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(54) **Titre : ANTICORPS DIRIGE CONTRE L'INTEGRINE α9 HUMAINE ET SON UTILISATION**
(54) **Title: ANTI-HUMAN α9 INTEGRIN ANTIBODY AND USE THEREOF**

(57) **Abrégé/Abstract:**
Disclosed are: an anti-human α9 integrin antibody, more specifically a monoclonal antibody, a chimeric antibody, a humanized antibody or a human antibody which can recognize human α9 integrin specifically; a hybridoma cell which can produce the monoclonal antibody; a method for producing the monoclonal antibody; a method for producing the hybridoma cell; a therapeutic agent comprising the anti-human α9 integrin antibody; a diagnostic agent comprising the anti-human α9 integrin antibody; a method for the screening of a compound capable of inhibiting the activity of human α9 integrin; and others.

ABSTRACT

The present invention relates to an anti-human α9 integrin antibody. More particularly, the present invention relates to: a monoclonal antibody, a chimeric antibody, a humanized antibody and a human antibody that specifically recognize human α9 integrin; a hybridoma cell that produces the monoclonal antibody; a method for producing the monoclonal antibody; a method for producing the hybridoma cell; a therapeutic agent comprising the anti-human α9 integrin antibody; a diagnostic agent comprising the human α9 integrin antibody; and a method for screening for a compound that inhibits the activity of human α9 integrin.

DESCRIPTION

ANTI-HUMAN α9 INTEGRIN ANTIBODY AND USE THEREOF

TECHNICAL FIELD

[0001] The present invention relates to an anti-human α9 integrin antibody and use thereof. More particularly, the present invention relates to: a monoclonal antibody, a chimeric antibody, a humanized antibody and a human antibody that specifically recognize human α9 integrin; a hybridoma cell that produces the monoclonal antibody; a method for producing the monoclonal antibody; a method for producing the hybridoma cell; a therapeutic agent comprising the anti-human α9 integrin antibody; a diagnostic agent comprising the anti-human α9 integrin antibody; and a method for screening for a compound that inhibits the activity of human α9 integrin.

BACKGROUND ART

[0002] Adhesion between a cell and the extracellular matrix is mediated by a transmembrane cell adhesion protein as typified by integrins. Integrins are heterodimers that have 1:1 of α- and β-chains. So far, 18 types of α-chains and 8 types of β-chains have been found where at least 24 types of combinations thereof have been identified and confirmed. Each integrin is known to recognize a specific extracellular matrix (ligand). Moreover, the transmembrane cell adhesion protein containing an integrin not only plays a role of adhering and anchoring a cell and the extracellular matrix but it has also been found to convert information from the extracellular matrix into intracellular signals for regulating cell proliferation, motility, cell death, differentiation and the like.

[0003] Integrins are classified, according to their specificities and functions with respect to ligands, into subfamilies, namely, collagen receptors, laminin receptors, RGD receptors that recognize Arg-Gly-Asp (RGD) sequence contained in fibronectin, vitronectin and the like, and leukocyte-specific receptors that exist only in leukocytes. Alpha-4 integrins and α9 integrins do not belong to any of these subfamilies and are called α4 integrin subfamily.

[0004] Ligands that are known to bind to α4 and α9 integrins include osteopontin (hereinafter, referred to as OPN), EDA domain of fibronectin, propeptide-von Willebrand factor (pp-vWF), tissue transglutaminase (tTG), coagulation factor XIII and Vascular Cell Adhesion Molecule-1 (VCAM-1). Furthermore, ligands that are known to be recognized

specifically by α4 integrin include CS-1 domain of fibronectin, MadCAM-1 (α4β7) and the like. Meanwhile, ligands that are known to be recognized specifically by α9 integrin include tenascin C, plasmin and the like.

[0005] OPN, one type of extracellular matrices (ECM), is a secreted acid phosphorylated acid glycoprotein with a molecular weight of about 41 kDa, which is a molecule generally acknowledged to be expressed in breast milk, urine, renal tubules, osteoclasts, osteoblasts, macrophage, activated T cell, tumor tissue and the like. OPN has cell adhesion sequence GRGDS in the middle, and SVVYGLR or SLAYGLR sequence for human or murine OPN, respectively, which is immediately followed by a thrombin cleavage site. OPN adheres to integrin of RGD receptor via the GRGDS sequence and to α4 (α4β1) and α9 (α9β1) integrins via the SVVYGLR sequence or the SLAYGLR sequence.

[0006] While α4β1 binds to OPN that is not cleaved with thrombin (non-cleaved OPN) as well as an N-terminal fragment cleaved with thrombin (cleaved OPN), α9β1 differs in that it binds only to cleaved OPN.

[0007] The amino acid sequences of α4,α9 and β1 integrin subunits are known and are registered with GenBank. The amino acid sequences are known to be highly similar among the species.

[0008] WO02/081522 discloses a therapeutic effect for rheumatoid arthritis and hepatitis by utilizing an OPN-deficient mouse and a neutralizing antibody against OPN to suppress the OPN functions. This publication also discloses that the SVVYGLR sequence, i.e., a sequence that recognizes α4 and α9 integrins, plays an important role upon onset of an inflammatory disease and that a receptor for OPN involved in inflammatory diseases is expressed in the immunocompetent cell or the like.

[Patent Document 1] WO02/081522

DISCLOSURE OF THE INVENTION

Problems to be solved by the invention

[0009] Currently, various therapeutic drugs for cancers, inflammatory diseases, infectious diseases, autoimmune diseases and bone diseases are known but development of a prophylactic drug and/or a therapeutic drug with improved therapeutic effects for cancers,

inflammatory diseases, infectious diseases, autoimmune diseases and bone diseases are desired.

[0010] Until today, the present inventors have conducted various studies focusing on integrins, in particular α9 integrin. As a result, they found that an antibody that specifically inhibits α9 integrin has a cancer suppressing effect as well as an anti-inflammatory effect and produced five types of hybridoma cells that produces the monoclonal antibody (1K11, 21C5, 24I11, 25B6 and 28S1) (each deposited with the International Patent Organism Depositary, the National Institute of Advanced Industrial Science and Technology (Central 6, Higashi 1-1-1, Tsukuba-shi, Ibaraki-ken, 305-8566) as FERM BP-10510, FERM BP-10511, FERM BP-10512, FERM BP-10513 and FERM BP-10832, respectively (the first four cells deposited on February 15, 2006 and the last one on May 29, 2007)).

[0011] Under such circumstances, there has been a need for a monoclonal antibody or an alternative monoclonal antibody with higher drug efficacy.

MEANS FOR SOLVING THE PROBLEM

[0012] In order to develop a monoclonal antibody or an alternative monoclonal antibody that has superior drug efficacy over the above-mentioned five monoclonal antibodies, the present inventors have gone through keen studies and succeeded in producing a hybridoma cell that produces such a novel monoclonal antibody, thereby accomplishing the present invention.

[0013] Thus, the present invention provides an anti-human α9 integrin antibody, a monoclonal antibody thereof, a cell that produces the antibody, a therapeutic agent comprising the antibody, and a method for screening for a compound that inhibits α9 integrin activity.

(1) An anti-human α9 integrin antibody comprising an amino acid sequence represented by any one of SEQ ID NOS:1-12.

(2) An anti-human α9 integrin antibody comprising an amino acid sequence represented by SEQ ID NO:1, 3, 5, 7, 9 or 11.

(3) An anti-human α9 integrin antibody comprising amino acid sequences represented by SEQ ID NOS:1, 3, 5, 7, 9 and 11.

(4) An anti-human α9 integrin antibody comprising an amino acid sequence represented by SEQ ID NO:2, 4, 6, 8, 10 or 12.

(5) An anti-human α9 integrin antibody comprising amino acid sequences represented by SEQ ID NOS:2, 4, 6, 8, 10 and 12.

(6) An anti-human α9 integrin antibody comprising the amino acid sequence represented by any one of SEQ ID NOS:1-6 as an amino acid sequence of the complementary determining region of the heavy chain (CDRH) and the amino acid sequence represented by any one of SEQ ID NOS:7-12 as an amino acid sequence of the complementary determining region of the light chain (CDRL).

(7) The anti-human α9 integrin antibody according to any one of (1)-(6) above, wherein the binding between the human α9 integrin and the ligand of α9 integrin is inhibited.

(8) The anti-human α9 integrin antibody according to any one of (1)-(7) above, wherein it is a monoclonal antibody.

(9) The anti-human α9 integrin antibody according to any one of (1)-(8) above, wherein it is a chimeric antibody.

(10) The anti-human α9 integrin antibody according to any one of (1)-(8) above, wherein it is a humanized antibody.

(11) The anti-human α9 integrin antibody according to any one of (1)-(8) above, wherein it is a human antibody.

(12) An anti-human α9 integrin antibody produced by a hybridoma cell assigned Accession No. FERM BP-10830 or FERM BP-10831.

(13) A therapeutic agent for a cancer, an inflammatory disease, an infectious disease,

an autoimmune disease or a bone disease, comprising the anti-human α9 integrin antibody according to any one of (1)-(12) above as an active component.

(14) A therapeutic agent for a cancer, an inflammatory disease, an infectious disease, an autoimmune disease or a bone disease, comprising the anti-human α9 integrin antibody according to any one of (1)-(12) above as well as an anti-human α4 integrin antibody as active components.

(15) A diagnostic agent for a cancer, an inflammatory disease, an infectious disease, an autoimmune disease or a bone disease, comprising the anti-human α9 integrin antibody according to any one of (1)-(12) above as an active component.

(16) A cell that produces the anti-human α9 integrin antibody according to any one of (1)-(12) above.

(17) A hybridoma cell assigned Accession No. FERM BP-10830 or FERM BP-10831.

(18) A method for screening for a compound that inhibits an activity of α9 integrin, comprising using a peptide comprising the amino acid sequence of α9 integrin.

EFFECT OF THE INVENTION

[0014] The present invention provides a novel anti-α-integrin antibody. The anti-α9-integrin antibody of the present invention shows an excellent suppression effect against a function of α9 integrin and exerts a therapeutic effect against cancers (for example, cancer cell proliferation or metastasis), inflammatory diseases (for example, rheumatoid arthritis, osteoarthritis, hepatitis, bronchial asthma, fibrosis, diabetes, arterial sclerosis, multiple sclerosis, inflammatory bowel diseases (ulcerative colitis, Crohn's disease, etc.)), infectious diseases (for example, hepatitis), autoimmune diseases (for example, systemic lupus erythematosus, polymyositis, autoimmune thyroid disease, tubulointerstitial nephritis, myasthenia gravis), bone diseases (for example, osteoporosis) and the like. Moreover, a pharmaceutical composition comprising the anti-α9-integrin antibody of the present invention as well as the anti-α4-integrin antibody exerts an improved therapeutic effect against cancers, inflammatory diseases and the like. The antibody of the present invention may also be utilized as a diagnostic drug since it is capable of pathologically detecting expression of α9 integrin in cells and tissues.

MODES FOR CARRYING OUT THE INVENTION

[0015] Background of the Invention

Tysabri (registered trademark) (natalizumab), an antibody against α4 integrin, from Biogen Idec Inc. (Massachusetts, USA) and Elan Corporation (Ireland), was approved as a drug for multiple sclerosistherapeutic by the Food and Drug Administration (FDA) in November 2004. Furthermore, Tysabri (registered trademark) is undergoing clinical development directing at diseases such as Crohn's disease and rheumatoid arthritis. An anti-human α4β1 integrin monoclonal antibody, called P4C2, is used in the laboratory.

[0016] Although a monoclonal antibody named Y9A2 that shows specificity to human and guinea pig α9 integrins (A.Wang et al, (1996) Am. J. Respir.,Cell Mol. Biol. 15, 664-672) has been subjected to experiments as an antibody against α9 integrin, its clinical use has not yet been realized.

[0017] According to the present invention, a novel antibody against human α9 integrin, which is expected to have superior drug efficacy, was obtained by carefully pursuing the following procedures.

[0018] (1) Production of human α9 integrin overexpressing strain

In order to produce an antibody against α9 integrin, gene transfection of mouse fibroblast NIH-3T3 cell was performed to establish a cell line that overexpresses human α9 integrin. This cell line is used as an antigen for immunizing mice.

[0019] (2) Screening for hybridoma

In order to efficiently obtain clones that react exclusively to human α9 integrin from various hybridoma resulting from cell fusion, human α4 integrin belonging to the same integrin family is expressed in CHO-K1 cells. The resulting cells are used to select clones that do not show cross reactivity with other integrins and that do not react with the cell surface antigen of the parent cell (CHO-K1), thereby efficiently obtaining an inhibition antibody that reacts specifically to human α9 integrin.

[0020] Anti-α9-integrin antibody of the present invention

The present invention provides a monoclonal antibody against human α9 integrin. According to the present invention, the term "antibody" refers to the whole antibody

molecule or a fragment thereof (for example, Fab, Fab' or $F(ab')_2$ fragment) that specifically binds to α9 integrin or the partial peptide thereof as an antigen, which may be either a polyclonal antibody or a monoclonal antibody. According to the present invention, the term preferably refers to a monoclonal antibody. The term "antibody" according to the present invention also encompasses a chimeric antibody, a humanized antibody and a human antibody.

[0021] According to the present invention, when an antibody "specifically binds" to a certain protein or a fragment thereof, it means that the antibody binds to a particular amino acid sequence of the certain protein or the fragment thereof with substantially higher affinity over other amino acid sequences. Herein, the phrase "substantially high affinity" refers to a level of affinity that is sufficient to distinguish and detect a particular amino acid sequence from other amino acid sequences with a desired measurement device or method. Typically, the phrase refers to binding affinity with an association constant (K_a) of at least 10^7M^{-1}, preferably at least 10^8M^{-1}, more preferably 10^9M^{-1}, still more preferably $10^{10}M^{-1}$, $10^{11}M^{-1}$, $10^{12}M^{-1}$ or higher, for example, up to $10^{13}M^{-1}$ or higher.

[0022] A "monoclonal antibody" according to the present invention refers to an antibody that is highly specific to recognize a single antigen.

[0023] According to the present invention, an "antibody fragment" refers to a part of a whole antibody, namely, an antigen-binding region or a variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments. These antibody fragments may be produced through a generally known technique such as papain digestion or pepsin digestion of the antibody.

[0024] The above-mentioned "chimeric antibody" refers to a chimeric human-mouse antibody obtained by genetically engineering the constant region of the anti-human α9 integrin antibody of the present invention to have the same constant region as that of the human antibody (see European Patent Publication No. EP0125023). A "humanized antibody" refers to an antibody obtained by genetically engineering the primary structure of the anti-human α9 integrin antibody of the present invention to have the corresponding primary structure of a human antibody except for the complementarity determining regions (CDRs) of the H- and L-chains. A "human antibody" refers to a monoclonal antibody produced by using a transgenic animal that has been transferred with a human gene

involved in human antibody production (see European Patent Publication No. EP0546073).

[0025] More specifically, the present invention provides an anti-human α9 integrin antibody that differs from the conventionally-produced anti-human α9 integrin antibodies. An antibody according to the preferred embodiment of the present invention comprises an amino acid sequence represented by SEQ ID NO:1, 3, 5, 7, 9 or 11. More preferable antibody is an anti-human α9 integrin antibody comprising two or more, three or more, four or more, five or more or six amino acid sequences selected from the group consisting of the amino acid sequences represented by SEQ ID NOS:1, 3, 5, 7, 9 and 11.

[0026] Furthermore, an antibody according to another embodiment of the present invention comprises an amino acid sequence represented by SEQ ID NO:2, 4, 6, 8, 10 or 12. More preferable antibody is an anti-human α9 integrin antibody comprising two or more, three or more, four or more, five or more or six amino acid sequences selected from the group consisting of the amino acid sequences represented by SEQ ID NOS:2, 4, 6, 8, 10 and 12.

[0027] Particularly preferable antibody of the present invention is an anti-human α9 integrin antibody produced by a hybridoma cell assigned Accession No. FERM BP-10830 or FERM BP-10831.

[0028] Hereinafter, production of an anti-α9-integrin monoclonal antibody will be described, although the production of the antibody should not be limited thereto.

Alpha-9 integrin (antigen)

[0029] Alpha-9 integrin as an antigen used with the present invention may be (1) any cell derived from human or other mammal that expresses α9 integrin or a protein derived from any tissue containing this cell, (2) gene DNA coding for α9 integrin, preferably a recombinant protein in which cDNA is introduced and expressed in a cell line such as a bacterium, an yeast or an animal cell, or (3) a synthetic protein.

[0030] Furthermore, α9 integrin of the present invention also comprises a polypeptide having an amino acid sequence of α9 integrin from any type of mammal, particularly preferably a polypeptide having substantially the same amino acid sequence as the amino acid sequence (SEQ ID NO:13) of human α9 integrin.

[0031] Here, the phrase "a polypeptide having substantially the same amino acid sequence" refers to a mutant polypeptide having an amino acid sequence with substantially the same biological nature as that of the amino acid sequence of natural α9 integrin, particularly preferably human-derived α9 integrin, where several amino acids, preferably 1-10 amino acids and particularly preferably one to several (for example, 1-5, 1-4, 1-3 or 1-2) amino acids are substituted, deleted and/or modified in said amino acid sequence, as well as a mutant polypeptide having an amino acid sequence having several amino acids, preferably 1-10 amino acids, particularly preferably one to several (for example, 1-5, 1-4, 1-3 or 1-2) amino acids added to the amino acid sequence of natural α9 integrin, particularly preferably human-derived α9 integrin. The polypeptide may also be a mutant polypeptide further having some of such substitution, deletion, modification and addition.

[0032] Alpha-9 integrin, particularly human-derived α9 integrin, of the present invention may be produced by appropriately employing a gene recombinant technique as well as a method known in the art such as chemical synthesis, cell culturing, or a modified method thereof.

[0033] Examples of methods for producing a mutant polypeptide include site-directed mutagenesis using a synthetic oligonucleotide (gapped duplex approach), introduction of random point mutation using nitrous acid or sulfurous acid treatment, production of a deficient mutant using, for example, Ba131 enzyme or the like, cassette mutagenesis, linker scanning technique, misincorporation technique, mismatch primer technique and synthesis of DNA segment.

[0034] In addition, α9 integrin of the present invention also comprises a "part" of said α9 integrin. Herein, the term "part" refers to a portion that includes a region necessary for binding to an α9 integrin ligand such as OPN, tenascin C or VCAM-1. The "part" of said α9 integrin may be produced by a later-described gene recombinant technique or chemical synthesis known in the art or a modified version thereof. Alternatively, it may be produced by appropriately cleaving α9 integrin, particularly preferably human-derived α9 integrin, that has been isolated through cell culturing with protease or the like.

[0035] An antigen may also be a cell itself that overexpresses α9 integrin on the cell membrane by recombinant technique, or a membrane fraction or the like thereof.

[0036] Alpha-9 integrin of the present invention also comprises a polypeptide having substantially the same amino acid sequence as the amino acid sequence (SEQ ID NO:13) of human α9 integrin. According to the present invention, a cell itself that overexpresses human α9 integrin on the cell membrane by a recombinant technique is particularly preferably used. Hence, a later-described cell that overexpresses human α9 integrin on the cell membrane or a cell membrane fraction thereof may be prepared to be used by itself as an antigen by cloning a gene (for example, cDNA) coding for human α9 integrin according to a known genetic engineering technique.

[0037] Preparation of antibody-producing cell

The antigen is administered alone or together with a carrier or a diluent to an animal to be immunized at a site that allows production of the antibody upon administration. In order to enhance the antibody producing capability upon administration, a complete Freund's adjuvant or an incomplete Freund's adjuvant may be administered. The administration takes place generally once in every 1-6 weeks for a total of about 2-10 times. Examples of the warm-blooded animal used include a mouse, monkey, rabbit, dog, guinea pig, rat, hamster, sheep, goat and chicken although a mouse is preferably used in the present invention.

[0038] When the subject of the treatment is human and the animal for producing the α9 integrin-inhibiting antibody is a mouse, a chimeric antibody from human and mouse or a humanized antibody is preferably used. Preferably, a transgenic animal such as a mouse that has been introduced with a human gene involved in antibody production is used to produce a humanized monoclonal antibody to be employed.

Cell fusion of antibody-producing cell and myeloma cell

[0039] As a myeloma cell, a cell derived from a mouse, rat, human or the like is used. Examples include mouse myeloma P3U1, P3X63-Ag8, P3X63-Ag8-U1, P3NS1-Ag4, SP2/0-Ag14 and P3X63-Ag8-653. Preferably, the antibody-producing cell and the myeloma cell are derived from animals of the same species, particularly animals of the same strain. The myeloma cell may be cryopreserved, or maintained by passage in a general medium supplemented with a fetal horse, rabbit or bovine serum. For cell fusion, cells in logarithmic growth phase are preferably used. According to the present invention, P3X63-Ag8-653 is preferably used.

[0040] Examples of a method for fusing the antibody-producing cell and the myeloma cell to form a hybridoma include a method using polyethylene glycol (PEG), a method using Sendai virus, and a method using an electric fusion device. For example, in the case of PEG method, a mixture ratio of 1-10:1, preferably 5-10:1 of spleen cells and myeloma cells are suspended in an appropriate medium or buffer containing about 30-60% of PEG (average molecular weight: 1000-6000) to allow reaction at a temperature of about 25-37°C under conditions of pH6-8 for about 30 seconds to about 3 minutes. At the end of the reaction, the PEG solution is removed and the resultant is resuspended in a medium and seeded in a cell well plate to continue the culturing.

[0041] Sorting of hybridoma cells

A hybridoma cell that produces the monoclonal antibody can be sorted according to a known method or a method corresponding thereto. In general, sorting can be performed in a medium for animal cells supplemented with HAT (hypoxanthine, aminopterin, thymidine). The medium used for sorting and breeding may be any medium as long as it allows growth of the hybridoma cell. For example, a RPMI 1640 medium containing 1-20%, preferably 10-20% fetal bovine serum, a GIT medium (Wako Pure Chemical Industries, Ltd.) containing 1-10% fetal bovine serum, or a serum-free medium for culturing hybridoma (SFM-101, Nissui Pharmaceutical Co., Ltd.) can be used. The temperature for culturing is generally 20-40°C, and preferably about 37°C. The time for culturing is generally 5 days to 3 weeks, and preferably 1-2 weeks. In general, culturing may be carried out under 5% CO_2.

[0042] Production of a monoclonal antibody of the present invention may be confirmed and screened by employing a cellular ELISA technique described in *New Experimental Methods of Clinical Immunology (part 3)*, Kagaku Hyoron-sha, 1997. When the cell used for immunization is used for screening, the background or the false positives may be expected to increase. In this case, a clone that reacts with human α9 integrin overexpressed in a cell other than the cell used for immunization, but that does not react with a cell that overexpresses human α4 integrin, may be used as an anti-human α9 integrin antibody. Limiting dilution method can be repeated once to five times, preferably twice to four times, on such a clone to prepare a monoclonal antibody.

[0043] Separation and purification of antibody

The resulting antibody may be purified to homogeneity. Separation and purification of the antibody may be performed by employing a separation and purification technique that is generally used for proteins. For example, but without limitation, a chromatography column such as affinity chromatography, a filter, ultrafiltration, salting-out, dialysis, SDS polyacrylamide gel electrophoresis, isoelectric point electrophoresis or the like may appropriately be selected or combined to separate and purify the antibody (*Antibodies: A Laboratory Manual*. Ed Harlow and David Lane, Cold Spring Harbor Laboratory, 1988). Examples of a column used for affinity chromatography include protein A column and protein G column. For example, a column using a protein A column may be Hyper D, POROS and Sepharose F. F. (Amersham Biosciences).

[0044] Labeling of antibody

The resulting antibody can be labeled according to various labeling (for example, biotin label, FITC label, APC label) by a known method or with commercially available kit. Preferably, a biotin label using Biotin labeling kit (Dojindo Laboratories) is used in the present invention.

[0045] The thus-obtained monoclonal antibody is purified, if necessary, and then formulated according to a common technique to be used as a prophylactic and/or therapeutic agent for cancers, inflammatory diseases, infectious diseases, autoimmune diseases and bone diseases. A formulation of these prophylactic and/or therapeutic agents may be a parenteral formulation such as an injectable agent or drip, or may be devised for use as an oral formulation. For formulation, a carrier, a diluent, an additive or the like suitable for the formulation may be used within a medically and pharmaceutically acceptable range.

[0046] Pharmacological effect of antibody

Integrin not only plays a role of attaching and anchoring a cell and the extracellular matrix (ECM), but also been found to take a role in converting information from the extracellular matrix into intracellular signals for regulating cell proliferation, motility, cell death, differentiation and the like. Since the resulting monoclonal antibody can interrupt the intracellular signaling of information from ECM by inhibiting the binding between ECM and α9 integrin, it can treat ECM-related diseases. Examples of ECM and α9 ligands that are known to bind to α9 integrin include OPN, fibronectin, propeptide-von Willebrand factor (pp-vWF), tissue transglutaminase (tTG), coagulation factor XIII,

Vascular Cell Adhesion Molecule-1 (VCAM-1), tenascin C and plasmin. Cells or cancer cells expressing these ECM and α9 integrins are used to observe binding inhibition *in vitro* in the presence of the resulting monoclonal antibody, thereby determining a disease targeted by the monoclonal antibody of the present invention.

[0047] Pharmaceutical agent comprising antibody

A formulation comprising an antibody (in particular, a monoclonal antibody) of the present invention as an active component may be used as a therapeutic agent or a prophylactic agent for cancers (for example, proliferation or metastasis of cancer cells), inflammatory diseases (for example, rheumatoid arthritis, osteoarthritis, hepatitis, bronchial asthma, fibrosis, diabetes, arterial sclerosis, multiple sclerosis or inflammatory bowel diseases (ulcerative colitis or Crohn's disease)), infectious diseases (for example, hepatitis), autoimmune diseases (for example, systemic lupus erythematosus, polymyositis, autoimmune thyroid disease, tubulointerstitial nephritis or myasthenia gravis), bone diseases (for example, osteoporosis) and the like.

[0048] The dosage differs depending on the subject of administration, the target disease, conditions, the administration route and the like. For example, in the case where it is used for preventing and/or treating a cancer patient, a single dose of generally about 0.01-20 mg/kg weight, preferably about 0.1-10 mg/kg weight and more preferably about 0.1-5 mg/kg weight of the antibody of the present invention is conveniently administered by intravenous injection for about 1-10 times a month, preferably about 1-5 times a month. The dosage for other parenteral or oral administration may be determined in accordance with the above-described dosage. If the condition is particularly severe, the dosage or the number of administration may be increased according to the condition.

[0049] The antibody of the present invention may be administered per se or as an appropriate pharmaceutical composition. A pharmaceutical composition used for the above-described administration comprises the antibody or a salt thereof as well as a pharmacologically-acceptable carrier, diluent or excipient. Such a composition is provided in a formulation appropriate for parenteral or oral administration.

[0050] Specifically, examples of compositions for parenteral administration include an injectable agent, nasal drops and a suppository, where the injectable agent includes formulations such as an intravenously-injectable agent, a subcutaneously-injectable agent,

intradermally-injectable agent, an intramuscularly injectable agent and an injectable drip. Such an injectable agent is prepared according to a known method, for example, by dissolving, suspending or emulsifying the antibody in a sterile aqueous or oily solution that is generally usable for an injectable agent. For example, physiological saline, glucose, sucrose, mannitol or an isotonic solution containing other adjuvants may be used as an aqueous solution for an injectable agent possibly in conjunction with an appropriate solubilizing aid such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a non-ionic surfactant [e.g., Polysorbate 80, Polysorbate20, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)] or the like. For example, sesame oil, soybean oil or the like may be used as an oily solution possibly in conjunction with a solubilizing aid such as benzyl benzoate, benzyl alcohol or the like. The prepared injectable solution is usually packaged in a suitable ampule, vial or syringe. A suppository used for rectal administration may be prepared by mixing the above-described antibody into a usual nasal drop base or suppository base. Moreover, a suitable excipient may be added to the above-described antibody to prepare a lyophilized formulation, which is dissolved in injectable water, physiological saline or the like upon use as an injectable solution. In general, since an oral administration of a protein such as an antibody is believed to be difficult as it is broken down by a digestive organ, oral administration may be possible by devising the antibody fragment or the modified antibody fragment and the formulation thereof.

[0051] The above-mentioned parenteral pharmaceutical composition is preferably prepared into a formulation in a dose unit adapted to the dosage of the active element. Examples of such a dose-unit formulation include an injectable agent (an ampule, a vial, a prefilled syringe), nasal drops and suppository, where each dose unit of the formulation preferably contains the above-described antibody for generally 5-500 mg, in particular 5-100 mg for an injectable agent, and 10-250 mg for other formulations.

[0052] Each of the above-mentioned composition may contain other active element as long as it does not cause any unfavorable interaction upon compounding with the above-described antibody. For example, a pharmaceutical formulation of the present invention may contain an anti-human α4 integrin antibody in addition to the above-described antibody. In this case, the mixture ratio is not particularly limited but, for example, the ratio of the anti-human α9 integrin antibody to the anti-human α4 integrin antibody may be adjusted within the range of 1:99-99:1.

[0053] Diagnostic agent comprising monoclonal antibody of the present invention

A pharmaceutical composition comprising a monoclonal antibody of the present invention may be used as a diagnostic agent for an inflammatory disease such as rheumatoid arthritis or hepatitis, bronchial asthma, fibrosis, diabetes, cancer metastasis, arterial sclerosis, multiple sclerosis or granuloma, or as a diagnostic agent for suppression of chronic rejection after organ transplantation, or for an autoimmune disease such as a systemic autoimmune disease, erythematosus, uveitis, Behcet's disease, polymyositis, proliferative glomerulonephritis or sarcoidosis. Since the monoclonal antibody of the present invention can specifically recognize α9 integrin, it may be used for quantitation of α9 integrin in a test solution, and in particular for quantitation employing sandwich immunoassay, a competition technique, an immunometric technique or the like. No particular condition, procedure or the like is required for adapting each of these immunological determination techniques to a measurement technique of the present invention. The measurement system may be configured by those skilled in the art by applying general technical consideration to usual conditions and procedures for each method. For details of these general technical procedures, reference may be made to articles, documents and the like.

[0054] Accordingly, an antibody of the present invention can be used to quantitate α9 integrin in a highly sensitive manner. Furthermore, an antibody of the present invention can be used to quantitate α9 integrin *in vivo* to diagnose various α9 integrin-related diseases. For example, when an increase or a decrease is detected in the α9 integrin level, diagnosis is that there is a high possibility of suffering or likelihood of suffering an α9 integrin-related disease such as an inflammatory disease. In addition, a monoclonal antibody of the present invention can be used for specifically detecting α9 integrin existing in the subject such as a body fluid or a tissue. It can also be used for preparing an antibody column used for purifying α9 integrin, for detecting α9 integrin contained in each fraction upon purification, or for analyzing the behavior of α9 integrin in a test cell or the like.

[0055] Method of screening for compound that inhibits activity of human α9 integrin

An epitope on a human α9 integrin that is recognized by an antibody of the present invention may be utilized to screen for a compound that can inhibit the activity of human α9 integrin. Specifically, the present invention provides a method for screening

for a low-molecular compound that inhibits the activity of human α9 integrin, the method comprising using a peptide comprising an amino acid sequence of human α9 integrin (hereinafter, referred to as "peptide A").

[0056] According to the screening method of the present invention, for example, comparison is made between (i) a case where peptide A and a ligand of human α9 integrin (for example, tenascin C, plasmin, etc.) are made to contact and (ii) a case where peptide A, the ligand and a test compound are made to contact. The cases (i) and (ii) are compared, for example, by determining binding between the ligand and peptide A. In order to readily compare the bindings, a ligand that has been labeled according to a known technique is preferably used. A candidate compound resulting from such a method is tested to confirm whether or not it inhibits the activity of human α9 integrin, thereby obtaining a compound that inhibits the activity of human α9 integrin.

[0057] The test substance used may be a polypeptide, a protein, a non-peptide compound derived from a living organism, a synthetic compound, a microbiological culture, a cell extract, a plant extract, an animal tissue extract or the like, which may be either a novel compound or a known compound.

[0058] As is the case with the antibody of the present invention, the selected compound may be used as a prophylactic and/or therapeutic agent for cancers, inflammatory diseases, infectious diseases, autoimmune diseases, bone diseases and the like.

[0059] Hereinafter, the present invention will be described in more detail by means of examples below, which do not limit the scope of the present invention.

EXAMPLES

[0060] (Example 1)

<u>Production of antibody against human α9 integrin</u>

An antibody against human α9 integrin was produced by immunizing three BALB/c mice as follows. First, 3×10^6 cells/animal of human-α9-integrin-expressing cells (human α9/NIH-3T3 cells) were intraperitoneally administered to the mice, and further 3×10^6 cells/animal of human α9/NIH-3T3 cells were intraperitoneally administered following one and two weeks. A week later, 2×10^6 cells/animal of human α9/NIH-3T3 cells were intravenously administered. Clones that reacted with human

α9/CHO-K1 cell and human melanoma cell line (G361 cells) endogenously expressing human α9 integrin but that did not react with CHO-K1 cell expressing human α4 integrin were determined as anti-α9-integrin antibodies. As a result, two clones of hybridoma cells (K33N, M35A) that produced anti-human α9 integrin antibody were established.

[0061] The thus-obtained hybridoma cells K33N and M35A were each deposited with the International Patent Organism Depositary, the National Institute of Advanced Industrial Science and Technology (Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, 305-8566) on May 29, 2007, and assigned Accession No. FERM BP-10830 and FERM BP-10831, respectively.

[0062] (Example 2)

Analysis of complementarity determining region (CDR) of anti-human α9 integrin antibody

mRNAs were extracted from hybridomas that produce the human α9 integrin antibodies (K33N, M35A), and subjected to reverse transcription to prepare cDNAs. These cDNAs were used as templates together with ScFv cloning primers (Light Primer Mix, Heavy Primer Mix; Amersham Bioscience) for PCR to elongate and amplify the variable region of each of the heavy and light chains of the antibodies. Subsequently, the PCR products were integrated into pCRII TOPO vectors by a common technique. The resultants were sequenced to determine the amino acid sequences. The above-described procedure was repeated for three times for each antibody.

[0063] As a result, the amino acid sequences of the variable regions and the CDR regions of heavy and light chains of K33N and M35A were determined as shown in Figures 1A and 1B. Specifically, the amino acid sequences of the CDR regions were as follows.

[0064] (Heavy chain)

[CDRH1]

K33N: SYYMN (SEQ ID NO:1)

M35A: SYWIH (SEQ ID NO:2)

[CDRH2]

K33N: WIFPGSGNTKYNEKFKG (SEQ ID NO:3)

M35A: EINPSSGRTNFIENFETK (SEQ ID NO:4)

[CDRH3]

K33N: SWVSYERGYYFDY (SEQ ID NO:5)

M35A: LAYGNYSWFAY (SEQ ID NO:6)

[0065] (Light chain)

[CDRL1]

K33N: RASENIYYSLA (SEQ ID NO:7)

M35A: RASETVDSYGNTFMH (SEQ ID NO:8)

[CDRL2]

K33N: NANSLED (SEQ ID NO:9)

M35A: LASNLES (SEQ ID NO:10)

[CDRL3]

K33N: KQAYDVPY (SEQ ID NO:11)

M35A: QQNNEDPYT (SEQ ID NO:12)

[0066] Figures 1A and 1B also show sequences (JN Bio and Takara) obtained by different analysis methods (using different sequence analysis softwares) from the above-described sequence analysis method (GTS). The details of each method are described below.

[0067] Sequence analysis method by JN Biosciences (K33N)

The hybridoma cell (K33N) was cultured in TIL Media I medium (Immuno-Biological Laboratories) containing 10% fetal bovine serum (FBS; HyClone) under 7.5% CO_2 at 37°C for amplification. Total RNA was extracted from about 3 x 10^6 hybridoma cells using TRIzol reagent (Invitrogen) according to the protocol of Invitrogen. Preparation of cDNA by reverse transcription reaction using oligo dT primers was performed with GeneRacer Kit (Invitrogen) according to the protocol provided by Invitrogen. cDNAs of the variable regions of the H- and L-chains were amplified by PCR using 3' primer and GeneRacer 5' primer (5'-CGACTGGAGCACGAGGACACTGA-3' (SEQ ID NO:14)) attached to GeneRacer Kit, each corresponding to mouse constant regions γl and κ, with Phusion DNA polymerase (New England Biolabs). The 3' primer used for PCR amplification of the H-chain variable region (VH) was 5'-GCCAGTGGATAGACAGATGG-3'(SEQ ID NO:15). The 3' primer used for PCR amplification of the L-chain variable region (VL) was 5'-GATGGATACAGTTGGTGCAGC-3'(SEQ ID NO:16). The amplified VH and VL cDNAs were subcloned into pCR4Blunt-TOPO vectors (Invitrogen) for sequencing.

DNA sequence analyses of the variable regions were carried out with Tocore (Menlo Park).

[0068] Sequence analysis method by Takara (M35A)

A hybridoma cell (M35A) was cultured and amplified. Then, the total RNA of the cell was extracted using RNAiso (Takara Bio Inc.) according to Acid Guanidine-Phenol-Chloroform method (AGPC method). The extracted RNA was subjected to DNase I treatment according to a common technique, then to phenol chloroform treatment to remove DNase I, and to ethanol precipitation for purification. The resulting RNA was again suspended in distilled water and used for analysis. About 1 μg of DNase-I-treated RNA as a template and Random Primers (9mer) were used with Reverse Transcriptase M-MLV (RNase H free) for reverse transcription reaction. For PCR amplification of the variable regions, a part of each reverse transcription reactant was used as a template, Heavy Primers 1 and 2 (Amersham Bioscience) for H-chain, Light Primer Mix (Amersham Bioscience) for L-chain, and TaKaRa LA Taq (Takara) as PCR enzyme were used.

[0069] The DNA fragment obtained by PCR was subjected to electrophoresis with agarose gel. The band was excised out and the gel was eluted to purify the DNA. The purified DNA was cloned into pMD20-T vector for TA cloning. For DNA sequence analysis of the variable regions, M13-47 primer sequence included in pMD20-T vector was used for gene sequencing. For sequencing reaction, BigDye Terminators v3.1 cycle sequencing kit (Applied Biosystems) was used with ABI3730 sequencer (Applied Biosystems) according to the protocol of the manufacturer.

[0070] As can be appreciated from Figures 1A and 1B, although there were slight differences in the resulting sequences depending on the analysis method (or the analysis software) employed, there was no difference in the amino acid sequences of CDRs due to different analysis methods.

[0071] (Example 3)

Effect of anti-human α9 integrin antibody in inhibiting cell adhesion

Since α9 integrin binds to a ligand containing extracellular matrix (ECM) such as OPN, fibronectin, tenascin C and VCAM-1 upon cell adhesion, inhibition of cell adhesion by the resulting novel anti-human α9 integrin antibody was examined as inhibition of binding between an α9-integrin-expressing cell (human melanoma cell G361) and the ligand.

[0072] SVVYGLR peptide bound to BSA (bovine serum albumin) was used as OPN peptide, and a protein obtained by expressing the third region of Fibronectin Type III repeat of human tenascin-C in *E.coli* (where RGD sequence within this region was replaced with RAA sequence) was used as TN-C fn3 (RAA).

[0073] OPN peptide or tenascin C fragment (TN-C fn3 (RAA)) (5 μg/mL) was left in a 96-well plate at 37°C for an hour, and then blocked with 0.5% BSA/PBS. The human melanoma cells G361 were prepared to be 1 x 10^5 cells/mL with 0.25% BSA/DMEM medium, and added with the anti-human α9 integrin antibody at various concentrations. Two-hundred μL each of the antibody-added G361 cells was placed into a solid-phased 96-well plate and allowed to react at 37°C for an hour. Washing was repeated twice with PBS and then the adhered cells were immobilized and stained with 0.5% crystal violet/20% methanol. The resultant was washed with distilled water for three times, dissolved in 20% acetic acid and absorbance thereof was determined at 590 nm. Meanwhile, a monoclonal antibody (5A1) against human osteopontin was used as a negative control while five types of anti-human α9 integrin antibodies prepared beforehand (1K11, 21C5, 24I11, 25B6 and 28S1) were used as positive controls.

[0074] Effect of the anti-human α9 integrin antibody on adhesion of the G361 cells to OPN peptide is shown in Figure 2 and the results with tenascin C fragments are shown in Figure 3.

[0075] Similar to negative control 5A1 and positive controls 1K11, 25B6 and 28S1, M35A had less effect in inhibiting cell adhesion of the G361 cells to OPN peptide. On the other hand, K33N inhibited cell adhesion with a smaller amount compared to the positive controls 21C5 and 24I11 and showed an effect of inhibiting cell adhesion to an equal level to that of Y9A2. As to the adhesion of G361 cells to tenascin C fragment, M35A had less effect in inhibiting the cell adhesion while K33N inhibited the cell adhesion with a smaller amount and showed an equal level of inhibition effect to that of Y9A2, in other words, it showed evidently stronger inhibition effect than the positive controls 21C5 and 24I11. Hence, K33N, in particular, exerted particularly remarkable effect in inhibiting cell adhesion as compared to other antibodies.

[0076] (Example 4)

Difference in recognition sites of anti-human α9 integrin antibodies

Since the behavior of newly prepared anti-human α9 integrin antibody K33N in inhibiting cell adhesion was similar to that of Y9A2, competitive reactions of these antibodies to human α9 integrin-expressing cell (hα9/CHO) were detected by FACS to examine the difference in the recognition sites.

[0077] To biotin-labeled K33N or Y9A2 (5 μg/mL, 100 μL), 100 times the amount thereof of K33N, Y9A2 or negative control IgG (0.5 mg/mL, 100 μL) was added and then allowed to react with human α9 integrin cells (hα9/CHO, 1 x 10^7/mL, 100 μL) (4°C, 30 minutes). The cells were washed with FACS buffer (0.5% BSA/PBS). Streptavidin-labeled APC (0.5 μg/mL, 100 μL) was added to the cell solution for reaction (4°C, 20 minutes). Again, the cells were washed with FACS buffer and the dead cells were stained with 7-AAD (0.05 mg/mL, 20 μL). Subsequently, the cells were again washed with FACS buffer and measured by FACS.

[0078] As can be appreciated from Figure 4, when biotin-labeled Y9A2 and non-labeled Y9A2 were allowed to competitively react with cells expressing human α9 integrin, fluorescence-bound cells were obviously decreased almost to the background level. In the presence of biotin-labeled Y9A2 and non-labeled K33N, however, fluorescence-bound cells were decreased but not as low as the background level. Meanwhile, when biotin-labeled K33N and non-labeled K33N were allowed to compete against each other upon addition to the cells expressing human α9 integrin, fluorescence-bound cells were obviously decreased but fluorescence-bound cells did not decrease upon addition of non-labeled Y9A2 as low as that upon addition of non-labeled K33N. Accordingly, since Y9A2 and K33N did not present complete competition against each other when they would show competition against each other if they recognize the same epitope, Y9A2 and K33N should recognize different epitopes, suggesting that the antibodies were not identical.

INDUSTRIAL APPLICABILITY

[0079] The anti-α9-integrin antibody of the present invention shows an excellent effect in suppressing a function of α9 integrin and exerts a therapeutic effect against cancers (for example, cancer cell proliferation or metastasis), inflammatory diseases (for example, rheumatoid arthritis, osteoarthritis, hepatitis, bronchial asthma, fibrosis, diabetes, arterial sclerosis, multiple sclerosis, inflammatory bowel diseases (ulcerative colitis, Crohn's disease, etc.)), infectious diseases (for example, hepatitis), autoimmune diseases (for

example, systemic erythematodes, polymyositis, autoimmune thyroid disease, tubulointerstitial nephritis, myasthenia gravis) and bone diseases (for example, osteoporosis) and the like. Moreover, a pharmaceutical composition comprising the anti-α9-integrin antibody of the present invention as well as the anti-α4-integrin antibody exerts an improved therapeutic effect against cancers, inflammatory diseases and the like. The antibody of the present invention may also be utilized as a diagnostic drug since it is capable of pathologically detecting expression of α9 integrin in cells and tissues.

BRIEF DESCRIPTION OF THE DRAWINGS

[0080]

[Figure 1A] A diagram showing the results from analysis of the amino acid sequence of the variable region containing the complementarity determining region (CDR) of the heavy chain of anti-human α9 integrin antibodies (K33N and M35A). The results shown were obtained by using two different sequence analysis softwares for K33N and M35A.

[Figure 1B] A diagram showing the results from analysis of the amino acid sequence of the variable region containing the complementarity determining region (CDR) of the light chain of anti-human α9 integrin antibodies (K33N and M35A). The results shown were obtained by using two different sequence analysis softwares for K33N and M35A.

[Figure 2] A diagram showing the results from examining the effects of anti-human α9 integrin antibodies (two clones of the present invention (K33N, M35A), additional five clones (1K11, 21C5, 24I11, 25B6 and 28S1) and Y9A2) in inhibiting cell adhesion by using a human-α9-integrin-expressing cell (human melanoma cell G361) and an α9-integrin-binding site peptide of OPN (SVVYGLR). A monoclonal antibody against human-osteopontin (5A1) was used as a negative control.

[Figure 3] A diagram showing the results from examining the effects of anti-human α9 integrin antibodies (two clones of the present invention (K33N, M35A), additional five clones (1K11, 21C5, 24I11, 25B6 and 28S1) and Y9A2) in inhibiting cell adhesion by using a human-α9-integrin-expressing cell (human melanoma cell G361) and an α9-integrin-binding site peptide of tenascin C fragment. A monoclonal antibody against human-osteopontin (5A1) was used as a negative control.

[Figure 4] A diagram showing the results from examining the competitive reaction of a

novel anti-human α9 integrin antibody (K33N) and Y9A2 to a human-α9-integrin-expressing cell.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 30179-213 SEQ 13-06-16 v2.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

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30179-213

CLAIMS:

1. An anti-human α9 integrin antibody comprising the amino acid sequences represented by SEQ ID NOS:1, 3, and 5 as CDRH1, CDRH2, and CDRH3, respectively, of the complementary determining region of the heavy chain (CDRH), and the amino acid sequences represented by SEQ ID NOS: 7, 9 and 11 as CDRL1, CDRL2, and CDRL3, respectively, of the complementary determining region of the light chain (CDRL).

2. The anti-human α9 integrin antibody according to claim 1, wherein the binding between the human α9 integrin and the ligand of α9 integrin is inhibited.

3. The anti-human α9 integrin antibody according to claim 1 or 2, wherein it is a monoclonal antibody.

4. The anti-human α9 integrin antibody according to claim 1, 2 or 3, wherein it is a chimeric antibody.

5. The anti-human α9 integrin antibody according to claim 1, 2 or 3, wherein it is a humanized antibody.

6. The anti-human α9 integrin antibody according to claim 1, 2 or 3, which, other than the CDRs, is a human antibody.

7. An anti-human α9 integrin antibody produced by a hybridoma cell assigned Accession No. FERM BP-10830.

8. A therapeutic agent for a cancer, an inflammatory disease, an infectious disease, an autoimmune disease or a bone disease, comprising the anti-human α9 integrin antibody according to any one of claims 1 to 7.

9. A therapeutic agent for a cancer, an inflammatory disease, an infectious disease, an autoimmune disease or a bone disease, comprising the anti-human α9 integrin antibody according to any one of claims 1 to 7 as well as an anti-human α4 integrin antibody as active components.

10. A cell that produces the anti-human α9 integrin antibody according to any one of claims 1 to 7.

11. A hybridoma cell assigned Accession No. FERM BP-10830.

Fig. 1A

Anti-human α9 integrin antibody Heavy chain CDR

K33N

```
GTS      1: -VKLQESGPELVKPGASVKISCKASGYSFTSYYMNWVKKRPGQGLEWIGW 50
JN Bio   1: QVQLQQSGPELVKPGASVKISCKASGYSFTSYYMNWVKKRPGQGLEWIGW 50

GTS     51: IFPGSGNTKYNEKFKGKATLTADTSSSTAYMQVSSLTSEDSAVYFCARSW 100
JN Bio  51: IFPGSGNTKYNEKFKGKATLTADTSSSTAYMQVSSLTSEDSAVYFCARSW 100

GTS    101: VSYERGYYFDYWCQGTTLTVSS 122
JN Bio 101: VSYERGYYFDYWGQGTSLTVSS 122
```

M35A

```
GTS      1: VKLQESGTKLVKPGASVRLSCKASGYTFTSYWIH-WVKQSPGQGLEWIGEI 50
Takara   1: VKLQESGTKLVKPGASVRLSCKASGYTFTSYWIH-WVKQSPGQGLEWIGEI 50

GTS     51: NPSSGRTNFIENFETKATLTVDRSSTTAYMQLT-SLTSEDSAVYYCARLAY 100
Takara  51: NPSSGRTNFIENFETKATLTVDRSSTTAYMQLT-SLTSEDSAVYYCARLAY 100

GTS    101: GNYSW---FAYWGQGTTVTVSS 119
Takara 101: GNYSW---FAYWGQGTTVTVSS 119
```

Fig. 1B

Anti-human α9 integrin antibody Light chain CDR

K33N

GTS 1: DIQMTQSPASLAASVGETVTLTCRASENIYYSLAWYQQKQGKSPQLLIYN

JN Bio 1: DIQMTQSPASLAASVGETVTLTCRASENIYYSLAWYQQKQGKSPQLLIYN 50

GTS 51: ANSLEDGVPSRFSGSGSGTQYSMKINSMQPEDTATYFCKQAYDVPYTFGG 100

JN Bio 51: ANSLEDGVPSRFSGSGSGTQYSMKINSMQPEDTATYFCKQAYDVPYTFGG 100

GTS 101: GTKLELK

JN Bio 101: GTKLEIK

M35A

GTS 1: QIVLTQSPASLAVSLGQRATISCRASETVDSYGNTFMHWYQQKPGQPPKL 50

Takara 1: DIVLTQSPASLAVSLGQRATISCRASETVDSYGNTFMHWYQQKPGQPPKL 50

GTS 51: LIYLASNLESGVPVRFSGSGSRTDFTLTIDPVEADDAATYYCQQNNED-PY 100

Takara 51: LIYLASNLESGVPVRFSGSGSRTDFTLTIDPVEADDAATYYCQQNNED-PY

GTS 101: TFGGGTNWKNGR 112

Takara 101: TFGGGTKLEIKR 112

Fig. 2

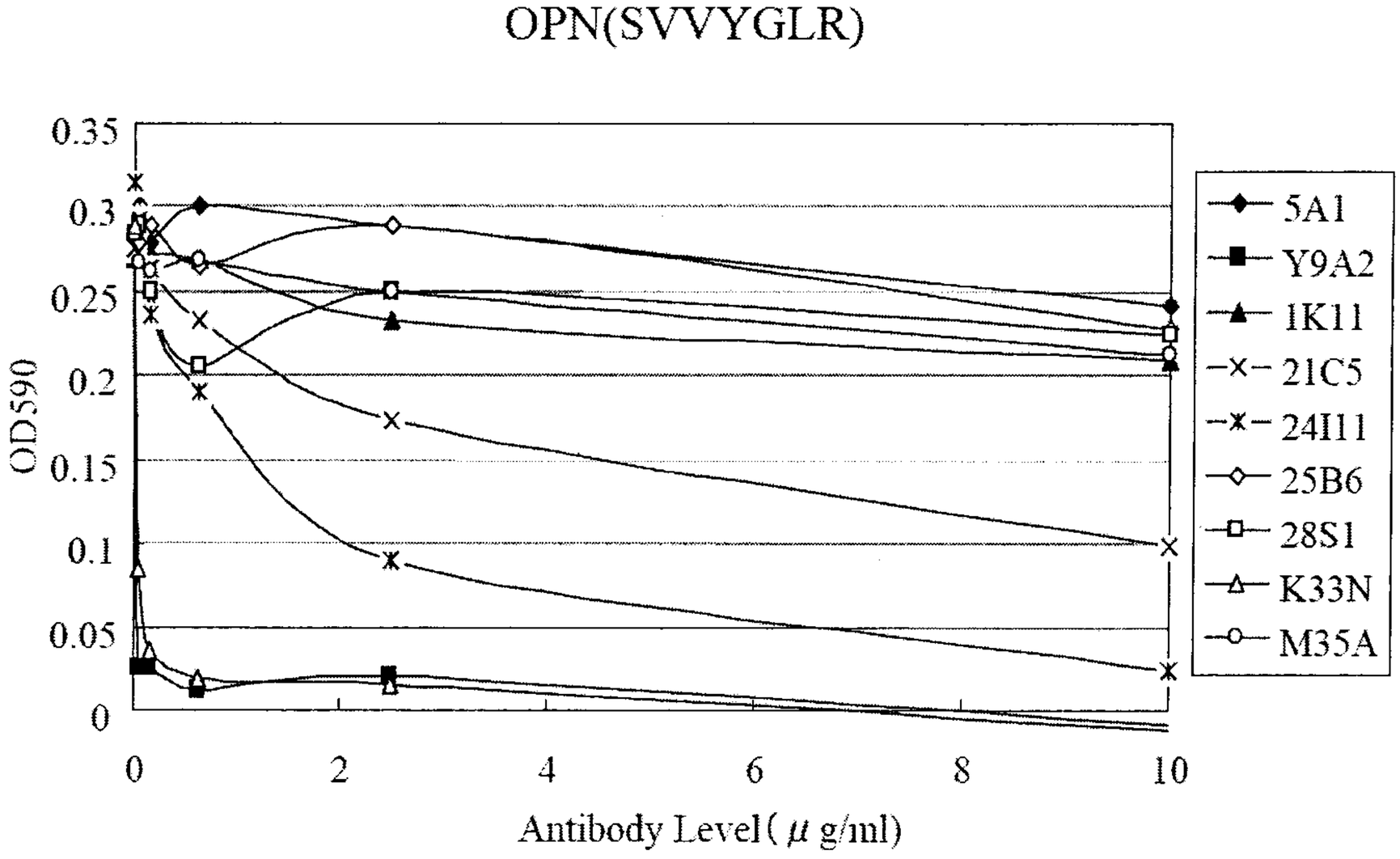

OPN(SVVYGLR)
OD590
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10
Antibody Level (μ g/ml)
5A1
Y9A2
1K11
21C5
24I11
25B6
28S1
K33N
M35A

Fig. 3

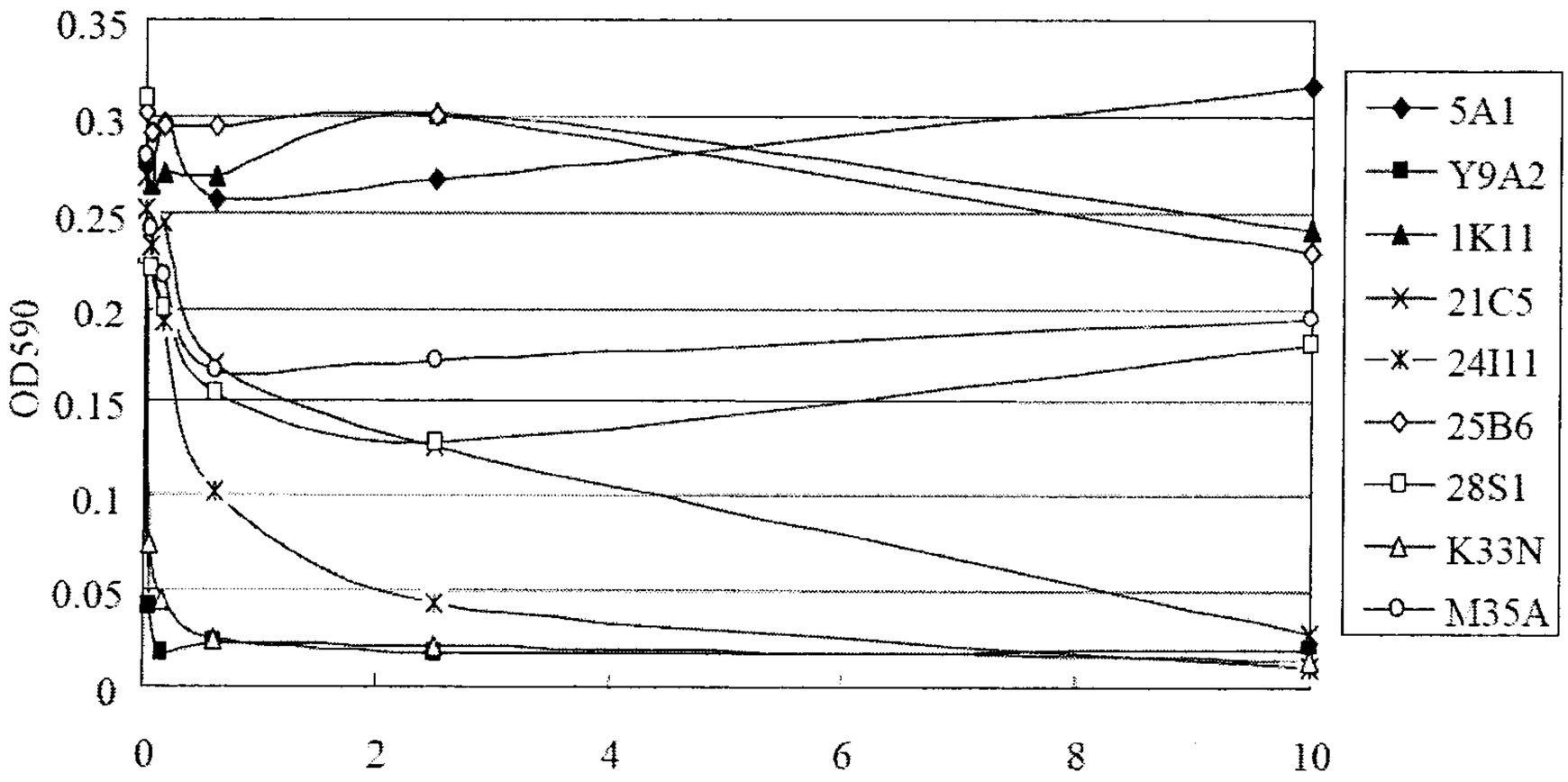
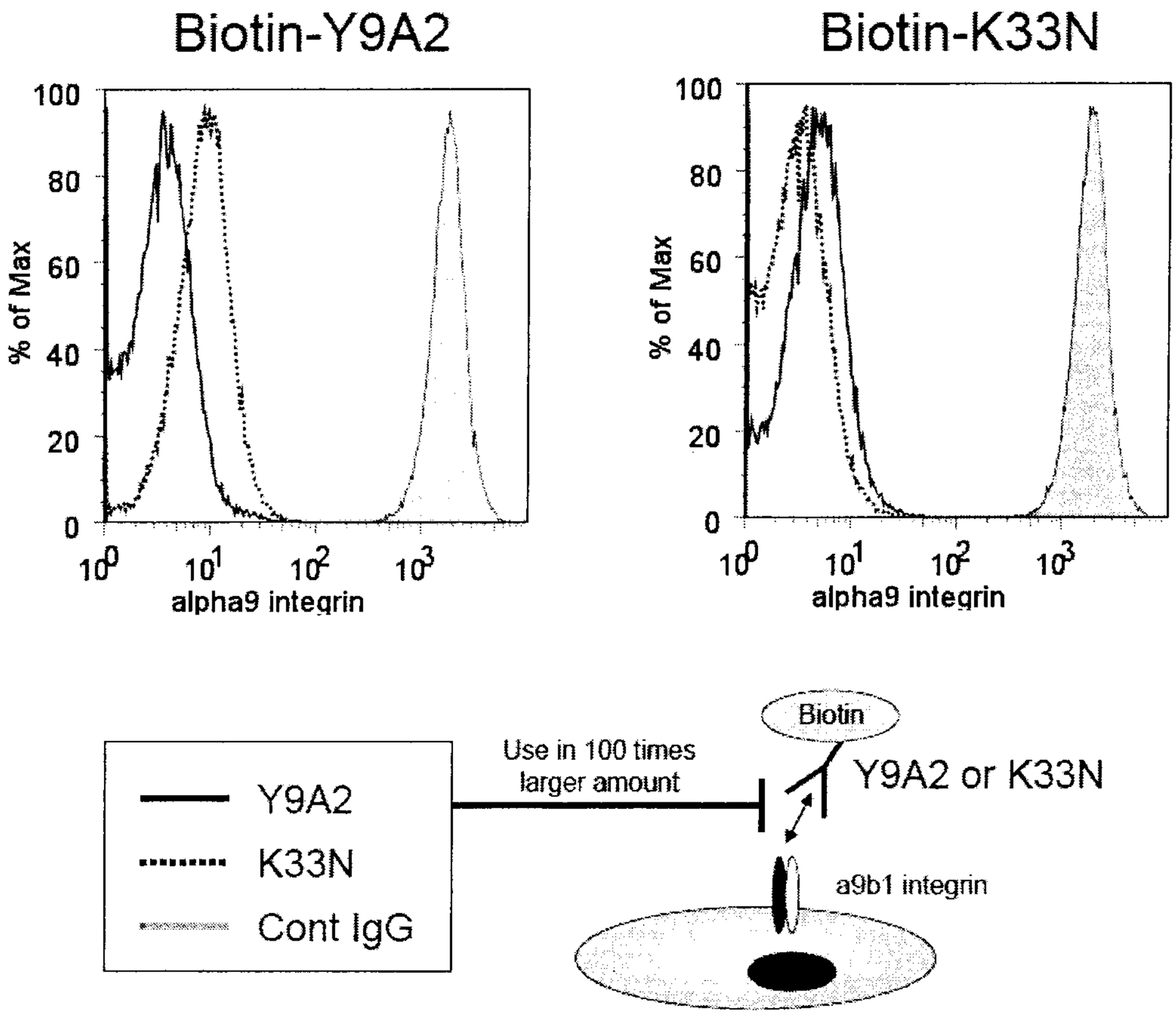

TN-C fn3(RAA)
OD590
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
2
4
6
8
10
Antibody Level (μ g/ml)
5A1
Y9A2
1K11
21C5
24I11
25B6
28S1
K33N
M35A

Fig. 4


Biotin-Y9A2
Biotin-K33N
100
80
60
40
20
0
% of Max
10^0
10^1
10^2
10^3
alpha9 integrin
Y9A2
K33N
Cont IgG
Use in 100 times larger amount
Biotin
Y9A2 or K33N
a9b1 integrin