(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 July 2009 (16.07.2009)

PCT

(10) International Publication Number
WO 2009/088105 A1

(51) International Patent Classification:
C12N 15/09 (2006.01) *C12N 1/21* (2006.01)
A61K 39/395 (2006.01) *C12N 5/10* (2006.01)
C07K 16/28 (2006.01) *C12P 21/08* (2006.01)
C12N 1/15 (2006.01) *G01N 33/563* (2006.01)
C12N 1/19 (2006.01) *G01N 33/577* (2006.01)

(21) International Application Number:
PCT/JP2009/050606

(22) International Filing Date: 13 January 2009 (13.01.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/020,527 11 January 2008 (11.01.2008) US

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

WO 2009/088105 A1

(54) Title: HUMANIZED ANTI-α9 INTEGRIN ANTIBODIES AND THE USES THEREOF

(57) Abstract: The present invention provides humanized antibodies that immunospecifically recognize human α9 integrin. Some of these antibodies inhibit the biological functions of the α9 integrin, thereby exhibiting therapeutic effects on various disorders or diseases that are associated with α9 integrin, including cancer, e.g., the growth and metastasis of a cancer cell, and inflammatory diseases, e.g., rheumatoid arthritis, osteoarthritis, hepatitis, bronchial asthma, fibrosis, diabetes, arteriosclerosis, multiple sclerosis, granuloma, an inflammatory bowel disease (ulcerative colitis and Crohn's disease), an autoimmune disease, and so forth.

DESCRIPTION

HUMANIZED ANTI-α9 INTEGRIN ANTIBODIES AND THE USES THEREOF

1. FIELD OF THE INVENTION

The present invention relates to humanized antibodies that immunospecifically recognize human α9 integrin and to their therapeutic and diagnostic uses for various diseases or disorders that are associated with or involve α9 integrin, including cancer, inflammatory diseases, autoimmune diseases, and the like.

2. BACKGROUND OF THE INVENTION

Cells adhere to extracellular matrix (hereinafter abbreviated as ECM) mediated by a group of cell surface receptors which are termed integrins. Integrins perform their functions by forming 1 : 1 heterodimers of α and β chains. At least 18 types of α chain, 8 types of β chain and 24 types of αβ heterodimer have been identified and confirmed so far. It is known that each integrin recognizes a specific ligand. Integrins are classified into subfamilies depending upon their ligand specificities or functions, and divided into collagen receptors, laminin receptors, RGD receptors recognizing an Arg-Gly-Asp (RGD) sequence present in fibronectin, vitronectin, etc., and leukocyte-specific receptors present only in leukocytes (Hynes, R. O., 2002, Integrins: Bidirectional, Allosteric Signaling Machines. *Cell* 110: 673-87; Miyasaka, M., 2000, New edition of *Adhesion Molecule Handbook*, Shujunsya). The α4 and α9 integrins are members of a subfamily that does not belong to any of these types and called the α4 integrin subfamily (Elise L. Palmer, Curzio Rfiegg, Ronald Ferrando, Robert Pytela, Sheppard D., 1993, Sequence and Tissue Distribution of the Integrin α9 Subunit, a Novel Partner of β1 That Is Widely Distributed in Epithelia and Muscle. *The Journal of Cell Biology*, 123: 1289-97). Meanwhile, ECM used to be considered so far to serve as a mere cementing substance between cells. It has now become clear that the integrin-mediated ECM-cell interaction is significantly involved in regulating the growth, adhesion, movement, etc. of cells and associated with the onset of diseases including a

progression of cancer, an exacerbation of inflammation, and the like.

For example, osteopontin (hereinafter abbreviated as OPN) which is one of the ECMs is a secreted, acidic phosphorylated glycoprotein with a molecular weight of about 41 kDa and is a molecule, whose expression is widely observed in breast milk, urine, renal tubules, osteoclasts, osteoblasts, macrophages, activated T cells, tumor tissues, and so forth. OPN has the adhesion sequences, GRGDS (SEQ ID NO:1) at the center of its molecule, the SVVYGLR (SEQ ID NO:2) sequence in human OPN or the SLAYGLR (SEQ ID NO:3) sequence in mouse OPN, and a thrombin-cleavage site in close proximity thereto, and binds through the GRGDS (SEQ ID NO:1) sequence to the RGD integrin or to the α4 (α4β1) and α9 (α9β1) integrins through the SVVYGLR (SEQ ID NO:2) sequence or the SLAYGLR (SEQ ID NO:3) sequence.

WO 02/081522 discloses a therapeutic effect on rheumatoid arthritis or hepatitis by inhibiting the OPN functions using OPN knockout mice or neutralizing antibodies against OPN. Moreover, this publication discloses that the SVVYGLR (SEQ ID NO:2) sequence is essential as recognizing the α9 and α4 integrins for pathogenesis of an inflammatory disease and that receptors for OPN are expressed in immunocompetent cells or the like and associated with an inflammatory disease.

Differences in binding profile have been found in that α4β1 binds both to OPN not cleaved with thrombin (uncleaved OPN) and to the N-terminal fragment of thrombin-cleaved OPN (cleaved OPN), whereas α9β1 binds only to the cleaved OPN (Y. Yokosaki, et al., (1999) *The Journal of Biological Chemistry*, 274: 36328-36334; P. M. Green, et al., (2001) *FEBS Letters*, 503: 75-79; S. T. Barry, et al., (2000) *Experimental Cell Research*, 258: 342-351).

The α4 and α9 integrins share many common ligands other than OPN. Known ligands are the EDA domain of fibronectin, propeptide-von Willebrand factor (pp-vWF), tissue transglutaminase (tTG), blood coagulation factor XIII, vascular cell adhesion molecule-1(VCAM-1), etc. In addition, the CS-1 domain of fibronectin, MadCAM-1 (α4β7), etc. are known as the ligands specifically recognized by the α4 integrin. Tenascin-C, plasmin, etc. are known as the ligands specifically recognized by the α9 integrin.

The amino acid sequences for the integrin subunits α9, α4 and β1 are

publicly known. For instance, human α9 is registered as NM_002207, mouse α9 as NM_133721, human α4 as NM_000885, mouse α4 as NM_010576, human β1 as X07979, and mouse β1 as NM_010578, at the GenBank. These integrins are also known to have high similarities between species in amino acid sequence.

3. SUMMARY OF THE INVENTION

While a variety of drugs are known at present for the treatment of cancer, inflammatory diseases and autoimmune diseases, it has been desired to develop a preventive and/or therapeutic agent, etc. having more improved therapeutic effects on cancer, inflammatory diseases and autoimmune diseases. The present invention is based, in part, on the discovery by the present inventors that a specific inhibitory antibody against the α9 integrin has cancer-suppressing and anti-inflammatory effects.

Previously, the present inventors isolated mouse monoclonal antibodies that immunospecifically recognize human α9 integrin and are produced by hybridoma clones, 1K11, 21C5, 24I11, 25B6 and 28S1 (Depository Accession Nos. FERM BP-10510, FERM BP-10511, FERM BP-10512, FERM BP-10513 and FERM BP-10832, respectively), and those that immunospecifically recognize mouse α9 integrin and are produced by hybridoma clones, 18R18D, 12C4'58, 11L2B and 55A2C (Depository Accession Nos. FERM ABP-10195, FERM ABP-10196, FERM ABP-10197 and FERM ABP-10198, respectively). Herein, the hybridoma clone designations are interchangeably used as the designations of the monoclonal antibodies produced by the clones. All of these mouse anti-human α9 integrin antibodies were of IgG1 isotype. Some of these monoclonal antibodies inhibit the binding between human and/or mouse α9 integrin and a ligand of α9 integrin, such as osteopontin. Thus, these anti-α9 integrin antibodies inhibit the α9 integrin functions and exhibit therapeutic effects on cancer, *e.g.*, the growth or metastasis of cancer cells, and on inflammatory diseases, *e.g.*, rheumatoid arthritis, osteoarthritis, hepatitis, bronchial asthma, fibrosis, diabetes mellitus, arteriosclerosis, multiple sclerosis, granuloma, an inflammatory bowel disease (ulcerative colitis and Crohn's disease), an autoimmune disease, and the like.

Furthermore, the anti-α9 integrin antibodies of the present invention can be

used as an *in vivo* diagnostic agent to detect the presence and the level of α9 integrin expression in a subject, thereby diagnosing a disorder or a disease involving α9 integrin.

However, since these monoclonal antibodies are of mouse origin, possible adverse effects due to their immunogenicity in humans have hampered their direct applications to diagnostic or therapeutic uses in humans. In order to reduce the immunogenicity, the present inventors have prepared a humanized antibody that have biological activities corresponding to those exhibited by the original mouse anti-α9 integrin antibody from which said humanized antibody was derived.

Accordingly, the present invention provides a humanized antibody or an antigen-binding fragment thereof, which immunospecifically recognizes human α9 integrin, said antibody comprising an antigen-binding region partially derived from a non-human origin and partially derived from a human origin. In a specific embodiment, the humanized antibody or an antigen-binding fragment thereof of the present invention comprises a complementarity determining region (CDR) derived from a non-human source (donor), such as 1K11, 21C5, 24I11, 25B6 and 28S1 monoclonal antibodies, and a framework region (FR) derived from a human source (acceptor). In one embodiment, said humanized antibody or an antigen-binding fragment thereof inhibits the binding between human α9 integrin and a ligand of human α9 integrin.

In a specific embodiment, said humanized antibody or an antigen-binding fragment thereof that immunospecifically recognizes human α9 integrin comprises: (i) a heavy chain (H-chain) comprising at least one H-chain FR (FRH) derived from a variable region (V-region) of a human H-chain, and at least one H-chain complementarity determining region (CDRH) derived from at least one of the CDRHs of a non-human antibody that immunospecifically recognizes human α9 integrin; or (ii) a light chain (L-chain) comprising at least one L-chain FR (FRL) derived from a V-region of a human L-chain, and at least one L-chain complementarity determining region (CDRL) derived from at least one of the CDRLs of a non-human antibody that immunospecifically recognizes human α9 integrin; or both (i) and (ii) above. For example, said non-human antibody, from which at least one of the CDRHs and/or at least one of the CDRLs of the humanized

antibody of the invention is derived, is a monoclonal antibody produced by a hybridoma selected from the group consisting of Accession Nos. FERM BP-10510, FERM BP-10511, FERM BP-10512, FERM BP-10513 and FERM BP-10832.

In a preferred specific embodiment, the humanized antibody or an antigen-binding fragment thereof, of the present invention comprises: (i) at least one FRH derived from a human FRH, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:4, 5 and 6; or (ii) at least one FRL derived from a human FRL, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:11, 12 and 13; or (iii) both (i) and (ii) above. Said humanized antibody or an antigen-binding fragment thereof, of the present invention may comprise CDRH1, CDRH2 and CDRH3, which comprise the amino acid sequences of SEQ ID NOS:4, 5 and 6, respectively. In the alternative, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS: 11, 12 and 13, respectively. In a preferred embodiment, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS:4, 5, 6, 11, 12 and 13, respectively. In another alternative, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises a FRH derived from a variable region of a human H-chain encoded by GenBank Accession No. X65891 (SEQ ID NO:18), or a FRL derived from a variable region of a human κ-L-chain encoded by GenBank Accession No. X72441 (SEQ ID NO:23). In a preferred embodiment, the FRH of the humanized antibody of the present invention comprises at least one amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:19, 20, 21 and 22 (FRH1, FRH2, FRH3 and FRH4, respectively, encoded by the corresponding portions of X65891). In another preferred embodiment, the FRL of the humanized antibody of the present invention comprises at least one amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:24, 25, 26 and 27 (FRL1, FRL2, FRL3 and FRL4, respectively, encoded by the corresponding portions of X72441). In a most preferred embodiment, the humanized antibody or an antigen-binding fragment

thereof, of the present invention comprises: (i) a H-chain variable region (VH region) comprising the amino acid sequence of SEQ ID NO:29; or (ii) a L-chain variable region (VL region) comprising the amino acid sequence of SEQ ID NO:31; or (iii) both (i) and (ii) above.

In another preferred specific embodiment, the humanized antibody or an antigen-binding fragment thereof, of the present invention comprises: (i) at least one FRH derived from a human FRH, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:32, 33 and 34; or (ii) at least one FRL derived from a human FRL, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:37, 38 and 39; or (iii) both (i) and (ii) above. Said humanized antibody or an antigen-binding fragment thereof, of the present invention may comprise CDRH1, CDRH2 and CDRH3, which comprise the amino acid sequences of SEQ ID NOS:32, 33 and 34, respectively. In the alternative, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS:37, 38 and 39, respectively. In a preferred embodiment, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS:32, 33, 34, 37, 38 and 39, respectively.

In another preferred specific embodiment, the humanized antibody or an antigen-binding fragment thereof, of the present invention comprises: (i) at least one FRH derived from a human FRH, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:42, 43 and 44; or (ii) at least one FRL derived from a human FRL, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:47, 48 and 49; or (iii) both (i) and (ii) above. Said humanized antibody or an antigen-binding fragment thereof, of the present invention may comprise CDRH1, CDRH2 and CDRH3, which comprise the amino acid sequences of SEQ ID NOS:42, 43 and 44, respectively. In the alternative, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRL1, CDRL2 and CDRL3, which comprise the

amino acid sequences of SEQ ID NOS:47, 48 and 49, respectively. In a preferred embodiment, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS: 42, 43, 44, 47, 48 and 49, respectively.

In another preferred specific embodiment, the humanized antibody or an antigen-binding fragment thereof, of the present invention comprises: (i) at least one FRH derived from a human FRH, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:52, 53 and 54; or (ii) at least one FRL derived from a human FRL, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:57, 58 and 59; or (iii) both (i) and (ii) above. Said humanized antibody or an antigen-binding fragment thereof, of the present invention may comprise CDRH1, CDRH2 and CDRH3, which comprise the amino acid sequences of SEQ ID NOS:52, 53 and 54, respectively. In the alternative, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS:57, 58 and 59, respectively. In a preferred embodiment, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS:52, 53, 54, 57, 58 and 59, respectively.

In another preferred specific embodiment, the humanized antibody or an antigen-binding fragment thereof, of the present invention comprises: (i) at least one FRH derived from a human FRH, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:62, 63 and 64; or (ii) at least one FRL derived from a human FRL, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:67, 68 and 69; or (iii) both (i) and (ii) above. Said humanized antibody or an antigen-binding fragment thereof, of the present invention may comprise CDRH1, CDRH2 and CDRH3, which comprise the amino acid sequences of SEQ ID NOS:62, 63 and 64, respectively. In the alternative, said humanized antibody or an antigen-binding fragment thereof, of

the present invention comprises CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS:67, 68 and 69, respectively. In a preferred embodiment, said humanized antibody or an antigen-binding fragment thereof, of the present invention comprises CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3, which comprise the amino acid sequences of SEQ ID NOS:62, 63, 64, 67, 68 and 69, respectively.

The present invention further provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the humanized antibody or an antigen-binding fragment thereof of the present invention which immunospecifically recognizes human α9 integrin. Specifically, the present invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a humanized H-chain comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOS:4, 5, 6, 32, 33, 34, 42, 43, 44, 52, 53, 54, 62, 63 and 64, or a humanized L-chain comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOS:11, 12, 13, 37, 38, 39, 47, 48, 49, 57, 58, 59, 67, 68 and 69, or both said humanized H-chain and said humanized L-chain. In a preferred specific embodiment, such an isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:28, which encodes a VH region, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:29. In another preferred specific embodiment, such an isolated nucleic acid molecule comprises the nucleotide sequence of SEQ ID NO:30, which encodes a VL region, or a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:31. In yet another preferred specific embodiment, the isolated nucleic acid molecule of the present invention comprises the nucleotide sequences of both SEQ ID NO:28 and 30. In yet another preferred specific embodiment, the isolated nucleic acid molecule of the present invention further comprises a nucleotide sequence encoding a signal peptide of donor origin, such as the amino acid sequences of SEQ ID NOS:10 and 17, respectively, or of heterologous origin.

The present invention further provides a vector, *e.g.*, an expression vector, comprising a nucleotide sequence encoding a H-chain or a L-chain, or both, of the humanized antibody or an antigen-binding fragment thereof of the present invention that immunospecifically recognizes human α9 integrin. In such a vector, the nucleotide sequence of the present invention may be operably linked to one or more

regulatory elements. The nucleotide sequence of the present invention may include a nucleotide sequence encoding a signal peptide native to a non-human donor antibody from which a CDR is derived, or a signal peptide of heterologous origin.

Furthermore, the present invention provides a host cell comprising the nucleic acid molecule of the present invention, including a vector comprising the nucleic acid molecule of the present invention. In one embodiment, the present invention provides an isolated host cell comprising a first nucleic acid molecule encoding a humanized H-chain of the present invention and a second nucleic acid molecule encoding a humanized L-chain of the present invention, said first and second nucleic acid molecules are each operably linked to a regulatory element in such a way that the biologically functional humanized antibody or antigen-binding fragment thereof of the present invention is expressed.

Accordingly, the present invention further provides a method for preparing the humanized antibody of the present invention, comprising culturing the host cell of the invention under conditions so that the humanized antibody is expressed; and collecting the produced humanized antibody.

The present invention further provides a composition comprising at least one of the humanized antibodies of the present invention. In addition, the present invention provides a pharmaceutical composition for preventing or treating a disorder or disease that is associated with α9 integrin, comprising at least one of the humanized antibodies of the present invention, and a pharmaceutically acceptable carrier. Either of said compositions can further comprise another active compound that can additively or synergistically ameliorate the disorder or disease. Such an active compound includes, but not by way of limitation, anti-inflammatory compounds, chemotherapeutic compounds, and the like, as well as an antibody or an antigen-binding fragment thereof, such as an antibody that can immunospecifically bind human α4 integrin.

In another aspect, the present invention provides a method for preventing or treating a disorder or disease that is associated with or involves α9 integrin, said method comprising administering a prophylactically or therapeutically effective amount of at least one of the humanized antibodies of the present invention to a subject in need thereof. For such uses, the humanized antibody of the present

invention may be conjugated to a therapeutic moiety that enhances the biological effect of the humanized antibody. Examples of such a therapeutic moiety include another antibody, such as anti-α4 antibody (*e.g.*, to form a bispecific antibody), cytotoxins that are cytostatic or cytocidal, radioactive elements, and/or other therapeutic agents, including anti-inflammatory agents, antibiotics, and the like.

In yet another aspect, the present invention provides a method for diagnosing a disorder or disease, in a subject, that is associated with or involves α9 integrin, said method comprising administering a diagnostically effective amount of the humanized antibody of the present invention to a subject to be examined. For such diagnostic uses, the humanized antibody of the present invention may be labeled with detectable markers, such as radioactive elements.

3.1. Definitions

As used herein, the term "antibody" refers to an antibody molecule capable of immunospecifically binding to a desired antigen, such as the α9 integrin, and encompasses an antibody molecule as a whole or a fragment thereof, including an antigen-binding fragment.

The term "immunospecifically recognize" used herein refers to an ability of an antibody or an antigen-binding fragment thereof to bind specifically to a target polypeptide or protein, in particular, human α9 integrin. Such an antibody does not non-specifically bind to other polypeptides or proteins. However, an antibody or an antigen-binding fragment thereof that immunospecifically binds to the target polypeptide or protein (*e.g.*, human α9 integrin) may cross-react with other antigens. For example, the humanized antibody or an antigen-binding fragment of the present invention that immunospecifically recognizes human α9 integrin may cross-react with, for example, murine α9 integrin. Preferably, an antibody or an antigen-binding fragment thereof that immunospecifically binds to human α9 integrin does not cross-react with other antigens.

The term "an antigen-binding fragment" used herein refers to any fragment of an antibody that retains an ability to immunospecifically bind to a target polypeptide or protein, in particular, human α9 integrin and/or mouse α9 integrin, and includes single chain antibodies, Fab fragments, $F(ab')_2$ fragments, disulfide-linked Fvs, and fragments containing either a variable region of a light chain (VL) and/or a variable

region of a heavy chain (VH) or even a complementary determining region (CDR) that specifically binds to a target polypeptide or protein. Thus, such antigen-binding fragments of humanized antibody may or may not include partial or full-length human constant regions. Various methods for obtaining the antibody fragments described above are well known in the art.

The term "derived from a human source" or "derived from a non-human source" used herein refers to an antibody portion whose amino acid sequence is derived from a corresponding portion of a human antibody or of a non-human antibody.

The term "an acceptor sequence" used herein refers to a nucleotide sequence or an amino acid sequence of framework regions from a human antibody VH or VL region that serves as an acceptor for CDRs from a donor antibody, which is usually a non-human antibody.

4. BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the nucleotide sequence (SEQ ID NO:7) of mouse 24I11 VH cDNA along with the deduced amino acid sequence (SEQ ID NO:8). Amino acid residues are shown in single letter code. The signal peptide sequence (SEQ ID NO:10) is in italic. The N-terminal amino acid residue (E) of the mature VH is double-underlined. CDR sequences according to the definition of Kabat *et al.* (Sequences of Proteins of Immunological Interests, Fifth edition, NIH Publication No. 91-3242, U.S. Department of Health and Human Services, 1991) are underlined.

Fig. 2 shows the nucleotide sequence (SEQ ID NO:14) of mouse 24I11 VL cDNA along with the deduced amino acid sequence (SEQ ID NO:15). Amino acid residues are shown in single letter code. The signal peptide sequence (SEQ ID NO:17) is in italic. The N-terminal amino acid residue (D) of the mature VL is double-underlined. CDR sequences according to the definition of Kabat et al. (1991) are underlined.

Fig. 3 shows the nucleotide sequence (SEQ ID NO:72) of the designed 24I11 VH gene flanked by SpeI and HindIII sites (underlined), along with the deduced amino acid sequence (SEQ ID NO:8). Amino acid residues are shown in single letter code. The signal peptide sequence (SEQ ID NO:10) is in italic. The

N-terminal amino acid residue (E) of the mature VH is double-underlined. CDR sequences according to the definition of Kabat et al. (1991) are underlined. The intron sequence is in italic.

Fig. 4 shows the nucleotide sequence (SEQ ID NO:73) of the designed 24I11 VL gene flanked by NheI and EcoRI sites (underlined), along with the deduced amino acid sequence (SEQ ID NO:15). Amino acid residues are shown in single letter code. The signal peptide sequence (SEQ ID NO:17) is in italic. The N-terminal amino acid residue (D) of the mature VL is double-underlined. CDR sequences according to the definition of Kabat et al. (1991) are underlined. The intron sequence is in italic.

Fig. 5 shows the schematic structure of pCh24I11 and pHu24I11 (collectively Expression Vector). Proceeding clockwise from the SalI site at the top, the plasmid contains the heavy chain transcription unit starting with the human cytomegalovirus (CMV) major immediate early promoter and enhancer (CMV promoter) to initiate transcription of the antibody heavy chain gene. The CMV promoter is followed by the VH exon, a genomic sequence containing the human gamma-1 heavy chain constant region including the CH1, hinge, CH2 and CH3 exons with the intervening introns, and a polyadenylation site of the gamma-1 gene for mRNA processing following CH3. After the heavy chain gene sequence, the light chain transcription unit begins with the CMV promoter, followed by the VL exon and a genomic sequence containing the human kappa chain constant region exon (CL) with part of the intron preceding it, and a poly A signal of the kappa gene. The light chain gene is then followed by the SV40 early promoter (SV40 promoter), the E. coli xanthine guanine phosphoribosyl transferase gene (gpt), and a segment containing the SV40 polyadenylation site (SV40 poly(A) site). Finally, the plasmid contains a part of the plasmid pUC19, comprising the bacterial origin of replication (pUC ori) and beta-lactamase gene (beta lactamase).

Fig. 6 shows the alignment of the amino acid sequences of 24I11 VH (SEQ ID NO:9), humanized 24I11 (Hu24I11) VH (SEQ ID NO:29) and FRH1 (SEQ ID NO:19), FRH2 (SEQ ID NO:20), FRH3 (SEQ ID NO:21) and FRH4 (SEQ ID NO:22) of human acceptor sequences, derived from the amino acid sequence encoded by the nucleotide sequence of GenBank Accession No. X65891. Amino acid residues are shown in single letter code. Numbers above the sequences

indicate the positions according to Kabat *et al.* (1991). CDR sequences defined by Kabat *et al.* (1991) are underlined. Double-underlined residues were predicted to contact with the CDRs and the mouse residues were retained at these locations in the humanized form. CDR residues in X65891 are omitted in the figure.

Fig. 7 shows the alignment of the amino acid sequences of 24I11 VL (SEQ ID NO:16), humanized 24I11 (Hu24I11) VL (SEQ ID NO:31) and FRL1 (SEQ ID NO:24), FRL2 (SEQ ID NO:25), FRL3 (SEQ ID NO:26) and FRL4 (SEQ ID NO:27) of human acceptor sequences, derived from the amino acid sequence encoded by the nucleotide sequence of GenBank Accession No. X72441. Amino acid residues are shown in single letter code. Numbers above the sequences indicate the positions according to Kabat et al. (1991). CDR sequences defined by Kabat et al. (1991) are underlined. Double-underlined residues were predicted to contact with the CDRs and the mouse residues were retained at these locations in the humanized form. CDR residues in X72441 are omitted in the figure.

Fig. 8 shows the oligonucleotides used for construction of the Hu24I11 VH gene.

Fig. 9 shows the oligonucleotides used for construction of the Hu24I11 VL gene.

Fig. 10 shows the oligonucleotides used for construction of the Hu24I11 VH gene flanked by SpeI and HindIII sites (SEQ ID NO:74 with 5'-GGG tail at 5'-terminal and CCC-3' tail at 3'-terminal). An arrow denotes the position and orientation (5' to 3') of each oligonucleotide. Amino acid residues of the signal peptide (SEQ ID NO:10) and the VH region (SEQ ID NO:29) are shown in single letter code.

Fig. 11 shows oligonucleotides used for construction of the Hu24I11 VL gene flanked by NheI and EcoRI sites (SEQ ID NO:75 with 5'-GGG tail at 5'-terminal and CCC-3' tail at 3'-terminal). An arrow denotes the position and orientation (5' to 3') of each oligonucleotide. Amino acid residues of the signal peptide (SEQ ID NO:17) and the VL region (SEQ ID NO:31) are shown in single letter code.

Fig. 12 shows the nucleotide sequence (SEQ ID NO:74) of the Hu24I11 VH gene flanked by SpeI and HindIII sites (underlined), along with the deduced amino acid sequence of the signal peptide (SEQ ID NO:10; shown in italic) and the VH

region (SEQ ID NO:29). Amino acid residues are shown in single letter code. The N-terminal amino acid residue (Q) of the mature VH is double-underlined. CDR sequences according to the definition of Kabat et al. (1991) are underlined. The intron sequence is in italic.

Fig. 13 shows the nucleotide sequence (SEQ ID NO:75) of the Hu24I11 VL gene flanked by NheI and EcoRI sites (underlined), along with the deduced amino acid sequence of the signal peptide (SEQ ID NO:17; shown in italic) and the VL region (SEQ ID NO:31). Amino acid residues are shown in single letter code. The N-terminal amino acid residue (D) of the mature VL is double-underlined. CDR sequences according to the definition of Kabat et al. (1991) are underlined. The intron sequence is in italic.

Fig. 14 shows the comparison of the affinity of chimeric and humanized 24I11 antibodies to human α9 integrin. The binding of chimeric and humanized 24I11 at 1 and 0.5 μg/ml to CHO/α9 cells was examined by cell ELISA. Experiments were carried out in triplicate. The mean absorbance value with SEM is shown in the figure.

Fig. 15 shows the result of the FACS analysis of the binding of mouse, chimeric and humanized 24I11 antibodies to human α9 integrin. Each antibody was tested at 1, 0.33, 0.11, 0.037 and 0.012 μg/ml for binding to CHO/huα9 cells. Geometric mean channel fluorescence values (MCF; Y-axis) are plotted at each antibody concentration tested (X-axis) in the figure.

Fig. 16 shows the result of cell adhesion inhibitory activity of anti-human α9 integrin antibodies, between Human α9/CHO-K1 cells and hOPN(RAA)N-half, Tenascin-C, VCAM-1, or human fibronectin.

Fig. 17 shows the result of cell adhesion inhibitory effects of anti-human α9 integrin antibodies on human melanoma cells in the presence of anti-human α4 integrin.

Fig. 18 shows therapeutic effects on hepatitis by the anti-α4 integrin antibodies and the anti-α9-integrin antibodies. In the Figure, NHG indicates normal hamster antibody and NRG indicates normal rat antibody.

Fig. 19 shows that growth of the B16-BL6 cells was inhibited by the anti-α9

integrin antibodies.

Fig. 20 shows the results of FACS analysis using anti-human α9 integrin antibodies for Human α9/CHO-K1 cells (Fig. 20a), Human α4/CHO-K1 (Fig. 20b) and human neutrophils (Fig. 20c).

Fig. 21 shows the cell growth inhibition of the B16-BL6 cells by the anti-α9 integrin antibodies using immobilized VCAM-1 as an ECM.

Fig. 22 shows the therapeutic effect of anti-α9 integrin in a mouse rheumatoid arthritis model.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. Preparation of Antibodies Against Human α9 Integrin

Antibodies that immunospecifically recognize human α9 integrin or any epitope thereof may be generated by any suitable method known in the art.

The α9 integrin used as an antigen in the present invention may be (1) proteins derived from all cells from human that express α9 integrin, or all tissues where these cells are present, (2) recombinant proteins in which the α9 integrin-encoding gene DNA, preferably cDNA, is transfected into bacteria, yeast, cell lines including animal cells, etc. and expressed, or (3) synthetic proteins.

The α9 integrin includes polypeptides comprising substantially the same amino acid sequences as the amino acid sequences of human α9 integrins (SEQ ID NO:76, wherein 1-29 residues are the signal peptide).

Herein, the term "polypeptides comprising substantially the same amino acid sequence" means variant polypeptides comprising an amino acid sequence, in which multiple amino acids, preferably 1 to 10 amino acids and more preferably 1 to several (e.g., 1 to 5) amino acids are substituted, deleted and/or modified, as long as these variant polypeptides have biological properties substantially equivalent to the naturally occurring human α9 integrin; and variant polypeptides comprising an amino acid sequence, wherein multiple amino acids, preferably 1 to 10 amino acids and more preferably 1 to several (e.g., 1 to 5) amino acids are added to the amino acid sequence of naturally occurring human α9 integrin. Furthermore, the variant

polypeptides may be those having a plurality of these substitutions, deletions, modifications and additions of amino acids.

The human α9 integrin as an antigen in the present invention can be produced by methods well known in the art, such as chemical synthesis method, cell culture method, etc., or their modifications, in addition to the gene recombinant techniques.

Examples of the methods for producing variant polypeptides include a synthetic oligonucleotide site-directed mutagenesis (gapped duplex method), a point mutagenesis method which involves introducing a point mutation at random by treatment with nitrite or sulfite, a method which involves preparing a deletion mutant with Bal31 enzyme, or other enzymes, a cassette mutagenesis, a linker scanning method, a miss incorporation method, a mismatch primer method, a DNA segment synthesis method, and the like.

The human α9 integrin to be used as an antigen in the present invention also includes a "part" of said α9 integrin. As used herein, the "part" refers to a part comprising a region required for binding to a ligand of the α9 integrin, for example, OPN, VCAM-1, tenascin-C, etc.; specifically, a part comprising the 14th-980th amino acid residues, and a part comprising the 11th-981st amino acid residues, of the mature human α9 integrin (the 30th-1035th amino acid residues of SEQ ID NO:76). The "part" of said α9 integrin can be produced by gene recombination or chemical synthesis according to methods known in the art described below, or modifications thereof, or can be produced by appropriately digesting the human α9 integrin isolated by the cell culture method with a proteolytic enzyme or the like.

As an antigen, a cell per se that overexpresses the α9 integrin on the cell membrane, or a membrane fraction thereof, can be also used. Cells overexpressing human α9 integrin can be prepared by recombinant DNA technologies well known in the art.

Using appropriate antigens prepared as described above, antibodies specific for human α9 integrin or any epitope thereof may be prepared by various methods well known in the art. Polyclonal antibodies to human α9 integrin can be produced by various procedures well known in the art. For example, an antigen of interest can be administered to various host animals including, but not limited to, rabbits,

mice, rats, etc., to induce the production of antisera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete) adjuvant, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful adjuvants for humans such as BCG (Bacille Calmette-Guerin) and Corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas, pp. 563-681 (Elsevier, N.Y., 1981) (both of which are incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with an antigen of interest or a cell expressing such an antigen. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells (*e.g.*, P3U1, P3X63-Ag8, P3X63-Ag8-U1, P3NS1-Ag4, SP2/0-Ag14, P3X63-Ag8-653, etc.). Hybridomas are selected and cloned by limiting dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding the antigen. Ascites fluid, which generally contains high levels of antibodies, can be generated by inoculating mice intraperitoneally with positive hybridoma clones.

Antibody fragments what recognize specific epitopes may be generated by known techniques. For example, Fab and $F(ab')_2$ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). $F(ab')_2$ fragments contain the complete light chain, and the variable region, the CH1 region and the hinge region of the heavy chain.

The antibodies of the invention or an antigen-binding fragment thereof can be also produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

The nucleotide sequence encoding an antibody may be obtained from any information available to those skilled in the art (i.e., from Genbank, the literature, or by routine cloning and sequence analysis). If a clone containing a nucleic acid encoding a particular antibody or an epitope-binding fragment thereof is not available, but the sequence of the antibody molecule or epitope-binding fragment thereof is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

5.2. Preparation of Recombinant Antibodies

Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., supra; and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in

their entireties), to generate antibodies having a different amino acid sequence by, for example, introducing amino acid substitutions, deletions, and/or insertions into the epitope-binding domain regions of the antibodies or any portion of antibodies which may enhance or reduce biological activities of the antibodies.

Recombinant expression of an antibody requires construction of an expression vector containing a nucleotide sequence that encodes the antibody. Once a nucleotide sequence encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art as discussed in the previous sections. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The nucleotide sequence encoding the heavy-chain variable region, light-chain variable region, both the heavy-chain and light-chain variable regions, an epitope-binding fragment of the heavy- and/or light-chain variable region, or one or more complementarity determining regions (CDRs) of an antibody may be cloned into such a vector for expression. Such a sequence may be fused with a polynucleotide encoding a signal peptide native to the original antibody or a heterologous signal peptide. Thus-prepared expression vector can be then introduced into appropriate host cells for the expression of the antibody. Accordingly, the invention includes host cells containing a polynucleotide encoding a humanized antibody or an antigen-binding fragment thereof that immunospecifically recognizes human α9 integrin.

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides or different selectable markers to ensure maintenance of both plasmids. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

In another embodiment, antibodies can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains, such as Fab and Fv or disulfide-bond stabilized Fv, expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage, including fd and M13. The antigen binding domains are expressed as a recombinantly fused protein to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the immunoglobulins, or fragments thereof, of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods, 182:41-50, 1995; Ames et al., J. Immunol. Methods, 184:177-186, 1995; Kettleborough et al., Eur. J. Immunol., 24:952-958, 1994; Persic et al., Gene, 187:9-18, 1997; Burton et al., Advances in Immunology, 57:191-280, 1994; PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired fragments, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques, 12(6):864-869, 1992; and Sawai et al., AJRI, 34:26-34, 1995; and Better et al., Science, 240:1041-1043, 1988 (each of which is incorporated by reference in its entirety). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos.

4,946,778 and 5,258,498; Huston et al., Methods in Enzymology, 203:46-88, 1991; Shu et al., PNAS, 90:7995-7999, 1993; and Skerra et al., Science, 240:1038-1040, 1988.

Once an antibody molecule of the invention has been produced by any methods described above, it may then be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A or Protein G purification, and sizing column chromatography), centrifugation, differential solubility, or by any other standard techniques for the purification of proteins. Further, the antibodies of the present invention or fragments thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. Chimeric antibodies and humanized antibodies are discussed in details in Section 5.3, *infra*.

Antibodies fused or conjugated to other compounds or heterologous polypeptides may be used in *in vitro* immunoassays, in purification methods (e.g., affinity chromatography), as well as *in vivo* therapeutic or diagnostic uses. See e.g., PCT publication Number WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett., 39:91-99, 1994; U.S. Patent 5,474,981; Gillies et al., PNAS, 89:1428-1432, 1992; and Fell et al., J. Immunol., 146:2446-2452, 1991, which are incorporated herein by reference in their entireties. For example, antibodies can be labeled in various ways using a known method or commercially available kit (e.g., biotin labeling, FITC labeling, APC labeling). As another example, antibodies may be conjugated to a therapeutic moiety that enhances the biological effect of the antibodies *in vivo*. Examples of such a therapeutic moiety include another antibody, cytotoxins that are cytostatic or cytocidal, radioactive element, and/or other therapeutic agents, including anti-inflammatory agents, antibiotics, and the like. In the present invention, the humanized anti-human α9 integrin may be conjugated to another antibody, such as anti-α4 antibody (*e.g.*, to form a bispecific antibody). As another example, the humanized antibody of the present invention may be labeled with detectable markers, such as radioactive elements, for *in vivo* diagnostic uses.

5.3. Chimeric and Humanized Antibodies

A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a constant region derived from a human immunoglobulin. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science, 229:1202, 1985; Oi et al., BioTechniques, 4:214 1986; Gillies et al., J. Immunol. Methods, 125:191-202, 1989; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entireties.

A humanized antibody is a molecule that binds a desired antigen and comprises a variable region containing one or more complementarity determining regions (CDRs) derived from a non-human species and one or more framework regions derived from a human immunoglobulin molecule. The typical methods for humanizing non-human antibodies have been described in various references, such as those: by Queen et al., 1989, *Proc. Natl. Acad. Sci. USA* 86:10029-10033 and U.S. Patent Nos. 5,585,089 and 5,693,762; by Riechmann et al., *Nature*, 332:323, 1988; and by Tsurushita *et al.*, *Methods* 36:69-83, 2005, all of which are incorporated herein by reference in their entireties). For example, the reference by Tsurushita *et al.* (2005, *supra*; hereafter "*Tsurushita*") provides a practical and instructive protocol for the humanization of mouse monoclonal antibodies based on the antibody-humanization method originally developed by Queen *et al.* (1989, *supra*). The general protocol disclosed in *Tsurushita* is briefly summarized below.

5.3.1. General protocol for preparing humanized antibodies

Cloning and sequencing of mouse V genes

Various methods are available for cloning cDNAs encoding the VH and VL regions of a target mouse monoclonal antibody. For example, 5' RACE (rapid amplification of cDNA ends) method using SMART RACE cDNA Amplification Kit (BD Biosciences, CA) or the GeneRacer Kit (Invitrogen, CA) has been commonly used. A gene-specific primer for 5' RACE can be prepared based on the isotypes of the H-chain and the L-chain of the target monoclonal antibody so that it can bind immediately downstream of the variable region for each of the H-chain and L-chain. Thus, 5' RACE primer may be designed to be specific for each subtype in mouse,

such as γ1, γ2a, γ2b or γ3. Alternatively, a common primer for all subtypes may be designed based on the consensus or highly homologous region among the subtypes. In *Tsurushita*, the following 5' RACE primers are disclosed as examples:

(i) 5'-GCCAGTGGATAGACTGATGG- (SEQ ID NO:129) (for cloning of mouse γ1, γ2a, γ2b and γ3 H-chains)

(ii) 5'-GATGGATACAGTTGGTGCAGC- (SEQ ID NO:130) (for cloning of mouse κ light chains).

PCR-amplified V gene fragments can be directly cloned into a plasmid vector, for example, using the Zero Blunt TOPO PCR Cloning Kit (Invitrogen), and their DNA sequences determined. The obtained sequences should be confirmed by, for example, comparing their encoding amino acid sequences with those of the target monoclonal antibody determined by the N-terminal amino acid sequencing, using, for example a Model 241 Protein Sequencer (Hewlett-Packard, CA). Typically, the determination of at least 15-20 amino acid residues at the N-terminus of the target antibody, for example, by Edman degradation, is sufficient to confirm the authenticity of the cloned DNA sequences. *Tsurushita* cautions that when glutamine, which is one of the two most common N-terminal amino acid in mouse, is the N-terminal amino acid, it might have been converted to pyroglutamine and blocks the sequencing at the N-terminus. In that case, it is necessary to deblock the N-terminus to obtain the sequence.

Three-dimensional modeling of V regions

Based on the sequences of the VH and VL regions, the framework residues of the target antibody that are potentially important for maintaining the conformational structure of the CDRs, are first identified by the method, for example, described by R. Levy *et al.*, 1989, *Biochemistry* 28:7168-7175; and by B. Zilber *et al.*, 1990, *Biochemistry* 29:10032-10041. Typically, each of the VH and VL regions is divided into 14 structurally meaningful segments, which are β strands and loop-like structures comprising the domain structure of the immunoglobulin superfamily. The amino acid sequence of each of the segments from the target antibody is aligned with the corresponding segments of antibodies of known structures, in the PDB database (*see* H.M. Berman *et al.*, 2000, *Nucleic Acids Res.* 28:235-342). By multiple sequence alignment, a corresponding segment having the

highest sequence homology to each of the target segment is selected and the three-dimensional model of the V-region is constructed. In order to optimize the structure, the model is subjected to multiple cycles of conjugate gradient energy minimization (*e.g.*, using ENCAD, or as described by Press *et al.*, 1990, in "*Numerical Recipes*, Cambridge University Press, Cambridge; AMBER by Weiner *et al.*, 1981, *J. Comp. Chem.* 2:287-303; 3D-JIG-SAW available at BioMolecularModelling or "BMM" web site run by Cancer Research UK; or SWISS-MODEL available at ExPASy Proteomics Server web site run by Swiss Institute of Bioinformatics, Geneva).

Selection of human frameworks

In parallel with modeling the structure of the V regions, the amino acid sequences deduced from the cDNA cloning of the mouse VH and VL regions, respectively, are compared to human V region sequences in the databases, for example, the Kabat database (*see* Johnson *et al.*, 2000, *Nucleic Acids Res.* 28:214-218.), GenBank, and so forth. Human framework regions that have overall sequence identity of at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least 95% identity, with the mouse sequence, can be searched using, for example, the Smith-Waterman algorithm (by Gusfield, 1997, in "*Algorithms on Strings, Trees, and Sequences*", Cambridge University Press, Cambridge), or BLAST (by Karlin *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-2268), and the like. These human sequences may be based on cDNA-based and protein-derived sequences; however, the use of germline is often preferable as it may be useful in eliminating potential immunogenicity associated with somatic hypermutations in cDNA-based, protein-derived sequences. In the alternative, as described in Queen *et al.* (1989, *supra*), the use of a consensus framework sequence can also identify and remove such hypermutated residues in the framework obtained from cDNA-based or protein-derived sequences. In the case where a germline VH segment is used as an acceptor framework, VH segments encoded on chromosome 14, rather than 15 and 16, should be used as only those on chromosome 14 produce functional VH regions.

Design of humanized V regions

According to Queen *et al.* (1989, *supra*), it is necessary to identify framework

amino acids within about 4-6 Å of the CDRs as these residues are considered to be potential key framework residues that support the correct CDR structures. Such a process can be achieved using a computer program, such as RASMOL available at Molecular Visualization Freeware web site supported by National Science Foundation (NSF), that calculates interatomic distances from the atomic coordinates or, through manual inspection of a computer model. If amino acids at key framework positions are different between mouse donor and human acceptor sequences, those of mouse donor usually replace the human residues. However, if such residues have minimal contribution to support the CDR structures, the corresponding human residues are typically used. Also, if the selected human acceptor contains "atypical" amino acids, which occur in less than about 10-20% of the V region sequences, they may be the result of somatic hypermutation during affinity maturation and should be replaced with the donor residues in order to avoid potential immunogenicity in humans.

In addition, other factors, such as residues of potential N-linked glycosylation signals, need to be carefully considered in order to design humanized V regions (*see Tsurushita* for details).

Humanized antibodies may contain a human constant region or a portion thereof from the human κ or λ light chain, and/or the γ1, γ2, γ3, γ4, μ, α1, α2, δ, or ε heavy chain of human antibodies, or variants thereof, depending on the effector functions required or to be eliminated for therapeutic uses. For example, a Fc portion of the constant region containing a mutation may be fused to the variable region of the chimeric or humanized antibody of the present invention so as to reduce the binding of the antibody to Fc receptors and/or to reduce its ability to fix complement (*see*, for example, Winter *et al.*, GB 2,209,757 B; Morrison *et al.*, WO 89/07142, Morgan *et al.*, WO 94/29351). Such manipulations of antibody molecules can be carried out by recombinant DNA technology as described in Section 5.2.

Preferably the resulting chimeric or humanized antibody has the same specificity as the non-human donor antibody and an affinity similar to or at least about 1/3, at least about 1/2, or at least about 2/3, of that of the non-human donor antibody. In another aspect, the resulting chimeric or humanized antibody has an affinity constant of at least about 1 x 10^7 M^{-1}, preferably at least about 1 x 10^8 M^{-1},

and most preferably at least about 1 x 10^9 M^{-1}.

In addition to the general protocol described above, antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101 and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology, 28(4/5):489-498, 1991; Studnicka et al., Protein Engineering, 7(6):805-814, 1994; Roguska et al., Proc Natl. Acad. Sci. USA, 91:969-973, 1994), and chain shuffling (U.S. Patent No. 5,565,332), all of which are hereby incorporated by reference in their entireties.

5.3.2. Additional considerations for preparing humanized antibodies as pharmaceuticals

To offer humanized antibodies as pharmaceuticals, an efficient and consistent production system therefor needs to be prepared. For example, an appropriate expression vector for humanized antibodies is prepared by inserting H- and L-chain sequences, and a high-productivity cell line transfected with the expression vector can be obtained as a seed cell for a master cell bank (MCB), which serves as a stable and semi-permanent source for a working cell bank (WCB). Humanized antibodies can be then prepared by culturing working cells from the WCB and collecting the culture medium.

Various expression vectors with appropriate regulatory genes can be used for the preparation of such a production cell line. As a host cell, those commonly used for expressing mammalian proteins can be used for the expression of humanized antibodies. Examples of such host cells include, but are not limited to, Chinese Hamster Ovary (CHO) cells, SP2/0-Ag14.19 cells, NSO cells, and the like. The productivity of humanized antibodies can be maximized by selecting the best combination of an expression vector and a host cell. Furthermore, the composition of culture media should be explored in order to select suitable media, from various serum-free culture media and supplements, so that the expression of humanized antibodies by the host cell can be optimized.

Based on the efficiency and the final yield, the humanized antibodies produced by the host cell can be purified from the culture supernatant using various methods well known in the art, including affinity chromatography, ion-exchange

chromatography, hydrophobic interaction chromatography, and the like.

5.4. Pharmaceutical Composition and Therapeutic Uses

The present invention provides a pharmaceutical composition comprising the humanized antibody or an antigen-binding fragment thereof, described above, that immunospecifically recognizes human α9 integrin. The pharmaceutical composition comprising the humanized antibody of the present invention as an active ingredient can be used as an agent for preventing and/or treating a disorder or disease that is associated with α9 integrin, including, but not limited to, cancer, *e.g.*, the growth or metastasis of cancer cells, and an inflammatory disease, *e.g.*, rheumatoid arthritis, osteoarthritis, hepatitis, bronchial asthma, fibrosis, diabetes mellitus, arteriosclerosis, multiple sclerosis, granuloma, an inflammatory bowel disease (ulcerative colitis and Crohn's disease), an autoimmune disease, and the like.

The pharmaceutical composition comprising the humanized antibody of the present invention can also be used to treat chronic rejection after organ transplantation, and an autoimmune disease such as systemic autoimmune disease, erythematosus, uveitis, Behcet's disease, polymyositis, glomerular proliferative nephritis, sarcoidosis, and the like.

The preventive and/or therapeutic agent for preventing or treating the disorders or diseases described above, comprising the humanized antibody of the present invention, has low toxicity and can be administered to humans orally or parenterally, directly as a liquid preparation by mixing in a suitable solvent, or as a pharmaceutical composition in an appropriate dosage form.

The pharmaceutical composition used for the administration described above contains the aforesaid antibody or salts thereof and pharmaceutically acceptable carriers, diluents or excipients. Such a composition is provided in a dosage form suitable for oral or parenteral administration.

The dose may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibody is used for preventing and/or treating, for example, rheumatoid arthritis in an adult patient, it is advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, preferably about 0.1 to about 10 mg/kg body weight, and more preferably

about 0.1 to about 5 mg/kg body weight, approximately 1 to 5 times per day, preferably approximately 1 to 3 times per day. In other parenteral administration and oral administration, the antibody can be administered in a dose corresponding to the dose given above. When the condition is especially severe, the dose may be increased according to the condition.

Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429 4432). Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, by means of nasal spray, or by means of an implant, said implant being of a porous, non porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) infected tissues.

In another embodiment, the pharmaceutical composition can be delivered in a vesicle, in particular a liposome (see Langer, 1990, Science 249:1527-1533; Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid. , pp. 317-327; see generally ibid.).

In yet another embodiment, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see

Langer, supra; Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al.,1980, Surgery 88:507; and Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

Examples of the composition for oral administration include solid or liquid dosage forms, specifically, tablets (including dragees and film-coated tablets), pills, granules, powdery preparations, capsules (including soft capsules), syrup, emulsions, suspensions, etc. Such a composition is manufactured by publicly known methods and contains a vehicle, a diluent or an excipient conventionally used in the field of pharmaceutical preparations. Examples of the vehicle or excipient for tablets are lactose, starch, sucrose, magnesium stearate, and the like.

The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. The injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl

benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule. The suppository used for rectal administration may be prepared by blending the aforesaid antibody or its salt with conventional bases for suppositories.

Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to 100 mg and in about 10 to 250 mg for the other dosage forms.

Each composition described above may further contain other active components unless formulation causes any adverse interaction with the antibodies described above.

The present invention also relates to an inhibitor and/or promoter for cell and/or tissue remodeling, which comprises an α9 integrin-binding functional molecule (e.g., OPN, VCAM-1, tenascin-C, fibronectin, pp-vWF, tTG, etc.) as an active ingredient; and a method for inhibiting and/or promoting cell and/or tissue remodeling, which comprises contacting the α9 integrin-expressing cell and/or tissue (e.g., a tumor cell, neutrophil, smooth muscle, etc.) with the α9 integrin-binding functional molecule. The dose, method for administration, pharmaceutical preparation, etc. of the active ingredient in such a therapeutic agent can be appropriately determined by referring to the foregoing description of medicaments comprising the humanized antibodies of the present invention.

As described above, the present invention further provides a method for preventing or treating a disorder or disease that is associated with or involves α9 integrin, said method comprising administering an effective amount of at least one of the humanized antibodies of the present invention to a subject in need thereof.

5.5. Diagnostic Uses

The pharmaceutical composition comprising the humanized antibody of the present invention can be used as a diagnostic agent for cancer, e.g., the growth or

metastasis of cancer cells, and an inflammatory disease, e.g., rheumatoid arthritis, osteoarthritis, hepatitis, bronchial asthma, fibrosis, diabetes mellitus, cancer metastasis, arteriosclerosis, multiple sclerosis, granuloma, etc., or as a diagnostic agent for chronic rejection after organ transplantation, an autoimmune disease such as systemic autoimmune disease, erythematosus, uveitis, Behcet's disease, polymyositis, glomerular proliferative nephritis, sarcoidosis, and so forth. The humanized antibodies of the present invention are capable of specifically recognizing the α9 integrin and hence can be used to quantify the α9 integrin in a test fluid, especially for quantification by the sandwich immunoassay, competitive assay, immunometry, nephrometry, etc., immunostaining, or the like. In applying these immunological methods to the assay methods of the present invention, it is not required to set forth any particular conditions, procedures, etc. It is sufficient to construct assay systems by adding ordinary technical consideration in the art to conventional conditions and procedures. For details of these general technical means, reference can be made to reviews, texts or the like.

As described above, the α9 integrin can be quantified with high sensitivity by using the antibodies of the present invention. The humanized antibodies of the present inventions are particularly useful for diagnosing various diseases associated with the α9 integrin by applying the method for quantifying the α9 integrin *in vivo*. For instance, where an increase or decrease in the expression level of the α9 integrin is detected, it can be diagnosed that it is highly likely that one now suffers from diseases associated with the α9 integrin, e.g., cancer or an inflammatory disease, or it is highly likely that one will suffer from these diseases in the future. Thus, the present invention also provides a method for diagnosing a disorder or disease associated with or involve α9 integrin in a subject, said method comprising administering an effective amount of at least one of the humanized antibodies of the present invention or both to a subject in need thereof. Required dosages for such an *in vivo* diagnosis may be less than those required for therapeutic uses and can be determined by one skilled in the art according to routine procedures.

The humanized antibodies of the present invention can also be used for specifically detecting the α9 integrin present in a test fluid such as a body fluid, a tissue, etc. The humanized antibodies can also be used for preparation of antibody columns for purification of the α9 integrin, for detection of the α9 integrin contained

in each fraction upon purification or for analysis of behaviors of the α9 integrin in cells to be tested.

6. EXAMPLES

The following examples illustrate the preparation of monoclonal antibodies that immunospecifically recognize human and/or mouse α9 integrin, the sequencing of the variable regions of the monoclonal antibodies, the epitope mapping and other characterization of the antibodies and the chimerization and the humanization of such antibodies, as well as the characterization of the resulting chimeric and humanized antibodies. These examples should not be construed as limiting.

6.1. Preparation of Mouse Antibody Against Human α9 Integrin

Mouse monoclonal antibodies against human α9 integrin were prepared according to the subtractive immunization method (by Williams C.V., *et al.*, 1992, *Biotechniques* 12:842-847). Briefly, three Balb/c mice were injected intraperitoneally with CHO-K1 cells at $4x10^6$ per mouse. In the following two days, the mice received 4 mg/mouse of cyclophosphamide intraperitoneally. At two weeks after the cyclophosphamide injection, the mice were injected intraperitoneally with $2x10^6$ cells/mouse of CHO-K1 cells expressing human α9 integrin (Human α9/CHO-K1 cells), followed by another intraperitoneal injection of the same cells at $3x10^6$ cell/mouse two weeks later. Hybridomas were prepared by the methods well known in the art (*see*, for example, Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas, pp. 563-681 (Elsevier, N.Y., 1981). Hybridoma clones producing monoclonal antibodies that were immunospecifically reactive with Human α9/CHO-K1 cells but not with CHO K1 cells expressing human α4 integrin were established and five hybridoma clones (*i.e.*, 1K11, 21C5, 24I11, 25B6 and 28S1) producing monoclonal antibodies immunospecifically recognizing human α9 integrin were isolated.

6.2. Epitope Analysis for Anti-Human α9 Integrin Monoclonal Antibodies

Twelve-residue polypeptides, starting from N-terminal of human α9 integrin

and every three residues thereafter (*i.e.*, amino acid residues 1-12, 4-15, 7-18, and so forth) were prepared and coupled to a cellulose membrane via C6-spacer and 2 βAla residues at 5 nmol/spot. The membrane was blocked with a blocking buffer (milk/0.05% Tween20 in PBS) and reacted with 10 ml of solution containing 1.0 μg/ml of each of the anti-human α9 integrin monoclonal antibodies (*i.e.*, 1K11, 21C5, 24I11 and 25 B6, respectively) labeled with peroxidase for three hours at room temperature. After washing with T-TBS, the membrane was reacted with enhanced chemiluminescence (ECL) detecting reagent for 1 minute at room temperature. The luminescence emitted as a result of the enzymatic reaction is measured and epitopes of the antibodies were determined based on the luminescence intensities. As a control, Y9A2 (*see* Wang *et al.*, 1996, *Am J Respir Cell Mol Biol* 15, 664-672), a commercially available monoclonal antibody against human α9 integrin, was used.

Table 1 below shows the result of the epitope mapping, which indicated that the monoclonal antibodies isolated by the present inventors have epitopes that are distinct from that of Y9A2.

Table 1

Human α9 integrin sequences	1K11	21C5	24I11	25B6	Y9A2
FQGPADSFFGYA (SEQ ID NO:77)	-	-	-	++	-
KSPGAVFKCRVHTNPDRR (SEQ ID NO:78)	++	+++	++++	-	+
WMGVSLARQPKADGRVLA (SEQ ID NO:79)	+	+++	+	-	+
CAHRWKNIYYEADHI (SEQ ID NO:80)	+	+	+++	+	+
GFCYIIPSNLQAKGRTLI (SEQ ID NO:81)	+++	+++++	+++++	+++++	+++++
VMGAPGSFYWAGTIKVLN (SEQ ID NO:82)	+	+	+	+++	+
VIMNRRYTYLGYAVT (SEQ ID NO:83)	+++++	++	+++++	+++++	+++
VYIFRADRRSGTLIKIFQ (SEQ ID NO:84)	++++	+++++	+++	-	+
QYSMKLSGQKINPVLRMFGQSISG (SEQ ID NO:85)	+	++++	-	-	+
VVLLRARPVITVDVSIFL (SEQ ID NO:86)	++	++	-	-	+++++
RHYVAHVKRRVQDVISPI (SEQ ID NO:87)	+++	+++	+++++	+	++
ELPPLTPVLRWKKGQKIAQKNQTVFERNCR (SEQ ID NO:88)	+	+++++	+	-	++
YLALGAVKNISL (SEQ ID NO:89)	+	+	-	++	+++++
CSVGFPFMRSKSKYEFSV (SEQ ID NO:90)	+	++	++	-	+++
SSSVIQFMSRAKVKVDPALRV (SEQ ID NO:91)	+	++	+++	-	+

6.3. CDR Analysis of Anti-Human α9 Integrin Antibodies

The amino acid sequences of CDRs of the monoclonal antibodies (*i.e.*, 1K11, 21 C5, 24I11, 25B6 and 28S1) were determined by reverse transcription of the mRNA extracted from the corresponding hybridomas to prepare cDNAs. Using the cDNAs as templates, the variable regions of the H-chains and L-chains were extended and amplified by PCR using ScFv-cloning primers (Light Primer Mix and Heavy Primer Mix; by Amersham Biosciences Corp., IL). The PCR products were cloned into pCRII TOPO vector, sequenced and the amino acid sequences were determined. This process was repeated three times for each antibody. The results are shown in Table 2.

Table 2

CDRs	1K11	21C5	24H1	25B6	28S1
CDRH1	DYNMD (SEQ ID NO:32)	DYYMY (SEQ ID NO:42)	DTYVH (SEQ ID NO:4)	SYGVH (SEQ ID NO:52)	GYGVN (SEQ ID NO:62)
CDRH2	DINPNNGGTIYNQKFQG (SEQ ID NO:33)	TISDGGNYTYYPDSVKG (SEQ ID NO:43)	NIDPANGNTKYDPKFQG (SEQ ID NO:5)	VIWSGGSTNYNSALMS (SEQ ID NO:53)	MIWGDGITEYNSALKS (SEQ ID NO:63)
CDRH3	SGVISTDY (SEQ ID NO:34)	DRDGSSLFAY (SEQ ID NO:44)	WLRHFYYAMDY (SEQ ID NO:6)	DYGNYPWFAY (SEQ ID NO:54)	RDASSGYGFA (SEQ ID NO:64)
CDRL1	RASQEISGYLI (SEQ ID NO:37)	KASQDVNIAVA (SEQ ID NO:47)	RASENIYSLA (SEQ ID NO:11)	KASQDVNTAVA (SEQ ID NO:57)	TASSSVSSSYLH (SEQ ID NO:67)
CDRL2	AASTLDS (SEQ ID NO:38)	WASTRHT (SEQ ID NO:48)	NANSLED (SEQ ID NO:12)	SASYRYT (SEQ ID NO:58)	STSNLAS (SEQ ID NO:68)
CDRL3	LQYANYPPT (SEQ ID NO:39)	QQHYNTPW (SEQ ID NO:49)	KQAYDVPYT (SEQ ID NO:13)	QQHYSTPCA (SEQ ID NO:59)	HQYHRSPYT (SEQ ID NO:69)

6.4. Cell Adhesion Inhibitory Activity

(1) Since it is known that cell adhesion involves the binding of α9 integrin to

its ligands, *i.e.*, various ECMs, including OPN, fibronectin , Tenascin-C, VCAM-1, and the like, the isolated anti-human α9 integrin antibodies were examined for their cell adhesion inhibitory activity.

Briefly, hOPN(RAA)N-half was prepared as a Glutathione S-transferase (GST)-fusion protein by isolating from *E. coli* host cells an N-terminal portion down to the thrombin-cleavage site of OPN, in which the GRD sequence has been replaced with the RAA sequence, and cleaving the GST portion with Precision protease (Amersham Biosciences). VCAM-1 was purchased from R&D Systems, Inc. (Minneapolis, MN). Tenascin-C and human fibronectin were prepared by synthesizing polypeptides containing AEIDGIEL (SEQ ID NO:92); the α9 integrin-binding region of Tenascin-C) and CPEDGIHELFP (SEQ ID NO:93); the α9 integrin-binding region of human fibronectin), respectively, and subsequently attaching them to bovine serum albumin (BSA). For human α9 integrin, CHO-K1 cells that abundantly expressed human α9 integrin (Human α9/CHO-K1) were used.

Fifty microliters of Tenascin-C, fibronectin, VCAM-1 or hOPN(RAA)N-half were added to a 96-well plate at 1.25-5.0 μg/ml and incubated at 37°C for 1 hour to coat the plate. After blocking the plate with a blocking solution (0.5% BSA/PBS) and washing it with PBS once, the mixture of human α9/CHO-K1 cells ($1.0x10^5$ cells/ml) and the isolated monoclonal antibodies (10 g/ml) in 0.25% BSA-Minimum Essential Media (MEM) was added to the plate at 200 μl/well and incubated at 37°C for 1 hour under 5% CO_2. Non-adherent cells were rinsed off with PBS and adherent cells were fixed and stained with 0.5% Crystal Violet (by WAKO, Osaka, Japan)/20% methanol. The stained cells were allowed to stand at room temperature for 30 minutes and 20% acetic acid solution was added thereto to effect dissolution. The adhesion activity was quantified by measuring OD at 590 nm wavelength.

As shown in Fig. 16, the cell adhesion involving Tenascin-C was inhibited by 21C5, 24I11, 25B6 and 28S1, but not by 1K11. The cell adhesion involving fibronectin was inhibited by 21C5, 25B6 and 28S1 and by 24I11 to a less degree, but no inhibition was observed with 1K11. The cell adhesion involving VICAM-1 was inhibited by 21C5, 24I11, 25B6 and 28S1, but not by 1K11. Likewise, the cell adhesion involving hOPN(RAA)N-half was inhibited by 21C5, 24I11, 25B6 and 28S1, but not by 1K11.

(2) Since α4 integrin and α9 integrin have many common ECM ligands, the presence of both anti-α4 integrin and anti-α9 integrin antibodies is expected to enhance the cell adhesion inhibitory activity. Thus, the effect of the both types of antibodies in combination was examined *in vitro* for a possible inhibitory effect on metastatic cancer, based on the cell adhesion between human melanoma cells (G361) expressing α4 integrin as well as α9 integrin and VCAM-1 (1.25 μg/ml) as an ECM; a rat monoclonal antibody, P1H4 (Cat. No. MAB16983Z, Chemicon International Inc., CA) was used as an anti-human α4 integrin antibody.

As shown in Fig. 17, the adhesion involving VCAM-1 was not inhibited by any of the anti-human α9 integrin antibodies alone, but was inhibited by the positive control (Y9A2), 21C5 and 24I11, in the co-presence of the anti-human α4 integrin antibody. Since cells expressing many α9 integrin molecules usually express α4 integrin molecules also, this result indicates that the inhibition of cell adhesion can be effectively achieved by the combination use of anti-human α9 integrin antibody and anti-human α4 integrin antibody, thereby enhancing the suppression of various disorders and diseases, including metastatic cancer involving these integrin molecules.

6.5. Use of Anti-Human α9 Integrin Antibodies in FACS Analysis

Whether the anti-human α9 integrin antibodies were usable for FACS was examined using Human α9/CHO-K1 cells, CHO-K1 cells and human neutrophils endogenously expressing the α9 integrin. In human neutrophils, FACS analysis was conducted at the cell count of 1.0×10^5 and antibodies were reacted on ice. Non-specific reaction with the Fc receptor was blocked with 50% goat serum. FITC-labeled anti-mouse IgG antibody was used as a secondary antibody. As a result (*see* Figs. 20a, 20b and 20c), all of the anti-human α9 integrin antibodies could detect the α9 integrin on Human α9/CHO-K1 and human neutrophils. None of the antibodies reacted with Human α4/CHO-K1 cells (*see* Figs. 20a, 20b and 20c). These results revealed that all of the anti-human α9 integrin antibodies could detect the human α9 integrin proteins expressed on cells using FACS.

6.6. Therapeutic Effects of Anti-α9 Integrin Antibody

Therapeutic effects of anti-α9 integrin antibodies were examined in a mouse system.

The anti-mouse α9 integrin monoclonal antibodies (11L2B, 12C4'58, 18R18D and 55A2C) were prepared substantially in the same manner as described for mouse anti-human α9 integrin antibodies (*see* Section 6.1, *supra*), except that hamsters were immunized with CHO-K1 cells expressing mouse α9 integrin (mouse α9/CHO-K1 cells) and the resulting monoclonal antibodies that reacted with mouse α9/NIH3T3 cells but not with mouse α4/NIH3T3 were selected.

6.6.1. Therapeutic effect on hepatitis

WO 02/081522 discloses that hepatitis can be treated by inhibiting the OPN functions. Accordingly, therapeutic effects of anti-α9 integrin antibody was studied in a mouse hepatitis model using a hamster anti-mouse α9 integrin antibody, 11L2B, and a rat anti-mouse α4 integrin antibody, R1-2 (Pharmingen). The blood AST and ALT levels in the mice were measured using GPT/ALT-PIII and GOT/AST-PIII (Fuji Film), 12 hours after an intravenous injection of 200 μg of concanavalin A (Con A) (Vector). Three hours before the Con A injection, 200 μg of the antibody were administered. As shown in Fig. 18, the AST and ALT levels were found to be decreased by the anti-α9 integrin antibody, and the therapeutic effects could be noted. In addition, the therapeutic effects could be boosted by concomitant use with the anti-α4 integrin antibody. The results revealed that hepatitis could be treated by the anti-α9 integrin antibody.

6.6.2. Effect of anti-α9 integrin antibodies on growth of mouse cancer cell line

Murine melanoma cell line B16-BL6 expresses abundant α9 integrin. Accordingly, cell growth inhibitory activities of the established anti-mouse α9 integrin antibodies against cancer cells were examined.

The B16-BL6 cells were prepared on a 96-well plate for cell culture (Becton Dickinson) at 5 x 10^4 cells/mL in 10% FCS/DMEM. After 10 μg/ml of the anti-mouse α9 integrin antibody and anti-mouse α4 integrin antibody were added,

100 μL each of the cell-antibody suspension was added to each well. Incubation was conducted at 37°C for 24 hours under 5% CO_2, and 10 μL each of Cell Counting Kit 8 (Dojin Kagaku Kenkyu-sho) was added, followed by incubation at 37°C for an hour under 5% CO_2. Absorbance at O.D. 450 was measured and the cell count was quantitatively analyzed. As shown in Fig. 19, 12C4'58 gave the highest inhibitory activity and inhibited the growth of B16-BL6 cells by about 35%. Both 55A2C and R1-2 could inhibit the growth by about 20%.

Next, for analysis of inhibitory effects against cell growth under conditions closer to the in vivo conditions, VCAM-1 was immobilized on a solid phase and assayed in a similar fashion. VCAM-1 is a ligand for α9 integrin and a recombinant soluble form of VCAM-1 protein, rhVCAM-1-Fc chimera (Roche), was used. Using the rhVCAM-1-Fc chimera immobilized on a solid phase with 10 μg/mL, non-specific reaction was blocked with 0.5% BSA/PBS. The chimera was added in a concentration of 10 μg/ml in single use of the antibody, and in 5 μg each/ml in concomitant use. Thereafter, the same procedures as in Fig. 19 were followed. As a result, the effect was not obtained at all or only an imperceptible effect was obtained by single administration of 12C4'58 and by single use of the α4 inhibitory antibody clone R1-2, whereas in simultaneous administration of 12C4'58 with R1-2 the cell growth inhibitory effect showed a marked increase by about 20%, as shown in Fig. 21.

6.6.3. Therapeutic effect of anti-α9 integrin in mouse rheumatoid arthritis model

Seven-week old female mice (Balb/c) (3 mice per group) were injected intraperitoneally with the hamster anti-mouse α9 integrin antibody (55A2C), or normal hamster IgG (NHG) at 400 μg/mouse. After 24 hours, 2 mg/mouse of the arthritis-inducing cocktail of type II collagen-specific monoclonal antibody (Chondrex Inc.) was injected intravenously. After 72 hours, 400 μg/mouse of 55A2C or NHG as well as 50 μg/mouse of LPS were injected intraperitoneally. The mice were observed from 3 days before the LPS injection until 6 days after the LPS injection and the levels of arthritis were scored according to the method by Wood *et al.* (1969, *Int. Arch. Allergy Appl. Immunol.* 35:456). The result is shown in Fig. 22. The mice injected with control NHG had a high score and developed rheumatoid

arthritis, whereas in those injected with anti-mouse α9 integrin antibody, the development of rheumatoid arthritis was completely blocked. Thus, anti-α9 integrin antibodies were indicated to have prophylactic and therapeutic effects on rheumatoid arthritis.

6.7. Humanization of Non-Human Antibodies

6.7.1. Cloning and sequencing of mouse 24I11 V genes

Mouse 24I11 hybridoma cells were grown in TIL Media I (Immuno-Biological Laboratories, Gunma, Japan) containing 10% fetal bovine serum (FBS; HyClone, Logan, UT) at 37°C in a 7.5% CO_2 incubator. Total RNA was extracted from approximately 3 x 10^6 hybridoma cells using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the supplier's protocol. Oligo dT-primed cDNA was synthesized using the GeneRacer Kit (Invitrogen) following the supplier's protocol. The variable region cDNAs for 24I11 heavy and light chains were amplified by polymerase chain reaction (PCR) with Phusion DNA polymerase (New England Biolabs, Beverly, MA) using primers that anneal respectively to the mouse gamma-1 and kappa chain constant regions, and a GeneRacer 5' primer (5'-CGACTGGAGCACGAGGACACTGA-) (SEQ ID NO:94) provided in the GeneRacer Kit. For PCR amplification of heavy chain variable region (VH), the primer has the sequence 5'-GCCAGTGGATAGACAGATGG- (SEQ ID NO:95). For PCR amplification of light chain variable region (VL), the primer has the sequence 5'-GATGGATACAGTTGGTGCAGC- (SEQ ID NO:96). The amplified VH and VL cDNAs were subcloned into the pCR4Blunt-TOPO vector (Invitrogen) for sequence determination. DNA sequencing of the variable regions was carried out at Tocore (Menlo Park, CA). Several heavy and light chain clones were sequenced and unique sequences homologous to typical mouse heavy and light chain variable regions were identified. The consensus cDNA sequences along with deduced amino acid sequences of 24I11 VH and VL are shown in Figs. 1 and 2, respectively.

6.7.2. Construction of chimeric 24I11 IgG1/κ antibody

A gene encoding 24I11 VH was generated as an exon including a splice donor signal and appropriate flanking restriction enzyme sites by PCR using 24I11 VH

cDNA as a template, 5'-GGGACTAGTACCACCATGAAATGCAGCTGGGTTATCTTC- (SEQ ID NO:97) (SpeI site is underlined) as a 5' primer, and 5'-GGGAAGCTTAGAGGCCATTCTTACCTGAGGAGACGGTGACTGAGGTTC C- (SEQ ID NO:98) (HindIII site is underlined) as a primer (Fig. 3). Likewise, a gene encoding 24I11 VL was generated as an exon including a splice donor signal and appropriate flanking restriction enzyme sites by PCR using 24I11 VL cDNA as a template, 5'-GGGGCTAGCACCACCATGAGTGTGCCCACTCAACTCCTG- (SEQ ID NO:99) (NheI site is underlined) as a 5' primer, and 5'-GGGGAATTCTGAGAAGACTACTTACGTTTTATTTCCAGCTTGGTCCCCCC- (SEQ ID NO:100) (EcoRI site is underlined) as a primer (Fig. 4). The splice donor signals of the 24I11 VH and VL exons were derived from the mouse germline JH4 and Jκ2 sequences, respectively. PCR-amplified fragments were gel-purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA), digested with SpeI and HindIII (for VH) or NheI and EcoRI (for VL), and cloned into a mammalian expression vector carrying human gamma-1 and kappa constant regions for production of chimeric 24I11 IgG1/κ antibody. The schematic structure of the resulting expression vector, pCh24I11, is shown in Fig. 5.

6.7.3. Generation of humanized 24I11 V genes

Humanization of the 24I11 variable regions was carried out as outlined by Queen et al. (Proc. Natl. Acad. Sci. USA 86: 10029-10033, 1989). First, a molecular model of the 24I11 variable regions was constructed with the aid of computer programs. Next, based on a homology search against human variable region sequences, the human amino acid sequence encoded by the nucleotide sequence of GenBank Accession No. X65891, which has a high homology [72.4% (63/87) amino acid identity in the FRHs] to 24I11 VH, was chosen as an acceptor to provide the frameworks for humanized 24I11 VH. Likewise, the human amino acid sequence encoded by the nucleotide sequence of GenBank Accession No. X72441 [77.5% (62/80) amino acid identity in the FRLs] was chosen as an acceptor for humanization of 24I11 VL.

At framework positions where the computer model suggested significant contact with the complementarity determining regions (CDRs), the amino acids from the 24I11 variable regions were substituted for the human framework amino acids.

This was done at positions 27, 28, 29, 30, 48, 66, 67 and 71 (according to Kabat numbering system; *see*, Kabat *et al.*, Sequences of Proteins of Immunological Interests, Fifth edition, NIH Publication No. 91-3242, U.S. Department of Health and Human Services, 1991) to generate humanized 24I11 (Hu24I11) VH (Fig. 6). For the light chain, replacements were made at residues 70 and 71 to generate humanized 24I11 (Hu24I11) VL (Fig. 7). The alignments of 24I11, designed Hu24I11, and the human acceptor amino acid sequence are shown for VH in Fig. 6 and for VL in Fig. 7.

A gene encoding each of Hu24I11 VH and VL was designed as an exon including a signal peptide, a splice donor signal, and appropriate restriction enzyme sites for subsequent cloning into a mammalian expression vector. The splice donor signals of the Hu24I11 VH and VL exons were derived from the human germline JH4 and Jκ1 sequences, respectively. The signal peptide sequences in the Hu24I11 VH and VL exons were derived from the corresponding mouse 24I11 VH and VL sequences, respectively. The Hu24I11 VH and VL genes were constructed by extension and PCR amplification of several overlapping synthetic oligonucleotide primers using ThermalAce DNA polymerase (Invitrogen) as outlined by He et al. (J. Immunol. 160: 1029-1035, 1998). The oligonucleotides used for construction of Hu24I11 VH and VL genes are listed in Figs. 8 and 9, respectively. The location of the oligonucleotides in the Hu24I11 VH and VL genes is shown in Figs. 10 and 11, respectively. PCR-amplified fragments were gel-purified using QIAquick Gel Extraction Kit (Qiagen) and cloned into pCR4Blunt-TOPO vector for sequence determination. After digestion with SpeI and HindIII (for VH) or NheI and EcoRI (for VL), Hu24I11 VH and VL genes were subcloned into corresponding sites in a mammalian expression vector for production in the human IgG1/κ form. The schematic structure of the resulting expression vector, pHu24I11, is shown in Fig. 5. The nucleotide sequences of the obtained Hu24I11 VH and VL genes along with deduced amino acid sequences are shown in Figs. 12 and 13, respectively.

6.7.4. Transient expression of chimeric and humanized 24I11 IgG1/κ

Chimeric and humanized 24I11 IgG1/κ antibodies were transiently expressed by transfecting pCh24I11 and pHu24I11 plasmid DNA, respectively, to HEK293 cells using polyethylenimine according to Durocher et al. (Nucl. Acids Res. 30: e9,

2002). Transiently transfected HEK293 cells were maintained for four days in DMEM containing 10% FBS at 37°C in a 7.5% CO_2 incubator. The expression level of each of Ch24I11 and Hu24I11 IgG1/κ antibodies in culture supernatant was measured by sandwich ELISA. An ELISA plate was coated overnight at 4°C with 100 μl/well of 1/2,000-diluted goat anti-human IgG Fcγ-chain-specific polyclonal antibodies (SouthernBiotech, Birmingham, AL) in PBS, washed with Wash Buffer (PBS containing 0.05% Tween 20), and blocked for 1 hr at room temperature with 300 μl/well of Blocking Buffer (PBS containing 2% Skim Milk and 0.05% Tween 20). After washing with Wash Buffer, 100 μl/well of samples appropriately diluted in ELISA Buffer (PBS containing 1% Skim Milk and 0.025% Tween 20) were applied to the ELISA plate. Human IgG1/κ antibody purified from human myeloma serum (SouthernBiotech) was used as a standard. After incubating the ELISA plate for 2 hr at room temperature and washing with Wash Buffer, bound antibodies were detected using 100 μl/well of 1/2,000-diluted horse radish peroxidase (HRP)-conjugated goat anti-human kappa chain polyclonal antibodies (SouthernBiotech). After incubating for 1 hr at room temperature and washing with Wash Buffer, color development was performed by adding 100 μl/well of ABTS substrate (bioWORLD, Dublin, OH). Color development was stopped by adding 100 μl/well of 2% oxalic acid. Absorbance was read at 405 nm.

6.7.5. Characterization of humanized 24I11

Binding of chimeric and humanized 24I11 antibodies to human α9 integrin was examined by cell ELISA. CHO-K1 stable transfectants expressing recombinant human α9 integrin on the surface (CHO/huα9; provided by Gene Techno Science) were seeded at 2 x 10^5 cells/well in 50 μl of F12/DMEM (HyClone) containing 10% FBS in a 96-well tissue culture plate and grown overnight at 37°C in a 7.5% CO_2 incubator. For testing of binding to human α9 integrin, 50 μl of chimeric 24I11, humanized 24I11 or irrelevant human IgG1/κ myeloma antibody (SouthernBiotech) in F12/DMEM containing 10% FBS was added to each well. After incubating for 1 hr at 4°C and washing cells twice with ice-cold PBS, 100 μl of 1/1,000-diluted HRP-conjugated goat anti-human IgG polyclonal antibodies (SouthernBiotech) was added to each well. After incubating for 1 hr at 4°C, cells were washed three times with ice-cold PBS. For color development, 100 μl of

ABTS substrate was added. Color development was stopped by adding 100 μl of 2% oxalic acid. Absorbance was read at 405 nm. The result showed that the binding of chimeric 24I11 antibody to human α9 integrin was almost same as that of humanized 24I11 antibody at both 0.5 and 1 μg/ml (Fig. 14).

Antigen binding of mouse, chimeric and humanized 24I11 monoclonal antibodies was also examined in a FACS binding assay using CHO/huα9 cells. Purified mouse 24I11 monoclonal antibody was provided by Gene Techno Sciences. Approximately 8 x 10^5 CHO/huα9 cells/test were washed with FACS Binding Buffer (PBS containing 0.5% BSA and 0.05% NaN_3) and suspended in 200 μl of FACS Binding Buffer containing various amounts of test antibody. After 30 min on ice, the cells were washed twice with FACS Binding Buffer. The cells stained with mouse 24I11 were then suspended in 200 μl of 1/200-diluted FITC-labeled goat anti-mouse IgG polyclonal antibody (SouthernBiotech) in FACS Binding Buffer. The cells stained with chimeric or humanized 24I11 were suspended in 200 μl of 1/200-diluted FITC-labeled goat anti-human IgG polyclonal antibody (SouthernBiotech) in FACS Binding Buffer. After 30 min on ice, the cells were washed with FACS Binding Buffer, suspended in 200 μl of FACS Binding Buffer, and analyzed using a FACSCan flow cytometer (BD Biosciences, Franklin Lakes, NJ). The binding of chimeric and humanized 24I11 antibodies to CHO/huα9 cells was very similar to each other in this analysis (Fig. 15).

The results of the cell ELISA and FACS experiments using transiently expressed antibodies suggest that humanization of mouse 24I11 antibody is successful.

Humanization of the other mouse anti-human α9 antibodies disclosed herein (*i.e.*, 1K11, 21C5, 25B6 and 28S1) can be also carried out by employing the same procedure described herein. The DNA sequences and the amino acid sequences of the VH and VL regions, respectively, of these mouse monoclonal antibodies are summarized below.

Mouse monoclonal antibodies	DNA sequence of VH region (SEQ ID NO:)[1]	Deduced amino acid sequence of mature VH (SEQ ID NO:)[2]	DNA sequence of VL region (SEQ ID NO:)[1]	Deduced amino acid sequence of mature VL (SEQ ID NO:)
1K11	35	36	40	41
21C5	45	46	50	51
25B6	55	56	60	61
28S1	65	66	70	71

[1] The V genes of each antibody were cloned by a method using Amersham's degenerate primers.

[2] The deduced amino acid sequence starts from the 2nd residue of VH region (according to the Kabat numbering system) for each clone.

7. DEPOSITION

The hybridomas designated herein as 1K11, 21C5, 24I11, 25B6 and 28S1 producing mouse anti-human α9 integrin monoclonal antibodies were deposited on February 15, 2006 with International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, located at AIST Tsukuba Central 6, 1-1, Higashi 1-chome Tsukuba-shi, Ibaraki-ken 305-8566 Japan in accordance with the Budapest Treaty on the Deposit of Microorganisms, and accorded Accession Nos. FERM BP-10510, FERM BP-10511, FERM BP-10512, FERM BP-10513 and FERM BP-10832, respectively, all of which are incorporated herein by reference in their entireties.

8. INDUSTRIAL APPLICABILITY

The humanized monoclonal antibodies of the present invention inhibit the function of α9 integrin to exhibit therapeutic effects on cancer, e.g., the growth or metastasis of cancer cells, and an inflammatory disease, e.g., rheumatoid arthritis, osteoarthritis, hepatitis, bronchial asthma, fibrosis, diabetes mellitus, cancer metastasis, arteriosclerosis, multiple sclerosis, granuloma, an inflammatory bowel disease (ulcerative colitis and Crohn's disease), an autoimmune disease, and the like. The pharmaceutical composition comprising both the anti-α9 integrin antibody and anti-α4 integrin antibody of the present invention exerts more improved therapeutic effects on cancer and an inflammatory disease.

9. LIST OF SEQUENCES

The sequences referenced throughout the specification are summarized below.

SEQ ID NO.	TYPE	DESCRIPTION	SEQUENCE
1	AA	OPN adhesion sequence	GRGDS
2	AA	HuOPN's α4β1/α9β1-binding site	SVVYGLR
3	AA	MuOPN's α4β1/α9β1-binding site	SLAYGLR
4	AA	CDRH1 of 24I11 (FERM BP-10512)	DTYVH
5	AA	CDRH2 of 24I11 (FERM BP-10512)	NIDPANGNTKYDPKFQG
6	AA	CDRH3 of 24I11 (FERM BP-10512)	WLRHFYYAMDY
7	DNA	VH of 24I11 (FERM BP-10512) including sequence encoding signal peptide (1-57)	ATGAAATGCAGCTGGGTTATCTTCTTCCTGATG GCAGTGGTTACAGGGGTCAATTCAGAGGTTCA GCTGCAGCAGTCTGGGGCAGAGCTTGTGAAG CCAGGGGCCTCAGTCAAGTTGTCCTGCACAG CTTCTGGCTTCAACATTAAAGACACCTATGTG CACTGGGTGAAGCAGAGGCCTGAACAGGGCC TGGAGTGGATTGGAAATATTGATCCTGCGAAT GGTAATACTAAATATGACCCGAAGTTCCAGGG CAAGGCCACTATAACAGCAGACACATCCTCCA ACACAGCCTACCTGCACCTCAGCAGCCTGACA TCTGAGGACACTGCCGTCTATTACTGTGCTAG ATGGTTACGACATTTTTACTATGCTATGGACTA CTGGGGTCAAGGAACCTCAGTCACCGTCTCCT CA
8	AA	VH of 24I11 (FERM BP-10512) including signal peptide (1-19)	*MKCSWVIFFLMAVVTGVNS*EVQLQQSGAELVKP GASVKLSCTASGFNIKDTYVHWVKQRPEQGLE WIGNIDPANGNTKYDPKFQGKATITADTSSNTAY LHLSSLTSEDTAVYYCARWLRHFYYAMDYWGQ GTSVTVSS

9	AA	Mature VH of 24I11 (FERM BP-10512)	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTY VHWVKQRPEQGLEWIGNIDPANGNTKYDPKFQ GKATITADTSSNTAYLHLSSLTSEDTAVYYCARW LRHFYYAMDYWGQGTSVTVSS
10	AA	Signal peptide of 24I11 H-chain	MKCSWVIFFLMAVVTGVNS
11	AA	CDRL1 of 24I11 (FERM BP-10512)	RASENIYYSLA
12	AA	CDRL2 of 24I11 (FERM BP-10512)	NANSLED
13	AA	CDRL3 of 24I11 (FERM BP-10512)	KQAYDVPYT
14	DNA	VL of 24I11 (FERM BP-10512) including sequence encoding signal peptide (1-60)	ATGAGTGTGCCCACTCAACTCCTGGGGTTGCT GCTGCTGTGGCTTACAGACGCAGGATGTGACA TCCAGATGACTCAGTCTCCAGCCTCCCTGGCT GCATCTGTGGGAGAAACTGTCACCGACATCCA GATGACTCAGTCTCCAGCCTCCCTGGCTGCAT CTGTGGGAGAAACTGTCACCGGGAAATCTCCT CAGCTCCTGATCTATAATGCAAACAGCTTGGA AGATGGTGTCCCATCGAGGTTCAGTGGCAGTG GATCTGGGACACAGTATTCTATGAAGATCAAC AGCATGCAGCCTGAAGATACCGCAACTTATTT CTGTAAACAGGCTTATGACGTTCCGTACACGT TCGGAGGGGGGACCAAGCTGGAAATAAAA
15	AA	VL of 24I11 (FERM BP-10512) including signal peptide (1-20)	*MSVPTQLLGLLLLWLTDAGC*DIQMTQSPASLAAS VGETVTITCRASENIYYSLAWYQQKQGKSPQLL IYNANSLEDGVPSRFSGSGSGTQYSMKINSMQP EDTATYFCKQAYDVPYTFGGGTKLEIK
16	AA	Mature VL of 24I11 (FERM BP-10512)	DIQMTQSPASLAASVGETVTITCRASENIYYSLA WYQQKQGKSPQLLIYNANSLEDGVPSRFSGSGS GTQYSMKINSMQPEDTATYFCKQAYDVPYTFG GGTKLEIK
17	AA	Signal peptide of 24I11 L-chain	MSVPTQLLGLLLLWLTDAGC
18	DNA	X65891	ATGGACTGGACCTGGAGGGTCCTCTTTTTGGT GGCAGCAGCCACAGGTGCCCACTCCCAGGTC CAGCTTGTGCAGTCTGGGGCTGAGGTGAAGA AGCCTGGGGCCTCAGTGAAGGTTTCCTGCAA GGCTTCTGGATACACCTTCACTAGCTATGCTAT GCATTGGGTGCGCCAGGCCCCCGGACAAAGG

			CTTGAGTGGATGGGATGGATCAACGCTGGCAA TGGTAACACAAAATATTCACAGAAGTTCCAGG GCAGAGTCACCATTACCAGGGACACATCCGCG AGCACAGCCTACATGGAGCTGAGCAGCCTGA GATCTGAAGACACGGCTGTGTATTACTGTGCG AGAATACCCCGTATTAGCAGTGGCTGGTTGGG GGACTACTTTGACTACTGGGGCCAGGGAACCC TGGTCACCGTCTCCTCA
19	AA	FRH1 of X65891	QVQLVQSGAEVKKPGASVKVSCKASGYTFT
20	AA	FRH2 of X65891	WVRQAPGQRLEWMG
21	AA	FRH3 of X65891	RVTITRDTSASTAYMELSSLRSEDTAVYYCAR
22	AA	FRH4 of X65891	WGQGTLVTVSS
23	DNA	X72441	CGCTCAGCTCCTGGGGCTCCTGCTACTCTGGC TCCGAGGTGCCAGATGTGACATCCAGATGACC CAGTCTCCATCCTCCCTGTCTGCATCTGTAGGA GACAGAGTCACCATCACTTGCCGGGCAAGTC AGAGCATTAGCAGCTATTTAAATTGGTATCAGC AGAAACCAGGGAAAGCCCCTAAGCTCCTGAT CTATGCTGCATCCAGTTTGCAAAGTGGGGTCC CATCAAGGTTCAGTGGCAGTGGATCTGGGACA GATTTCACTCTCACCATCAGCAGTCTGCAACC TGAAGATTTTGCAACTTACTACTGTCAACAGA GTTACAGTACCCCTCGGACGTTCGGCCAAGGG ACCAAGGTGGAAATCAAA
24	AA	FRL1 of X72441	DIQMTQSPSSLSASVGDRVTITC
25	AA	FRL2 of X72441	WYQQKPGKAPKLLIY
26	AA	FRL3 of X72441	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
27	AA	FRL4 of X72441	FGQGTKVEIK
28	DNA	VH of Hu24I11	**CAGGTTCAGCTGGTGCAGTCTGGGGCAGA GGTGAAGAAGCCAGGGGCCTCAGTCAAGG TTTCCTGCAAGGCTTCTGGCTTCAACATTA** AAGACACCTATGTGCAC**TGGGTGCGCCAGG CCCCTGGACAGAGGCTGGAGTGGATTGGA** AATATTGATCCTGCGAATGGTAATACTAAATAT GACCCGAAGTTCCAGGGC**AAGGCCACTATAA CAGCAGACACATCCGCGAGCACAGCCTACA TGGAGCTCAGCAGCCTGAGATCTGAGGAC**

			ACTGCCGTCTATTACTGTGCTAGATGGTTAC GACATTTTTACTATGCTATGGACTACTGGGGTC AAGGAACCCTGGTCACCGTCTCCTCA
29	AA	VH of Hu24I11	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDT YVHWVRQAPGQRLEWIGNIDPANGNTKYDPKF QGKATITADTSASTAYMELSSLRSEDTAVYYCAR WLRHFYYAMDYWGQGTLVTVSS
30	DNA	VL of Hu24I11	GACATCCAGATGACTCAGTCTCCATCCTCCCT GTCTGCATCTGTGGGAGACAGAGTCACCATCA CATGTCGAGCAAGTGAGAACATTTACTACAGT TTAGCATGGTATCAGCAGAAGCCAGGGAAAG CCCCTAAGCTCCTGATCTATAATGCAAACAGCT TGGAAGATGGTGTCCCATCGAGGTTCAGTGGC AGTGGATCTGGGACACAGTATACTCTCACCAT CAGCAGCCTGCAGCCTGAAGATTTTGCAACTT ATTACTGTAAACAGGCTTATGACGTTCCGTAC ACGTTCGGACAAGGGACCAAGGTGGAAATCA AA
31	AA	VL of Hu24I11	DIQMTQSPSSLSASVGDRVTITCRASENIYYSLA WYQQKPGKAPKLLIYNANSLEDGVPSRFSGSGS GTQYTLTISSLQPEDFATYYCKQAYDVPYTFGQ GTKVEIK
32	AA	CDRH1 of 1K11 (FERM BP-10510)	DYNMD
33	AA	CDRH2 of 1K11 (FERM BP-10510)	DINPNNGGTIYNQKFQG
34	AA	CDRH3 of 1K11 (FERM BP-10510)	SGVISTDY
35	DNA	VH of 1K11 (FERM BP-10510)	GTGCAGCTGCAGGAGTCAGGACCTGAGCTGG TGAAGCCTGGGGCTTCAGTGAAGATACCCTGC AAGGCTTCTGGATACACATTCACTGACTACAA CATGGACTGGGTGAAGCAGAGCCATGGAAAG AGCCTTGAGTGGATTGGAGATATTAATCCTAAC AACGGTGGTACAATCTACAACCAGAAGTTCCA GGGCAAGGCCACATTGACTGTAGACAAGTCC TCCAGCACAGCCTACATGGAGCTCCGCAGCCT GACATCTGAGGACACTGCAGTCTATTACTGTG CAAGATCGGGGGTTATTAGTACGGACTACTGG GGCCAAGGGACCACGGTCACCGTCTCCTCA
36	AA	Mature VH of 1K11 (FERM BP-10510) starting from	?VQLQESGPELVKPGASVKIPCKASGYTFTDYN MDWVKQSHGKSLEWIGDINPNNGGTIYNQKFQ GKATLTVDKSSSTAYMELRSLTSEDTAVYYCARS GVISTDYWGQGTTVTVSS

		2nd residue according to Kabat numbering	
37	AA	CDRL1 of 1K11 (FERM BP-10510)	RASQEISGYLI
38	AA	CDRL2 of 1K11 (FERM BP-10510)	AASTLDS
39	AA	CDRL3 of 1K11 (FERM BP-10510)	LQYANYPPT
40	DNA	VL of 1K11 (FERM BP-10510)	GACATCCAGATGACACAGTCTCCACCCTCCCT ATCTGCCTCTCTGGGAGAAAGAGTCAGTCTCA CTTGTCGGGCAAGTCAGGAAATTAGTGGTTAC TTAATCTGGCTTCAACAGAAACCAGATGGAAC TATTCAACGCCTGATCTACGCCGCATCCACTTT AGATTCTGGTGTCCCAAAAAGGTTCAGTGGCA GTAGGTCTGGGTCAGATTATTCTCTCACCATCA GCAGCCTTGAGTCTGAAGATTTTGCAGACTAT TACTGTCTACAATATGCTAATTATCCTCCGACG TTCGGTGGAGGCACCAAGCTGGAAATCAAAC GG
41	AA	Mature VL of 1K11 (FERM BP-10510)	DIQMTQSPPSLSASLGERVSLTCRASQEISGYLIW LQQKPDGTIQRLIYAASTLDSGVPKRFSGSRSGS DYSLTISSLESEDFADYYCLQYANYPPTFGGGTK LEIKR
42	AA	CDRH1 of 21C5 (FERM BP-10511)	DYYMY
43	AA	CDRH2 of 21C5 (FERM BP-10511)	TISDGGNYTYYPDSVKG
44	AA	CDRH3 of 21C5 (FERM BP-10511)	DRDGSSLFAY
45	DNA	VH of 21C5 (FERM BP-10511)	GTGCAGCTGCAGGAGTCTGGGGGAGGCTTAG TGAAGCCTGGAGGGTCCCTGAAACTCTCCTGT GCAGCCTCTGGATTCACTTTCAGTGACTATTAC ATGTATTGGGTTCGCCAGACTCCGGAAAAGAG GCTGGAGTGGGTCGCAACCATTAGTGATGGTG GTAATTACACCTACTATCCAGACAGTGTGAAG GGGCGATTCACCATCTCCAGAGACAATGCCAA GAATAACCTGTACCTGCAAATGAGCAGTCTGA AGTCTGAGGACACAGCCATGTATTACTGTGCA

			AGAGATCGGGACGGTAGTAGCCTGTTTGCTTA CTGGGGCCAAGGGACCACGGTCACCGTCTCC TCA
46	AA	Mature VH of 21C5 (FERM BP-10511) starting from 2nd residue according to Kabat numbering	?VQLQESGGGLVKPGGSLKLSCAASGFTFSDYY MYWVRQTPEKRLEWVATISDGGNYTYYPDSVK GRFTISRDNAKNNLYLQMSSLKSEDTAMYYCA RDRDGSSLFAYWGQGTTVTVSS
47	AA	CDRL1 of 21C5 (FERM BP-10511)	KASQDVNIAVA
48	AA	CDRL2 of 21C5 (FERM BP-10511)	WASTRHT
49	AA	CDRL3 of 21C5 (FERM BP-10511)	QQHYNTPW
50	DNA	VL of 21C5 (FERM BP-10511)	CATCCAGATGACACAGTCTCCAAATTCATGTC CACATCAGTAGGAGACAGGGTCAGCATCACCT GCAAGGCCAGTCAGGATGTGAATATTGCTGTA GCCTGGTATCAACAAAGACCAGGGCAATCTCC TAAACTACTGATTTACTGGGCATCCACCCGGC ACACTGGAGTCCCTGATCGCTTCACAGGCAGT GGATCTGGGACAGATTATACTCTCACCATCAG CAGTGTGCAGGCTGAAGACCTGGCACTTTATT ACTGTCAGCAACATCATAACACTCCGTGGACG TTCGGTGGAGGCACCAAGCTGGAAATCAAAC GG
51	AA	Mature VL of 21C5 (FERM BP-10511)	HPDDTVSKFMSTSVGDRVSITCKASQDVNIAVA WYQQRPGQSPKLLIYWASTRHTGVPDRFTGSGS GTDYTLTISSVQAEDLALYYCQQHYNTPWTFGG GTKLEIKR
52	AA	CDRH1 of 25B6 (FERM BP-10513)	SYGVH
53	AA	CDRH2 of 25B6 (FERM BP-10513)	VIWSGGSTNYNSALMS
54	AA	CDRH3 of 25B6 (FERM BP-10513)	DYGNYPWFAY

55	DNA	VH of 25B6 (FERM BP-10513)	GTCAAGCTGCAGCAGTCTGGACCTGGCCTGG TGGCGCCCTCACAGAGCCTGTCCATCACTTGC ACTGTCTCTGGGTTTTCATTAACCAGTTATGGT GTACACTGGGTTCGCCAGCCTCCAGGAAAGG GTCTGGAGTGGCTGGGAGTAATATGGTCTGGT GGAAGCACAAATTATAATTCGGCTCTCATGTCC AGACTGAGCATCAGTAAAGACAATTTTAAGAG CCAAGTTTTCTTAAAAATGAACAGTCTGCAAA CTGATGACACAGCCATATACTACTGTGCCAGA GACTATGGTAACTACCCCTGGTTTGCTTACTGG GGCCAAGGGACCACGGTCACCGTCTCCTCA
56	AA	Mature VH of 25B6 (FERM BP-10513) starting from 2nd residue according to Kabat numbering	?VKLQQSGPGLVAPSQSLSITCTVSGFSLTSYGV HWVRQPPGKGLEWLGVIWSGGSTNYNSALMS RLSISKDNFKSQVFLKMNSLQTDDTAIYYCARD YGNYPWFAYWGQGTTVTVS
57	AA	CDRL1 of 25B6 (FERM BP-10513)	KASQDVNTAVA
58	AA	CDRL2 of 25B6 (FERM BP-10513)	SASYRYT
59	AA	CDRL3 of 25B6 (FERM BP-10513)	QQHYSTPCA
60	DNA	VL of 25B6 (FERM BP-10513)	CATCCAGATGACACAGTCTCCAAATTCATGTC CACATCAGTAGGAGACAGGGTCAGCATCACCT GCAAGGCCAGTCAGGATGTGAATACTGCTGTG GCCTGGTATCAACAGAAACCAGGACAATCCCC TAAACTACTGATTTACTCGGCATCCTACCGGTA CACTGGAGTCCCTGATCGCTTCACTGGCAGTG GATCTGGGACGGATTTCACTTTCACCATCAGC AGTGTGCAGGCTGAAGACCTGGCAGTTTATTA CTGTCAGCAACATTATAGTACTCCGTGCGCGTT CGGAGGGGGGACAAAGTTGGAAATAAAACGG
61	AA	Mature VL of 25B6 (FERM BP-10513)	HPDDTVSKFMSTSVGDRVSITCKASQDVNTAVA WYQQKPGQSPKLLIYSASYRYTGVPDRFTGSGS GTDFTFTISSVQAEDLAVYYCQQHYSTPCAFGG GTKLEIKR
62	AA	CDRH1 of 28S1 (FERM BP- 10832)	GYGVN

63	AA	CDRH2 of 28S1 (FERM BP- 10832)	MIWGDGITEYNSALKS
64	AA	CDRH3 of 28S1 (FERM BP- 10832)	RDASSGYGFA
65	DNA	VH of 28S1 (FERM BP-10832)	AGGTGAAGCTGCAGGAGTCAGGACCTGGCCT GGTGGCGCCCTCACAGAGCCTGTCCATCACAT GCACCGTCTCAGGGTTCTCATTAACCGGCTAT GGTGTAAACTGGGTTCGCCAGCCTCCAGGAA AGGGTCTGGAGTGGCTGGGAATGATATGGGGT GATGGAATCACAGAGTATAATTCAGCTCTCAA ATCCAGACTGAGCATCAGCAAGGACAACTCC AAGAGCCAAGTTTTCTTAAAAATGAACAGTCT GCAAACTGATGACACAGCCAGGTACTACTGTG CCAGAGATGCCAGCTCGGGCTACGGGTTTGCT TACTGGGGCCAAGGGACCACGGTCACCGTCT CCTCA
66	AA	Mature VH of 28S1 (FERM BP- 10832) starting from 2nd residue according to Kabat numbering	?VKLQESGPGLVAPSQSLSITCTVSGFSLTGYGV NWVRQPPGKGLEWLGMIWGDGITEYNSALKSR LSISKDNSKSQVFLKMNSLQTDDTARYYCARDA SSGYGFAYWGQGTTVTVSS
67	AA	CDRL1 of 28S1 (FERM BP-10832)	TASSSVSSSYLH
68	AA	CDRL2 of 28S1 (FERM BP-10832)	STSNLAS
69	AA	CDRL3 of 28S1 (FERM BP-10832)	HQYHRSPYT
70	DNA	VL of 28S1 (FERM BP-10832)	TACATTGTGCTGACCCAGTCTCCAGCAATCAT GTCTGCATCTCTAGGGGAACGGGTCACCATGA CCTGCACTGCCAGCTCAAGTGTAAGTTCCAGT TACTTGCACTGGTACCAGCAGAAGCCAGGATC CTCCCCCAAACTCTGGATTTATAGCACATCCAA CCTGGCTTCTGGAGTCCCAGCTCGCTTCAGTG GCAGTGGGTCTGGGACCTCTTACTCTCTCACA ATCAGCAGCATGGAGGCTGAAGATGCTGCCAC TTATTACTGCCACCAGTATCATCGTTCCCCGTA CACGTTCGGAGGGGGGACAAAGTTGGAAATA AAACGG

71	AA	Mature VL of 28S1 (FERM BP- 10832)	YIVLTQSPAIMSASLGERVTMTCTASSSVSSSYL HWYQQKPGSSPKLWIYSTSNLASGVPARFSGSG SGTSYSLTISSMEAEDAATYYCHQYHRSPYTFG GGTKLEIKR
72	DNA	VH of 24I11 (FERM BP-10512) including sequence encoding signal peptide (1-57), flanked by SpeI and HindIII sites	ACTAGTACCACCATGAAATGCAGCTGGGTTAT CTTCTTCCTGATGGCAGTGGTTACAGGGGTCA ATTCAGAGGTTCAGCTGCAGCAGTCTGGGGC AGAGCTTGTGAAGCCAGGGGCCTCAGTCAAG TTGTCCTGCACAGCTTCTGGCTTCAACATTAA AGACACCTATGTGCACTGGGTGAAGCAGAGG CCTGAACAGGGCCTGGAGTGGATTGGAAATAT TGATCCTGCGAATGGTAATACTAAATATGACCC GAAGTTCCAGGGCAAGGCCACTATAACAGCA GACACATCCTCCAACACAGCCTACCTGCACCT CAGCAGCCTGACATCTGAGGACACTGCCGTCT ATTACTGTGCTAGATGGTTACGACATTTTTACT ATGCTATGGACTACTGGGGTCAAGGAACCTCA GTCACCGTCTCCTCAGGTAAGAATGGCCTCTA AGCTT
73	DNA	VL of 24I11 (FERM BP-10512) including sequence encoding signal peptide (1-60), flanked by NheI and EcoRI sites	GCTAGCACCACCATGAGTGTGCCCACTCAACT CCTGGGGTTGCTGCTGCTGTGGCTTACAGACG CAGGATGTGACATCCAGATGACTCAGTCTCCA GCCTCCCTGGCTGCATCTGTGGGAGAAACTGT CACCGACATCCAGATGACTCAGTCTCCAGCCT CCCTGGCTGCATCTGTGGGAGAAACTGTCACC GGGAAATCTCCTCAGCTCCTGATCTATAATGCA AACAGCTTGGAAGATGGTGTCCCATCGAGGTT CAGTGGCAGTGGATCTGGGACACAGTATTCTA TGAAGATCAACAGCATGCAGCCTGAAGATACC GCAACTTATTTCTGTAAACAGGCTTATGACGTT CCGTACACGTTCGGAGGGGGGACCAAGCTGG AAATAAAACGTAAGTAGTCTTCTCAGAATTC
74	DNA	Fig. 10 (w/ 5'-GGG & CCC-) and Fig. 12 Hu24I11 VH gene flanked by SpeI and HindIII sites	ACTAGTACCACCATGAAATGCAGCTGGGTTAT CTTCTTCCTGATGGCAGTGGTTACAGGGGTCA ATTCACAGGTTCAGCTGGTGCAGTCTGGGGCA GAGGTGAAGAAGCCAGGGGCCTCAGTCAAGG TTTCCTGCAAGGCTTCTGGCTTCAACATTAAA GACACCTATGTGCACTGGGTGCGCCAGGCCCC TGGACAGAGGCTGGAGTGGATTGGAAATATTG ATCCTGCGAATGGTAATACTAAATATGACCCGA AGTTCCAGGGCAAGGCCACTATAACAGCAGA CACATCCGCGAGCACAGCCTACATGGAGCTCA GCAGCCTGAGATCTGAGGACACTGCCGTCTAT TACTGTGCTAGATGGTTACGACATTTTTACTAT GCTATGGACTACTGGGGTCAAGGAACCCTGGT CACCGTCTCCTCAGGTGAGTCCTCACAAAAGC TT
75	DNA	Fig. 11 (w/	GCTAGCACCACCATGAGTGTGCCCACTCAACT

		5'-GGG & CCC-) and Fig. 13 Hu24I11 VL gene flanked by NheI and EcoRI sites	CCTGGGGTTGCTGCTGCTGTGGCTTACAGACG CACGATGTGACATCCAGATGACTCAGTCTCCA TCCTCCCTGTCTGCATCTGTGGGAGACAGAGT CACCATCACATGTCGAGCAAGTGAGAACATTT ACTACAGTTTAGCATGGTATCAGCAGAAGCCA GGGAAAGCCCCTAAGCTCCTGATCTATAATGC AAACAGCTTGGAAGATGGTGTCCCATCGAGGT TCAGTGGCAGTGGATCTGGGACACAGTATACT CTCACCATCAGCAGCCTGCAGCCTGAAGATTT TGCAACTTATTACTGTAAACAGGCTTATGACGT TCCGTACACGTTCGGACAAGGGACCAAGGTG GAAATCAAACGTGAGTAGAATTTAAAGAATTC
76	AA	Human α9 integrin (signal peptide; 1-29 residues; in italic)	*MGGPAAPRGAGRLRALLLALVVAGIPAGA*YNLDPQ RPVHFQGPADSFFGYAVLEHFHDNTRWVLVGAP KADSKYSPSVKSPGAVFKCRVHTNPDRRCTELD MARGKNRGTSCGKTCREDRDDEWMGVSLARQ PKADGRVLACAHRWKNIYYEADHILPHGFCYII PSNLQAKGRTLIPCYEEYKKKYGEEHGSCQAGI AGFFTEELVVMGAPGSFYWAGTIKVLNLTDNTY LKLNDEVIMNRRYTYLGYAVTAGHFSHPSTIDV VGGAPQDKGIGKVYIFRADRRSGTLIKIFQASGK KMGSYFGSSLCAVDLNGDGLSDLLVGAPMFSEI RDEGQVTVYINRGNGALEEQLALTGDGAYNAH FGESIASLDDLDNDGFPDVAIGAPKEDDFAGAV YIYHGDAGGIVPQYSMKLSGQKINPVLRMFGQS ISGGIDMDGNGYPDVTVGAFMSDSVVLLRARP VITVDVSIFLPGSINITAPQCHDGQQPVNCLNVT TCFSFHGKHVPGEIGLNYVLMADVAKKEKGQM PRVYFVLLGETMGQVTEKLQLTYMEETCRHYV AHVKRRVQDVISPIVFEAAYSLSEHVTGEEEREL PPLTPVLRWKKGQKIAQKNQTVFERNCRSEDCA ADLQLQGKLLLSSMDEKTLYLALGAVKNISLNI SISNLGDDAYDANVSFNVSRELFFINMWQKEEM GISCELLESDFLKCSVGFPFMRSKSKYEFSVIFDT SHLSGEEEVLSFIVTAQSGNTERSESLHDNTLVL MVPLMHEVDTSITGIMSPTSFVYGESVDAANFI QLDDLECHFQPINITLQVYNTGPSTLPGSSVSISF PNRLSSGGAEMFHVQEMVVGQEKGNCSFQKNP TPCIIPQEQENIFHTIFAFFTKSGRKVLDCEKPGIS CLTAHCNFSALAKEESRTIDIYMLLNTEILKKDS SSVIQFMSRAKVKVDPALRVVEIAHGNPEEVTV VFEALHNLEPRGYVVGWIIAISLLVGILIFLLLAV LLWKMGFFRRRYKEIIEAEKNRKENEDSWDWV QKNQ
77	AA	Partial aa sequence of huα9 integrin	FQGPADSFFGYA
78	AA	Partial aa sequence of	KSPGAVFKCRVHTNPDRR

		huα9 integrin	
79	AA	Partial aa sequence of huα9 integrin	WMGVSLARQPKADGRVLA
80	aa	Partial aa sequence of huα9 integrin	CAHRWKNIYYEADHI
81	aa	Partial aa sequence of huα9 integrin	GFCYIIPSNLQAKGRTLI
82	aa	Partial aa sequence of huα9 integrin	VMGAPGSFYWAGTIKVLN
83	aa	Partial aa sequence of huα9 integrin	VIMNRRYTYLGYAVT
84	aa	Partial aa sequence of huα9 integrin	VYIFRADRRSGTLIKIFQ
85	aa	Partial aa sequence of huα9 integrin	QYSMKLSGQKINPVLRMFGQSISG
86	aa	Partial aa sequence of huα9 integrin	VVLLRARPVITVDVSIFL
87	aa	Partial aa sequence of huα9 integrin	RHYVAHVKRRVQDVISPI
88	aa	Partial aa sequence of huα9 integrin	ELPPLTPVLRWKKGQKIAQKNQTVFERNCR
89	aa	Partial aa sequence of huα9 integrin	YLALGAVKNISL
90	aa	Partial aa sequence of huα9 integrin	CSVGFPFMRSKSKYEFSV
91	aa	Partial aa sequence of huα9 integrin	SSSVIQFMSRAKVKVDPALRV
92	aa	Tenascin-C huα9-binding site	AEIDGIEL

93	aa	Human fibronectin huα9-binding site	CPEDGIHELFP
94	DNA	GeneRacer 5' primer	CGACTGGAGCACGAGGACACTGA
95	DNA	VH primer	GCCAGTGGATAGACAGATGG
96	DNA	VL primer	GATGGATACAGTTGGTGCAGC
97	DNA	VH 5' primer w/ SpeI site	GGGACTAGTACCACCATGAAATGCAGCTGGGT TATCTTC
98	DNA	VH primer w/ HindIII site	GGGAAGCTTAGAGGCCATTCTTACCTGAGGA GACGGTGACTGAGGTTCC3
99		VL 5' primer w/ NheI site	GGGGCTAGCACCACCATGAGTGTGCCCACTCA ACTCCTG
100		VL 5' primer w/ EcpRI site	GGGGAATTCTGAGAAGACTACTTACGTTTTAT TTCCAGCTTGGTCCCCCC
101	DNA	JNJ120	GGGACTAGTACCACCATGAAATGCAGC
102	DNA	JNJ137	GGGACTAGTACCACCATGAAATGCAGCTGGGT TATCTTCTTCCTGATGGCAGTGGTT
103	DNA	JNJ138	AGACTGCACCAGCTGAACCTGTGAATTGACCC CTGTAACCACTGCCATCAGGAAGAA
104	DNA	JNJ139	CAGGTTCAGCTGGTGCAGTCTGGGGCAGAGG TGAAGAAGCCAGGGGCCTCAGTCAAG
105	DNA	JNJ140	GTCTTTAATGTTGAAGCCAGAAGCCTTGCAGG AAACCTTGACTGAGGCCCCTGGCTT
106	DNA	JNJ141	TCTGGCTTCAACATTAAAGACACCTATGTGCA CTGGGTGCGCCAGGCCCCTGGACAGAGG
107	DNA	JNJ142	ACCATTCGCAGGATCAATATTTCCAATCCACTC CAGCCTCTGTCCAGGGGCCTGGCG
108	DNA	JNJ143	AATATTGATCCTGCGAATGGTAATACTAAATAT GACCCGAAGTTCCAGGGCAAGGCCACT
109	DNA	JNJ144	CATGTAGGCTGTGCTCGCGGATGTGTCTGCTG TTATAGTGGCCTTGCCCTGGAACTT
110	DNA	JNJ145	TCCGCGAGCACAGCCTACATGGAGCTCAGCA GCCTGAGATCTGAGGACACTGCCGTC
111	DNA	JNJ146	ATAGTAAAAATGTCGTAACCATCTAGCACAGT AATAGACGGCAGTGTCCTCAGA
112	DNA	JNJ147	TGGTTACGACATTTTTACTATGCTATGGACTAC TGGGGTCAAGGAACCCTGGTCACC
113	DNA	JNJ148	GGGAAGCTTTTGTGAGGACTCACCTGAGGAG

			ACGGTGACCAGGGTTCCTTGACC
114	DNA	JNJ149	GGGAAGCTTTTGTGAGGACTC
115	DNA	JNJ150	GGGGCTAGCACCACCATGAGT
116	DNA	JNJ126	GGGGCTAGCACCACCATGAGTGTGCCCACTCA ACTCCTGGGGTTGCTGCTGCTGTGG
117	DNA	JNJ127	AGACTGAGTCATCTGGATGTCACATCGTGCGT CTGTAAGCCACAGCAGCAGCAACCCCAG
118	DNA	JNJ128	GACATCCAGATGACTCAGTCTCCATCCTCCCT GTCTGCATCTGTGGGAGACAGA
119	DNA	JNJ129	GTAAATGTTCTCACTTGCTCGACATGTGATGGT GACTCTGTCTCCCACAGATGCAGA
120	DNA	JNJ130	CGAGCAAGTGAGAACATTTACTACAGTTTAGC ATGGTATCAGCAGAAGCCAGGGAAA
121	DNA	JNJ131	CAAGCTGTTTGCATTATAGATCAGGAGCTTAG GGGCTTTCCCTGGCTTCTGCTGATA
122	DNA	JNJ132	ATCTATAATGCAAACAGCTTGGAAGATGGTGT CCCATCGAGGTTCAGTGGCAGTGGA
123	DNA	JNJ133	CAGGCTGCTGATGGTGAGAGTATACTGTGTCC CAGATCCACTGCCACTGAACCTCGA
124	DNA	JNJ134	ACTCTCACCATCAGCAGCCTGCAGCCTGAAGA TTTTGCAACTTATTACTGTAAACAG
125	DNA	JNJ135	GGTCCCTTGTCCGAACGTGTACGGAACGTCAT AAGCCTGTTTACAGTAATAAGTTGC
126	DNA	JNJ136	TACACGTTCGGACAAGGGACCAAGGTGGAAA TCAAACGTGAGTAG
127	DNA	JNJ101	GGGGAATTCTTTAAATTCTACTCACGTTTGATT TCCA
128	DNA	JNJ117	GGGGAATTCTTTAAATTCTA
129	DNA	Primer for mouse γ1, γ2a, γ2b and γ3 H-chains	GCCAGTGGATAGACTGATGG
130	DNA	Primer for mouse κ L-chain primer	GATGGATACAGTTGGTGCAGC

CLAIMS

1. A humanized antibody or an antigen-binding fragment thereof that immunospecifically recognizes human α9 integrin, comprising:

(i) a H-chain comprising at least one FRH derived from a VH region of a human antibody, and at least one CDRH derived from at least one of CDRHs of a non-human antibody that immunospecifically recognizes human α9 integrin; or

(ii) a L-chain comprising at least one FRL derived from a VL region of a human antibody, and at least one CDRL derived from at least one of CDRLs of a non-human antibody that immunospecifically recognizes human α9 integrin; or

(iii) both (i) and (ii) above.

2. The humanized antibody or an antigen-binding fragment thereof of claim 1, wherein said non-human antibody is a mouse monoclonal antibody produced by a hybridoma selected from the group consisting of Depository Accession Nos. FERM BP-10510, FERM BP-10511, FERM BP-10512, FERM BP-10513 and FERM BP-10832.

3. The humanized antibody or an antigen-binding fragment thereof of claim 1, wherein at least one CDRH of said humanized antibody comprises an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:4, 5 and 6, and at least one CDRL of said humanized antibody comprises an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:11, 12 and 13.

4. The humanized antibody or an antigen-binding fragment thereof of claim 2 or 3, wherein said VH region of a human antibody comprises an amino acid sequence derived from the amino acid sequence encoded by the nucleotide sequence of GenBank Accession No. X65891 (SEQ ID NO:18), and said VL region of a human antibody comprises an amino acid sequence derived from the amino aid sequence encoded by the

nucleotide sequence of GenBank Accession No. X72441 (SEQ ID NO:23).

5. The humanized antibody or an antigen-binding fragment thereof of claim 4, wherein said H-chain comprises the amino acid sequence of SEQ ID NO:29 and said L-chain comprises the amino acid sequence of SEQ ID NO:31.

6. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:29.

7. The nucleic acid molecule of claim 6, wherein said nucleotide sequence has the nucleotide sequence of SEQ ID NO:28.

8. The nucleic acid molecule of claim 6 further comprising a nucleotide sequence encoding a signal peptide.

9. The nucleic acid molecule of claim 8, wherein said signal peptide comprises the amino acid sequence of SEQ ID NO:10.

10. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:31.

11. The nucleic acid molecule of claim 10, wherein said nucleotide sequence has the nucleotide sequence of SEQ ID NO:30.

12. The nucleic acid molecule of claim 10 further comprising a nucleotide sequence encoding a signal peptide.

13. The nucleic acid molecule of claim 12, wherein said signal peptide comprises the amino acid sequence of SEQ ID NO:17.

14. A vector comprising the nucleic acid molecule of claim 6 or 10, or both, wherein said nucleic acid molecule is operably linked to one or more regulatory elements.

15. An isolated host cell comprising the vector of claim 14.

16. A method for preparing a humanized antibody or an antigen-binding fragment thereof, comprising culturing the host cell of claim 15 under conditions so that the humanized antibody or an antigen-binding fragment thereof is expressed, and

collecting the expressed humanized antibody.

17. A pharmaceutical composition comprising the humanized antibody or an antigen-binding fragment thereof of any one of claims 1, 2 or 5, and a pharmaceutically acceptable carrier.

18. A method for preventing or treating a disorder or disease associated with α9 integrin, said method comprising administering an effective amount of the humanized antibody or an antigen-binding fragment thereof of claim 5 to a subject in need thereof.

19. A method for diagnosing a disorder or disease associated with α9 integrin *in vivo*, said method comprising administering an effective amount of the humanized antibody or an antigen-binding fragment thereof of claim 1 or 2 to a subject to be examined.

20. A humanized antibody or an antigen-binding fragment thereof that immunospecifically recognizes human α9 integrin, comprising:

(i) a H-chain comprising at least one FRH derived from a VH region of a human antibody, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:32, 33 and 34; or

(ii) a L-chain comprising at least one FRL derived from a VL region of a human antibody, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS:37, 38 and 39; or

(iii) both (i) and (ii) above.

21. A humanized antibody or an antigen-binding fragment thereof that immunospecifically recognizes human α9 integrin, comprising:

(i) a H-chain comprising at least one FRH derived from a VH region of a human antibody, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid

sequences of SEQ ID NOS:42, 43 and 44; or

(ii) a L-chain comprising at least one FRL derived from a VL region of a human antibody, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 47, 48 and 49; or

(iii) both (i) and (ii) above.

22. A humanized antibody or an antigen-binding fragment thereof that immunospecifically recognizes human α9 integrin, comprising:

(i) a H-chain comprising at least one FRH derived from a VH region of a human antibody, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 52, 53 and 54; or

(ii) a L-chain comprising at least one FRL derived from a VL region of a human antibody, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 57, 58 and 59; or

(iii) both (i) and (ii) above.

23. A humanized antibody or an antigen-binding fragment thereof that immunospecifically recognizes human α9 integrin, comprising:

(i) a H-chain comprising at least one FRH derived from a VH region of a human antibody, and at least one CDRH comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 62, 63 and 64; or

(ii) a L-chain comprising at least one FRL derived from a VL region of a human antibody, and at least one CDRL comprising an amino acid sequence selected from the group consisting of the amino acid sequences of SEQ ID NOS: 67, 68 and 69; or

(iii) both (i) and (ii) above.

Fig. 1

ATGAAATGCAGCTGGGTTATCTTCTTCCTGATGGCAGTGGTTACAGGGGTCAATTCAGAG
M K C S W V I F F L M A V V T G V N S E

GTTCAGCTGCAGCAGTCTGGGGCAGAGCTTGTGAAGCCAGGGGCCTCAGTCAAGTTGTCC
V Q L Q Q S G A E L V K P G A S V K L S

TGCACAGCTTCTGGCTTCAACATTAAAGACACCTATGTGCACTGGGTGAAGCAGAGGCCT
C T A S G F N I K D T Y V H W V K Q R P

GAACAGGGCCTGGAGTGGATTGGAAATATTGATCCTGCGAATGGTAATACTAAATATGAC
E Q G L E W I G N I D P A N G N T K Y D

CCGAAGTTCCAGGGCAAGGCCACTATAACAGCAGACACATCCTCCAACACAGCCTACCTG
P K F Q G K A T I T A D T S S N T A Y L

CACCTCAGCAGCCTGACATCTGAGGACACTGCCGTCTATTACTGTGCTAGATGGTTACGA
H L S S L T S E D T A V Y Y C A R W L R

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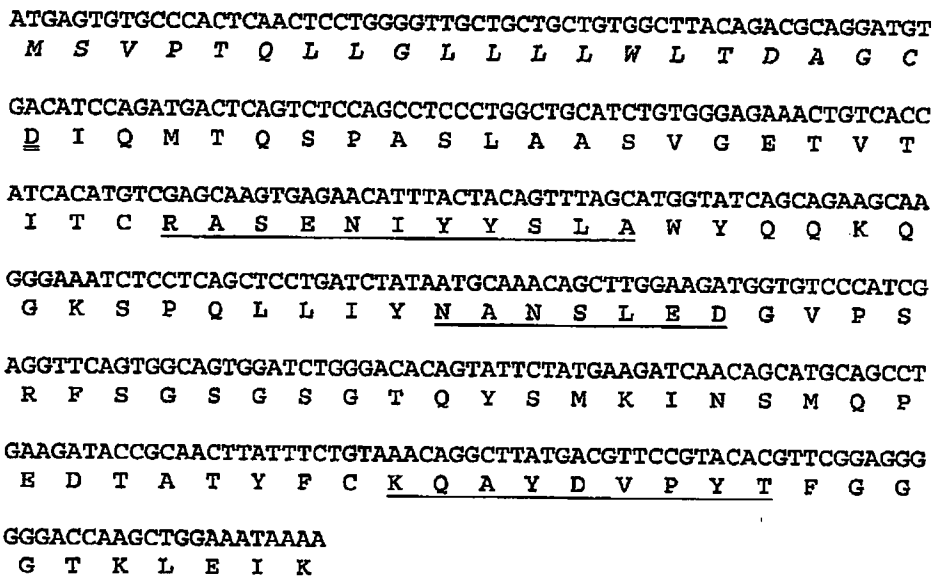
H F Y Y A M D Y W G Q G T S V T V S S

Fig. 2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D I Q M T Q S P A S L A A S V G E T V T
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I T C R A S E N I Y Y S L A W Y Q Q K Q
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G K S P Q L L I Y N A N S L E D G V P S
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R F S G S G S G T Q Y S M K I N S M Q P
GAAGATACCGCAACTTATTTCTGTAAACAGGCTTATGACGTTCCGTACACGTTCGGAGGG
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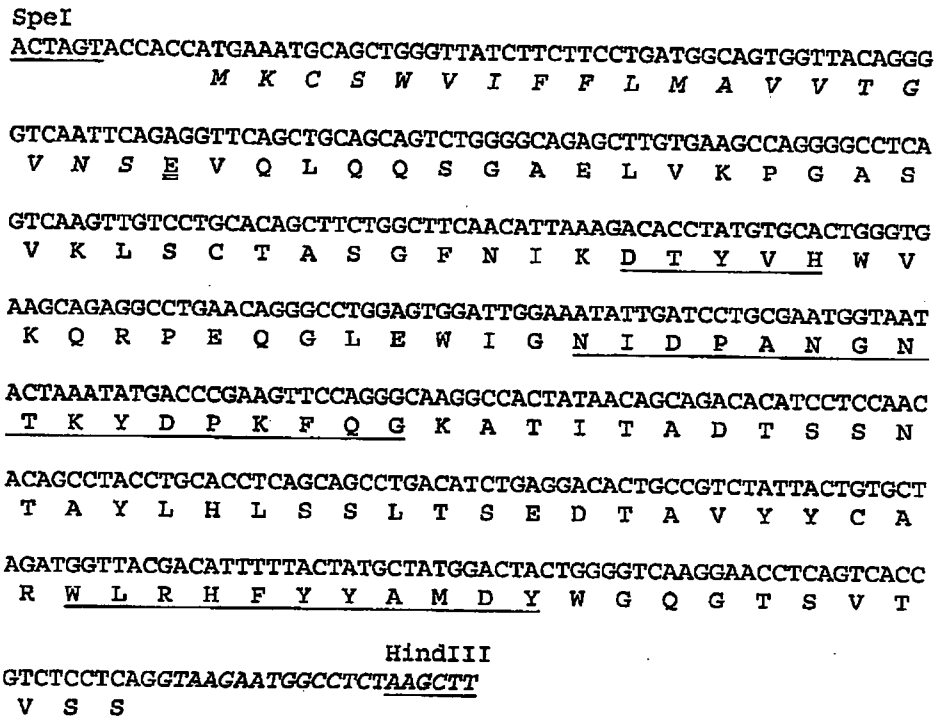
G T K L E I K

Fig. 3


SpeI
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V N S E V Q L Q Q S G A E L V K P G A S
GTCAAGTTGTCCTGCACAGCTTCTGGCTTCAACATTAAAGACACCTATGTGCACTGGGTG
V K L S C T A S G F N I K D T Y V H W V
AAGCAGAGGCCTGAACAGGGCCTGGAGTGGATTGGAAATATTGATCCTGCGAATGGTAAT
K Q R P E Q G L E W I G N I D P A N G N
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T K Y D P K F Q G K A T I T A D T S S N
ACAGCCTACCTGCACCTCAGCAGCCTGACATCTGAGGACACTGCCGTCTATTACTGTGCT
T A Y L H L S S L T S E D T A V Y Y C A
AGATGGTTACGACATTTTTACTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACC
R W L R H F Y Y A M D Y W G Q G T S V T
HindIII
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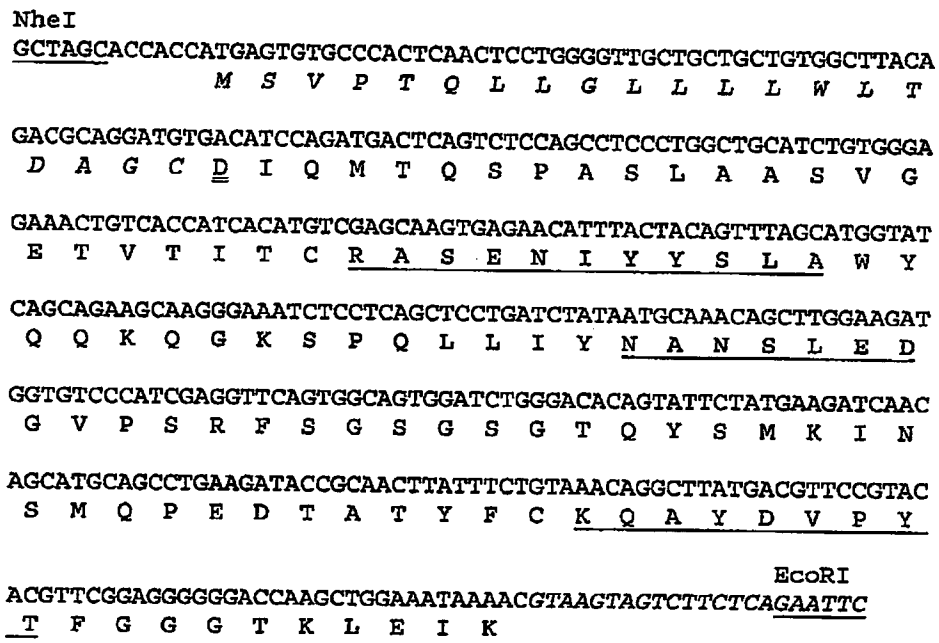
V S S

Fig. 4


NheI
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M S V P T Q L L G L L L L W L T
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D A G C D I Q M T Q S P A S L A A S V G
GAAACTGTCACCATCACATGTCGAGCAAGTGAGAACATTTACTACAGTTTAGCATGGTAT
E T V T I T C R A S E N I Y Y S L A W Y
CAGCAGAAGCAAGGGAAATCTCCTCAGCTCCTGATCTATAATGCAAACAGCTTGGAAGAT
Q Q K Q G K S P Q L L I Y N A N S L E D
GGTGTCCCATCGAGGTTCAGTGGCAGTGGATCTGGGACACAGTATTCTATGAAGATCAAC
G V P S R F S G S G S G T Q Y S M K I N
AGCATGCAGCCTGAAGATACCGCAACTTATTTCTGTAAACAGGCTTATGACGTTCCGTAC
S M Q P E D T A T Y F C K Q A Y D V P Y
EcoRI
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T F G G G T K L E I K

Fig. 5

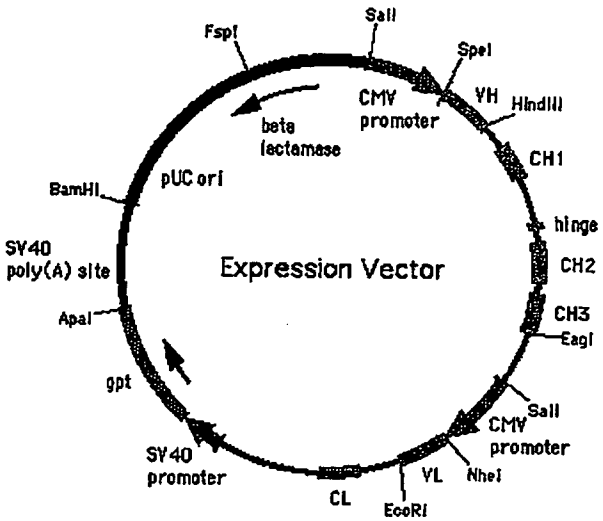
FspI
SalI
SpeI
CMV promoter
VH
HindIII
CH1
hinge
CH2
CH3
EagI
SalI
CMV promoter
NheI
VL
EcoRI
CL
SV40 promoter
gpt
ApaI
SV40 poly(A) site
BamHI
pUC ori
beta lactamase
Expression Vector

Fig. 6

```
                    1          2          3
          123456789 0123456789 0123456789 0123456789
24I11 VH  EVQLQQSGA ELVKPGASVK LSCTASGFNI KDTYVHWVKQ
Hu24I11 VH QVQLVQSGA EVKKPGASVK VSCKASGFNI KDTYVHWVRQ
X65891    QVQLVQSGA EVKKPGASVK VSCKASGYTF T-----WVRQ

          4          5          6          7
          0123456789 01223456789 0123456789 0123456789
                       a
24I11 VH  RPEQGLEWIG NIDPANGNTKY DPKFQGKATI TADTSSNTAY
Hu24I11 VH APGQRLEWIG NIDPANGNTKY DPKFQGKATI TADTSASTAY
X65891    APGQRLEWMG ---------- ------RVTI TRDTSASTAY

                                  1          1
          8          9          0          1
          0122223456789 0123456789 0000123456789 0123
             abc
24I11 VH  LHLSSLTSEDTAV YYCARWLRHF YYAMDYWGQGTSV TVSS
Hu24I11 VH MELSSLRSEDTAV YYCARWLRHF YYAMDYWGQGTLV TVSS
X65891    MELSSLRSEDTAV YYCAR----- ------WGQGTLV TVSS
```

Fig. 7

```
                    1          2          3
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24I11 VL  DIQMTQSPA SLAASVGETV TITCRASENI YYSLAWYQQK
Hu24I11 VL DIQMTQSPS SLSASVGDRV TITCRASENI YYSLAWYQQK
X72441    DIQMTQSPS SLSASVGDRV TITC------ -----WYQQK

          4          5          6          7
          0123456789 0123456789 0123456789 0123456789
24I11 VL  QGKSPQLLIY NANSLEDGVP SRFSGSGSGT QYSMKINSMQ
Hu24I11 VL PGKAPKLLIY NANSLEDGVP SRFSGSGSGT QYTLTISSLQ
X72441    PGKAPKLLIY -------GVP SRFSGSGSGT DFTLTISSLQ

                               1
          8          9          0
          0123456789 0123456789 01234567
24I11 VL  PEDTATYFCK QAYDVPYTFG GGTKLEIK
Hu24I11 VL PEDFATYYCK QAYDVPYTFG QGTKVEIK
X72441    PEDFATYYC- --------FG QGTKVEIK
```

Fig. 8

OLIGO-NUCLEO-TIDES	SEQUENCE	SEQ ID NO.
JNJ120	GGGACTAGTACCACCATGAAATGCAGC	101
JNJ137	GGGACTAGTACCACCATGAAATGCAGCTGGGTTATCTTCTTCCTGATGGCAGTGGTT	102
JNJ138	AGACTGCACCAGCTGAACCTGTGAATTGACCCCTGTAACCACTGCCATCAGGAAGAA	103
JNJ139	CAGGTTCAGCTGGTGCAGTCTGGGGCAGAGGTGAAGAAGCCAGGGGCCTCAGTCAAG	104
JNJ140	GTCTTTAATGTTGAAGCCAGAAGCCTTGCAGGAAACCTTGACTGAGGCCCCTGGCTT	105
JNJ141	TCTGGCTTCAACATTAAAGACACCTATGTGCACTGGGTGCGCCAGGCCCCTGGACAGAGG	106
JNJ142	ACCATTCGCAGGATCAATATTTCCAATCCACTCCAGCCTCTGTCCAGGGGCCTGGCG	107
JNJ143	AATATTGATCCTGCGAATGGTAATACTAAATATGACCCGAAGTTCCAGGGCAAGGCCACT	108
JNJ144	CATGTAGGCTGTGCTCGCGGATGTGTCTGCTGTTATAGTGGCCTTGCCCTGGAACTT	109
JNJ145	TCCGCGAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGACACTGCCGTC	110
JNJ146.	ATAGTAAAAATGTCGTAACCATCTAGCACAGTAATAGACGGCAGTGTCCTCAGA	111
JNJ147	TGGTTACGACATTTTTACTATGCTATGGACTACTGGGGTCAAGGAACCCTGGTCACC	112
JNJ148	GGGAAGCTTTTGTGAGGACTCACCTGAGGAGACGGTGACCAGGGTTCCTTGACC	113
JNJ149	GGGAAGCTTTTGTGAGGACTC	114

Fig. 9

OLIGO-NUCLEO-TIDES	SEQUENCE	SEQ ID NO.
JNJ150	GGGGCTAGCACCACCATGAGT	115
JNJ126	GGGGCTAGCACCACCATGAGTGTGCCCACTCAACTCCTGGGGTTGCTGCTGCTGTGG	116
JNJ127	AGACTGAGTCATCTGGATGTCACATCGTGCGTCTGTAAGCCACAGCAGCAGCAACCCCAG	117
JNJ128	GACATCCAGATGACTCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGAGACAGA	118
JNJ129	GTAAATGTTCTCACTTGCTCGACATGTGATGGTGACTCTGTCTCCCACAGATGCAGA	119
JNJ130	CGAGCAAGTGAGAACATTTACTACAGTTTAGCATGGTATCAGCAGAAGCCAGGGAAA	120
JNJ131	CAAGCTGTTTGCATTATAGATCAGGAGCTTAGGGGCTTTCCCTGGCTTCTGCTGATA	121
JNJ132	ATCTATAATGCAAACAGCTTGGAAGATGGTGTCCCATCGAGGTTCAGTGGCAGTGGA	122
JNJ133	CAGGCTGCTGATGGTGAGAGTATACTGTGTCCCAGATCCACTGCCACTGAACCTCGA	123
JNJ134	ACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTAAACAG	124
JNJ135	GGTCCCTTGTCCGAACGTGTACGGAACGTCATAAGCCTGTTTACAGTAATAAGTTGC	125
JNJ136	TACACGTTCGGACAAGGGACCAAGGTGGAAATCAAACGTGAGTAG	126
JNJ101	GGGGAATTCTTTAAATTCTACTCACGTTTGATTTCCA	127
JNJ117	GGGGAATTCTTTAAATTCTA	128

Fig. 10

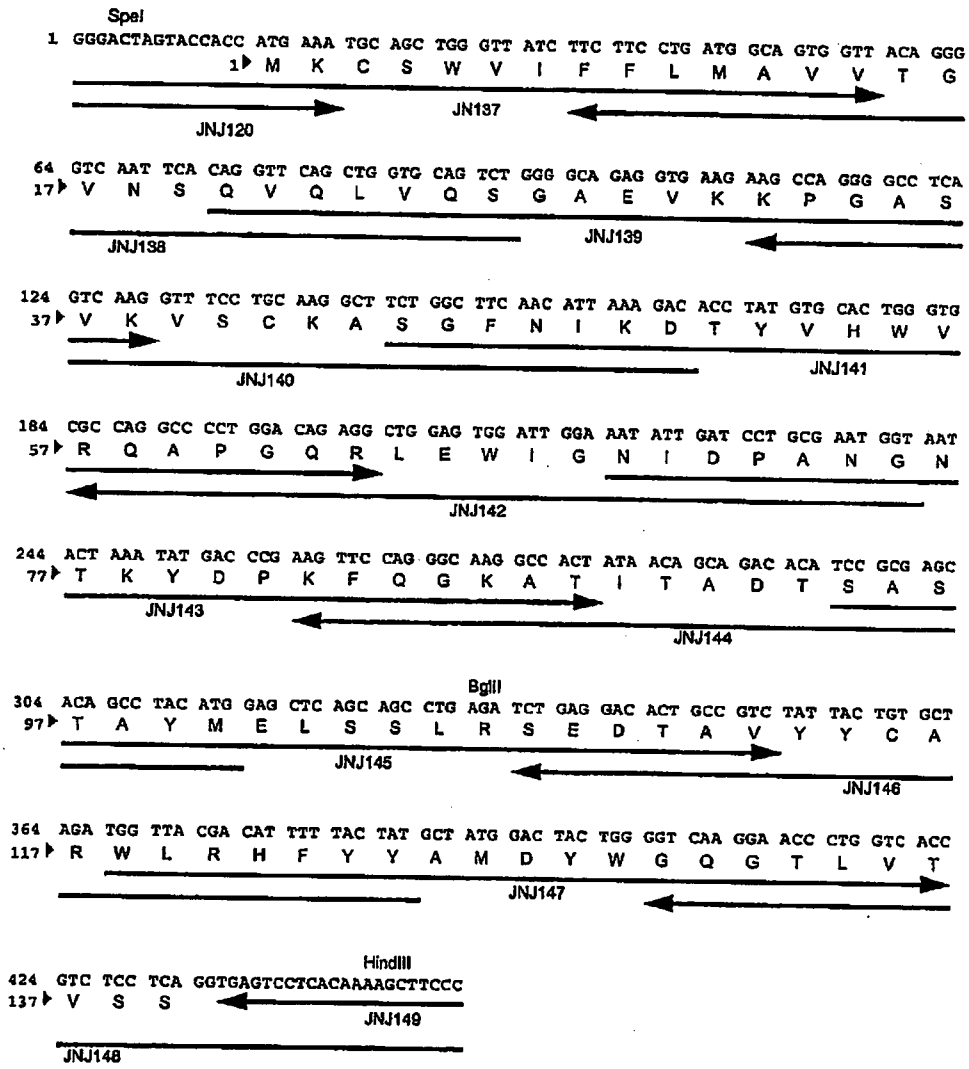

SpeI
1 GGGACTAGTACCACC ATG AAA TGC AGC TGG GTT ATC TTC TTC CTG ATG GCA GTG GTT ACA GGG
1 M K C S W V I F F L M A V V T G
JNJ120
JN137
64 GTC AAT TCA CAG GTT CAG CTG GTG CAG TCT GGG GCA GAG GTG AAG AAG CCA GGG GCC TCA
17 V N S Q V Q L V Q S G A E V K K P G A S
JNJ138
JNJ139
124 GTC AAG GTT TCC TGC AAG GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT GTG CAC TGG GTG
37 V K V S C K A S G F N I K D T Y V H W V
JNJ140
JNJ141
184 CGC CAG GCC CCT GGA CAG AGG CTG GAG TGG ATT GGA AAT ATT GAT CCT GCG AAT GGT AAT
57 R Q A P G Q R L E W I G N I D P A N G N
JNJ142
244 ACT AAA TAT GAC CCG AAG TTC CAG GGC AAG GCC ACT ATA ACA GCA GAC ACA TCC GCG AGC
77 T K Y D P K F Q G K A T I T A D T S A S
JNJ143
JNJ144
BglII
304 ACA GCC TAC ATG GAG CTC AGC AGC CTG AGA TCT GAG GAC ACT GCC GTC TAT TAC TGT GCT
97 T A Y M E L S S L R S E D T A V Y Y C A
JNJ145
JNJ146
364 AGA TGG TTA CGA CAT TTT TAC TAT GCT ATG GAC TAC TGG GGT CAA GGA ACC CTG GTC ACC
117 R W L R H F Y Y A M D Y W G Q G T L V T
JNJ147
HindIII
424 GTC TCC TCA GGTGAGTCCTCACAAAAGCTTCCC
137 V S S
JNJ149
JNJ148

Fig. 11

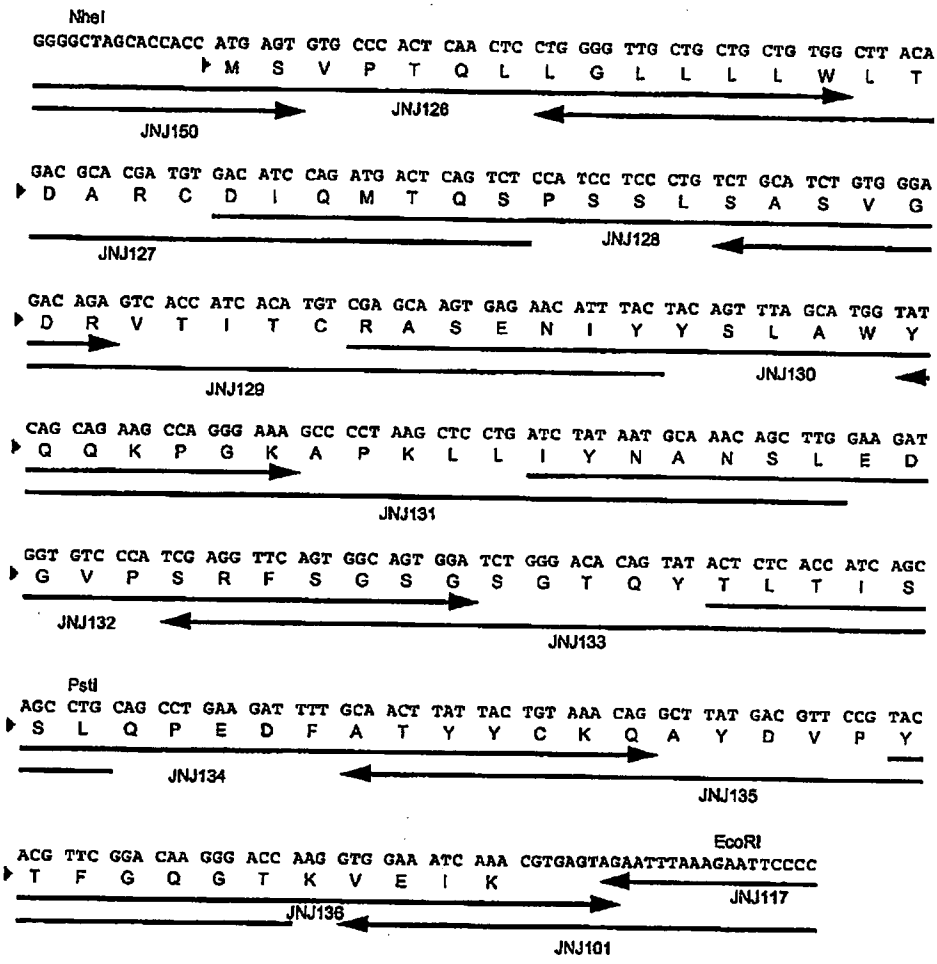

NheI
GGGGCTAGCACCACC ATG AGT GTG CCC ACT CAA CTC CTG GGG TTG CTG CTG CTG TGG CTT ACA
M S V P T Q L L G L L L L W L T
JNJ150
JNJ126
GAC GCA CGA TGT GAC ATC CAG ATG ACT CAG TCT CCA TCC TCC CTG TCT GCA TCT GTG GGA
D A R C D I Q M T Q S P S S L S A S V G
JNJ127
JNJ128
GAC AGA GTC ACC ATC ACA TGT CGA GCA AGT GAG AAC ATT TAC TAC AGT TTA GCA TGG TAT
D R V T I T C R A S E N I Y Y S L A W Y
JNJ129
JNJ130
CAG CAG AAG CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT AAT GCA AAC AGC TTG GAA GAT
Q Q K P G K A P K L L I Y N A N S L E D
JNJ131
GGT GTC CCA TCG AGG TTC AGT GGC AGT GGA TCT GGG ACA CAG TAT ACT CTC ACC ATC AGC
G V P S R F S G S G S G T Q Y T L T I S
JNJ132
JNJ133
PstI
AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGT AAA CAG GCT TAT GAC GTT CCG TAC
S L Q P E D F A T Y Y C K Q A Y D V P Y
JNJ134
JNJ135
EcoRI
ACG TTC GGA CAA GGG ACC AAG GTG GAA ATC AAA CGTGAGTAGAATTTAAAGAATTCCCC
T F G Q G T K V E I K
JNJ117
JNJ136
JNJ101

Fig. 12

SpeI
ACTAGTACCACCATGAAATGCAGCTGGGTTATCTTCTTCCTGATGGCAGTGGTTACAGGG
M K C S W V I F F L M A V V T G

GTCAATTCACAGGTTCAGCTGGTGCAGTCTGGGGCAGAGGTGAAGAAGCCAGGGGCCTCA
V N S Q V Q L V Q S G A E V K K P G A S

GTCAAGGTTTCCTGCAAGGCTTCTGGCTTCAACATTAAAGACACCTATGTGCACTGGGTG
V K V S C K A S G F N I K D T Y V H W V

CGCCAGGCCCCTGGACAGAGGCTGGAGTGGATTGGAAATATTGATCCTGCGAATGGTAAT
R Q A P G Q R L E W I G N I D P A N G N

ACTAAATATGACCCGAAGTTCCAGGGCAAGGCCACTATAACAGCAGACACATCCGCGAGC
T K Y D P K F Q G K A T I T A D T S A S

ACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGACACTGCCGTCTATTACTGTGCT
T A Y M E L S S L R S E D T A V Y Y C A

AGATGGTTACGACATTTTTACTATGCTATGGACTACTGGGGTCAAGGAACCCTGGTCACC
R W L R H F Y Y A M D Y W G Q G T L V T

HindIII
GTCTCCTCAG*GTGAGTCCTCACAAAAGCTT*
V S S

Fig. 13

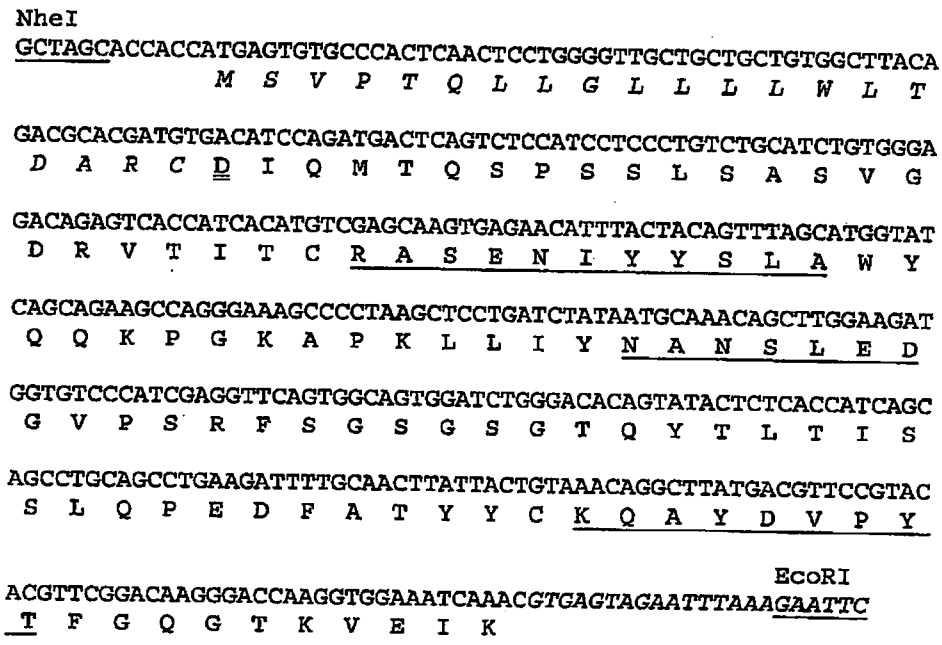

NheI
GCTAGCACCACCATGAGTGTGCCCACTCAACTCCTGGGGTTGCTGCTGCTGTGGCTTACA
M S V P T Q L L G L L L L W L T
GACGCACGATGTGACATCCAGATGACTCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGA
D A R C D I Q M T Q S P S S L S A S V G
GACAGAGTCACCATCACATGTCGAGCAAGTGAGAACATTTACTACAGTTTAGCATGGTAT
D R V T I T C R A S E N I Y Y S L A W Y
CAGCAGAAGCCAGGGAAAGCCCCTAAGCTCCTGATCTATAATGCAAACAGCTTGGAAGAT
Q Q K P G K A P K L L I Y N A N S L E D
GGTGTCCCATCGAGGTTCAGTGGCAGTGGATCTGGGACACAGTATACTCTCACCATCAGC
G V P S R F S G S G S G T Q Y T L T I S
AGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTAAACAGGCTTATGACGTTCCGTAC
S L Q P E D F A T Y Y C K Q A Y D V P Y
EcoRI
ACGTTCGGACAAGGGACCAAGGTGGAAATCAAACGTGAGTAGAATTTAAAGAATTC
T F G Q G T K V E I K

Fig. 14

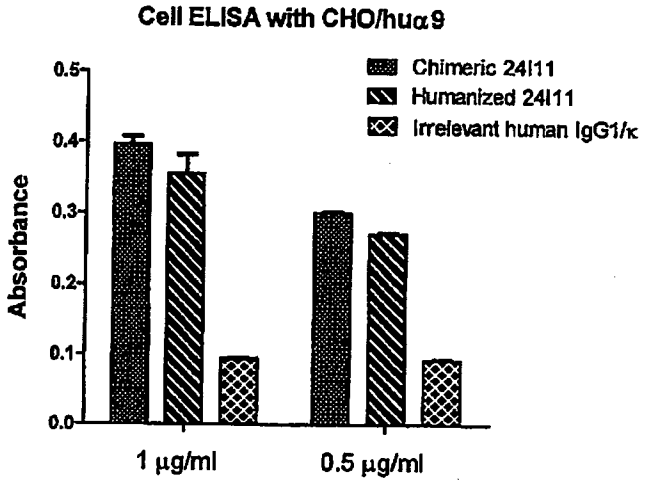

Cell ELISA with CHO/huα9
Chimeric 24I11
Humanized 24I11
Irrelevant human IgG1/κ
Absorbance
0.5
0.4
0.3
0.2
0.1
0.0
1 μg/ml
0.5 μg/ml

Fig. 15

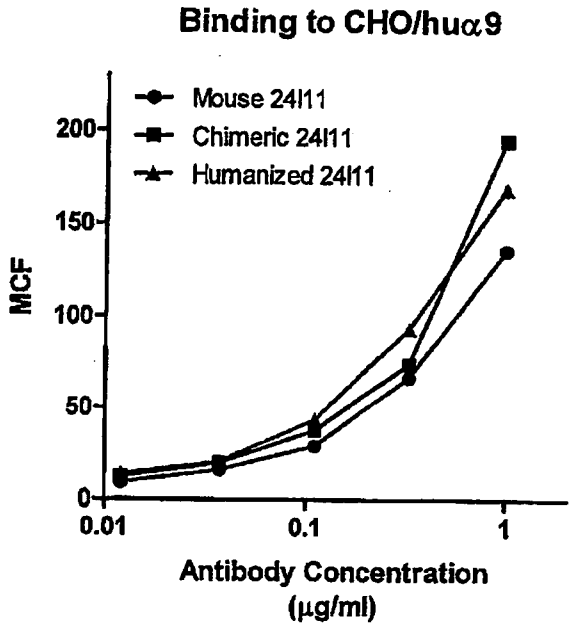

Binding to CHO/huα9
Mouse 24I11
Chimeric 24I11
Humanized 24I11
MCF
200
150
100
50
0
0.01
0.1
1
Antibody Concentration
(μg/ml)

Fig. 16

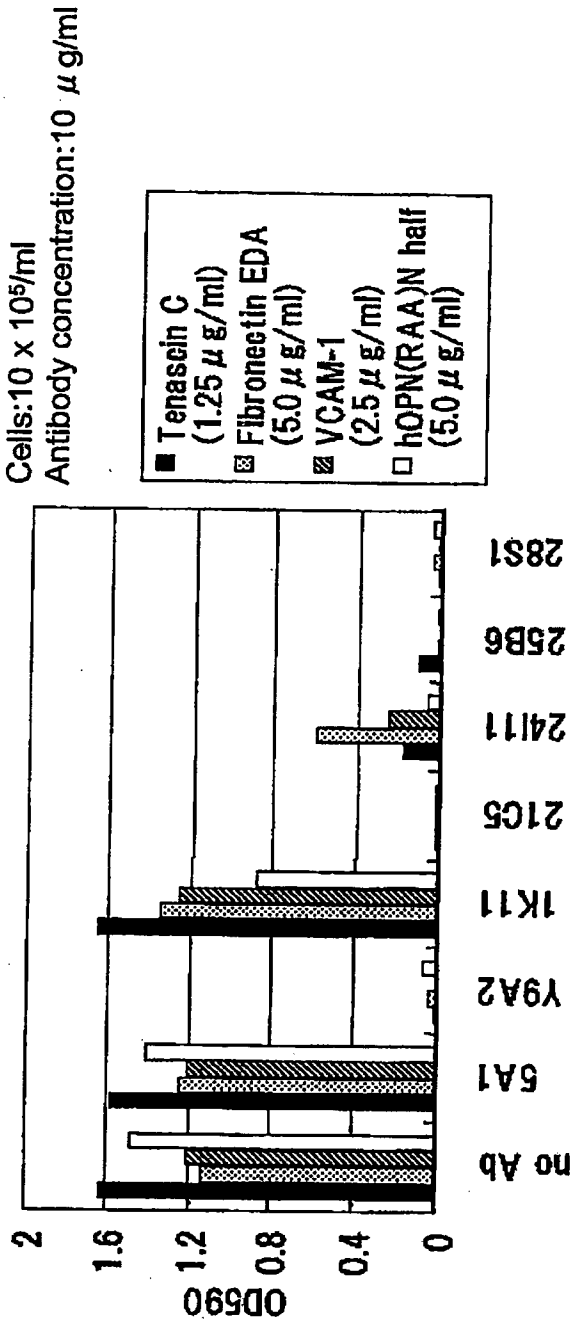

Cells:10 x 10⁵/ml
Antibody concentration:10 μg/ml
Tenascin C (1.25 μg/ml)
Fibronectin EDA (5.0 μg/ml)
VCAM-1 (2.5 μg/ml)
hOPN(RAA)N half (5.0 μg/ml)
OD590
2
1.6
1.2
0.8
0.4
0
no Ab
5A1
Y9A2
1K11
21C5
24I11
25B6
28S1

Fig. 17

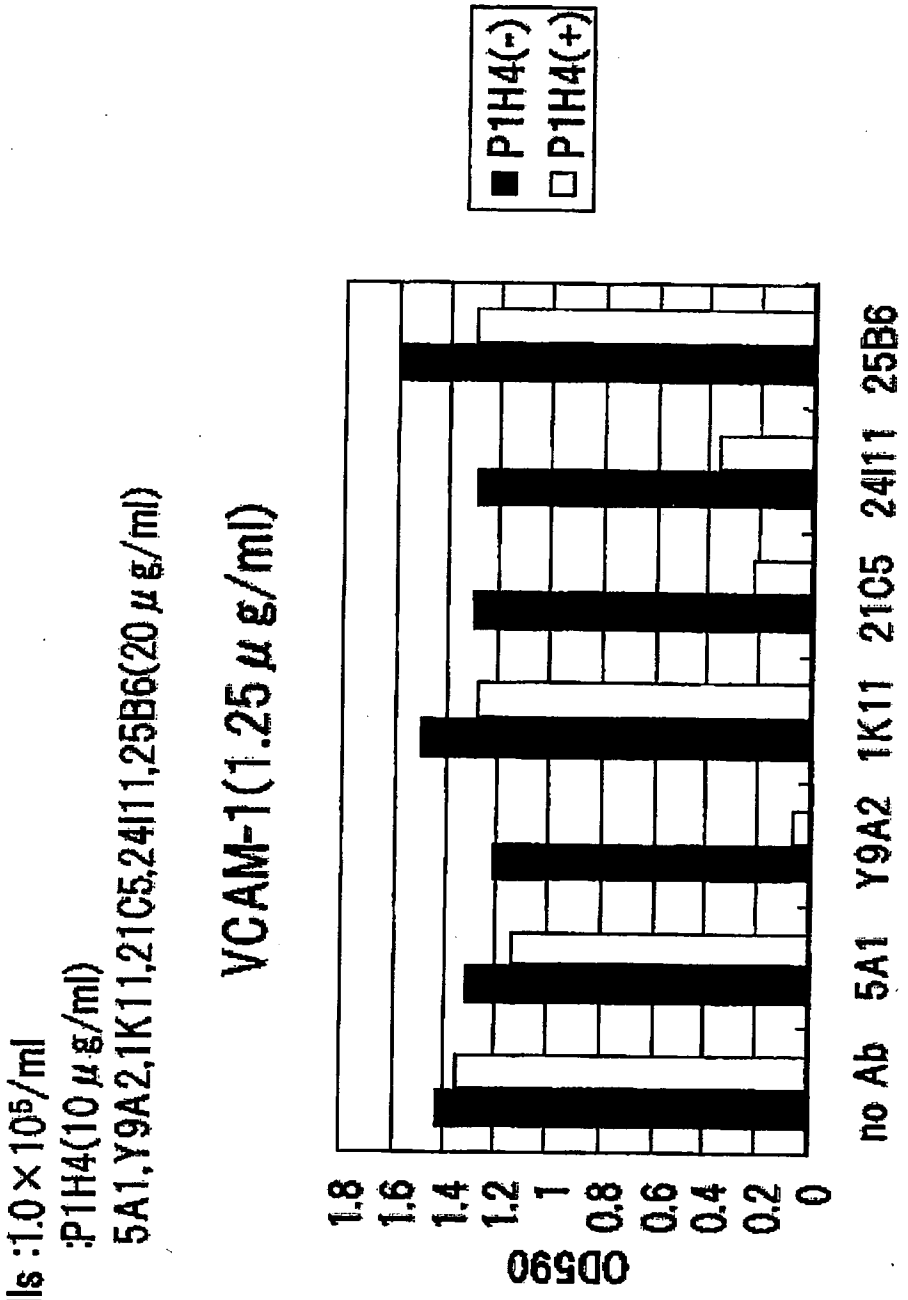

Cells :1.0×10⁵/ml
Ab :P1H4(10μg/ml)
5A1,Y9A2,1K11,21C5,24I11,25B6(20μg/ml)
VCAM-1(1.25μg/ml)
■ P1H4(-)
□ P1H4(+)
OD590
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
no Ab
5A1
Y9A2
1K11
21C5
24I11
25B6

Fig. 18

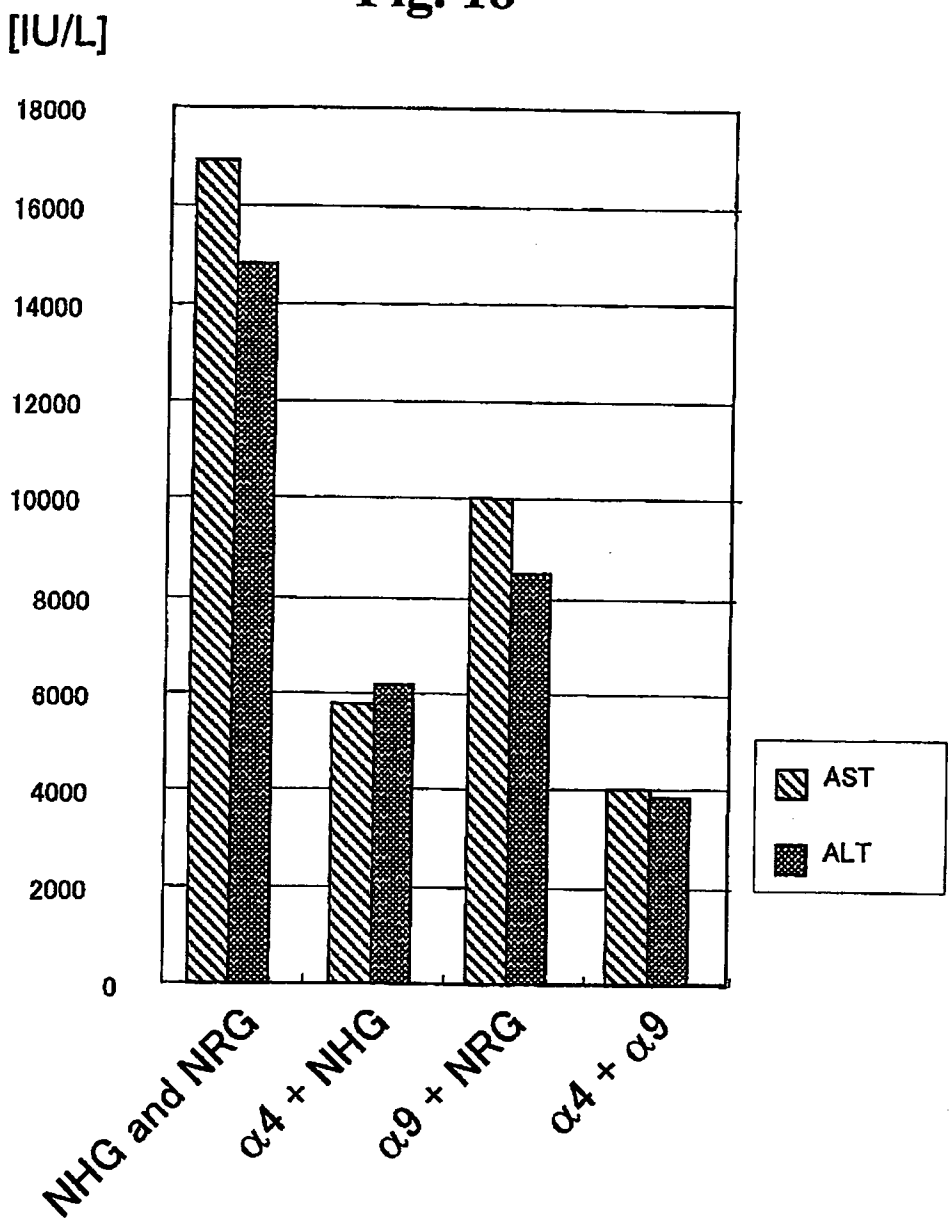

[IU/L]
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
AST
ALT
NHG and NRG
α4 + NHG
α9 + NRG
α4 + α9

Fig. 19

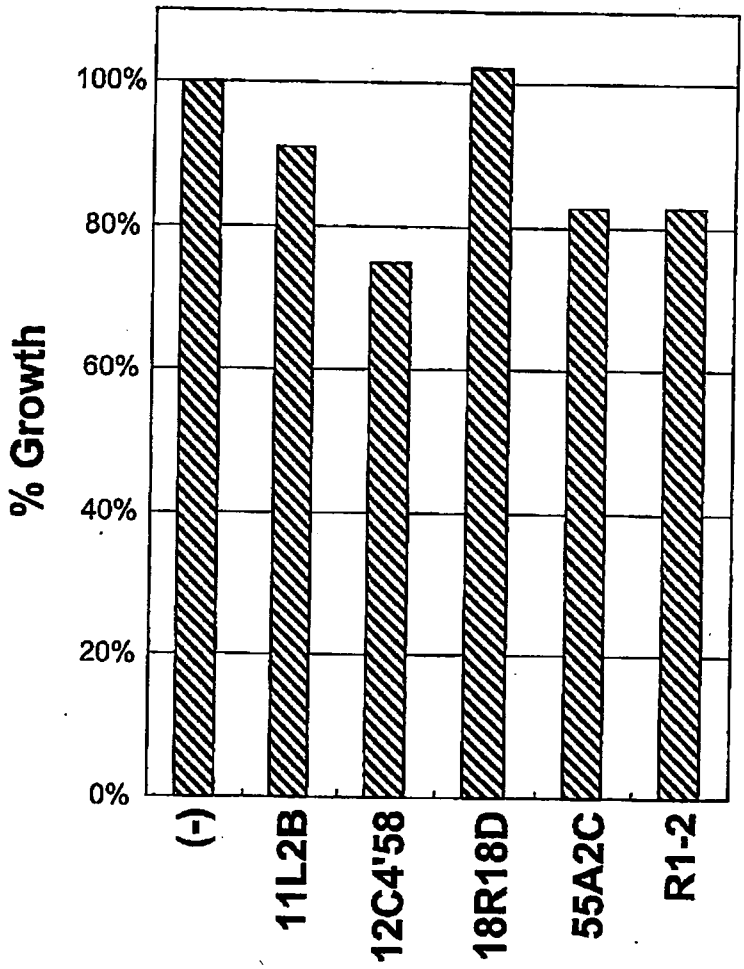


Fig. 20a

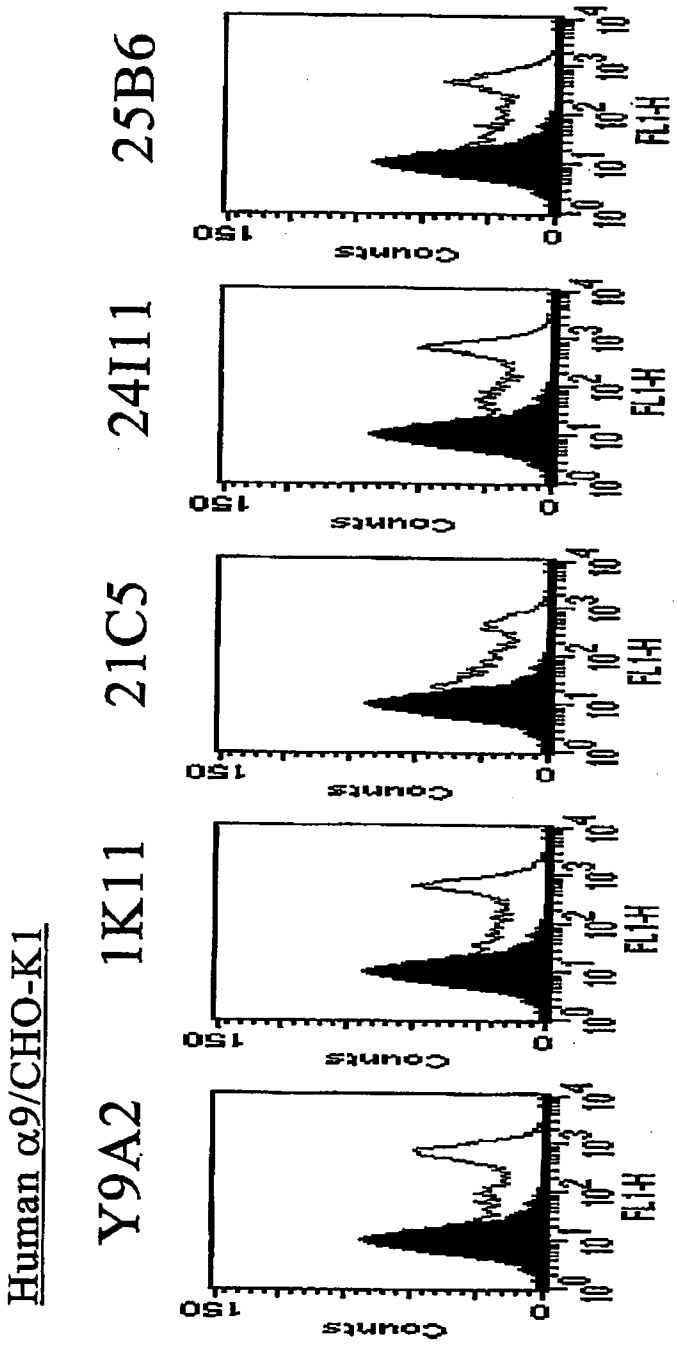


Fig. 20b

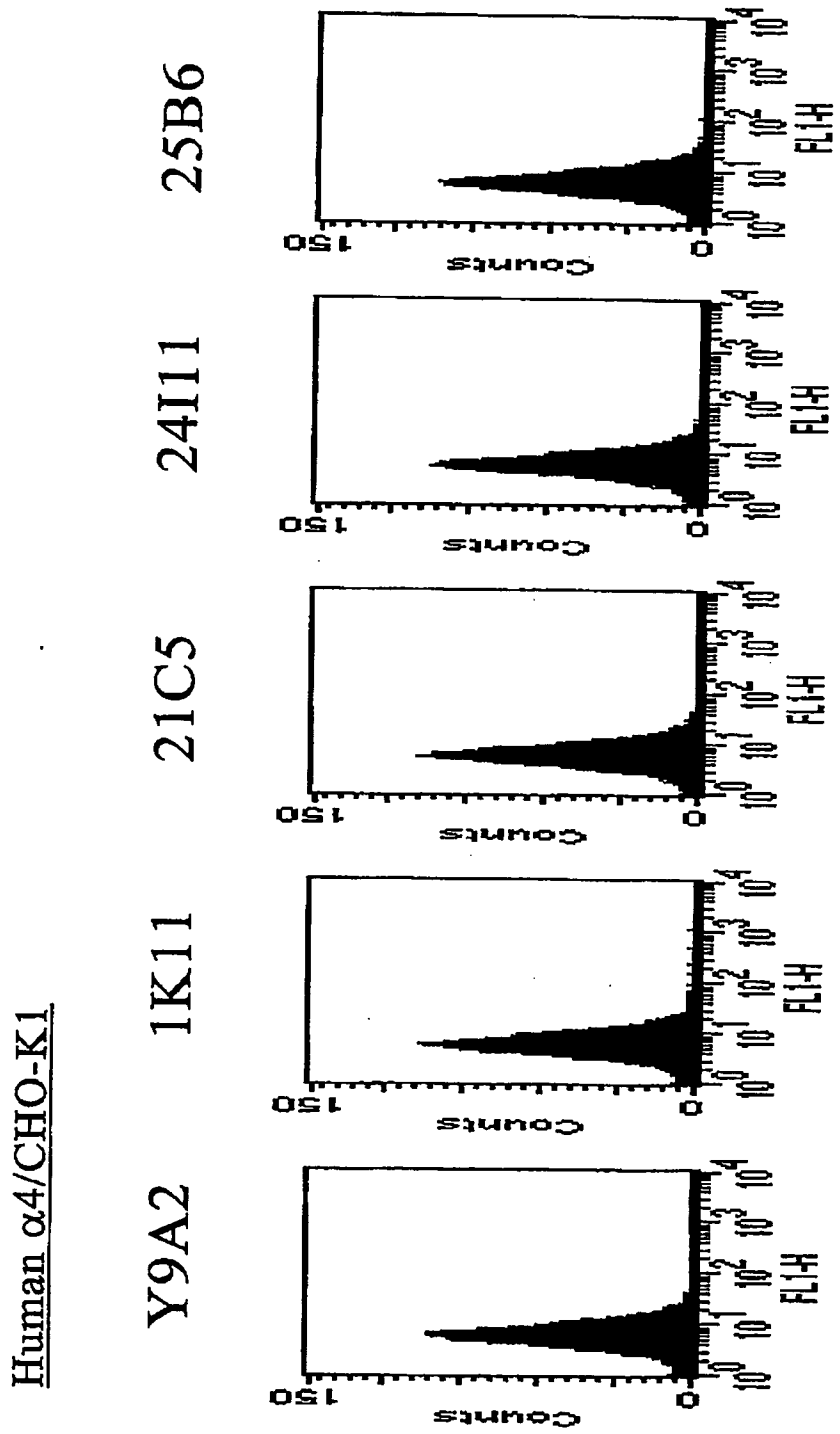


Fig. 20c

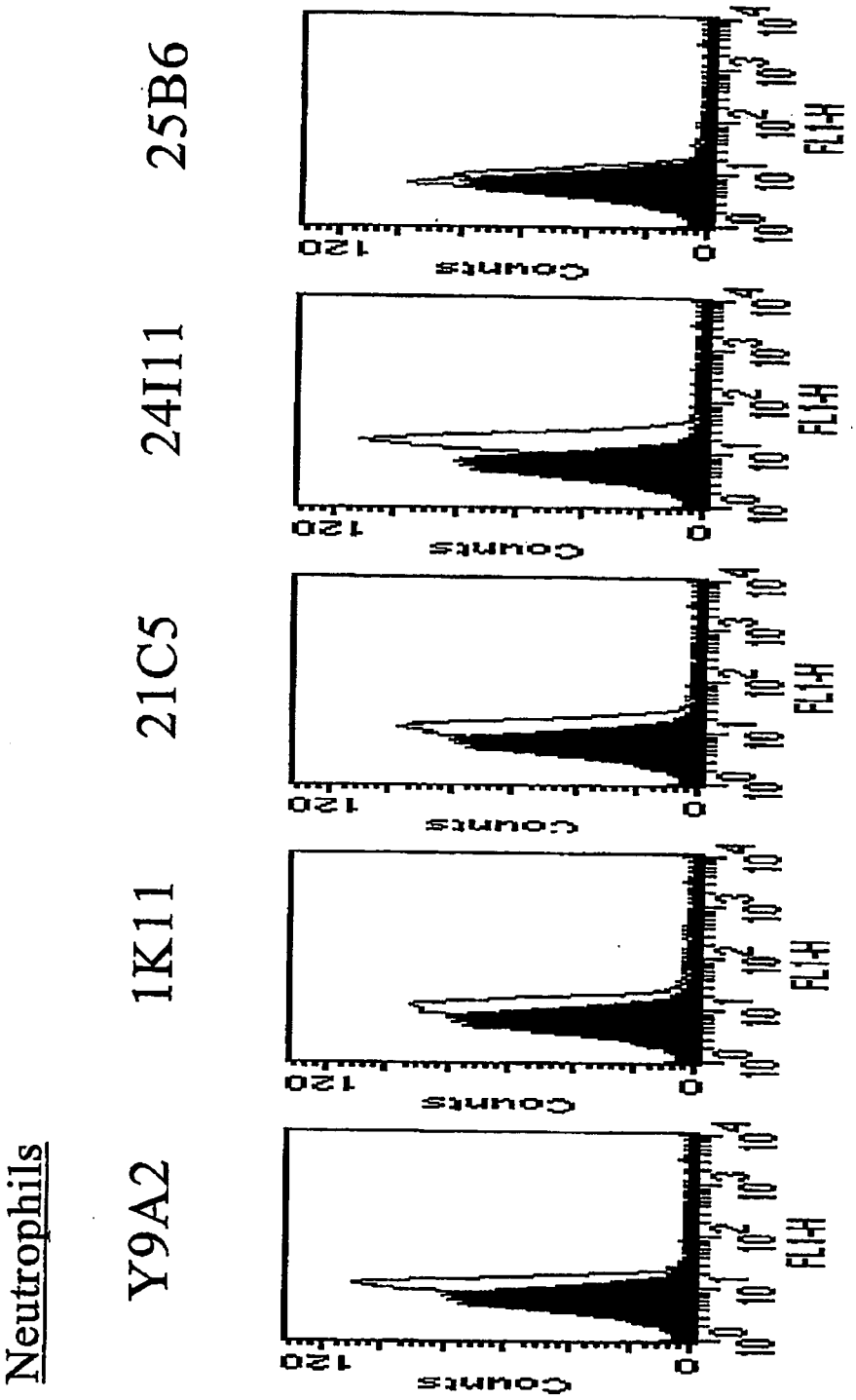


Fig. 21

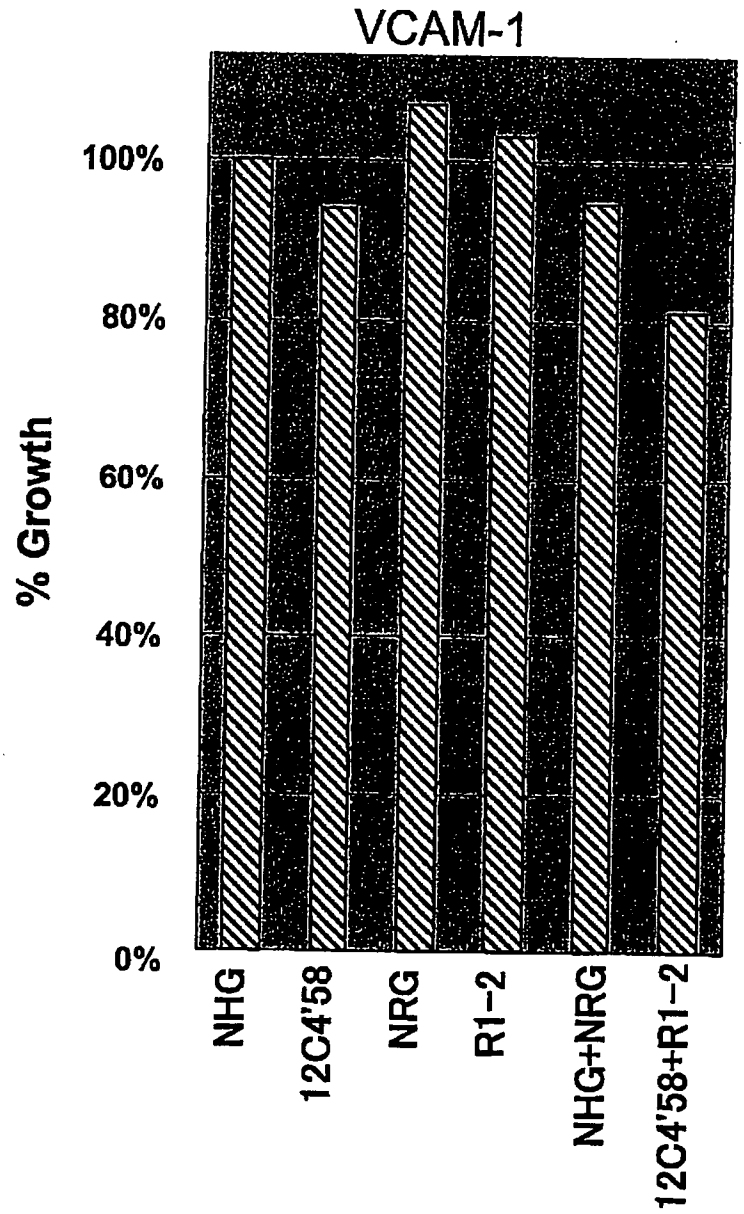
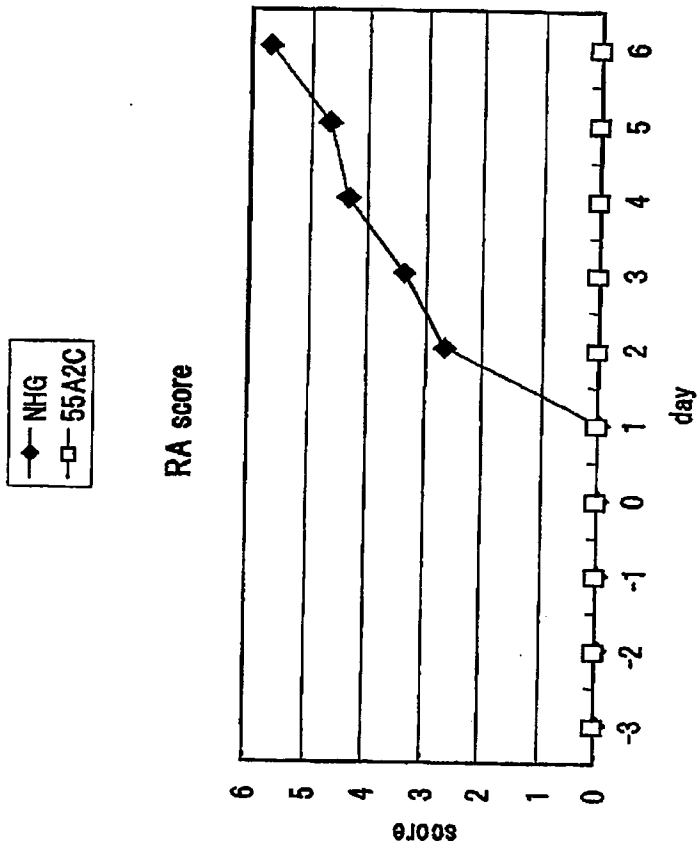

VCAM-1
% Growth
100%
80%
60%
40%
20%
0%
NHG
12C4'58
NRG
R1-2
NHG+NRG
12C4'58+R1-2

Fig. 22



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/050606

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. See extra sheet

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)

Int.Cl. C07K14/00-19/00

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 practicable, search terms used)

BIOSIS/MEDLINE/WPIDS(STN), GenBank/EMBL/DDBJ/GeneSeq, SwissProt/PIR/GeneSeq, JSTPlus(JDreamII), JMEDPlus(JDreamII), Igaku Yakugaku Yokoshu Zenbun Data Base

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 2006/075784 A1 (GENE TECHNO SCIENCE CO., LTD.), 20 July 2006, Full text, for example, Background Art, Example3, Figure 19 & EP 1840135 A1 & IN 2007KN02471 P2 & CN 101103044 A & JP 2006-553034 A & KR 2007-115884 A & US 2008/0152653 A1	1-4,6-17,20-23 5
P,X P,A	WO 2008/007804 A1 (GENE TECHNO SCIENCE CO., LTD.), 17 January 2008, Full text (No Family)	1-4,6-17,20-23 5

☐ Further documents are listed in the continuation of Box C.

☐ 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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02.03.2009	10.03.2009

Name and mailing address of the ISA/JP	Authorized officer
Japan Patent Office 3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	Shinji SHIMIZU Telephone No. +81-3-3581-1101 Ext. 3448

4B 3535

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/050606

CLASSIFICATION OF SUBJECT MATTER
C12N15/09(2006.01)i, A61K39/395(2006.01)i, C07K16/28(2006.01)i,
C12N1/15(2006.01)i, C12N1/19(2006.01)i, C12N1/21(2006.01)i,
C12N5/10(2006.01)i, C12P21/08(2006.01)i, G01N33/563(2006.01)i,
G01N33/577(2006.01)i

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/050606

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

a. type of material

- [x] a sequence listing
- [ ] table(s) related to the sequence listing

b. format of material

- [ ] on paper
- [x] in electronic form

c. time of filing/furnishing

- [ ] contained in the international application as filed
- [x] filed together with the international application in electronic form
- [ ] furnished subsequently to this Authority for the purposes of search

2. [x] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/050606

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: 18-19
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 18-19 are deemed to be concerned with a therapeutic or diagnostic method for the human body. Consequently, these claims are relevant to methods for treatment of the human body by surgery or therapy and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.