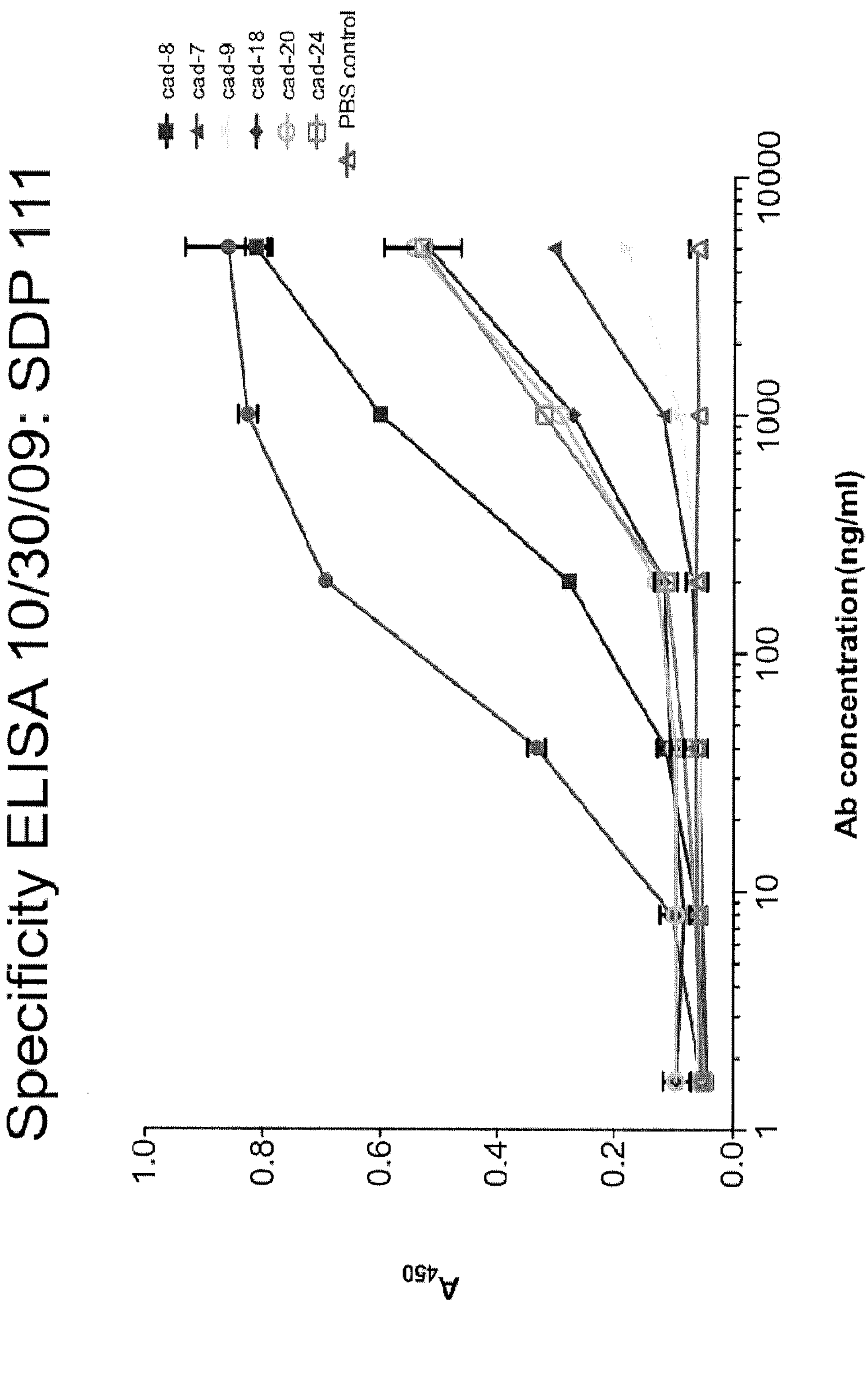

Specificity ELISA 10/30/09: SDP 111
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
A_{450}
0.0
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39K

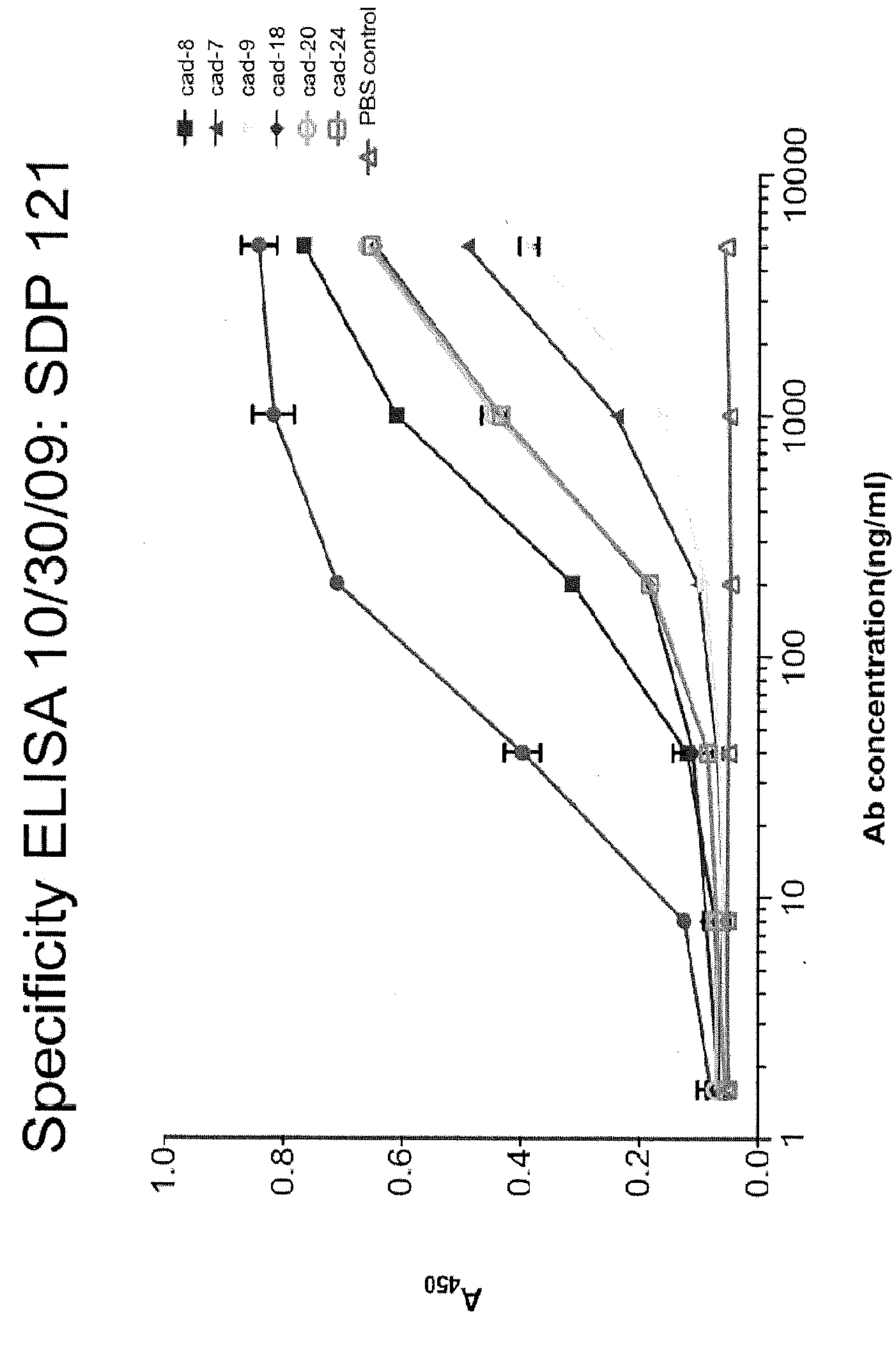
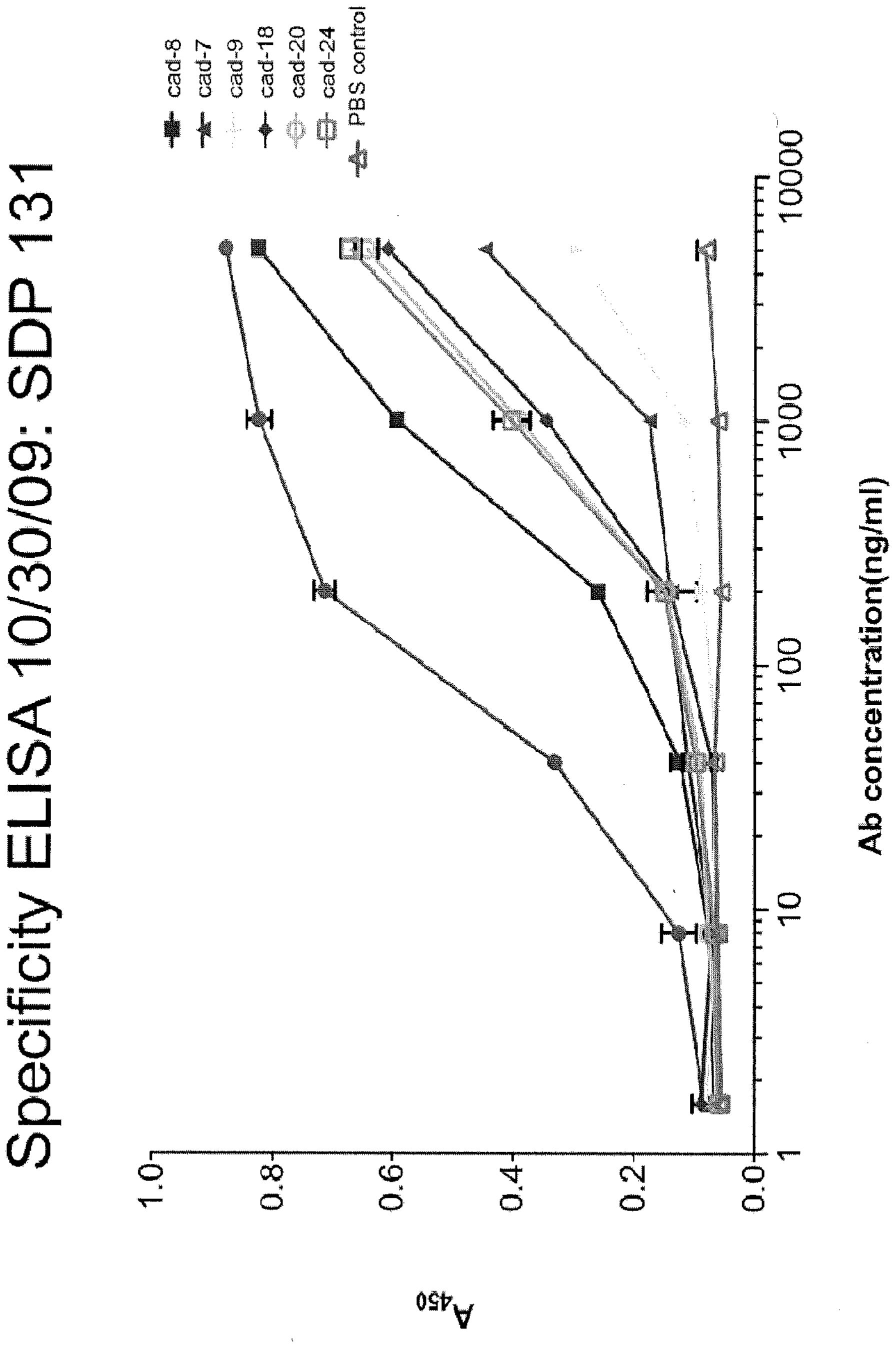

Specificity ELISA 10/30/09: SDP 121
A450
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control

FIG. 39L



Specificity ELISA 10/30/09: SDP 131
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
A_{450}
0.0
0.2
0.4
0.6
0.8
1.0
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39M

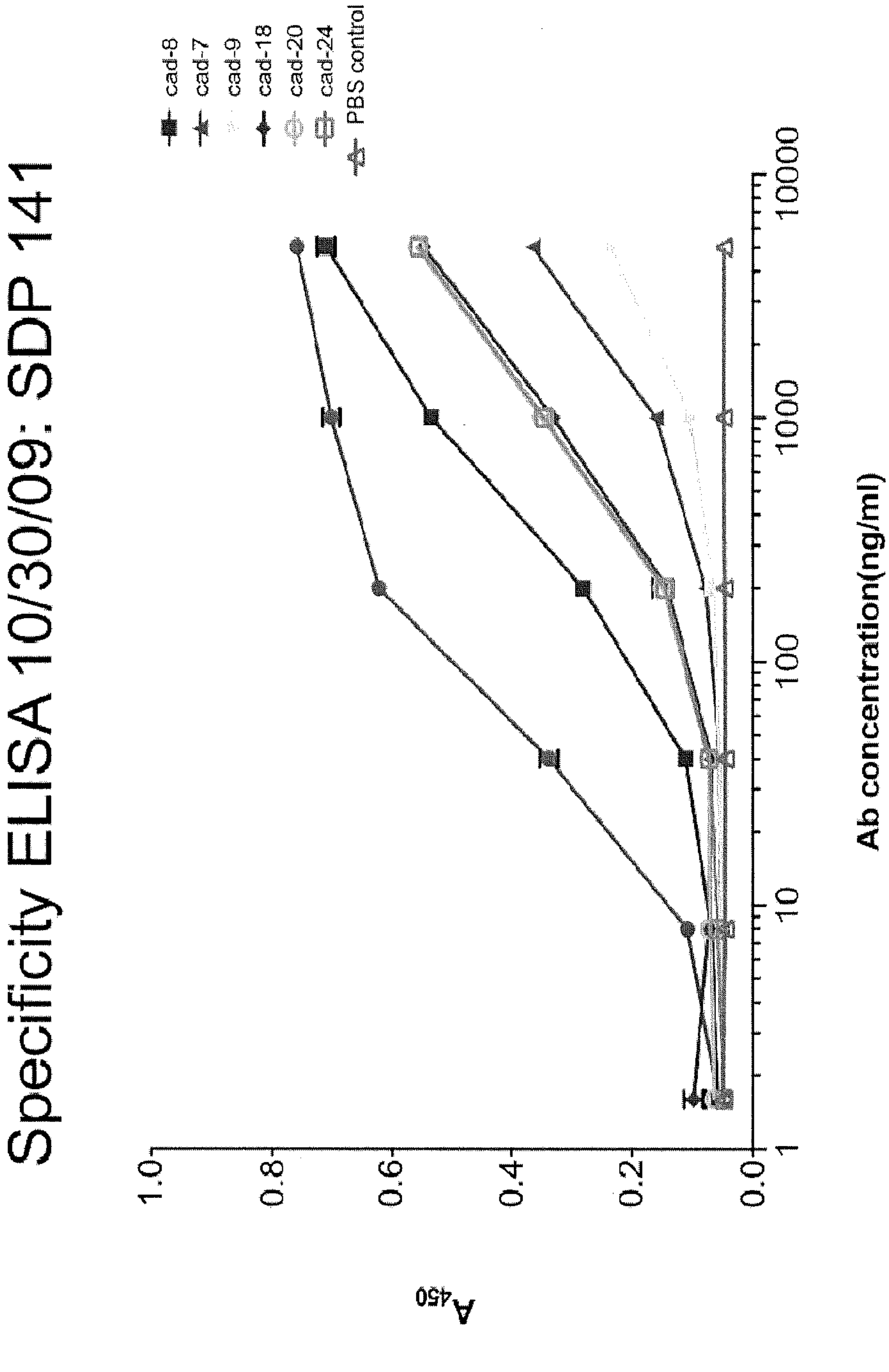

Specificity ELISA 10/30/09: SDP 141
A450
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control

FIG. 39N

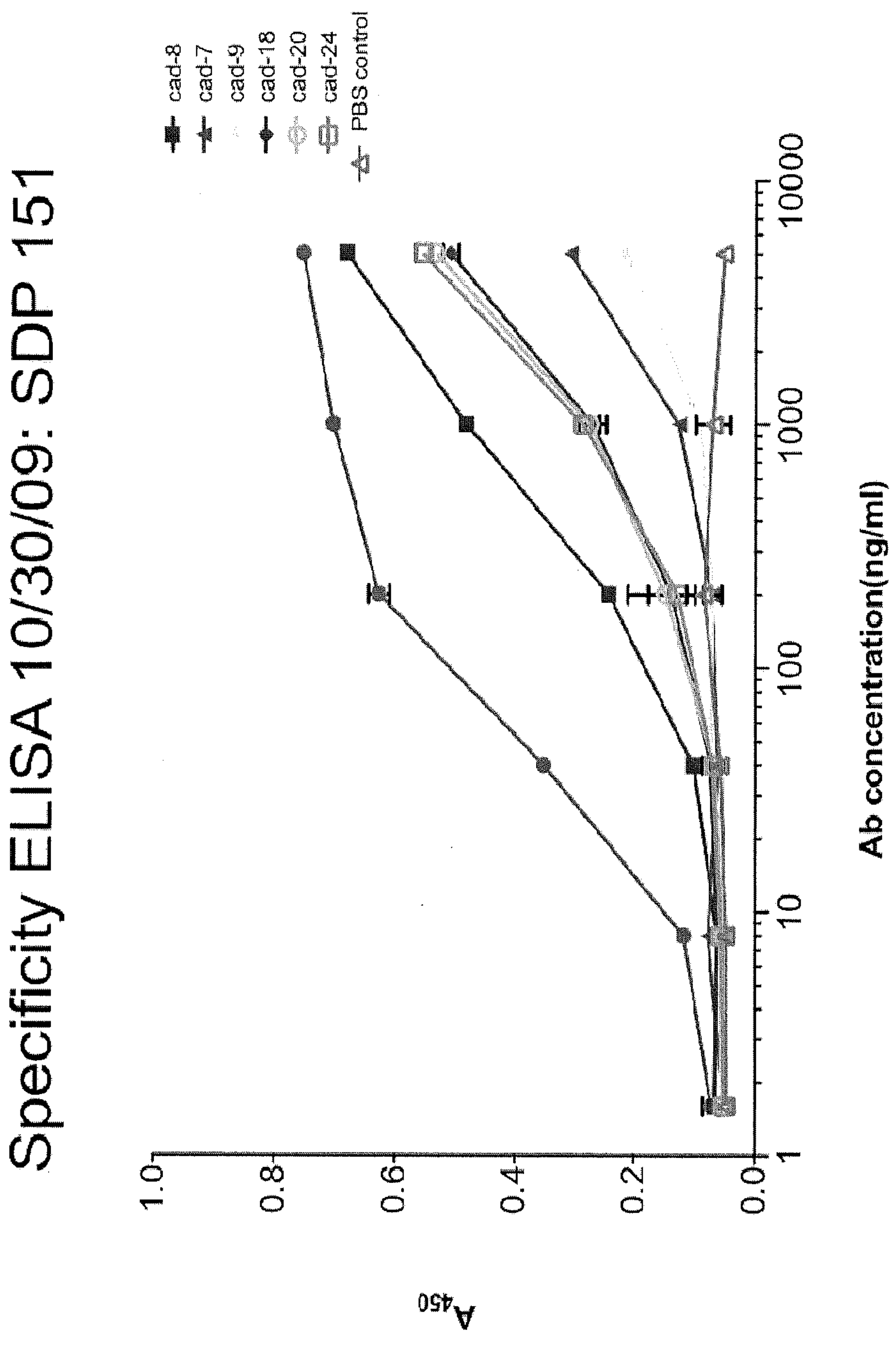

Specificity ELISA 10/30/09: SDP 151
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
A_{450}
1.0
0.8
0.6
0.4
0.2
0.0
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 39O

>SDP031_LIGHT CHAIN

DVLMTQTPLSSPVTLGQPASISCRSSQSIIHSNGNTYLEWYLQKPGQSPQLLIYKVS
NRFSGVPDRFTGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPWTFGQGTKLEIK
(SEQ ID NO:71)

>SDP031_HEAVY CHAIN

EVQLVQSGAEVKKPGASVKISCKASGYSFTGYFMNWVRQAPGQGLEWIGRINPYT
GDTFYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCGRLGSRYWYFDVWG
QGTTVTVSS (SEQ ID NO:65)

FIG. 40A

>SDP051_ LIGHT CHAIN

DVLMTQTPLSSPVTLGQPASISCRSSQSIIHSNGNTYLEWYLQKPGQSPQLLIYKVSN
RFSGVPDRFTGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPWTFGQGTKLEIK
(SEQ ID NO:71)

>SDP051_ HEAVY CHAIN

EVQLVQSGAEVKKPGASVKVSCKASGYSFTGYFMNWVRQAPGQGLEWIGRINPYT
GDTFYNQKFKGRVTITVDKSTSTAYMELSSLRSEDTAVYYCGRLGSRYWYFDVWGQ
GTTVTVSS (SEQ ID NO:69)

FIG. 40B

>SDP061_ LIGHT CHAIN

DVLMTQTPLSSPVTLGQPASISCRSSQSIIHSNGNTYLEWYLQKPGQSPQLLIYKVSNRF
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPWTFGQGTKLEIK (SEQ ID NO:73)

>SDP061_ HEAVY CHAIN

EVQLVQSGPELKKPGASVKISCKASGYSFTGYFMNWVKQAHGQGLEWIGRINPYTGDT
FYNQKFKGRATLTVDKSSSTAYMELVSLSSEDSAVYYCGRLGSRYWYFDVWGQGTTVT
VSS (SEQ ID NO:61)

FIG. 40C

>SDP071_ LIGHT CHAIN

DVLMTQTPLSSPVTLGQPASISCRSSQSIIHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPD
RFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPWTFGQGTKLEIK (SEQ ID NO:73)

>SDP071_ HEAVY CHAIN

EVQLVQSGAEVKKPGASVKISCKASGYSFTGYFMNWVKQAPGQGLEWIGRINPYTGDTFYNQK
FKGRATLTVDKSTSTAYMELSSLRSEDTAVYYCGRLGSRYWYFDVWGQGTTVTVSS (SEQ ID NO:63)

FIG. 40D

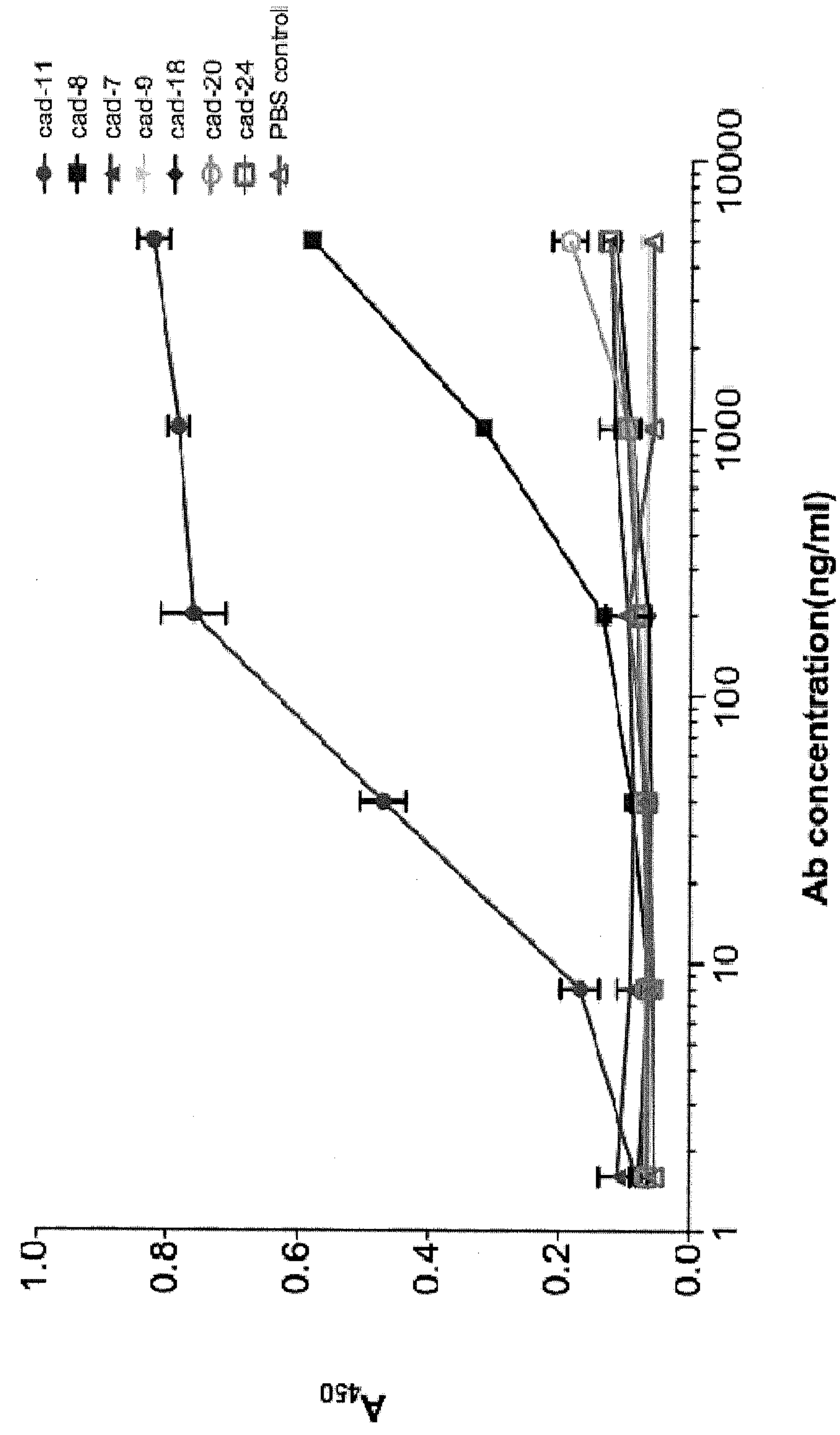

cad-11
cad-8
cad-7
cad-9
cad-18
cad-20
cad-24
PBS control
1.0
0.8
0.6
0.4
0.2
0.0
A_{450}
1
10
100
1000
10000
Ab concentration(ng/ml)

FIG. 41A

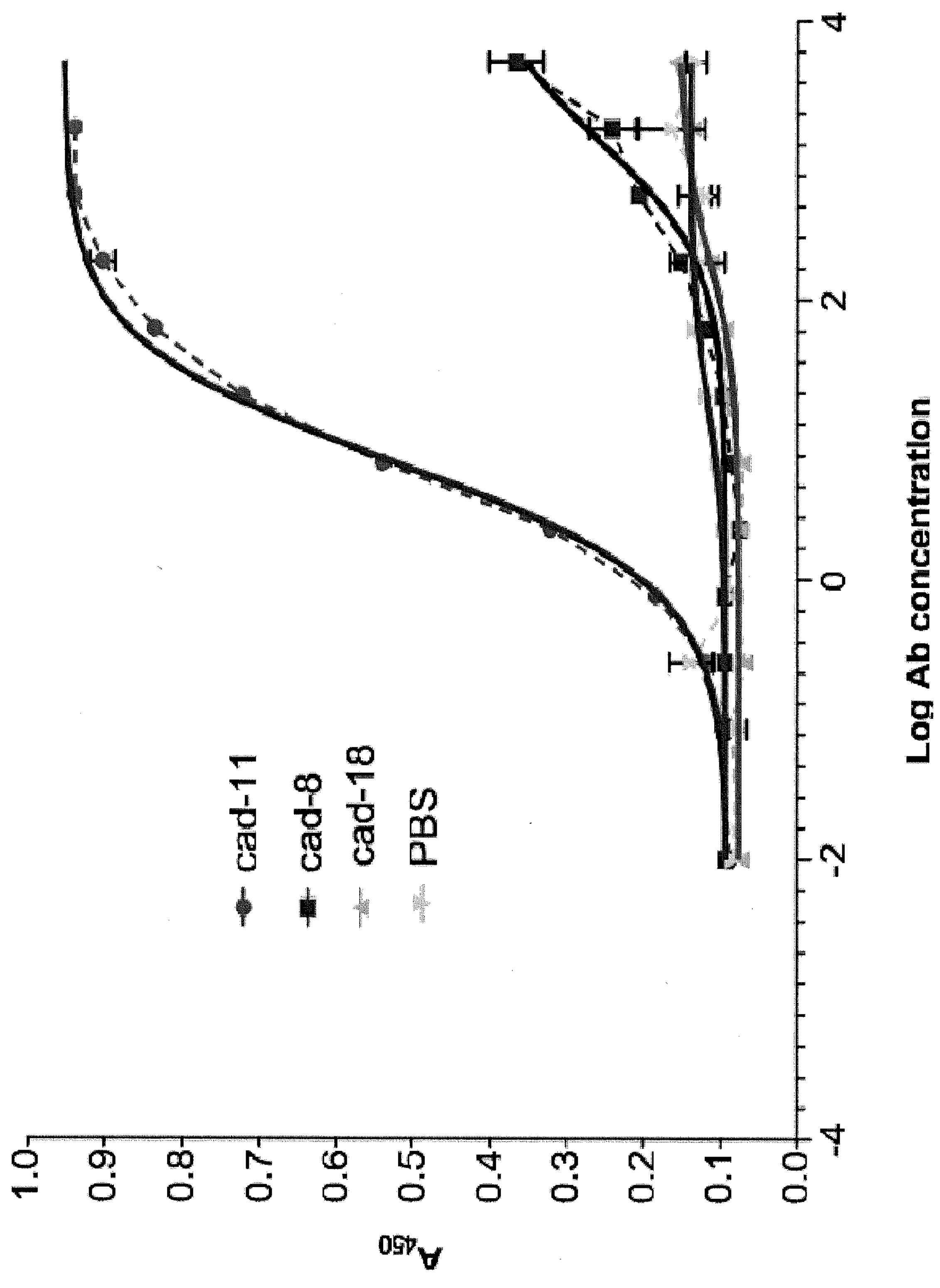

cad-11
cad-8
cad-18
PBS
A450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-4
-2
0
2
4
Log Ab concentration

FIG. 41B

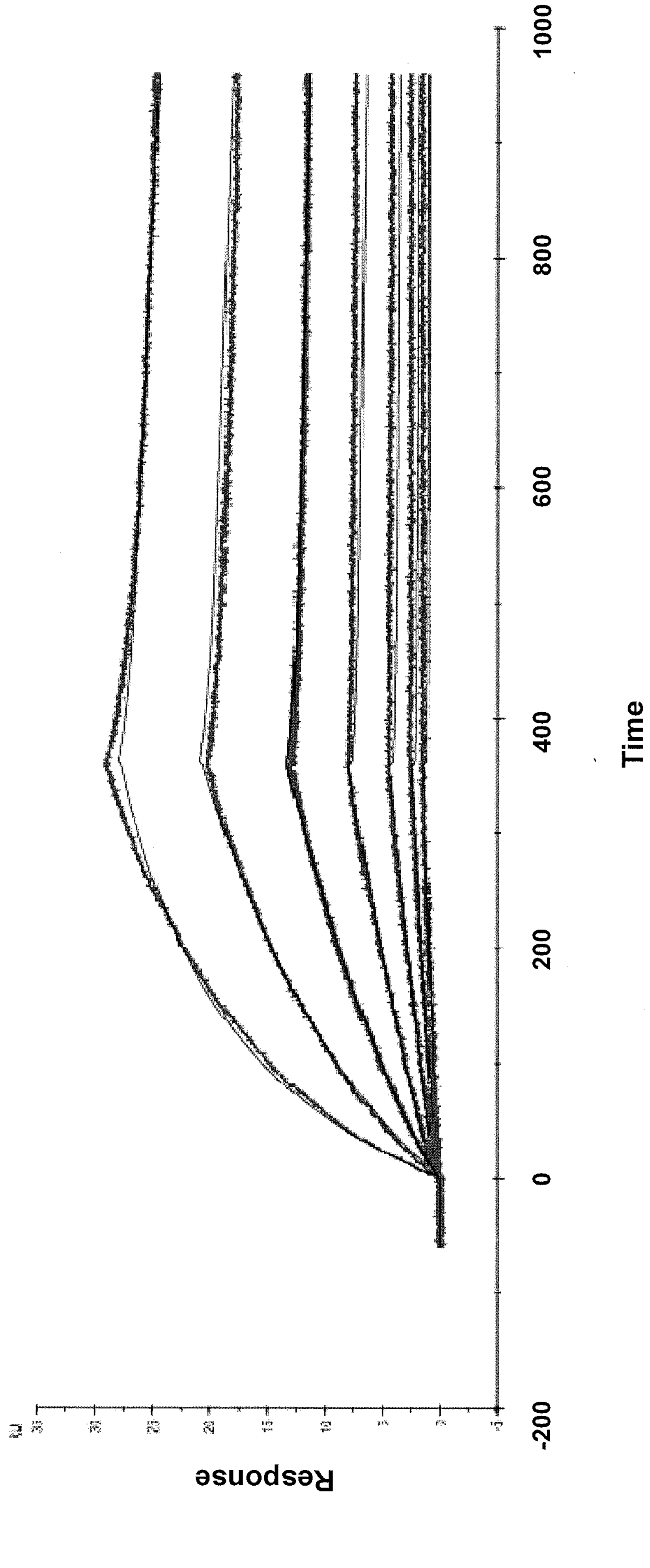


FIG. 42

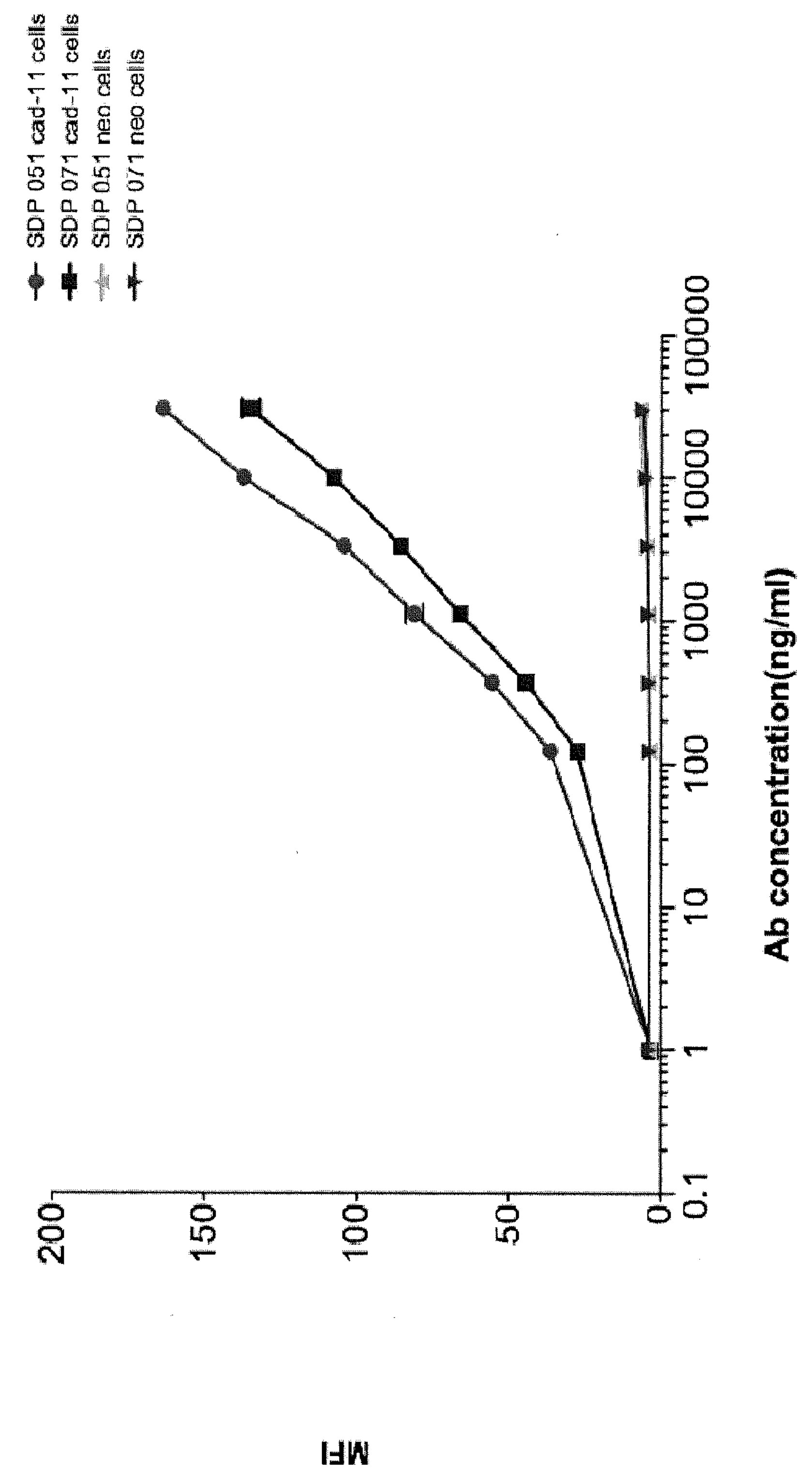

SDP 051 cad-11 cells
SDP 071 cad-11 cells
SDP 0.51 neo cells
SDP 071 neo cells
200
150
100
50
0
0.1
1
10
100
1000
10000
100000
Ab concentration(ng/ml)
MFI

FIG. 43

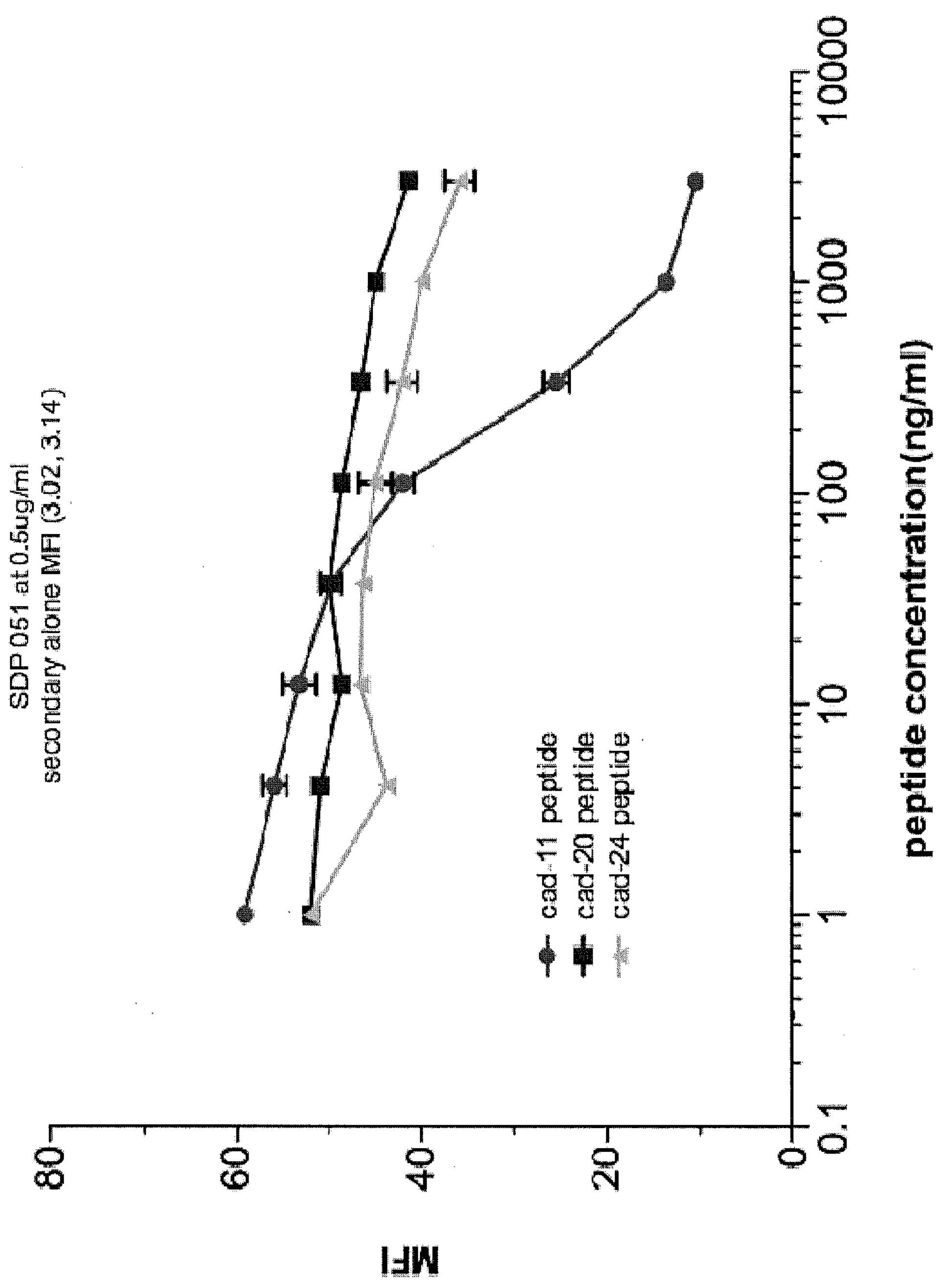

SDP 051 at 0.5ug/ml
secondary alone MFI (3.02, 3.14)
MFI
80
60
40
20
0
0.1
1
10
100
1000
10000
peptide concentration(ng/ml)
cad-11 peptide
cad-20 peptide
cad-24 peptide

FIG. 44A

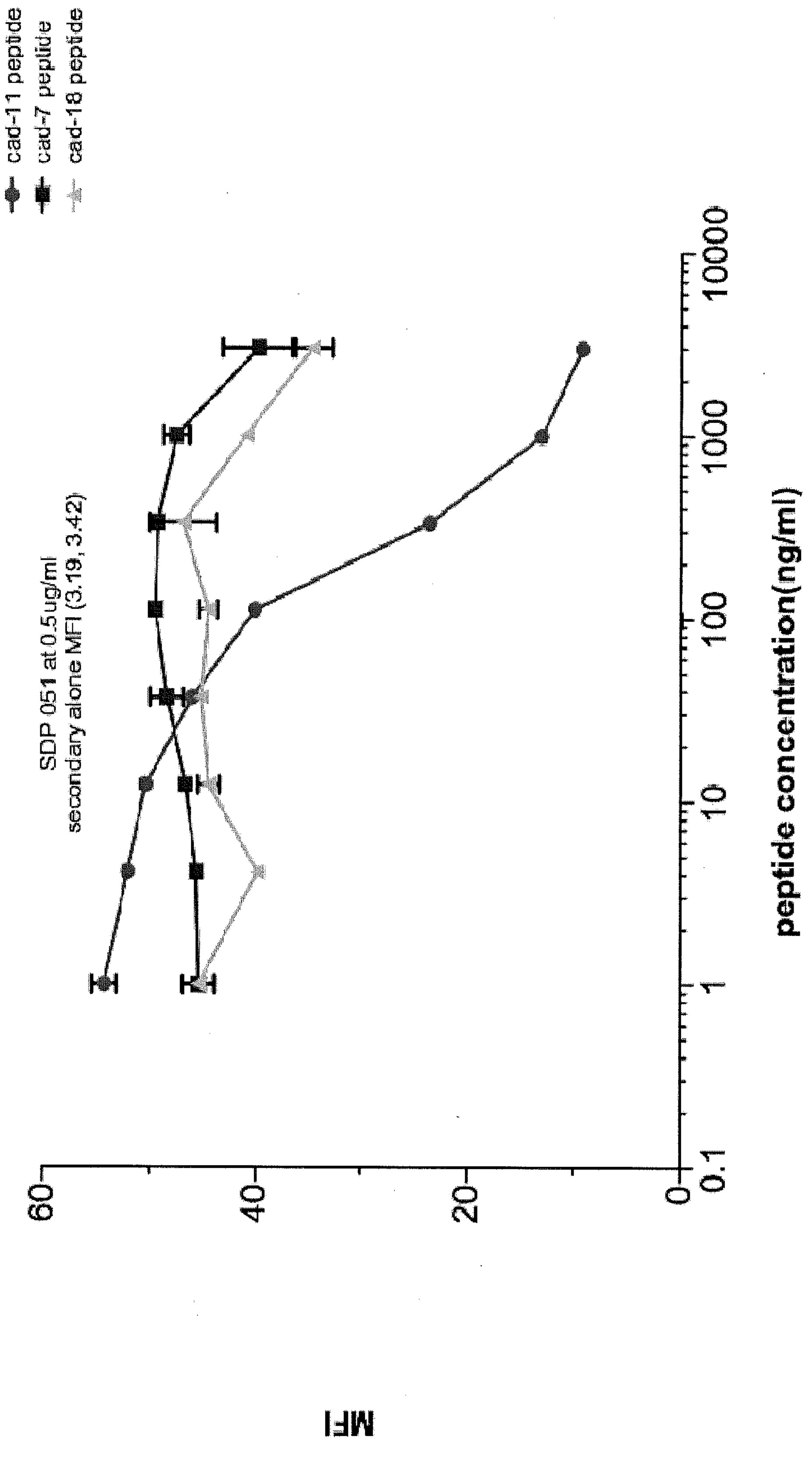

cad-11 peptide
cad-7 peptide
cad-18 peptide
SDP 051 at 0.5ug/ml
secondary alone MFI (3.19, 3.42)
MFI
0
20
40
60
0.1
1
10
100
1000
10000
peptide concentration(ng/ml)

FIG. 44B

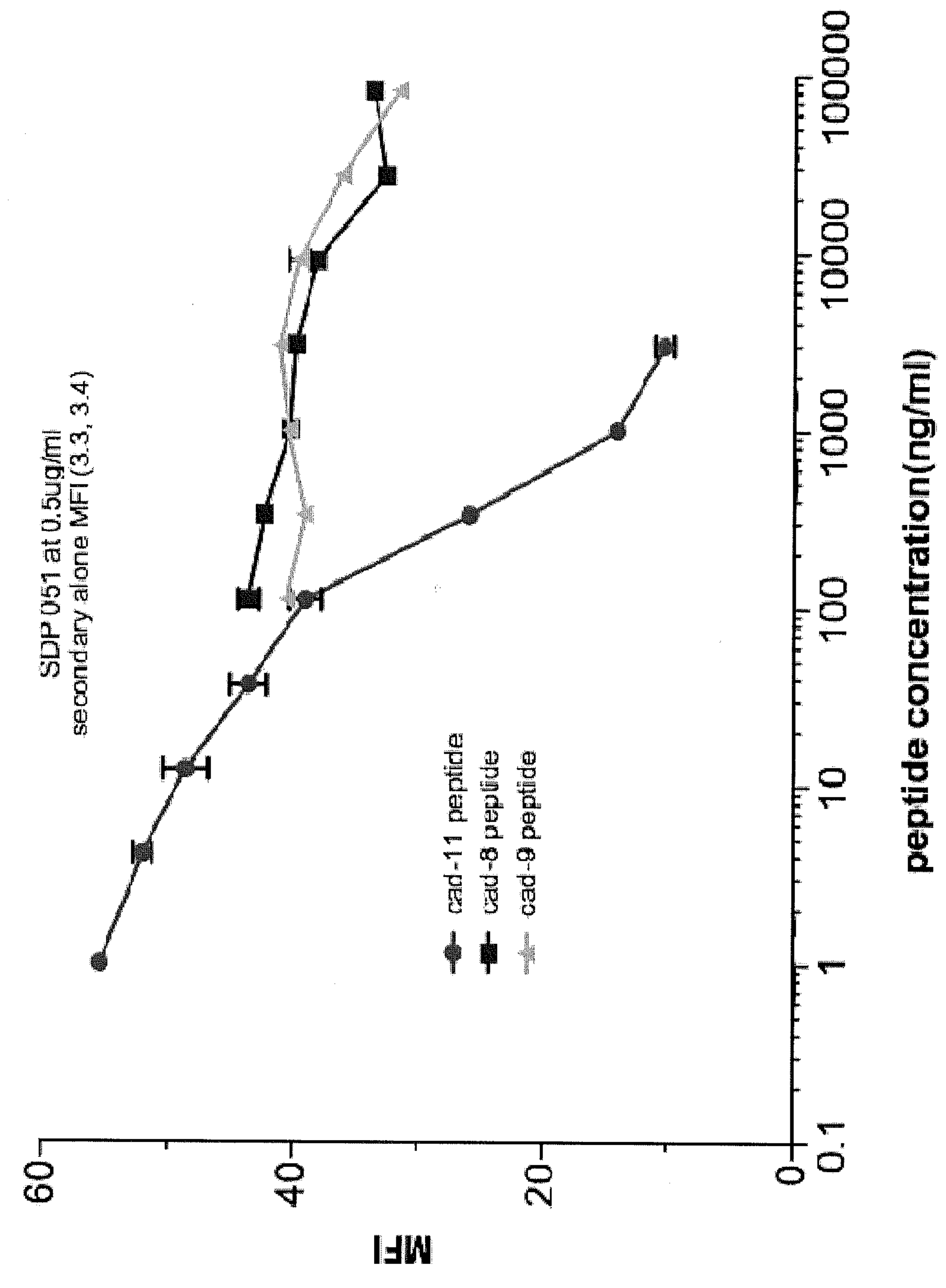

SDP 051 at 0.5ug/ml
secondary alone MFI (3.3, 3.4)
cad-11 peptide
cad-8 peptide
cad-9 peptide
MFI
60
40
20
0
0.1
1
10
100
1000
10000
100000
1000000
peptide concentration(ng/ml)

FIG. 44C

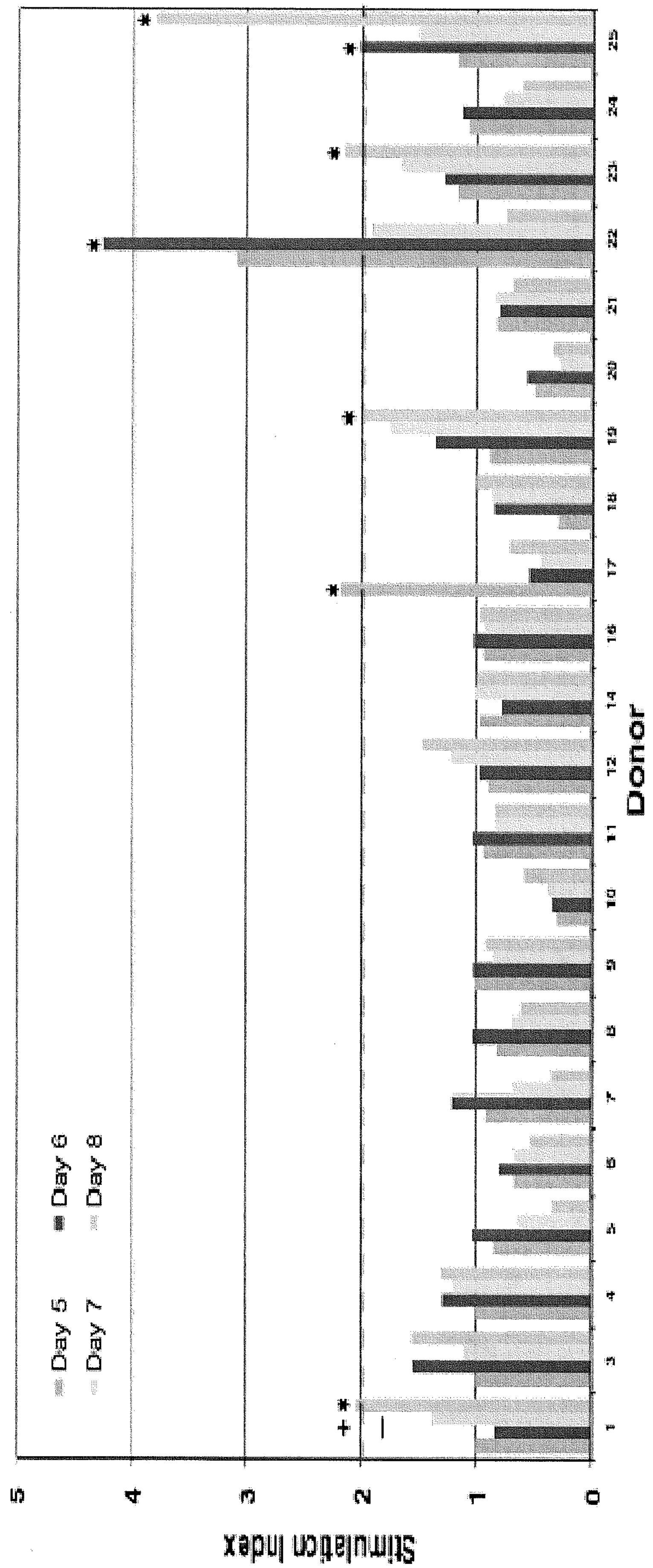

Day 5
Day 6
Day 7
Day 8
Stimulation Index
Donor
0
1
2
3
4
5
1
3
4
5
6
7
8
9
10
11
12
14
16
17
18
19
20
21
22
23
24
25
+
—
*

FIG. 45A

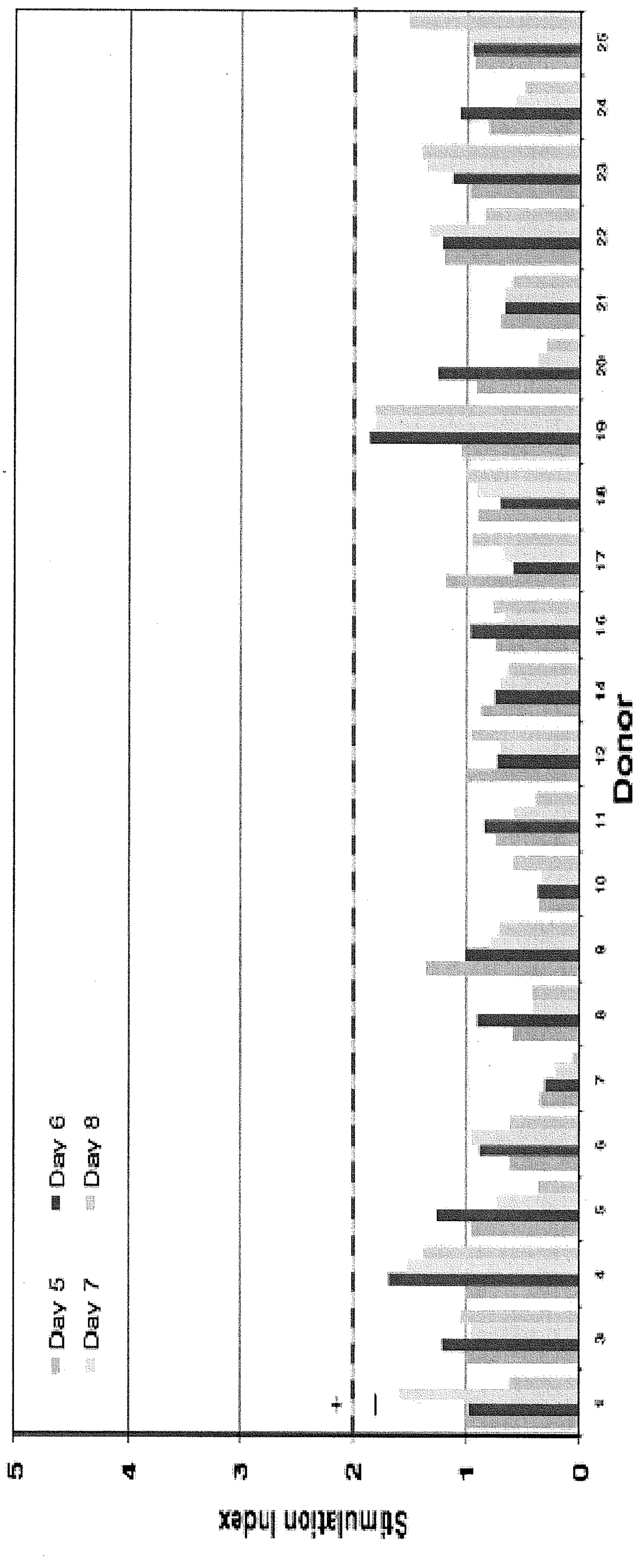

Stimulation Index
0
1
2
3
4
5
Donor
2
3
4
5
6
7
8
9
10
11
12
14
16
17
18
19
20
21
22
23
24
25
Day 5
Day 6
Day 7
Day 8
+
–

FIG. 45B

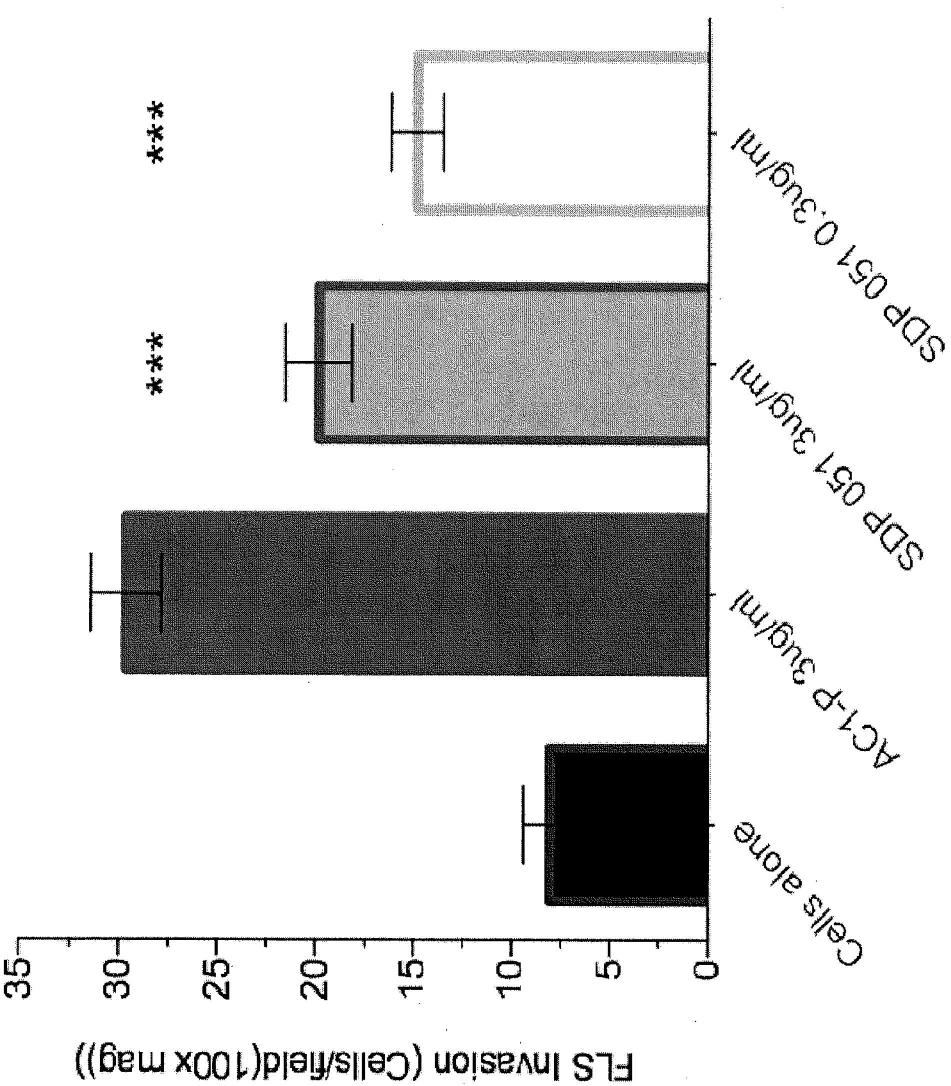

FLS Invasion (Cells/field(100x mag))
35
30
25
20
15
10
5
0
Cells alone
AC1-P 3ug/ml
SDP 051 3ug/ml
SDP 051 0.3ug/ml
***
***

FIG. 46

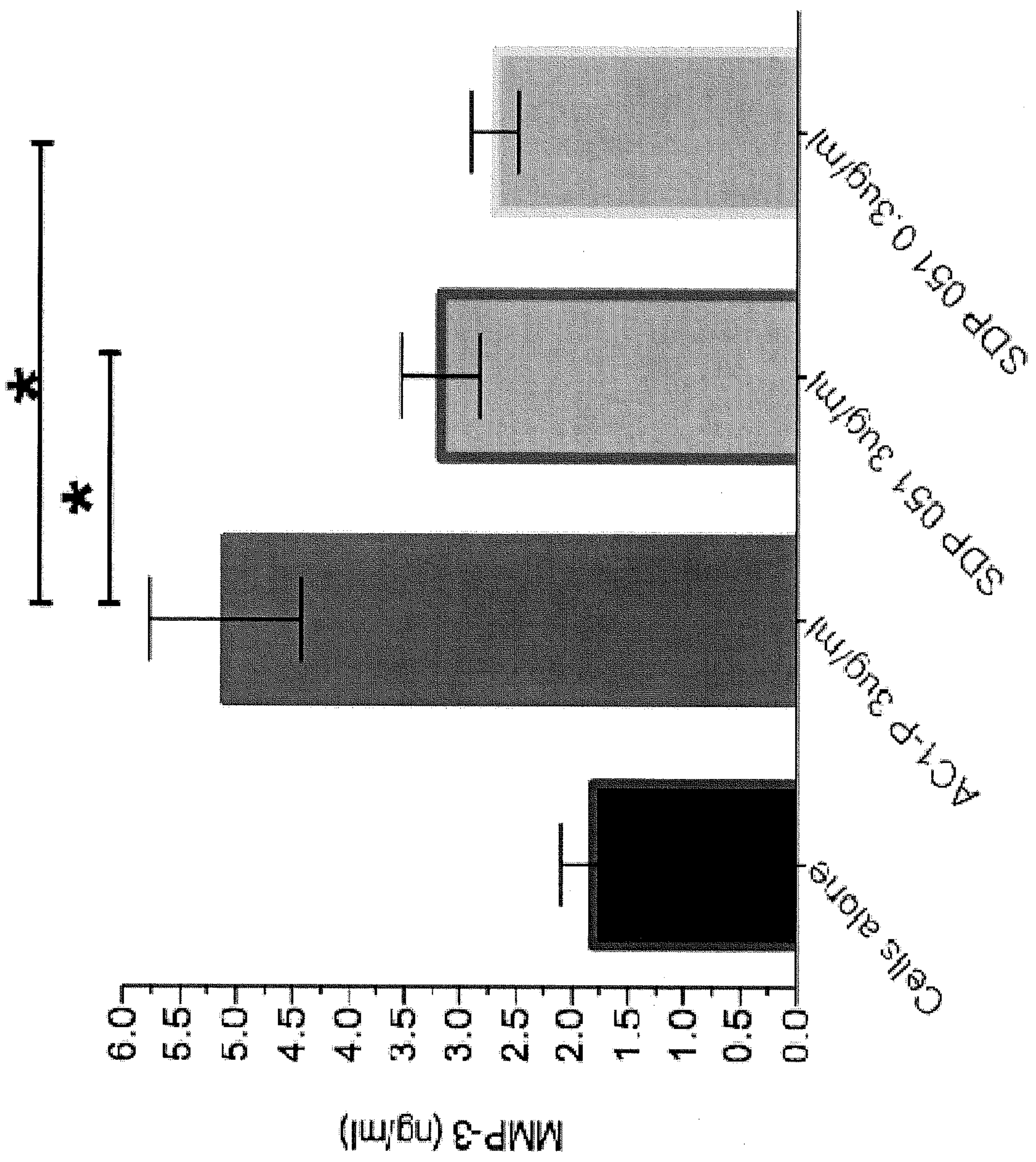
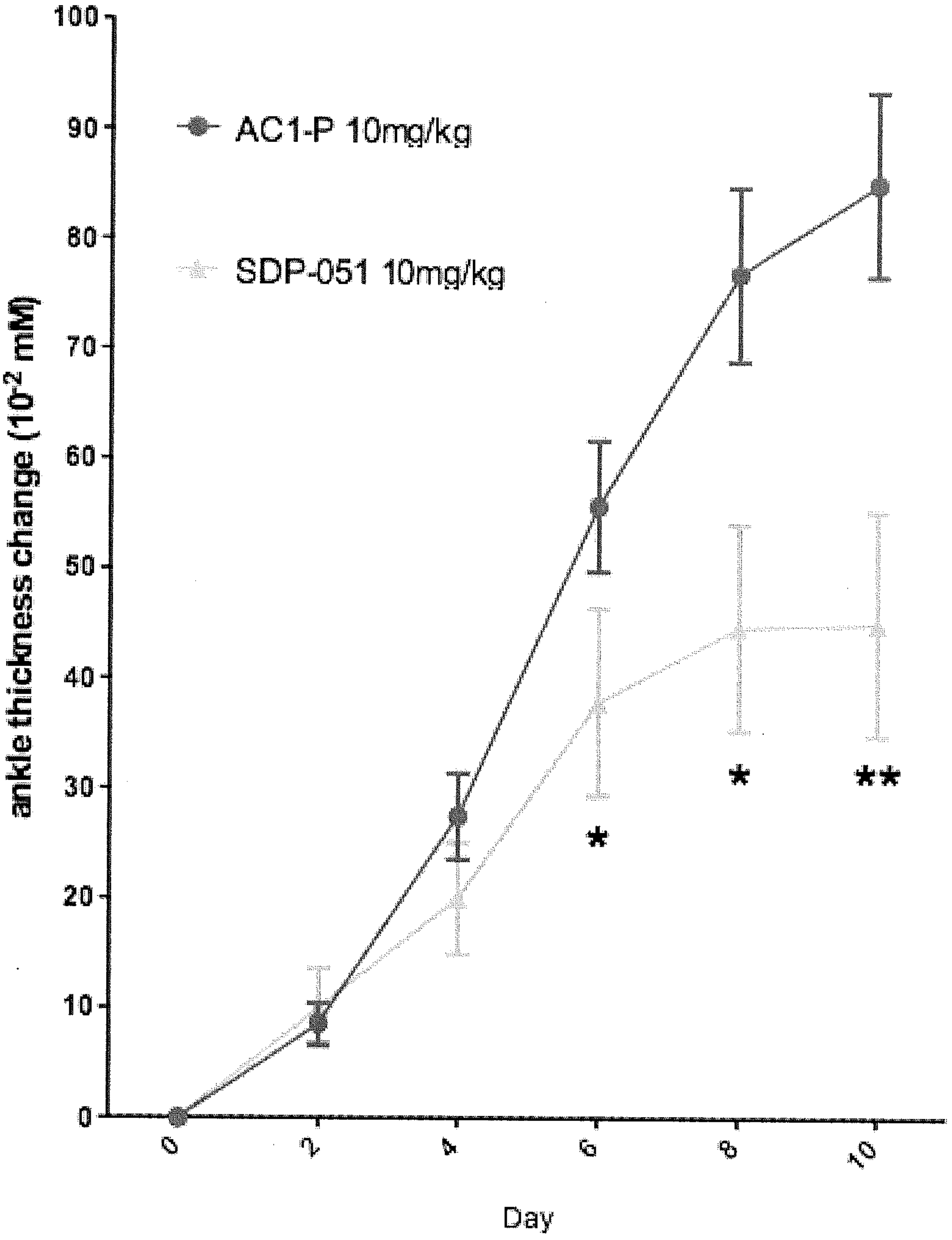

MMP-3 (ng/ml)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Cells alone
AC1-P 3ug/ml
SDP 051 3ug/ml
SDP 051 0.3ug/ml
*
*

FIG. 47



100
90
80
70
60
50
40
30
20
10
0
ankle thickness change (10^{-2} mM)
AC1-P 10mg/kg
SDP-051 10mg/kg
*
*
**
0
2
4
6
8
10
Day

FIG. 48

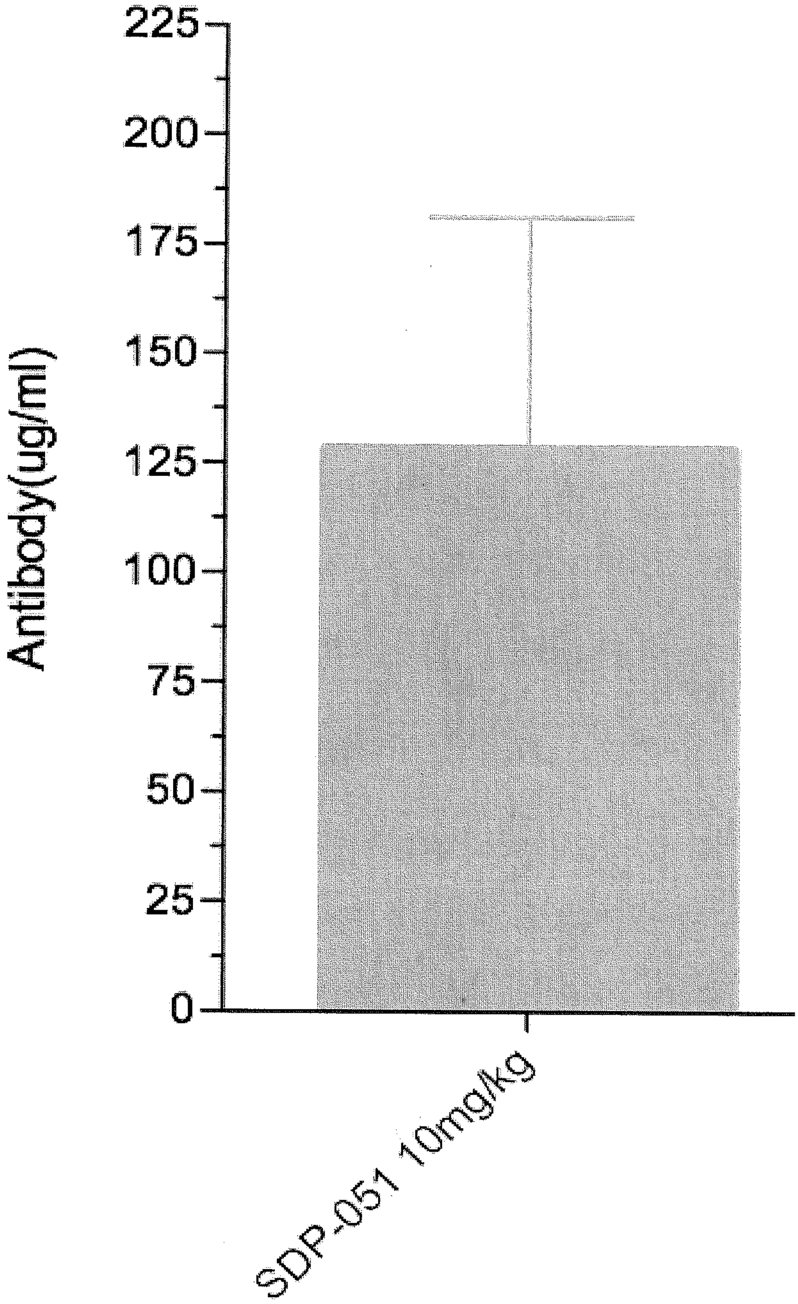
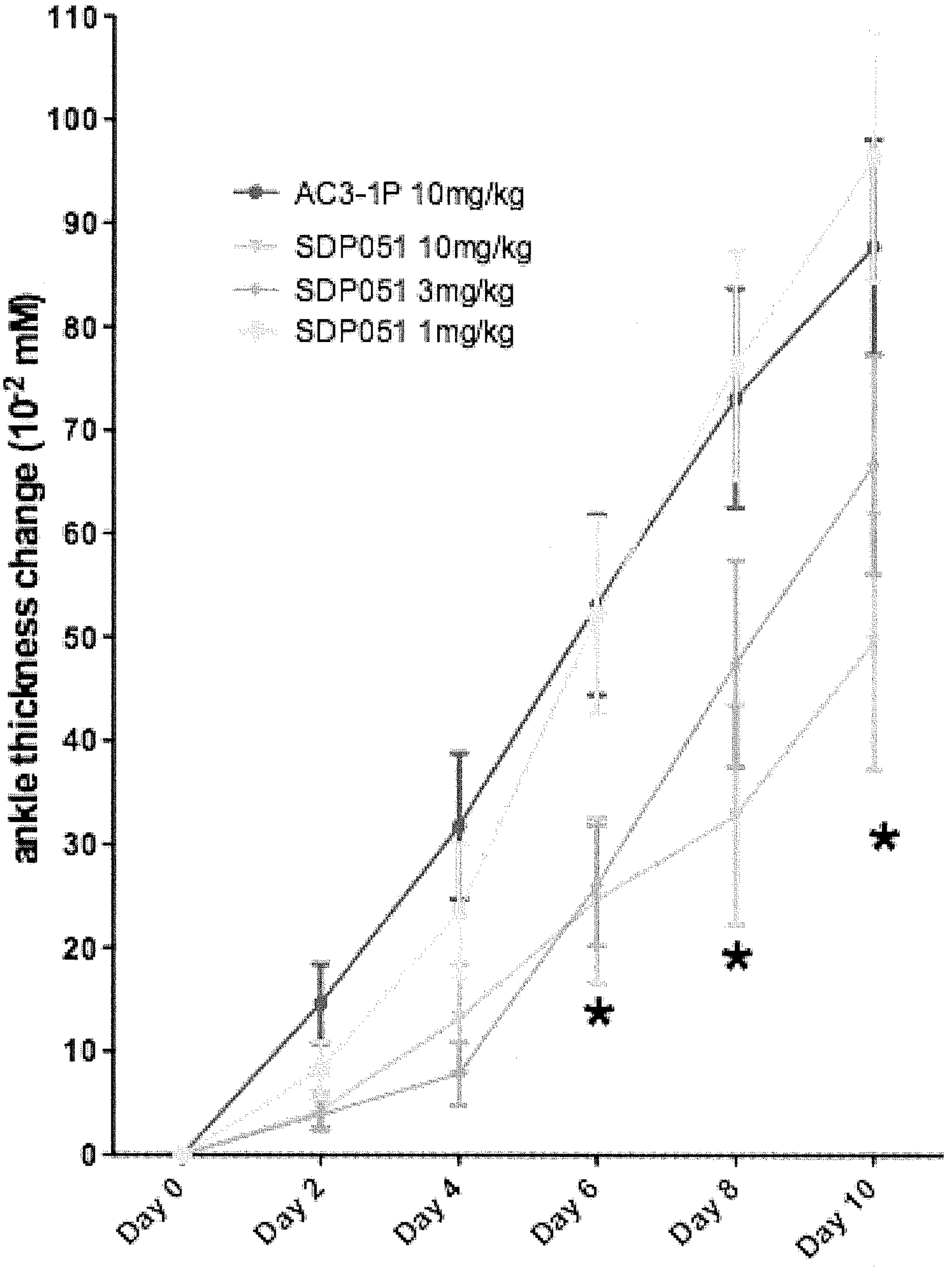


FIG. 49



ankle thickness change (10^{-2} mM)
AC3-1P 10mg/kg
SDP051 10mg/kg
SDP051 3mg/kg
SDP051 1mg/kg
0
10
20
30
40
50
60
70
80
90
100
110
Day 0
Day 2
Day 4
Day 6
Day 8
Day 10
*
*
*

* = $p<0.05$

FIG. 50

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/044172

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 C07K16/28 C07K16/46
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/253200 A1 (MCARTHUR JAMES G [US]) 8 October 2009 (2009-10-08) the whole document	1-63
X	WO 2009/089062 A2 (SYNOVEX CORP [US]; MCARTHUR JAMES G [US]) 16 July 2009 (2009-07-16) the whole document	1-63
A	WO 01/17557 A1 (BRIGHAM & WOMENS HOSPITAL [US]) 15 March 2001 (2001-03-15) the whole document	1-63

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 November 2011	18/11/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Merlos, Ana Maria

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/044172

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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			CN	101952316	A	19-01-2011
			EP	2242769	A2	27-10-2010
			JP	2011509094	A	24-03-2011
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