DESCRIPTION

Title of Invention: ANTI-HUMAN RECEPTOR-TYPE PROTEIN TYROSINE PHOSPHATASE σ ANTIBODY

Technical Field

[0001]

The present invention relates to an antibody that binds to human receptor-type protein tyrosine phosphatase σ. Hereinafter "protein tyrosine phosphatase" is abbreviated as PTP, "receptor-type protein tyrosine phosphatase" is abbreviated as RPTP or PTPR, "receptor-type protein tyrosine phosphatase σ" is sometimes abbreviates as RPTP-σ, PTP-σ or PTPRS, and "human" and "mouse" are sometimes represented by the prefixes h and m, respectively.

Background Art

[0002]

Interferons (hereinafter "interferon" is sometimes abbreviated as IFN) are the most important cytokines in antiviral immune response. An interferon-producing cell (IPC: IPC is an undifferentiated lymphocytic dendritic cell that is positioned as a precursor cell of a dendritic cell (DC). IPC is also sometimes called a plasmacytoid dendritic cell or a plasma cell-like dendritic cell (plasmacytoid dendritic cell: pDC). Hereinafter IPC and pDC are considered to have the same meaning herein, and are hereinafter standardized by the term pDC as a general rule. A pDC in human blood expresses a major histocompatibility complex Class II protein together with CD4. However, since the number of such cells is small and the cells rapidly cause apoptosis and lack a lineage marker, those cells have not been isolated or characterized in detail until now. It was proved that pDC is a CD4+CD11c-2-type dendritic cell precursor cell, and that it produces IFN by 200 to 1,000 times greater than that produced by other blood cells after stimulation by a microorganism. Therefore, pDC2 is a decisive immune system effector cell in antiviral and antitumor immune responses.

IFNα and IFNβ are known as Type I IFNs having an antiviral activity or antitumor activity. On the other hand, it was clarified that IFNα relates to autoimmune diseases. For example, abnormal production of IFNα was reported in patients suffering from the following autoimmune diseases. Furthermore, possibility of alleviation of an autoimmune

condition by neutralizing IFNα was suggested.

Systemic erythematosus (Shiozawa et al., Arthr. & Rheum. 35, 412, 1992) and chronic rheumatoid arthritis (Hopkins et al., Clin. Exp. Immunol 73, 88, 1988), and furthermore, examples in which a condition of an autoimmune disease was expressed or deteriorated by administering recombinant IFNα2 or IFN were reported (Wada et al., Am. J. Gastroenterol. 90, 136, 1995; Perez et al., Am. J. Hematol. 49, 365, 1995; Wilson LE et al., Semin Arthritis, Rheum. 32, 163-173, 2002).

[0003]

Furthermore, it was also clarified that IFNα induces the differentiation of a dendritic cell (DC). Since a dendritic cell is also an antigen presenting cell, it is considered that the induction of differentiation of a dendritic cell constitutes an important mechanism in autoimmune diseases. In fact, it was suggested that the induction of differentiation of a dendritic cell of IFNα is intimately related to the onset of systemic erythematosus (Blanco et al., Science, 16: 294, 1540-1543, 2001). Therefore, the antitumor activity and intimate relation with autoimmune diseases of IFNα have been pointed out. Furthermore, IFNα also intimately relates to the onset of psoriasis (Nestle FO et al., J. Exp. Med. 202, 135-143, 2005).

[0004]

Only a small amount of pDC is present in blood. It is considered that the ratio of pDC in peripheral blood lymphocyte is 1% or less. However, pDC has an extremely high ability of producing IFN. The ability of pDC to produce IFN reaches, for example, 3,000 pg/mL/10^4 cells. Namely, it can be considered that, although the number of cells is small, the major part of IFNα or IFNβ in blood is produced while viral infection is brought by pDC.

[0005]

pDC differentiates into a dendritic cell by viral stimulation to induce the production of IFN-γ and IL-10 by a T cell. Furthermore, pDC also differentiates into a dendritic cell by the stimulation of IL-3. The dendritic cell differentiated by the stimulation of IL-3 induces the production of Th2 cytokines (IL-4, IL-5, IL-10) by a T cell. Thus, pDC has a characteristic that it differentiates into different dendritic cells depending on the difference of stimulation.

[0006]

Therefore, pDC is a cell having two aspects: one is an aspect as an IFN-producing cell and other is an aspect as a precursor cell for a dendritic cell. Both cells play important roles in an immune system. Namely, pDC is one of important cells that support an immune

system from various aspects.

[0007]

For the control of the activity of a humoral factor such as IFN, administration of an antibody that recognizes the factor is effective. For example, an attempt to treat an autoimmune disease by an antibody against interleukin (IL)-1 or IL-4 was put to practical use (Guler et al., Arthritis Rheum, 44, S307, 2001). Furthermore, it is considered that a neutralized antibody may become a therapeutic drug for autoimmune diseases also in interferons (IFNs) (Stewart, TA. Cytokine Growth Factor Rev. 14; 139-154, 2003). It can be expected that a similar approach would be effective for IFNs produced by pDC. However, such approach is based on the inhibition of the action of the produced humoral factor. If the production of an objective humoral factor can be controlled directly, a more essential therapeutic effect can be achieved.

[0008]

An antibody that recognizes human pDC was reported. For example, an anti-BDCA-2 monoclonal antibody is a monoclonal antibody that is specific to human pDC (Dzionek A. et al., J. Immunol 165: 6037-6046, 2000). It was clarified that the anti-BDCA-2 monoclonal antibody has an action of suppressing the IFN production of human pDC (J. Exp. Med. 194: 1823-1834, 2001). Furthermore, it was also reported that a monoclonal antibody that recognizes a mouse interferon-producing cell suppresses the production of interferons (Blood 2004 Jun 1; 103/11: 4201-4206. Epub 2003 Dec). It was reported that a monoclonal antibody against mouse pDC decreased the number of dendritic cells (J. Immunol. 2003, 171: 6466-6477).

[0009]

It is useful if an antibody that similarly recognizes human pDC and may control the activity thereof is provided. For example, the present inventors have already clarified that an antibody that recognizes Ly49Q specifically binds to mouse pDC. However, an antibody against Ly49Q did not interfere the activity of mouse pDC (Blood, 1 Apri1 2005, vol. 105, No. 7, pp. 2787-2792: WO2004/13325A1).

[0010]

Protein phosphatases are dephosphorylating enzymes that were found in the studies of glycogen metabolism. Besides protein tyrosine phosphatase (PTP), protein serine/threonine phosphatase, phospholipid-specific phosphatase and the like have been found, and these form a superfamily of protein phosphatases. Of these, protein tyrosine phosphatase is an enzyme that is responsible for dephosphorylation among reversible phosphorylation modifications that are observed in tyrosine residues of proteins. On the other hand, protein

tyrosine kinase (PTK) is exemplified as an enzyme that is responsible for phosphorylation among reversible phosphorylation modifications that are observed in tyrosine residues of proteins.

[0011]

Protein tyrosine phosphatase (PTP) converts the binding information of a ligand in an extracellular domain thereof to the phosphatase activity of an intracellular domain, and it is considered that protein tyrosine kinase (PTK) is activated by the binding of a ligand, whereas protein tyrosine phosphatase (PTP) is generally inactivated by the binding of a ligand. Therefore, in both of protein tyrosine phosphatase (PTP) and protein tyrosine kinase (PTK), stimulation of a ligand leads to increase in the phosphorylation level, whereas a great difference is expected in the signal properties. In the case of protein tyrosine kinase (PTK), positive feedback control in which receptors are phosphorylated with each other and activated is conducted, and the topical activation of the protein tyrosine kinase (PTK) molecules transmits to other protein tyrosine kinase (PTK) molecules on a cell membrane, thereby phosphorylation is increased over a wide range. On the other hand, only molecules to which ligands have bound are inactivated in protein tyrosine phosphatase (PTP), and the phosphorylation of the substrate is increased only topically. Protein tyrosine phosphatase (PTP) that is involved in many physiological functions and cellular functions gets a lot of attention in broad areas of brain neurobiochemistry, immunology, cancers, diabetes mellitus and the like (copy of the home page of the Division of Molecular Neurobiology, National Institute for Basic Biology, http://niwww3.nibb.ac.jp/RPTP.pdf).

[0012]

The protein tyrosine phosphatase family can be classified into a receptor type having a cell membrane penetrating region and a non-receptor type. There are 21 molecules of receptor type protein tyrosine phosphatases (also abbreviated as RPTP or PTPR) in mammals, which are classified into eight subfamilies and each subfamily has an inherent extracellular structure in which a immunoglobulin-like domain, a fibronectin type III-like domain, a carbonate dehydratase-like domain, an MAM domain and the like are observed (Nat Rev Mol Cell Biol., Vol. 7, 833-846, 2006).

[0013]

Human receptor-type protein tyrosine phosphatase σ (this is abbreviated as hRPTP-σ, hPTP-σ or hPTPRS, and the abbreviation hPTPRS that is mainly used herein) belongs to a R2A subfamily together with LAR (leukocyte antigen-related protein tyrosine phosphatase) and receptor-type protein tyrosine phosphatase δ (PTP-δ). Enzymes of the PTPR family are

expressed in various tissues including nerve systems from initiation of generation to after maturation of animals, but few physiological functions thereof have been clarified since identification of ligand molecules and substrate molecules is not easy.

Dendritic cells (DCs) are major antigen presenting cells in a living body, which are present in blood, lymphoid tissues and the like and are roughly classified into myeloid dendritic cell (mDCs) and plasmacytoid dendritic cells (pDCs). pDC selectively expresses TLR7 and TLR9 as Toll-like receptors on the cell surfaces thereof, and produce Type I interferons α and β, specifically interferon α.

The recent studies have clarified various ligand molecules that act on dendritic cells to control their maturation and activation, and the intracellular signal transmission mechanisms from the receptors thereof have been becoming clear. However, there are many unclear points about the mechanisms of modification and control of the functions of dendritic cells. Similarly to the clarification in many other cells, it is considered that the phosphorylation of proteins plays an important role also in dendritic cells for the control of signal transmission from receptors, of motion/migration of cells, and the like.

Protein phosphatases that are negative controlling factors for protein phosphorylation are dominant candidates as factors for maintaining suitable intensities and lengths of signals to modulate the activation and functions of dendritic cells. (Nobuhiro Tanuma (Institute for Genetic Medicine, Hokkaido University), "Functional Analysis Of Tyrosine Phosphatase Induced in Maturing of Dendritic Cells" in the homepage of the Northern Advancement Center for Science & Technology (abbreviation: NOASTEC), http://www.noastec.jp/kinouindex/data2005/pdf/01/01_20.pdf)

[0014]

International Publication No. WO95/9656A1 discloses RPTP-σ (PTPRS) and a nucleic acid coding therefor; however, the disclosed amino acid sequence is one derived from a rat, and the publication does not mention about an antibody specific to PTPRS. International Publication No. WO95/9656A1 also fails to disclose about an anti-human PTPRS antibody.

International Publication No. WO2007/41317A1 relates to an isolated antibody that specifically binds to at least RPTP-σ or RPTP-δ to suppress the immune response of an immune cell, or an antigen binding fragment thereof. The document describes that the binding of poxvirus polypeptide A41L and RPTP is competitively inhibited by using an antibody that specifically binds to RPTP, thereby suppression of the immune response of an immune cell is achieved. However, this document fails to disclose that the antibody that specifically binds to RPTP-σ (PTPRS) was actually obtained, and as far as the description of the Examples is called

into account, the Examples merely confirmed that RPTP expressed in an immune cell that binds to A41L is a part of RPTP-σ, RPTP-δ and LAR that belong to the same subtype R2A and prepared a fusion protein of the immunoglobulin-like domain of LAR and Fc (LAR (Ig domain)-Fc fusion protein). It is hardly to say that International Publication No. WO2007/41317A1 discloses an antibody specific to only RPTP-σ and the preparation therefor.

Citation List

Patent Literature

[0015]

PTL 1: WO2004/13325A1

PTL 2: WO95/9656A1

PTL 3: WO2007/41317A1

Non Patent Literature

[0016]

NPL 1: Shiozawa et al., Arthr. & Rheum. 35, 412, 1992

NPL 2: Hopkins et al.,Clin. Exp. Immunol. 73, 88, 1988

NPL 3: Wada et al., Am.J. Gastroenterol. 90, 136, 1995

NPL 4: Perez et al., Am. J. Hematol. 49, 365, 1995

NPL 5: Wilson LE et al, Semin Arthritis. Rheum. 32, 163-173, 2002

NPL 6: Blanco et al., Science, 16:294,1540-1543,2001

NPL 7: Nestle FO et al., J.Exp.Med. 202, 135-143, 2005

NPL 8: Guler et al., Arthritis Rheum., 44. S307, 2001

NPL 9: Stewart, TA. Cytokine Growth Factor Rev. 14; 139-154, 2003

NPL 10: Dzionek, A. et al. J.Immunol. 165: 6037-6046, 2000

NPL 11: J. Exp. Med.194:1823-1834, 2001

NPL 12: Blood 2004 Jun 1;103/11:4201-4206. Epub 2003 Dec

NPL 13: J. Immunol. 2003, 171:6466-6477

NPL 14: Blood, 1 April 2005, Vol. 105, No. 7, pp. 2787-2792

NPL 15: http://niwww3.nibb.ac.jp/RPTP.pdf

NPL 16: Nat Rev Mol Cell Biol., Vol. 7, 833-846, 2006

NPL 17: http://www.noastec.jp/kinouindex/data2005/pdf/01/01_20.pdf

Summary of Invention

Technical Problem

[0017]

The object of the present invention is to provide an antibody that binds to human receptor-type protein tyrosine phosphatase σ (human PTPRS, hRPTP-σ), and to detect, identify or isolate pDC. Furthermore, the object of the present invention is to modulate the activity of pDC.

[0018]

The present inventors confirmed through the studies relating to human pDC that the expression of PTPRS in pDC is specifically enhanced. Therefore, the present inventors tried to prepare an antibody of PTPRS and clarify the action thereof.

[0019]

In order to obtain an antibody that recognizes a trace amount of a protein derived from a living body, a protein prepared by a gene recombination technology is generally utilized as an immunogen. The present inventors have tried to express human PTPRS based on the base sequence of cDNA of human PTPRS, which has been already clarified, and the information on the amino acid sequence coded thereby (GenBank Accession No. NM_002856.3).

[0020]

In order to obtain an antibody of a protein, utilization of a partial amino acid sequence of a natural protein as an immunogen is often tried. However, in order for an antibody to recognize a molecule on a cellular surface, a region that constitutes a part that is recognized by an antibody as an epitope on a cellular surface should be selected. Therefore, it was considered that obtainment of an antibody that is specific to human PTPRS by using a fragment amino acid sequence as an immunogen is distant.

Solution to Problem

[0021]

Under such situation, the present inventors have clarified that an antibody that binds to pDC can be obtained by utilizing a special immunogen. Furthermore, they have also confirmed that the thus-obtained antibody specifically recognizes human pDC and has an action to modulate the activity thereof, and completed the present invention.

Therefore, the present invention relates to the following anti-human PTPRS antibody, the method for the production of the same, and the applications thereof.

The present invention is as follows.

(1) A monoclonal antibody or a fragment including an antigen-binding region thereof that binds, as confirmed by flow cytometry, to an extracellular domain of human

receptor-type protein tyrosine phosphatase σ (human PTPRS) on a human plasmacytoid dendritic cell, which is a recombinant antibody or antibody fragment including an antigen-binding region.

(2) A monoclonal antibody or a fragment including an antigen-binding region thereof according to the above-mentioned (1), which suppresses production of interferon (IFN) by the cell and/or survival of the cell, and which is a recombinant antibody or antibody fragment including an antigen-binding region.

(3) The antibody or the fragment according to the above-mentioned (1) or (2) which is a chimeric antibody, a humanized antibody, a single chain Fv, a diabody, a linear antibody, or a multispecific antibody formed from antibody fragments.

(4) The antibody or the fragment according to the above-mentioned (1) or (2), which is a humanized antibody.

(5) The antibody fragment according to the above-mentioned (1) or (2), which is a Fab or $F(ab')_2$.

(6) The antibody or the fragment according to the above-mentioned (3) or (4), which is a chimeric antibody or a humanized antibody comprising a constant region derived from IgG or IgM.

(7) The antibody or the fragment according to any one of the above-mentioned (1) to (6), which comprises an antigen-binding region of a monoclonal antibody produced by hybridoma 9H5-4 that was deposited as Accession No. FERM BP-11356, hybridoma 10F7-38 that was deposited as Accession No. FERM BP-11357, hybridoma 13G5-52 that was deposited as Accession No. FERM BP-11358, hybridoma 13G5-57 that was deposited as Accession No. FERM BP-11359, hybridoma 14A8-85 that was deposited as Accession No. FERM BP-11360, hybridoma 22H8-84 that was deposited as Accession No. FERM BP-11361, hybridoma 49F2-30 that was deposited as Accession No. FERM BP-11362 or hybridoma 55E7-79 that was deposited as Accession No. FERM BP-11363.

(8) A polynucleotide that codes for the chimeric antibody of the above-mentioned (3) or (6), or the humanized antibody of the above-mentioned (4) or (6).

(9) An expression vector comprising cDNA that codes for an antigen-binding region of the antibody from hybridoma 9H5-4 that was deposited as Accession No. FERM BP-11356, hybridoma 10F7-38 that was deposited as Accession No. FERM BP-11357, hybridoma 13G5-52 that was deposited as Accession No. FERM BP-11358, hybridoma 13G5-57 that was deposited as Accession No. FERM BP-11359, hybridoma 14A8-85 that was deposited as Accession No. FERM BP-11360, hybridoma 22H8-84 that was deposited as Accession No.

FERM BP-11361, hybridoma 49F2-30 that was deposited as Accession No. FERM BP-11362 or hybridoma 55E7-79 that was deposited as Accession No. FERM BP-11363.

(10) A method for the production of the antibody or the fragment according to any one of the above-mentioned (1) to (7).

(11) A method according to the above-mentioned (10), which:

(a) comprises gene recombination;

(b) comprises a method of gene engineering using a polynucleotide of the above-mentioned (8); or

(c) comprises expressing the vector of the above-mentioned (9) in a host cell.

(12) A method for the detection of a human plasmacytoid dendritic cell, which comprises contacting the antibody or the fragment according to any one of the above-mentioned (1) to (7) with a subject cell, and detecting the antibody or the fragment that has bound to the cell.

(13) Use of the antibody according to any one of the above-mentioned (1) to (7) in the *in vitro* diagnosis of diseases in which human plasmacytoid dendritic cells are involved.

(14) The antibody or the fragment according to any one of the above-mentioned (2) to (7) for use in the treatment of the human or animal body by therapy, wherein the antibody suppresses production of IFN by a human plasmacytoid dendritic cell and/or survival of the cell.

(15) The antibody or the fragment according to any one of the above-mentioned (2) to (7) for use in the treatment of an autoimmune disease in which the expression of IFNalpha is enhanced, wherein the antibody suppresses production of IFN by a human plasmacytoid dendritic cell and/or survival of the cell.

(16) The antibody or the fragment according to any one of the above-mentioned (2) to (7) for use in therapy.

(17) Use of the antibody or the fragment according to any one of the above-mentioned (2) to (7) in the manufacture of a medicament for the treatment of an autoimmune disease in which the expression of IFNα is enhanced, wherein the antibody suppresses production of IFN by a human plasmacytoid dendritic cell and/or survival of the cell.

(18) A pharmaceutical composition, comprising the antibody or the fragment according to any one of the above-mentioned (2) to (7) as an active ingredient.

Advantageous Effects of the Invention

[0022]

The present invention provides an antibody that specifically recognizes human PTPRS, and a method for the production of the anti-human PTPRS antibody. Human PTPRS is

a membrane protein that belongs to the RPTP family. The present inventors clarified that an antibody that specifically recognizes human PTPRS can be readily obtained. The anti-human PTPRS antibody that can be obtained by the present invention is an antibody having high specificity, which distinguishes human pDC from cells that express other RPTP families.

[0023]

The anti-human PTPRS antibody provided by the present invention binds to an extracellular domain of human PTPRS on a human pDC. Furthermore, the antibody of the present invention specifically recognizes human pDC. Therefore, it is useful for detection and isolation of pDC. pDC is a cell that produces the major part of Type 1 IFN. Therefore, the detection and isolation thereof are important in the diagnoses and studies of diseases in which pDC is involved such as autoimmune diseases.

[0024]

Furthermore, the anti-human PTPRS antibody provided by the present invention has an action to modulate the activity of human pDC in a preferable embodiment. Therefore, the anti-human PTPRS antibody of the present invention can be utilized for suppressing the activity of pDC. Therefore, if the suppression of the activity of pDC utilizing the antibody of the present invention is utilized, a therapeutic effect can be expected even in a patient with an autoimmune disease in which the expression of IFNα has enhanced.

[0025]

pDC produces a large amount of IFN with little cells. For neutralization of IFN, an antibody corresponding to the molecular number of IFN is necessary. However, in the present invention, the activity of the produced cell is suppressed directly. As a result, a stronger effect of suppressing IFN can be expected with a smaller amount of antibody as compared to neutralization by an anti-IFN antibody. Furthermore, in the case when IFN is produced persistently, it is expected that neutralization of IFN by an antibody is suppressed only transiently, whereas the activity of pDC is suppressed and thus an effect of suppressing production of IFN for a long term can be expected in the present invention.

Brief Description of Drawings

[0026]

FIG. 1 is the amino acid sequence of PTPRS (SEQ ID NO:1). PTPRS is a single transmembrane membrane protein having an immunoglobulin-like domain (Ig-like domain) and a fibronectin Type III-like domain in the extracellular region. Furthermore, it has two protein tyrosine phosphatase regions (PTP domains) in the intracellular region;

FIG. 2 is a graph showing the relative expression levels of PTPRS in various immune cells. It was shown that PTPRS expresses in a pDC-specific manner;

FIG. 3 is a graph showing the comparison of expression of PTPRS gene between tissues. PTPRS mRNA shows relatively high expression in the spleen and ovary, and also expresses broadly in other tissues;

FIG. 4 shows selection of the human PTPRS (hPTPRS)-expressing cell by FACS sorting;

FIG. 5 shows FACS screening of hybridomas using immunized hPTPRS/D2SC/1 cell. Thirteen hybridomas that produce an anti-hPTPRS antibody were obtained;

FIG. 6 shows FACS screening using CAL-1 cell;

FIG. 7 shows FACS screening using human peripheral blood pDC;

FIG. 8 is a graph showing the homology of hPTPRS with other PTPRs. PTPRS belongs to the PTPR family, of which the amino acid sequences of several family molecules have high homology against the amino acid sequence of PTPRS;

FIG. 9 is the test result showing whether or not the ten kinds of hybridoma cell culture supernatants (2G6, 4B2, 2G2, 9H5, 10F7, 22H8, 49F2, 14A8, 55E7, 13G5) that recognize PTPRS and produce an antibody that specifically binds to human pDC specifically bind to only PTPRS (hPTPRE did not express on the cellular surface). As a result thereof, 2G6 showed cross-reactivity with PTPRF (FIG. 9, D), and 4B2 showed cross-reactivity with PTPRD (FIG. 9, C). Other 9 kinds of antibodies showed PTPRS-specific binding (FIG. 9, A to D);

FIG. 10 is the test result of the cross-reactivity of the anti-PTPRS antibody with a monkey. All hybridoma cell culture supernatants specifically bonded to the pDC cell group (Lineage-CD123+ HLA-DR+) of a cynomolgus monkey;

FIG. 11 shows the singlization sorting of the hybridomas. Single cell sorting was conducted by using FACS Aria (BD), and D2SC/1 cell and hPTPRS/D2SC/1 cell (A and B), CAL-1 cell (C) and human pDC (D) were stained by using the cell culture supernatants of the hybridomas, and single hybridomas were selected;

FIG. 12 shows the concentrations of the endotoxin in the purified antibodies obtained from the culture supernatants of the hybridomas. All concentrations were the standard value 0.3 Eu/mg Ab or less;

FIG. 13 is the test result of the abilities of the purified antibodies to bind human PTPRS on the cellular surface. It could be confirmed that all of the antibodies maintained their binding ability;

FIG. 14 shows the specific binding of the purified antibodies to the pDC cell

groups (BDCA2+) of human peripheral blood;

FIG. 15 is the result of testing whether or not the anti-human PTPRS antibodies also bind to mouse PTPRS. 49F2-30, 13G5-52, 13G5-57 and 22H8-84 bound to mPTPRS/CHO;

FIG. 16 shows the complement-dependent cytotoxic activities of the anti-PTPRS antibodies against an hPTPRS-expressing cell. The complement-dependent cytotoxic activities of the anti-PTPRS antibodies against human PTPRS/CHO (FIG. 16A) and mouse PTPRS/CHO (FIG. 16B) were measured. As a result, 13G5-52 and 13G5-57 showed about 20% of CDC activity against the target of human PTPRS/CHO (A), whereas 13G5-52 and 13G5-57 showed about 100% of CDC activity against the target of mouse PTPRS/CHO (B);

FIG. 17 shows that ch49F2-30(FIG 17A),ch9H5-4,ch13G5-57 and ch22H8-84(FIG 17B) of an anti-hPTPRS chimeric antibody injure the target hPTPRS/CHO cell in an effector cell number-dependent manner; and

FIG. 18 shows that IFNα production is completely inhibited by the treatment of the anti-PTPRS chimeric antibody with ch49F2-30,ch9H5-4,ch13G5-57 and ch22H8-84 (FIG. 18A), and it was clarified that the pDC population was decreased more than in the Synagis treatment of the control antibody (FIG. 18B and FIG 18C).

Description of Embodiments

[0027]

Human PTPRS is a molecule whose specific expression is observed in a plasma cell-like dendritic cell pDC. However, any method for the production of an antibody that recognizes human PTPRS has not been established yet.

[0028]

Four isoforms of human PTPRS are known, which include isoform 1 that consists of 1,948 amino acid residues, isoform 2 that consists of 1,910 amino acid residues, isoform 3 that consists of 1,501 amino acid residues, and isoform 4 that consists of 1,505 amino acid residues. In the structures thereof, three immunoglobulin-like domains (first Ig domain, second Ig domain and third Ig domain), a fibronectin Type III-like domain, a transmembrane domain (transmembrane domain, TM region) as extracellular structures, and two phosphatase domains (D1 and D2 domains) as intracellular structures are observed. Only D1 domain that is close to the cell membrane has protein tyrosine phosphatase (PTP) activity. In FIG. 1, signal peptides and typical domains are marked in the amino acid sequence.

The isoform 3 of human PTPRS is a membrane penetrating protein including 831

to 851 of SEQ ID NO:1 (FIG. 1) as a transmembrane domain. Of the 1,501 amino acid residues including N terminus, 29 amino acid residues (1 to 29 in SEQ ID NO:1) constitute a signal sequence, and 30 to 830 constitute an extracellular domain. On the other hand, the C-terminus side is an intracellular domain. It is considered that the ligands in the extracellular environment control the activity in PTPRS.

[0029]

The present inventors have confirmed by a gene expression analysis that human PTPRS is specifically expressed in human pDC. They considered that, if an antibody that can distinguish human PTPRS from other molecules can be obtained, it would be useful for the studies of pDC. However, there are many molecules having similar structures in the PTP family including human PTPRS. Molecules such as PTPRS that is RPTP-σ and PTPRA (RPTP-α), PTPRD (RPTP-δ), PTPRE (RPTP-ε, PTPRF (RPTP-ζ) specifically include an amino acid sequence having high homology (FIG. 8). Therefore, they considered that it would be difficult to obtain an antibody that can differentiate these molecules from each other by using a domain peptide using the partial sequence of an amino acid sequence that constitutes an extracellular domain as an immunogen. Therefore, the present inventors tried to obtain an antibody against human PTPRS by using a cell that expresses human PTPRS as an immunogen.

[0030]

The present inventors have done intensive studies so as to obtain an antibody that recognizes human PTPRS and clarified that the objective antibody can be obtained by using a specific transforming cell as an immunogen, and completed the present invention. Namely, the present invention relates to a monoclonal antibody that binds to an extracellular domain of human PTPRS, or a fragment including an antigen-binding region thereof.

[0031]

In the present invention, human PTPRS can be defined as a natural molecule that expresses in human pDC, or a molecule that is immunologically equivalent to human PTPRS that expresses in human pDC. In the present invention, that the antibody binds to human PTPRS can be confirmed, for example, as follows.

- Confirmation based on reactivity with human cell:

According to the finding obtained by the present inventors, it is considered that human PTPRS can be utilized as a marker for pDC since expression specific to human pDC is observed.

[0032]

Based on such expression profile of human PTPRS, firstly, the activity of pDC to

bind to at least a part of subset is one of important characteristics of the antibody that binds to human PTPRS in the present invention. That a certain cell is pDC can be confirmed by a cell surface marker that is inherent to each cell group. For example, binding to the objective cell is confirmed by double staining with an antibody that binds to a cell surface marker and an antibody whose binding activity is to be confirmed. Namely, pDC in the present invention includes, for example, a cell that expresses BDCA2.

[0033]

- Confirmation based on reactivity with transforming cell that expresses human PTPRS gene:

The present inventors have confirmed that, when a human PTPRS gene is expressed under a specific condition, the immunological characteristics of human PTPRS expressed in human pDC is reconstituted. Therefore, the reactivity with human PTPRS can be confirmed based on the reactivity of an antibody against a cell to which a gene that codes for human PTPRS has been artificially introduced. Namely, the present invention relates to a monoclonal antibody that binds to a molecule including an amino acid sequence that constitutes an extracellular domain of human PTPRS as an extracellular domain, or a fragment including an antigen-binding region thereof. Meanwhile, the extracellular domain is constituted by the amino acid sequence corresponding to from 30 to 830 in SEQ ID NO:1 (FIG. 1) from the N-terminus of the amino acid sequence shown in SEQ ID NO:1.

For example, in a cell that has been transformed with an expression vector including a DNA that codes for human PTPRS, the immunological characteristics of PTPRS that expresses in human pDC are maintained. Therefore, a transforming cell that expresses human PTPRS is preferable as a cell for confirming the binding property of the antibody against an extracellular domain of human PTPRS in the present invention. When the reactivity of the antibody is confirmed by a transformation cell in the present invention, it is desirable to utilize a cell that has not been transformed as a control.

[0034]

Next, the antibody that binds to human PTPRS in the present invention may be an antibody whose cross-reactivity with a cell group that is known to express PTP family other than human PTPRS is observed or not observed. The antibody whose cross-reactivity is not observed is preferable as the antibody that binds to human PTPRS in the present invention. Specifically, an antibody whose binding with a cell group that is known to express PTP family other than human PTPRS under the same condition as the condition under which binding to pDC has been confirmed is preferable as the antibody that binds to human PTPRS in the present invention.

[0035]

Namely, a monoclonal antibody that binds to an extracellular domain of human PTPRS in the present invention preferably includes a monoclonal antibody having the following immunological characteristics.

a) it binds to human pDC,

b) under the condition in which it binds to human pDC, its binding to one kind or plural kinds selected from the group consisting of a monocyte, a macrophage, a B cell and a CD34 positive cell, and dendritic cells derived from these cells, cannot be confirmed.

Specifically, an antibody whose binding to one kind or plural kinds selected from the group consisting of a monocyte, a macrophage, a B cell and a CD34 positive cell, and dendritic cells derived from these cells cannot be confirmed under the condition in which the antibody binds to human pDC is preferable as the monoclonal antibody of the present invention.

[0036]

Alternatively, the monoclonal antibody that binds to the extracellular domain of human PTPRS in the present invention preferably includes a monoclonal antibody having the following immunological characteristics.

c) it binds to a transforming cell that has been transformed with an expression vector that expressibly retains a DNA that codes for human PTPRS,

d) under the condition for binding to the transformed cell in c), binding to a host cell before the transformation in c) cannot be confirmed.

[0037]

In the present invention, that the anti-human PTPRS monoclonal antibody does not cross-react with other molecules in the PTP family can be confirmed by using a cell in which each PTP family has been expressed forcibly. Namely, a cDNA that codes for an amino acid sequence of each PTP family is expressed forcibly by introducing into a suitable host cell. An anti-human PTPRS monoclonal antibody whose cross-reactivity is to be confirmed is contacted with the obtained transforming cell. Then, if the binding to a cell that expresses other PTP family molecule other than human PTPRS is not observed, it can be confirmed that the antibody can immunologically distinguish human PTPRS from other PTP family molecule. For example, in the Examples mentioned below, it was confirmed that most of the anti-human PTPRS monoclonal antibodies obtained by the present invention did not cross-react with PTPRA, PTPRD and PTPRF that specifically had high homology with PTPRS. Therefore, a monoclonal antibody that binds to human PTPRS and whose binding to PTPRA, PTPRD and PTPRF under the same condition is not detected is a preferable monoclonal antibody in the

present invention. If an antibody that can immunologically distinguish these PTP family molecules from PTPRS is utilized, the change in the expression of PTPRS can be detected specifically. In addition, it was proved that, among the molecules having high homology with PTPRS, the expression of PTPRE can be confirmed in a cell but PTPRE does not express out of the cell. Therefore, it does not bind to PTPRE as an antibody.

[0038]

The binding between a monoclonal antibody whose binding activity is to be confirmed and various cells can be confirmed by, for example, the principle of flow cytometry. In order to confirm the reactivity of the antibody by the principle of flow cytometry, it is advantageous to label the antibody in advance with a molecule or atomic group that generates a detectable signal. Generally, a fluorescence label or a light emission label is utilized. In order to analyze the binding between a fluorescence-labeled antibody and a cell by the principle of flow cytometry, a fluorescence-activated cell sorter (FACS) can be utilized. By utilizing the FACS, the binding between plural antibodies and cells can be confirmed effectively.

[0039]

Specifically, for example, an antibody A that has been clarified in advance to be able to identify pDC, and an antibody B whose property to bind to pDC is to be analyzed are simultaneously reacted with a group of cells including pDC. The antibody A and antibody B are labeled with fluorescence signals that can be distinguished from each other in advance. If the two signals are detected in the same cell group, it can be confirmed that those antibodies bind to the same cell group. Namely, it can be found that the antibody A and antibody B have the same binding property. If they bind to different cell groups, it is apparent that their binding properties are different.

[0040]

Examples of the preferable monoclonal antibody in the present invention may include a monoclonal antibody produced by hybridomas 9H5-4, 10F7-38, 13G5-52, 13G5-57, 14A8-85, 22H8-84, 49F2-30 or 55E7-79.

The hybridomas 9H5-4, 10F7-38, 13G5-52, 13G5-57, 14A8-85, 22H8-84, 49F2-30 and 55E7-79 were deposited as Accession Nos. FERM BP-11356, FERM BP-11357, FERM BP-11358, FERM BP-11359, FERM BP-11360, FERM BP-11361, FERM BP-11362 and FERM BP-11363 respectively, with the International Patent Organism Depositary (IPOD), National Institute of Technology and Evaluation on April 1, 2011. Hereinafter the content for specifying the deposit will be described.

(a) Name of depositary organization: International Patent Organism Depositary

(IPOD), National Institute of Advanced Industrial Science and Technology

Address: Tsukuba Central 6. 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan

(b) Date of deposition: April 1, 2011

(c) Accession No. FERM BP-11356 (hybridoma 9H5-4)

(c) Accession No. FERM: BP-11357 (hybridoma 10F7-38)

(c) Accession No. FERM BP-11358 (hybridoma 13G5-52)

(c) Accession No. FERM BP-11359 (hybridoma 13G5-57)

(c) Accession No. FERM BP-11360 (hybridoma 14A8-85)

(c) Accession No. FERM BP-11361 (hybridoma 22H8-84)

(c) Accession No. FERM BP-11362 (hybridoma 49F2-30)

(c) Accession No. FERM BP-11363 (hybridoma 55E7-79)

[0041]

The monoclonal antibody of the present invention may be a fragment including an antigen-binding region thereof. For example, an antibody fragment that includes an antigen binding site that is generated by enzymatic digestion of IgG can also be utilized as the antibody in the present invention. Specifically, an antibody fragment such as Fab or F(ab')2 can be obtained by digestion by papain or pepsin. It is well-known that these antibody fragments can be utilized as antibody molecules having binding affinity for an antigen. Alternatively, as long as necessary antigen binding activity is maintained, an antibody constructed by gene recombinant can also be used. Examples of the antibody constructed by gene recombination may include chimeric antibodies, CDR-transplanted antibodies, single chain Fv, diabodies, linear antibodies, and multispecific antibodies that are formed from antibody fragments. Methods for obtaining these antibodies based on monoclonal antibodies or antibody-producing cells that produce the monoclonal antibodies are known.

[0042]

The monoclonal antibody of the present invention can be obtained by using a specific transforming cell as an immunogen. Namely, the present disclosure relates to a method for the production of a cell that produces a monoclonal antibody that binds to an extracellular domain of human PTPRS, which includes:

(1) administering a cell that expresses an exogenous protein including an extracellular domain of human PTPRS to an animal to immunize the animal with the cell, and

(2) selecting an antibody-producing cell that produces the monoclonal antibody that binds to an extracellular domain of human PTPRS from the immunized animal.

By culturing the thus-obtained antibody-producing cell or the antibody-producing

cell that has been immortalized, the objective monoclonal antibody can be collected from the culture. Various methods are known for the method for immortalizing the antibody-producing cell.

[0043]

The transforming cell that is used as an immunogen in the present disclosure can be obtained by, for example, preparing the following cell that expressibly retains an exogenous polynucleotide (a) that codes for an amino acid sequence including an extracellular domain of human PTPRS.

In the present disclosure, the exogenous polynucleotide refers to that the polynucleotide has been introduced artificially in a host cell. In the case when a human cell is used as the cell, a human gene is introduced into a human cell. Also in such combination, the artificially-introduced polynucleotide is called an exogenous polynucleotide. Therefore, the ectopic expression of human PTPRS is encompassed in the expression of the exogenous polynucleotide.

[0044]

In the present invention, the extracellular domain of human PTPRS refers to the amino acid sequence from the 30 to 830 positions that correspond to the extracellular domain of the amino acid sequence described in SEQ ID NO:1. For example, an amino acid sequence that includes the respective regions in the order from the side of the N-terminus mentioned below is preferable as the amino acid sequence including an extracellular domain of human PTPRS in the present invention.

[Signal sequence + extracellular domain + transmembrane domain + intracellular region]

Alternatively, an amino acid sequence that partially lacks intracellular regions as follows is also encompassed in the amino acid sequence including an extracellular domain of human PTPRS in the present invention.

[Signal sequence + extracellular domain + transmembrane domain + part of intracellular region]

Furthermore, a structure that lacks an intracellular region as follows is also encompassed in the amino acid sequence including an extracellular domain of human PTPRS in the present invention.

[Signal sequence + extracellular domain + transmembrane domain]

[0045]

In the above-mentioned structures, the regions other than the extracellular domain may have a sequence selected from the amino acid sequence shown in SEQ ID NO:1, or may include other homologous amino acid sequence in combination. For example, an amino acid

sequence that constitutes a signal sequence, a transmembrane domain and an intracellular region can be an amino acid sequence of PTP family molecules other than human PTPRS. Alternatively, an amino acid sequence of a PTP family of a species other than human can be combined. Furthermore, the amino acid sequences that constitute the regions other than the extracellular domain can include mutation to the extent that the functions of the respective regions can be maintained. Furthermore, other region can be interposed between the respective regions. For example, an epitope tag such as FLAG can be inserted between the signal sequence and extracellular domain. Specifically, the signal sequence is a region that is translated into a protein, processed in the stage of transferring to the surface of a cell membrane, and removed. Therefore, any amino acid sequence that induces passage of the cell membrane of the translated protein can be utilized as the signal sequence. More specifically, the amino acid sequence of human PTPRS (SEQ ID NO:1) is preferable as an amino acid sequence including an extracellular domain of human PTPRS.

[0046]

Therefore, for the polynucleotide that constitutes the above-mentioned (a) in the present disclosure, any base sequence that codes for an amino acid sequence that constitutes the above-mentioned structure [signal sequence + extracellular domain + transmembrane domain + intracellular region] can be utilized. For example, the amino acid sequence of SEQ ID NO:1 is coded by the base sequence described in SEQ ID NO:2.

[0047]

In the present disclosure, in order to obtain a transforming cell to be used as an immunogen, it is only necessary to introduce an expression vector in which the above-mentioned polynucleotide (a) is expressibly retained in a suitable host cell.

[0048]

The host cell in the present disclosure is preferably a mammal cell. Specifically, a cell derived from a human, a monkey, a mouse or a rat can be utilized as a host cell. A HEK-293T cell is a human embryo-derived kidney cell line, which can be utilized as the host cell in the present disclosure. A HEK-293T cell is available as ATCC CRL-11268. Cells derived from the animal to be immunized can also be utilized as host cells. When a cell derived from an animal to be immunized is utilized as an immunogen, immune response against the host cell is small. Therefore, an antibody against an extracellular domain of human PTPRS that expresses exogenously can be obtained effectively. Therefore, for example, when a mouse is used as an animal to be immunized, a mouse-derived cell can also be used as a host cell.

[0049]

The above-mentioned polynucleotide can be transformed into a cell by mounting the polynucleotide on a vector that can induce expression in a host cell. A commercially available vector that can induce expression in a mammal cell may be utilized. Expression vectors such as pCMV-Script (R) Vector, pSG5 vector (manufactured by Stratagene) and pcDNA3.1 (manufactured by Invitrogen) can be utilized in the present invention.

[0050]

The thus-obtained transforming cell is administered to an animal to immunize the animal, together with additional components such as an adjuvant as necessary. As the adjuvant, Freund's complete adjuvant can be utilized. In the case when a mouse is utilized as an immunized animal, the transforming cell can be administered by from 10^4 to 10^9 cells, more specifically by from 10^4 to 10^6 cells. In general, the immunogen is administered plural times at intervals until an antibody titer increases. For example, in the case of a short-period immunization process, the transforming cell can be administered at intervals of from 2 to 4 days, specifically 3 days, and the antibody-producing cell can be collected after 2 to 3 times of administration. Alternatively, the antibody-producing cell can be collected after 5 to 6 times of administration at intervals of about once a week.

[0051]

In the present disclosure, the collected antibody-producing cell is cloned so as to obtain a monoclonal antibody. It is preferable for the cloning to immortalize the antibody-producing cell. For example, a cell fusion process such as a hybridoma process, or transformation by Epstein-Barr Virus (EBV) can be utilized as the process for the immortalization of the antibody-producing cell.

[0052]

In the antibody-producing cell, one cell produces one kind of antibody. Therefore, if a cell group derived from one cell can be established (i.e., cloning), a monoclonal antibody can be obtained. The hybridoma process refers to a process in which an antibody-producing cell is fused with a suitable cell strain, immortalized and cloned. The immortalized antibody-producing cell can be cloned by a technique such as a limiting dilution method. Many cell strains that are useful for the hybridoma process are known. These cell strains have various genetic markers that are excellent in immortalization efficiency of a lymphocyte-based cell and necessary for the selection of a cell that has succeeded in cell fusion. Furthermore, in the case when the obtainment of an antibody-producing cell is intended, a cell strain that lacks antibody-producing ability can also be used.

[0053]

For example, mouse myeloma P3 × 63Ag8.653 (ATCC CRL-1580) and P3 × 63Ag8U.1 (ATCC CRL-1597) are widely used as cell strains that are useful in cell fusion processes in mice and rats. In general, a hybridoma is prepared by fusing homologous cells, but a monoclonal antibody can be obtained from closely-related heterologous heterohybridomas.

[0054]

A specific protocol of cell fusion is known. Namely, an antibody-producing cell of an immunized animal is mixed with a suitable fusion partner to effect cell fusion. For the antibody-producing cell, for example, a spleen cell, a lymphocyte cell collected from a lymph node and a peripheral blood B cell are used. As the fusion partner, various cell strains that have been mentioned above can be utilized. For the cell fusion, a polyethylene glycol process or an electric fusion process is used.

Next, the cell that has succeeded in cell fusion is selected based on a selection marker possessed by the fusion cell. For example, in the case when an HAT-sensitive cell strain is used for cell fusion, the cell that has succeeded in cell fusion is selected by selecting the cell that grows in an HAT medium. Furthermore, that the antibody produced by the selected cell has intended reactivity is confirmed.

[0055]

Each hybridoma is screened based on the reactivity of the antibody. Namely, a hybridoma that produces an antibody that binds to human PTPRS is selected by the process as mentioned above. Preferably, the selected hybridoma is subcloned, and in the case when the production of the objective antibody is finally confirmed, it is selected as a hybridoma that produces the monoclonal antibody of the present invention.

[0056]

Specifically, the objective hybridoma can be selected based on the reactivity with a human cell or the reactivity with a transforming cell that expresses human PTPRS gene. The antibody that binds to the cell can be detected by the principle of an immunoassay. For example, ELISA utilizing a cell as an antigen can be utilized for the detection of the objective antibody. Specifically, a culture supernatant of a hybridoma is contacted with a support on which human pDC, or a transforming cell utilized as an immunogen is fixed. In the case when the culture supernatant includes the objective antibody, the antibody is captured by the cell fixed on the support. Then, the solid-phase is separated from the culture supernatant, and washed as necessary, thereby the antibody captured on the solid-phase can be detected. An antibody that recognizes the antibody can be utilized for the detection of the antibody. For example, a mouse antibody can be detected by an anti-mouse immunoglobulin antibody. If an antibody that

recognizes the antibody is labeled in advance, the detection thereof is easy. As the label, for example, an enzyme, a fluorescent pigment or a light emission pigment can be utilized.

On the other hand, as the support for fixing the cell, particles, or an inner wall of a microtiter plate can be utilized. The cell can be fixed by physical adsorption on the surface of particles or a container made of a plastic. For example, beads or a reaction container made of polystyrene can be utilized as the support for fixing the cell.

[0057]

In the selection of a hybridoma, production of an antibody against not human PTPRS but the host cell of the transforming cell used for the immunogen is expected in some cases. For example, as shown in Examples, when a human cell is used as an immunogen and a mouse is utilized as an animal to be immunized, the human cell is recognized as a foreign substance, and production of an antibody that binds thereto is expected. The present invention aims at obtaining an antibody that recognizes human PTPRS. Therefore, it is not necessary to obtain an antibody that recognizes a human cell antigen other than human PTPRS. In order to exclude a hybridoma that produces such antibody by screening, an antibody that is not intended can be absorbed in advance before confirmation of the reactivity of the antibody.

[0058]

The antibody that is not intended can be absorbed by an antigen to which an antibody whose presence is expected binds. Specifically, for example, antibodies against human cell antigens other than human PTPRS can be absorbed by cells in which the expression of human PTPRS cannot be detected. In the present disclosure, the host cell used as the immunogen is preferable as the antigen for absorbing the antibody that is not intended.

[0059]

Where necessary, the actual effect on the activity of pDC of the monoclonal antibody whose binding activity against the antigen has been confirmed is confirmed. The effect on pDC can be confirmed by, for example, the method as mentioned below.

[0060]

The monoclonal antibody of the present invention can be collected from a culture obtained by culturing a hybridoma that produces the monoclonal antibody. The hybridoma can be cultured in vitro or in vivo. The hybridoma can be cultured in vitro by using a known medium such as RPMI1640. In the culture supernatant, an immunoglobulin secreted by the hybridoma is accumulated. Therefore, the monoclonal antibody of the present invention can be obtained by collecting the culture supernatant and purifying as necessary. The purification of the immunoglobulin is easier in the case when serum is not added to the medium. However, for

the purposes of more rapid proliferation of the hybridoma and acceleration of the production of the antibody, about 10% of fetal bovine serum can be added to the medium.

[0061]

The hybridoma can also be cultured in vivo. Specifically, by inoculating the hybridoma in the abdominal cavity of a nude mouse, the hybridoma can be cultured in the abdominal cavity. The monoclonal antibody is accumulated in ascites fluid. Therefore, a desired monoclonal antibody can be obtained by collecting the ascites fluid and purifying as necessary. The obtained monoclonal antibody can be suitably modified or processed according to the purpose.

[0062]

The monoclonal antibody of the present invention can be expressed by obtaining cDNA that codes for an antigen-binding region of the antibody from the hybridoma, and inserting this into a suitable expression vector. A technique for obtaining a cDNA that codes for a variable region of an antibody and expressing in a suitable host cell is known. Furthermore, a technique for binding a variable region including an antigen-binding region to a constant region to form a chimeric antibody is also known.

[0063]

For example, as a preferable monoclonal antibody in the present invention, a monoclonal antibody produced by hybridoma 9H5-4 that was deposited as Accession No. FERM BP-11356, hybridoma 10F7-38 that was deposited as Accession No. FERM BP-11357, hybridoma 13G5-52 that was deposited as Accession No. FERM BP-11358, hybridoma 13G5-57 that was deposited as Accession No. FERM BP-11359, hybridoma 14A8-85 that was deposited as Accession No. FERM BP-11360, hybridoma 22H8-84 that was deposited as Accession No. FERM BP-11361, hybridoma 49F2-30 that was deposited as Accession No. FERM BP-11362 or hybridoma 55E7-79 that was deposited as Accession No. FERM BP-11363 can be represented.

As the chimeric antibody including a variable region, or the humanized antibody to which CDR that constitutes a variable region has been transplanted, an antibody having a constant region derived from IgG or IgM is encompassed in the preferable antibody in the present invention. The present inventors have confirmed that a monoclonal antibody against PTPRS has a CDC action against the PTPRS-expressing cell. Therefore, the antibody having a constant region derived from IgG or IgM has a cytotoxic action against a PTPRS-expressing cell by the CDC action. Such antibody is useful for suppressing the cell number of the PTPRS-expressing cell such as pDC.

The chimeric antibody that recognizes human PTPRS, or the humanized antibody

can be produced by gene engineering by using a polynucleotide that codes for the antibody.

[0064]

About four years have already passed since the structure of human PTPRS was clarified in WO2007/041317 (JP2009-510102A); however, an antibody that can specifically recognize human PTPRS has not been obtained yet. An antibody that recognizes human PTPRS was first provided by the immunogen of the present invention. Namely, the present disclosure provides an antibody that recognizes human PTPRS, which can be obtained by the following processes:

(1) administering to an animal a protein including an extracellular domain of human PTPRS to immunize the animal;

(2) selecting an antibody-producing cell that produces an antibody that binds to human PTPRS from the antibody-producing cell of the immunized animal; and

(3) culturing the antibody-producing cell selected in (2), and collecting from the culture an antibody that recognizes human PTPRS.

[0065]

It was clarified that human PTPRS is specifically expressed in human pDC. The specific expression in human pDC was also confirmed in the gene expression analysis by SAGE by the present inventors. However, in the past reports, the expression level of human PTPRS was analyzed based on mRNA in all cases. Since an antibody by which detection of human PTPRS is enabled was not provided, the expression state of a protein was not analyzed in the past. The antibody that binds to an extracellular domain of human PTPRS, which was provided by the present invention, realized the analysis of a human PTPRS protein.

[0066]

According to the actual confirmation by the present inventors, the monoclonal antibody that binds to an extracellular domain of human PTPRS based on the present invention specifically detected human pDC. Namely, the present invention relates to a method for the detection of a plasmacytoid dendritic cell, which includes contacting the monoclonal antibody or the fragment of the invention with a subject cell, and detecting the monoclonal antibody or the fragment that has bound to the cell.

[0067]

By detecting human PTPRS based on the present invention, whether or not a certain cell is pDC can be confirmed. Namely, the present invention provides a method for the identification of pDC using human PTPRS as an index. Alternatively, human pDC can be separated by separating the cell in which human PTPRS has been detected according to the

present invention. Namely, the present disclosure provides a method for the separation of pDC using human PTPRS as an index.

[0068]

In the present invention, a monoclonal antibody that binds to an extracellular domain of human PTPRS, or a fragment including an antigen-binding region thereof can be labeled in advance. For example, the antibody can be detected readily by labeling with a light emission pigment or a fluorescence pigment. More specifically, a fluorescence pigment-labeled antibody is contacted with a cell aggregate that possibly includes pDC, thereby a cell to which the antibody of the present invention has bound can be detected using the fluorescence pigment as an index. Furthermore, if the cell in which the fluorescence pigment has been detected is separated, pDC can be separated. The series of processes can be readily carried out by the principle of FACS.

[0069]

Alternatively, the antibody of the present invention can be bound to a solid-phase support such as magnetic particles in advance. The antibody bound to the solid-phase support recognizes human PTPRS, and pDC is captured by the solid-phase support. As a result, pDC can be detected and separate.

[0070]

The antibody required for the detection of pDC based on the present invention can be supplied as an agent for detecting pDC. Namely, the present disclosure provides an agent for detecting pDC including a monoclonal antibody that binds to an extracellular domain of human PTPRS, or a fragment including an antigen-binding region thereof. For the agent for detecting pDC, besides the antibody, a positive control or negative control can be combined. For example, the transforming cell that expresses an extracellular domain of human PTPRS, which was utilized as an immunogen, or pDC collected from a human can be utilized as the positive control. Generally, human pDC can be obtained only little from peripheral blood. Therefore, the transforming cell is specifically preferable as the positive control in the agent. On the other hand, any cell that does not express human PTPRS can be utilized for the negative control.

[0071]

Namely, the present disclosure provides a kit for detecting human pDC, which includes a monoclonal antibody that binds to an extracellular domain of human PTPRS, or a fragment including an antigen-binding region thereof.

[0072]

Furthermore, the present inventors have analyzed the effect of the antibody that binds to an extracellular domain of human PTPRS on pDC. As a result, they have confirmed that the antibody that binds to an extracellular domain of human PTPRS suppress the activity of pDC. Namely, the present disclosure relates to a method for suppressing the activity of an interferon-producing cell, which includes contacting any of the following components with pDC:

(a) a monoclonal antibody that binds to human PTPRS to suppress the activity of pDC, or a fragment including an antigen-binding region thereof, and

(b) an immunoglobulin to which a complementarity determining region of the monoclonal antibody of (a) has been transplanted, or a fragment including an antigen-binding region thereof.

[0073]

Alternatively, the present disclosure relates to a method for suppressing of the activity of pDC in a living body, which includes administering any of the following components to the living body:

(a) a monoclonal antibody that binds to human PTPRS to suppress the activity of pDC, or a fragment including an antigen-binding region thereof,

(b) an immunoglobulin to which a complementarity determining region of the monoclonal antibody of (a) has been transplanted, or a fragment including an antigen-binding region thereof, and

(c) a polynucleotide that codes for the component described in (a) or (b).

[0074]

In the present disclosure, pDC refers to a cell that has an ability to produce IFN, and expresses human PTPRS on a cellular surface. Hereinafter, unless otherwise indicated, pDC encompasses not only a cell that is a precursor cell of a dendritic cell but also a cell that has an ability to produce IFN and expresses human PTPRS on a cellular surface. A method for identifying such pDC is known. For example, pDC can be distinguished from other blood cells using several cellular surface markers as indices. Specifically, the profile of the cellular surface marker of human pDC is as follows (Shortman, K. and Liu, YJ, Nature Reviews 2: 151-161, 2002). It was also reported in recent years that BDCA-2 positive cell is positioned as pDC (Dzionek, A. et al. J. Immunol. 165: 6037-6046, 2000).

[Profile of cellular surface antigen of human pDC]

CD4 positive, CD123 positive, Lineage (CD3, CD14, CD16, CD19, CD20, CD56) negative, CD11c negative

Therefore, a cell having the expression profile of these known markers and also

having an ability to produce IFN can also be referred to pDCs. Furthermore, even a group of cells having a profile that is different from the expression pattern of the expression profile of these markers, a cell in a living body having an ability to produce IFN, the cells are encompassed in pDCs.

Furthermore, as characteristics that are commonly observed in human pDC, the following characteristics can be shown.

[Characteristics in form of cell]

- it resembles a plasma cell.
- it is a round cell having a smooth cellular surface.
- it has a relatively large nucleus. [Functional characteristics of cell]
- it produces a large amount of Type I IFN within a short period during viral infection.
- it differentiates into a dendritic cell after viral infection.

[0075]

In the present disclosure, suppression of the activity of pDC refers to suppression of at least one function possessed by pDC. As the functions of pDC, production of IFN and cell survival can be shown. In other word, cell survival can be said to be a cell number. Therefore, suppression of one or both of these functions refers to suppression of the activity of pDC. It was clarified that Type I IFN produced by pDC causes various diseases. Therefore, it is useful to suppress the cell number of pDC and the production of IFN as therapeutic strategies for those diseases.

For example, a relationship between the pathological conditions of autoimmune diseases and IFNα was pointed out. Most of IFNα is produced by pDC. Therefore, if the production thereof is suppressed, the pathological conditions brought by IFNα can be alleviated. Meanwhile, in the present invention, suppression of IFN production by pDC refers to suppression of production of at least one kind of IFN among IFNs produced by pDC. Type I IFNs are preferable IFNs in the present invention. Among these, IFNα is important.

[0076]

Namely, the present disclosure relates to an agent for suppressing the production of IFN, which includes an antibody that binds to an extracellular domain of human PTPRS as an active ingredient. Alternatively, the present disclosure provides a method for suppressing the production of IFN, which includes administering an antibody that binds to an extracellular domain of human PTPRS. Furthermore, the present disclosure relates to use of an antibody that binds to an extracellular domain of human PTPRS in the production of a pharmaceutical

composition for suppressing the production of IFN.

[0077]

pDC includes a cell that produces a large amount of IFN by a small number of cells. For example, a precursor cell of a dendritic cell that has been stimulated by, for example, a virus produces most of IFN produced by a living body. Suppression of the cell number of pDC that produces a large amount of IFN consequently leads to suppression of the production amount of IFN. Therefore, the pathological conditions brought by IFNα can also be alleviated by suppressing the cell number of pDC.

In a preferable embodiment of the present invention, it was confirmed that an anti-human PTPRS monoclonal antibody binds to a human PTPRS-expressing cell and imparts a cytotoxic action by a CDC (Complement Dependent Cytotoxicity) action. The CDC action is one of important mechanisms of action in antibody medicaments. The anti-human PTPRS monoclonal antibody of the present invention also has a strong cytotoxic action against human PTPRS-expressing cells such as pDC by the CDC action thereof. Namely, an effect of suppression of IFN production can be expected also by a cytotoxic action against pDC besides the mechanism of suppression of IFN production in a preferable embodiment.

[0078]

The antibody that recognizes an extracellular domain of human PTPRS used in the present invention can be obtained based on the method as previously mentioned. The antibody in the present invention may be of any class. Furthermore, the species of the organism from which the antibody is derived is also not limited. Furthermore, a fragment including an antigen-binding region of the antibody can be used as the antibody. For example, an antibody fragment that includes an antigen binding site that is generated by enzymatically digestion of IgG can also be used as the antibody in the present invention. Specifically, an antibody fragment such as Fab or F(ab')2 can be obtained by digestion by papain or pepsin. It is well-known that these antibody fragments can be utilized as antibody molecules having binding affinity for antigens. Alternatively, an antibody constructed by gene recombination can also be used as long as it maintains necessary antigen binding activity. Examples of the antibody constructed by gene recombination may include chimeric antibodies, CDR transplant antibodies, single chain Fv, diabodies and linear antibodies, and multispecific antibodies formed from antibody fragments. Methods for obtaining these antibodies based on monoclonal antibodies are known.

[0079]

In the present invention, the antibody can be modified as necessary. According

to the present invention, an antibody that recognizes an extracellular domain of human PTPRS has an action to suppress the activity of pDC. Namely, a possibility that the antibody itself has a cytotoxic action against pDC was considered. The subclass of the antibody showing a strong effector action is known. Alternatively, by modifying the antibody with a cytotoxic substance (a cytotoxic agent), the effect of suppressing the activity of pDC can further be enhanced. Examples of the cytotoxic substance may include the following substances.

Toxins: Pseudomonas Endotoxin (PE), diphtheriatoxin

Lysine

Radioisotope elements: Tc99m, Sr89, I131, Y90

Anticancer agents: calicheamicin, mytomycin, paclitaxel

Toxins composed of a protein can be bound to, for example, an antibody or a fragment thereof by a bifunctional agent. Alternatively, a gene that codes for toxins can be joined to a gene that codes for an antibody to give a fusion protein of the two genes. A method for binding a radioisotope element to an antibody is also known. For example, a method for labeling an antibody with a radioisotope element by utilizing a chelating agent is known. Furthermore, an anticancer agent can be bound to an antibody by utilizing a sugar chain or a bifunctional agent.

[0080]

In the present invention, an antibody whose structure has been modified artificially can also be utilized as an active ingredient. For example, various modification methods for ameliorating cytotoxic action and stability of an antibody are known. Specifically, an immunoglobulin in which a sugar chain of a heavy chain has been modified is known (Shinkawa, T. et al., J. Biol. Chem 278:3466-3473. 2003.). By modifying the sugar chain, the ADCC (Antibody Dependent Cell-mediated Cytotoxicity) activity of the immunoglobulin was enhanced.

[0081]

When the antibody that binds to an extracellular domain of human PTPRS is contacted with pDC, the activity thereof is suppressed. Therefore, these antibodies can be utilized for an agent or method for suppressing the activity of pDC. Namely, the present disclosure provides an agent for suppressing the activity of pDC, which includes at least one kind of component selected from the group consisting of the following (a)-(b) as an active ingredient. Alternatively, the present disclosure relates to a method for suppressing the activity of pDC, which includes administering at least one kind of component selected from the group consisting of the following (a)-(b). Furthermore, the present disclosure relates to use of the

component selected from the group consisting of the following (a)-(b) in the production of an agent for suppressing the activity of pDC.

(a) An antibody that binds to an extracellular domain of human PTPRS, or a fragment including an antigen-binding region thereof, and

(b) An immunoglobulin to which a complementarity determining region of the monoclonal antibody of (a) has been transplanted, or a fragment including an antigen-binding region thereof.

In the present invention, as the monoclonal antibody that suppresses the activity of pDC, a monoclonal antibody that recognizes an extracellular domain of human PTPRS can be utilized. In the present invention, one kind or plural kinds of monoclonal antibody can be utilized. For example, plural kinds of monoclonal antibodies that recognize an extracellular domain of human PTPRS can be incorporated and utilized in the present invention.

[0082]

That an antibody has an action of suppressing the IFN-producing activity of pDC can be confirmed as follows. pDC produces a large amount of IFN by the stimulation of a virus. By providing an antibody before or after the stimulation with the virus against pDC, or simultaneously with the stimulation with the virus, and using pDC to which the antibody is not provided as a control, the abilities of producing IFN are compared. The abilities of producing IFN can be evaluated by measuring IFN-α and IFN-β included in the culture supernatant of pDC. As a result of the comparison, when the amount of IFN in the supernatant is decreased significantly by adding the antibody, it can be confirmed that the tested antibody has an action of suppressing the ability of producing IFN. A method for measuring these IFNs is known. pDC is a cell that produces most of IFNs in a living body. Therefore, by suppressing the ability of producing IFN of pDC, the state of production of IFN in a living body can be modulated.

[0083]

In the present invention, the activity of pDC includes maintenance of the cell number of pDC. Therefore, suppression of the activity of pDC in the present invention includes suppression of the cell number of pDC. If that the cell number of pDC is suppressed in the presence of an antibody is confirmed, it is found that the antibody suppresses the activity of pDC. As a control for comparison, an inert immunoglobulin derived from the same animal species as that for an antibody whose activity is to be confirmed can be used as in the production of IFN. The cell number of pDC can be compared quantitatively by counting the number of the cell. The cell number can be counted by an FACS or microscope.

[0084]

Furthermore, it is also considered that pDC differentiates into a cell that induces Th2 called DC2 (Dendritic Cell 2) as a result of infection with, for example, a virus. If the production of IFN of pDC by stimulation with a virus can be suppressed, it is also possible that the differentiation into Th2 can be suppressed. Therefore, therapeutic effects on various allergy diseases can be expected for the monoclonal antibody of the present invention that suppresses IFN production.

[0085]

In the case when the antibody that recognizes an extracellular domain of human PTPRS is administered to a host that is different from an organism species from which the antibody is derived, it is desirable to process the antibody into a shape that is hardly recognized as a foreign substance for the host. For example, by processing into the following molecules, the immunoglobulin can become difficult to be recognized as a foreign substance. The technique for processing an immunoglobulin molecule as follows is known.

- A fragment including an antigen-binding region that lacks a constant region (Monoclonal Antibodies: Principles and Practice, third edition, Academic Press Limited. 1995; Antibody Engineering, A Practical Approach, IRL PRESS, 1996)

- A chimeric antibody that is constituted by an antigen-binding region of a monoclonal antibody and a constant region of an immunoglobulin of a host (Experimental Manual for Gene Expression, Kodansha Ltd., 1994 (edited by Isao Ishida and Tamie Ando))

- A CDR-substituted antibody obtained by substituting a complementarity determining region (CDR) in an immunoglobulin of a host with a CDR of a monoclonal antibody (Experimental Manual for Gene Expression, Kodansha Ltd., 1994 (edited by Isao Ishida and Tamie Ando)).

[0086]

Alternatively, an immunoglobulin variable region gene of a human can be acquired by a phage display process (McCafferty J. et al., Nature 348: 552-554, 1990; Kretzschmar T et. al., Curr Opin Biotechnol 2002 Dec: 13 (6): 598-602.). In the phage display process, a gene that codes for a human immunoglobulin variable region is incorporated into a phage gene. A phage library can be prepared by using various immunoglobulin genes as sources. A phage expresses the variable region as a fusion protein of a protein that constitutes the phage itself. The variable region on the surface of the phage, which is expressed by the phage, maintains the binding activity with the antigen. Therefore, by selecting a phage that binds to a cell that has expressed an antigen, a phage that has expressed a variable region having an intended binding activity can be screened from a phage library. Furthermore, a gene that

codes for a variable region having an intended binding activity is retained in the phage particles selected by such way. Namely, in the phage display process, a gene that codes for a variable region having an intended binding activity can be acquired by using the binding activity of the variable region as an index.

[0087]

In the agent or method for suppressing the activity of pDC, the antibody that recognizes an extracellular domain of human PTPRS, or an antibody fragment including at least an antigen-binding region thereof can be administered as a protein or a polynucleotide that codes for the protein. In order to administer the polynucleotide, it is desirable to utilize a vector to which a polynucleotide that codes for an intended protein has been disposed under the control of a suitable promoter so that an intended protein can be expressed. An enhancer or terminator can also be disposed on the vector. A vector that can retain genes of a heavy chain and a light chain that constitute immunoglobulin and can express an immunoglobulin molecule is known. The vector that can express an immunoglobulin can be administered by introducing into a cell. In administration to a living body, a vector that can be transmitted to a cell by administering to the living body can be administered as it is. Alternatively, a vector can be introduced in a lymphocyte that has been once separated from a living body and thereafter returned to the living body (ex vivo).

[0088]

In the agent or method for suppressing the activity of pDC, the amount of the monoclonal antibody to be administered to a living body as an immunoglobulin is generally from 0.5 mg to 100 mg, for example from 1 mg to 50 mg, preferably from 2 mg to 10 mg, per 1 Kg body weight. The intervals of administration of the antibody to a living body can be suitably modulated so that the effective concentration of the immunoglobulin in a living body during a therapeutic period can be maintained. Specifically, the antibody can be administered at intervals of from 1 to 2 weeks. The route of administration is optional. A person skilled in the art can suitably select an effective administration route for a therapy. Specifically, oral or parenteral administration can be shown. For example, the antibody can be administered systemically or topically by intravenous injection, intramuscular injection, peritoneal injection or subcutaneous injection. Examples of formulations that are suitable for the parenteral administration in the present invention may include an injection agent, a suppository and an aerosol. Furthermore, when the antibody is provided to a cell, an immunoglobulin of generally 1 μg/mL, preferably 10 μg/mL or more, more preferably 50 μg/mL or more, further preferably 0.5 mg/mL or more is provided.

[0089]

In the agent or method for suppressing the activity of pDC, the monoclonal antibody can be administered to a living body by any method. Generally, the monoclonal antibody is compounded with a pharmaceutically acceptable carrier. Where necessary, additives such as a thickening agent, a stabilizer, an antiseptic agent and a solubilizer can be incorporated into the monoclonal antibody. Examples of such carrier or additive may include lactose, citric acid, stearic acid, magnesium stearate, sucrose, starch, talc, gelatin, agar, vegetable oils and ethylene glycol. The term "pharmaceutically acceptable" refers to having been accepted by the supervisory of the government of each country, or being listed in the pharmacopoeia of each country or a generally-recognized pharmacopoeia with respect to use in animals, mammals, and specifically in humans. The agent for suppressing the activity of pDC can be provided in the form of a lyophilized powder or tablet including one dose or plural doses. The lyophilized powder or tablet can further be combined with injectable sterilized water, physiological saline or buffer for solving the composition so as to give a desired concentration prior to administration.

[0090]

Furthermore, when administered in the form of a vector that expresses an immunoglobulin, each plasmid can be administered by from 0.1 to 10 mg, for example from 1 to 5 mg per 1 kg body weight, considering that a heavy chain and a light chain are co-transfected as separate plasmids. Furthermore, for introducing into a cell in vitro, a vector of from 1 to 5 $\mu g/10^6$ cells is used.

Hereinafter the present invention will be explained more specifically with referring to the Examples, but the present invention is not construed to be limited by the Examples.

EXAMPLES

[0091]

Example 1

A. Analysis of expression of PTPRS

A-1) Analysis using SAGE library

Expressions of a gene in human monocyte, pDC, and pDC treated with herpes simplex virus (HSV) were compared and analyzed by an SAGE™ (Serial Analysis of Gene Expression) process. The analysis method is as follows.

A monocyte was isolated as a CD14 positive cell and pDC was separated as a BDCA-4 positive cell from human peripheral blood mononuclear cells by a cell sorter. Furthermore, pDC was cultured in the presence of HSV for 12 hours to prepare activated pDC. RNAs were obtained from the respective cells, and an SAGE library was prepared by using an I-SAGE™ kit (Invitrogen). The obtained base sequence data of about 100,000 tags was analyzed by SAGE Analysis Software (Invitrogen). As a result, as a gene having a score value of monocyte/pDC/pDC+HSV of 0/7/0, i.e., a gene that shows pDC-specific expression, a known gene: PTPRS (GenBank Acc#NM_002856.3) was found. PTPRS is coded by the base sequence shown in SEQ ID NO:2. Furthermore, it is a single transmembrane domain having an immunoglobulin-like domain (Ig-like domain) and a Fibronectin Type III-like domain in the extracellular region. In addition, it has two protein tyrosine phosphatase regions (PTP domains) in the intracellular region (FIG. 1).

[0092]

A-2) Analysis of expression of PTPRS mRNA in various human immune competent cells by quantitative RT-PCR

The expression of PTPRS in immune cells was analyzed in more detail. Each cell was isolated from human peripheral blood by a cell sorter. RNA was extracted from the isolated each cell population, and cDNA was synthesized. Using the obtained cDNA as a template, quantitative RT-PCR was conducted according to a general process to analyze the expression level of PTPRS mRNA. By normalization with the expression level of a GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene that is known to express constantly, the expression of the PTPRS gene was compared between immune cells.

The base sequences of the used primers, and the conditions for PCR are as follows.

Forward primer for PTPRS: 5' CAC GGC CTA TGA CCT CCA 3' (SEQ ID NO:3)

Reverse primer for PTPRS: 5' AAG TTC TTG GGC GAG ACT TG 3' (SEQ ID NO:4)

Forward primer for GAPDH: 5' CCA CCC ATG GCA AAT TCC 3' (SEQ ID NO:5)

Reverse primer for GAPDH: 5' TGG GAT TTC CAT TGA TGA CAA G 3' (SEQ ID NO:6)

1 cycle at 50°C for 2 minutes,

1 cycle at 95°C for 10 minutes, and

50 cycles [at 95°C for 15 seconds and at 60°C for 60 seconds].

A monocyte, pDC, pDC stimulated with HSV, a B-cell (CD19+cell), a T-cell (CD3+cell), an activated T-cell stimulated with PMA (Phorbol 12-myristate 13-acetate) and an NK cell (CD56+cell) were analyzed, and it was shown that PTPRS was expressed in a pDC-specific manner. Furthermore, it was found as a characteristic that the expression of PTPRS is decreased by the pDC stimulated with HSV (FIG. 2).

[0093]

A-3) Analysis of expression of PTPRS mRNA in human tissue by quantitative RT-PCR

Furthermore, expression in tissues was studied by quantitative PCR using ABI PRISM 7000 (Applied Biosystem). As cDNA panels, BD™ MTC multiple tissue cDNA panel (Human I; Cat. No. 636742, Human immune; Cat. No. 636748, Human blood fractions; Cat. No. 636750; all by Becton Dickinson) were used. The base sequences of the primers used are shown below.

Forward primer for PTPRS: 5' ACT CAC CCA CAC CCT ACA AGA 3'(SEQ ID NO:7)

Reverse primer for PTPRS: 5' CTT GGT GGT ACG GCC ATC 3'(SEQ ID NO:8)

Forward primer for GAPDH: 5' CCA CCC ATG GCA AAT TCC 3'(SEQ ID NO:5)

Reverse primer for GAPDH: 5' TGG GAT TTC CAT TGA TGA CAA G 3'(SEQ ID NO:6)

Using an SYBR green PCR master mix kit (Applied Biosystem), PCR was conducted by ABI PRISM 7000 available from the same company. Sequence Detection System Software available from the same company was used for the analysis. The reaction conditions are as follows.

Step 1: 1 cycle at 50°C for 2 minutes

Step 2: 1 cycle at 95°C for 10 minutes

Step 3: 40 cycles at 95°C for 15 seconds and at 60°C for 1 minute

By normalization with the expression level of a GAPDH (glyceraldehyde-3-

phosphate dehydrogenase) gene that is known to express constantly, the expression of the PTPRS gene was compared between tissues. As a result, PTPRS mRNA was expressed widely in the tissues (FIG. 3).

[0094]

B. Preparation of PTPRS expression vector

In order to express a PTPRS protein, preparation of an expression vector of a PTPRS gene was conducted. Only a PTPRS gene was taken out from a PTPRS cDNA Clone that had been incorporated in a pCR4-TOPO cloning vector (Open Biosystem cc# MHS1010-98052887) and incorporated into a pcDNA3.1 expression vector (PTPRS/pcDNA3.1). Using the obtained PTPRS/pcDNA3.1 plasmid as a template, the PTPRS gene was amplified with a primer including EcoRI, Not I and Kozak sequence (GCC GCC ACC) (the information on the primer is shown below). The PCR product was cloned into a pMX-IP retroviral vector at EcoRI and Not I sites (PTPRS/pMX-IP). For the PCR reaction, one unit of KOD Plus DNA polymerase (TOYOBO) was used, and the reaction conditions were 1 cycle at 94°C for 2 minutes and 25 cycles [at 94°C, 15 seconds and at 68°C for 4 minutes and 30 seconds].

Forward primer (SEQ ID NO:9): 5' aaa GAA TTC gcc gcc acc ATG GCG CCC ACC TGG GGC CCT3'

Reverse primer (SEQ ID NO:10): 5' aaa gcg gcc gcT TAG GTT GCA TAG TGG TCA AAG C3'

In the above-mentioned base sequences, the small characters represent the cleavage sites of the restriction enzyme EcoRI or the sites of Not I. The aaa at the 5'terminus is an additional base for enzymatic cleavage.

[0095]

C. Preparation of human PTPRS (hPTPRS)-expressing cell

To make a retro virus containing PTPRS gene, HEK-293T cell that is a kidney cell strain of a human embryo was transiently transfected with PTPRS/pMX-IP and a retro virus packaging vector PCL-ECO by FuGENE kit (Roche). Two days later, the cell culture supernatant in which a virus including a hPTPRS gene was collected and infected with a D2SC/1 cell that is a dendritic cell derived from the spleen of a BALB/c mouse (this was prepared based on Paglia et al., J. Exp. Med., 178, 1893-1901 (1993)). Since the pMX-IP retroviral vector includes a puromycin resistance gene, only a cell that expresses hPTPRS becomes possible to survive by culturing the infected D2SC/1 cell with puromycin, thereby selection becomes possible. hPTPRS-expressing D2SC/1 cells were selected by FACS sorting and cultured. In order to confirm the expression of hPTPRS, 10 μg/mL of goat IgG (SantaCruz) and a

commercially available hPTPRS polyclonal antibody (pAb; R&D) were added to the selected hPTPRS/D2SC/1 cells by 100 μL each, and the mixture was incubated at 4°C for 30 minutes. The cell was washed with PBS, and then a FITC-labeled anti-goat IgG antibody (SantaCruz) diluted by 100-fold was added by 50 μL, and the mixture was incubated at 4°C for 30 minutes. After washing with PBS, data was imported by FACSCalibur (BD) (FIG. 4).

[0096]

Example 2

A. Preparation of anti-human PTPRS monoclonal antibody

A-1) Immunization

As a cell used as an immunogen, the above-mentioned hPTPRS/D2SC/1 cell was used. BALB/c mice were anesthetized, and a Freund's Complete Adjuvant (CFA) emulsion was injected subcutaneously to the footpads by 50 μl per each foot. The total was 100 μl/mouse. On the next day, an emulsion was prepared by using a hPTPRS/D2SC/1 cell prepared as an immunogen and a Freund's Incomplete Adjuvant (IFA) and injected subcutaneously to the footpads (50 μl/foot, total 100 μl/mouse). Immunization was done every two days for three times in total, and the drawing lymph nodes were collected at 3 days after the last immunization.

[0097]

A-2) Cell fusion

The drawing lymph node cells were collected from the both feet of an immunized mouse and mixed with a mouse myeloma cell P3-X63-Ag8.563 that had been cultured in a RPMI1640 medium (SIGMA) including 10% FBS so that the ratio of the lymph node cells and the myeloma cells became 5:4, and the cells were collected by centrifugation. PEG1500 (Roche) was added to the mixed cells for cell fusion. The fused cell (hybridoma) was washed and cultured in 10% Fetal Bovine Serum (FBS) including a cell growth supplement+HAT (Sigma)-RPMI1640 medium (including 2 mM L-Glutamine, 100 Unit/ml Penicillin, 100 μg/ml Streptomycin, 10 mM HEPES, 1 mM Sodium Pyruvate, 50 μM 2-ME).

[0098]

A-3) FACS screening of hybridoma using immunized hPTPRS/D2SC/1 cell

An anti-CD16/32 (2.4G2) prepared to 2.5 μg/ml was added by 50 μL to 3×10^5/well of the D2SC/1 cell or hPTPRS/D2SC/1 cell to block an FC receptor. After washing with PBS, a goat IgG prepared to 10 μg/ml, a commercially available anti-hPTPRS pAb (R&D), a mouse IgG_{2ak} (BioLegend) and the culture supernatant of the cultured hybridoma were added

by 60 μl each, and the mixture was incubated at 4°C for 60 minutes. After washing with PBS, a 50-fold diluted FITC-labeled anti-goat IgG antibody and a 100-fold diluted PE-labeled anti-mouse IgG antibody (BD) were added to the cells by 50 μl each, and the mixture was incubated at 4°C for 30 minutes under light shielding. After washing with PBS, the cell was suspended in 200 μl of PBS. Data was collected by FACS Calibur (BD). The collected data was developed by dot plots of FSC and SSC to gate a living cell. The data was collected until the data of the cell in this gate reached 2,000 count. As a result, 13 hybridomas that produces an anti-hPTPRS antibody could be obtained (2G6, 28G10, 4B2, 2G2, 9H5, 10F7, 22H8, 49F2, 9D2, 14A8, 55E7, 13G5, 16H2) (FIG. 5).

[0099]

A-4) FACS screening using CAL-1 cell

3×10^5 of a human pDC-like cell strain CAL-1 cells were stained in 50 μl of the culture supernatant of the above-mentioned each hybridoma for 15 minutes at 4°C. The cells were washed once with FACS buffer (1% FBS+PBS) and then centrifuged to remove the supernatant. 2 μg/ml of a PE-labeled anti-mouse IgG antibody was then reacted at 4°C for 20 minutes. The cells were washed once with an FACS buffer and centrifuged. The cell pellet was re-suspended by an FACS buffer and analyzed by Calibur. As a result, 2G6, 4B2, 2G2, 9H5, 10F7, 22H8, 49F2, 14A8, 55E7, 13G5 and 16H2 in the hybridoma culture supernatant reacted well with CAL-1. On the other hand, 28G10 and 9D2 reacted little (FIG. 6).

[0100]

A-5) FACS screening using human peripheral blood pDC

[Isolation of human PBMC]

20 ml of peripheral blood was collected from a healthy human, and peripheral blood mononuclear cells (PBMCs) were isolated by specific gravity centrifugation using HISTOPAQUE-1077 (SIGMA). 1×10^6 of PBMCs were stained with each sample. The cells were washed with an FACS buffer, a Fc block reagent (Miltenyi) was added by 25 μl by 5-fold dilution, and a reaction was done at 4°C for 15 minutes. After washing with an FACS buffer, 50 μl of the cell culture supernatant of each hybridoma, 10 μg/ml of a goat IgG, an anti-hPTPRS pAb and a mouse IgG2a,κ were added, and a reaction was done at 4°C for 20 minutes. After washing with an FACS buffer, 8 μg/ ml of an FITC-labeled anti-goat IgG antibody or 2 μg/ ml of a PE-labeled anti-mouse IgG antibody was added, and a reaction was done at 4°C for 20 minutes. After washing with an FACS buffer, 50 μl of an APC-labeled anti-BDCA2 antibody by 10-fold dilution was reacted at 4°C for 20 minutes. After washing with an FACS buffer, the

cell was resuspended in 300 μl of a FACS buffer and analyzed by FACS calibur. As a result, 2G6, 28G10, 4B2, 2G2, 9H5, 10F7, 22H8, 49F2, 14A8, 55E7 and 13G5 showed a binding reaction specific to the pDC cell population. 9D2 showed binding to pDC, and also showed reactions with the cell group other than pDC (BDCA2-). 16H2 did not show a reaction for PBMCs (FIG. 7).

[0101]

Test on specificity of anti-PTPRS antibody

PTPRS belongs to the PTPR family, and the amino acid sequences of the several family molecules therefrom have high homology against the amino acid sequence of PTPRS (FIG. 8).

A-6) Whether or not the 10 kinds of hybridoma cell culture supernatants that generate an antibody that recognizes PTPRS and specifically binds to human pDC (2G6, 4B2, 2G2, 9H5, 10F7, 22H8, 49F2, 14A8, 55E7, 13G5) specifically binds to only PTPRS was examined. The transfected cells of PTPRA (40%), PTPRD (76%) and PTPRF (67%) that had specifically high homology with PTPRS were prepared by expressing an FLAG tag to the N terminus of the molecule, and stained. The expression of hPTPRE in the transfected cells was confirmed by Western Blot, but expression on the cellular surface could not be confirmed. Therefore, hPTPRE did not express on the cellular surface. As a result, 4B2 reacted with hPTPRD (FIG. 9C), and 2G6 showed cross-reactivity to hPTPRF (FIG. 9D). Other 8 kinds of antibodies showed PTPRS-specific binding (FIGS. 9A-D).

[0102]

A-7) Cross-reactivity of anti-PTPRS antibody to monkey

PBMCs of a cynomolgus monkey were isolated from peripheral blood (10 ml; Shin-Nippon Biomedical Laboratories, Ltd.) by specific gravity centrifugation using HISTOPAQUE-1077 (SIGMA). For FACS, 5×10^5 cells were used per one sample. The cells were washed with a FACS buffer, and 10 μl of 10% cynomolgus serum diluted with a FACS buffer was added thereto, and a reaction was conducted at 4°C for 20 minutes. After washing with a FACS buffer, 100 μl of the cell culture supernatant of each hybridoma and 10 μg/ ml of a mouse IgG2a,κ or mouse IgG1, κ (BioLegend) were added, and a reaction was conducted at 4°C for 15 minutes. After washing with a FACS buffer, 1 μg/ ml of an APC-labeled anti-mouse IgG antibody (BD) was added, and a reaction was conducted at 4°C for 20 minutes. After washing with a FACS buffer, an FITC-labeled anti-Lineagel antibody (BD), a PE-labeled anti-CD123 antibody (BD), and a PerCP7Cy5.5-labeled anti-HLA-DR antibody (BD) by 25 μl by 10-fold

dilution were reacted at 4°C for 15 minutes. After washing with a FACS buffer, the cells were resuspended in 300 μl of an FACS buffer and analyzed by FACS calibur. As the hybridoma culture supernatants used, 7 kinds: 49F2, 55E7, 14A8, 13G5, 10F7, 22H8 and 9H5 that are PTPRS-specific and bind well to a CAL-1 cell and human pDC were selected. As a result, all hybridoma cell culture supernatants specifically bound to the pDC population group (Lineage-CD123+HLA-DR+) of the cynomolgus monkey (FIG. 10).

[0103]

A-8) Singlization of hybridoma

The above-mentioned 7 kinds of hybridomas (49F2, 55E7, 14A8, 13G5, 10F7, 22H8 and 9H5) were each collected and suspended in a sorting buffer (1% FBS/PBS) so as to become 1×10^5 cells/ml. Using FACS Aria (BD), single cell sorting was conducted. The data was collected, and the collected data was developed by two-dimensional dot plot of X axis: FSC and Y axis: SSC. The live cells were gated on the dot plot. Gating for removing doublets from the cell in the living cell gate was conducted, and the cell population was dispensed to a 96-well flat bottom plate so as to be 1 cell/well. The cell subjected to the single cell sorting was cultured in an HAT medium (RPMI1640+2 mM L-Glutamine, 100 Unit/ml Penicillin, 100 μg/ml Streptomycin, 10 mM HEPES, 1 mM Sodium Pyruvate, and 50 μM 2-ME)+a hybridoma growth supplement HFCS (Roche). Thereafter D2SC cell and hPTPRS/D2SC cell (FIGS. 11A and B), CAL-1 cell (FIG. 11C) and human pDC (FIG. 11D) were stained by using the cell culture supernatant of the hybridoma, and a single hybridoma was selected.

[0104]

Example 3

Purification of antibody

Eight kinds of purified antibodies (9H5-4, 10F7-38, 13G5-52, 13G5-57, 14A8-85, 22H8-84, 49F2-30 and 55E7-79) were obtained from the culture supernatant of the hybridomas by purification using Protein G Sepharose FastFlow (GE Healthcare). Using Pierce rapid ELISA mouse mAb Isotyping Kit (Thermo Fisher Scientific), isotypes were determined. As a result, 13G5-52 and 13G5-57 were mouse IgG2b, κ, 55E7-79 had both mouse IgG2b, κ and mouse IgGI, κ, and others were mouse IgG1, κ. If the purified antibody includes endotoxin, it may affect the result of a property determination test. Therefore, the concentration of endotoxin was measured. The kits used were Endospecy ES-50M set, Toxicolor DIA-MP set and Endotoxin standard product CSE-L set (all by Seikagaku Biobusiness Corporation). As a result thereof, all purified antibodies had an endotoxin concentration equal to or less than the standard

value 0.3 EU/mg Ab (FIG. 12).

[0105]

Study on reactivity of purified antibody

The binding abilities of the purified antibodies were confirmed by a human pDC-like cell strain CAL-1 cell (FIG. 13). In addition, all of the antibodies maintained a binding ability against the human pDC population of human peripheral blood (BDCA2+) (FIG. 14).

[0106]

The homology of the amino acid sequence of human PTPRS against mouse PTPRS (mPTPRS) is about 96%. Since they are remarkably similar to each other, whether or not the prepared anti-human PTPRS antibody also binds to mouse PTPRS was studied. A CHO cell in which the gene of mPTPRS had been forcibly expressed (Chinese hamster ovary cell; hereinafter referred to as mPTPRS/CHO) was stained by 10 μg/ ml of each anti-PTPRS antibody. The cell number was 2×10^5 per one sample. After washing with a FACS buffer, a PE-labeled anti-mouse IgG antibody was diluted by 50-times and stained with 25 μl. As a result, 49F2-30, 13G5-52, 13G5-57 and 22H8-84 bound to mPTPRS/CHO (FIG. 15).

[0107]

Example 4

Complement-dependent cellular cytotoxicity of anti-PTPRS antibody to hPTPRS-expressing cell

Using baby rabbit serum as a source of complement, the complement-dependent cellular cytotoxicity (hereinafter referred to as CDC activity) of the anti-PTPRS antibody against a CHO cell that expresses human PTPRS (hereinafter referred to as hPTPRS/CHO) and a mouse PTPRS/CHO cell (hereinafter referred to as mPTPRS/ CHO) was measured. The activity was obtained by using cell toxicity that was calculated from a measured value of lactase dehydrogenase (LDH) released from the cell as an index. Each cell was dispensed to a 96-well U bottom plate by 2×10^4 cells/50 μl/well. A 18% Complement (CEDARLANE) was prepared by a CDC medium (RPMI1640+0.1%BSA+10 mM HEPES+2mM L-Glutamine+100 Unit/ml Penicillin+100 μg/ml Streptomycin). Two kinds: 3.3 μg/ ml and 30 μg/ml were prepared for a control antibody (mouse IgG1, κ or mouse IgG2b, κ) and an anti-PTPRS antibody. An assay was conducted by using a kit of CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega). As a result, 13G5-52 and 13G5-57 showed about 20% of CDC activity against the target of hPTPRS/CHO (FIG. 16A). On the other hand, 13G5-52 and 13G5-57 showed about 100% of CDC activity against the target of mPTPRS/CHO (FIG. 16B).

[0108]

Example 5

Preparation of chimerized antibody

As a hybridoma for the production of a mouse anti-PTPRS antibody, the following one was used.

Hybridoma 9H5-4 (Accession No.: FERM BP-11356)

Hybridoma 10F7-38 (Accession No.: FERM BP-11357)

Hybridoma 13G5-52 (Accession No.: FERM BP-11358)

Hybridoma 13G5-57 (Accession No.: FERM BP-11359)

Hybridoma 14A8-85 (Accession No.: FERM BP-11360)

Hybridoma 22H8-84 (Accession No.: FERM BP-11361)

Hybridoma 49F2-30 (Accession No.: FERM BP-11362)

1. Confirmation of isotype of constant region

The isotype of the constant region of each of mouse antibody produced from seven hybridomas(9H5-4,10F7-38,13G5-52, 13G5-57, 14A8-85, 22H8-84 and 49F2-30) was confirmed.

For the confirmation, a mouse monoclonal antibody isotyping kit (Catalog No.: MMT1; Serotec Product; Oxford, UK) or Pierce Rapid ELISA mouse mAb Isotyping Kit (Thermo Fisher Scientific),and such 9H5-4,10F7-38,13G5-52, 13G5-57, 14A8-85, 22H8-84 and 49F2-30 hybridoma culture supernatant as a sample were used.

As a result, the isotype of the antibodies produced by the 13G5-52 and 13G5-57 hybridomas was an isotype including mouse IgG2b as a heavy chain and κ as a light chain. On the other hand, the isotype of the antibodies produced by the 9H5-4, 10F7-38, 14A8-85, 22H8-84 and 49F2-30 hybridomas was an isotype including mouse IgG1 as a heavy chain and κ as a light chain.

[0109]

2. Cloning of cDNA that codes for variable region of mouse anti-PTPRS antibody

2-1) Isolation of total RNA

Using a commercially available kit "RNeasy Mini Kit" (Qiagen, Catalog No.: 74106), the total RNA was isolated from seven hybridomas according to the instruction attached to the kit. About 30 μg of the total RNA was obtained by preparation from the hybridoma cell strain of 5×10^6 cell number.

[0110]

2-2) Amplification and fragmentation of cDNA that codes for mouse heavy chain variable region

Using 5 μg from the total RNA isolated in 2-1), cDNA that codes for mouse

heavy chain variable region was amplified by the 5' RACE PCR process. In the amplification, a commercially available kit "5' RACE System for Rapid Amplification of cDNA ENDs, Version 2.0 Kit" (Invitrogen, Catalog No.: 18374-058) was used. The specifics are as follows. First, a first strand cDNA was synthesized from the total RNA obtained in 2-1) by a reverse transcriptase. At that time, the antisense primer (GSP1) shown below was used.

The GSP1 primer used for amplification of cDNA is used according to the isotype of each mouse heavy chain. For example, the following antisense primers are used for the cloning of the heavy chain variable region of the 9H5-4, 10F7-38, 14A8-85, 22H8-84 and 49F2-30 hybridomas including mouse IgG1 as a heavy chain.

GSP1 primer : mu IgG1 VH-GSP1

Sequence : 5'-CCA GGA GAG TGG GAG AGG CTC TTC TCA GTA TGG TGG-3' (36-mer) (SEQ ID NO: 39)

GSP2 primer : mu IgG1 VH-GSP2

Sequence : 5'-GGC TCA GGG AAA TAG CCC TTG ACC AGG CAT CC-3' (32-mer) (SEQ ID NO: 40)

Also, for example, the following antisense primers can be used for the cloning of the heavy chain variable region of the 9H5-4, 10F7-38, 14A8-85, 22H8-84 and 49F2-30 hybridomas including mouse IgG1 as a heavy chain.

GSP1 primer : mu IgGHγ1-GSP1

Sequence : 5'-TCC AGA GTT CCA GGT CAC TGT CAC-3' (24-mer) (SEQ ID NO:11)

GSP2 primer : mu IgG Hγ1-GSP2

Sequence : 5'-AGG GGC CAG TGG ATA GAC AGA TGG-3' (32-mer) (SEQ ID NO:13)

And the following antisense primers are used for the cloning of the heavy chain variable region of the 13G5-52 and 13G5-57 hybridomas including mouse IgG2b as a heavy chain.

GSP1 primer : mu IgGHγ 2B-GSP1

Sequence : 5'-TCC AGA GTT CCA AGT CAC AGT CAC-3' (24-mer) (SEQ ID NO: 41)

GSP2 primer : mu IgG Hγ 2B-GSP2

Sequence : 5'-AGG GGC CAG TGG ATA GAC TGA TGG-3' (24-mer) (SEQ ID NO: 42)

[0111]

Furthermore, using a terminal deoxynucleotidyl transferase (TdT) at the 3'-terminus of the first chain cDNA, a nucleotide homopolymer dC was added. Furthermore, using an anchor primer having a nucleotide polymer that is complementary to the dC (anchor sequence) (SEQ ID NO:12), and the antisense primer (GSP2), the cDNA was amplified by a

PCR process. Furthermore, using the obtained PCR product as a template, and using an AUAP primer (SEQ ID NO:14) and the antisense primer (GSP2), the cDNA was amplified by a Nested PCR process. Furthermore, this PCR product was purified by a 1.5% low melting point agalose process.

Anchor primer for 5'RACE (SEQ ID NO:12): 5'-GGC CAC GCG TCG ACT AGT ACG GGI IGG GII GGG IIG-3' (36-mer)

AUAP primer for 5'RACE (SEQ ID NO:14): 5'-GGC CAC GCG TCG ACT AGT AC-3' (20-mer)

[0112]

2-3) Amplification and fragmentation of cDNA that codes for mouse light chain variable region

From the total RNA isolated in 2-1), a cDNA that codes for mouse light chain variable region was amplified in a similar manner to 2-2).

Since these seven antibodies include mouse Ig κ light chain, the following antisense primers are used for the cloning of the light chain.

GSP1 primer : Mu IgVL5RACE-GSP1

Sequence : 5'-TTC ACT GCC ATC AAT CTT CCA CTT-3' (24-mer) (SEQ ID NO:15)

GSP2 primer : Mu IgVL5RACE-GSP2

Sequence : 5'-GAT GGA TAC AGT TGG TGC AGC-3' (21-mer) (SEQ ID NO:16)

The obtained PCR product was purified by a 1.5% low melting point agalose process.

[0113]

2-4) Confirmation of base sequence of cDNA and determination of CDR region

The cDNA fragments of the heavy chain variable region obtained in 2-2) and the light chain variable region obtained in 2-3) were each cloned to a pCR4Blunt-TOPO vector using a commercially available kit "Zero Blunt TOPO PCR Cloning Kit" (Invitrogen, Catalog No.: 1325137), according to the instruction attached to the kit, and introduced into an E. coli competent cell to give an E. coli transformant. A plasmid was obtained from this transformant and a plasmid DNA sample was sent to Operon Biotechnology Co. Ltd (Tokyo) for sequence analysis to confirm the cDNA base sequence in the plasmid. For the analyses of the sequences, "Sequencher DNA sequence assembly and analysis software version 4.2.2 (Gene Codes Corporation)" and "GENETYX-MAC Version 11. 1. 1" software (GENETYX CORPORATION)" were used.

[0114]

The transformants that became inactive RNAs since frame shifting, nonsense mutation and the like occurred around a complementary determination region (hereinafter

referred to as "CDR region") were excluded, and transformants having correct sequences were extracted. Furthermore, the Immunoglobulins Database(IgBLAST, URL:www.ncbi.nlm.nih.gov/igblast/) and homology were confirmed for the cDNA base sequence included in the plasmid to determine the sequences of the CDR region(CDRs; CDR1,CDR2,CDR3) in each variable region, Framework region and the sequence of the variable region were determined according to the analysis method using Kabat numbering system (Kabat et al., 1991, sequences of Proteins of Immunological Interest, National Institutes of Health Publication No. 91-3242, 5th ed., United States Department of Health and Human Services, Bethesda, MD).

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PTPRS mouse 9H5-4 antibody was SEQ ID NO: 43, and the amino acid sequence was SEQ ID NO: 44. The amino acid sequences of the CDR1, CDR2 and CDR3 in the heavy chain variable region of the mouse 9H5-4 antibody were SEQ ID NO: 45, SEQ ID NO: 46 and SEQ ID NO: 47, respectively.

The nucleic acid sequence of the light chain variable region of the obtained anti-PTPRS mouse 9H5-4 antibody is SEQ ID NO: 48, and the amino acid sequence is SEQ ID NO: 49. The amino acid sequences of the CDR1, CDR2 and CDR3 in the light chain variable region of the mouse 9H5-4 antibody are SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52, respectively.

And the nucleic acid sequences of the heavy chain variable region and the light chain variable region of obtained anti-PTPRS mouse 10F7-38 antibody and 14A8-85 antibody were the same as those of 9H5-4 antibody, including the sequences of the CDR1, CDR2 and CDR3.

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PTPRS mouse 13G5-57 antibody was SEQ ID NO: 53, and the amino acid sequence was SEQ ID NO: 54. The amino acid sequences of the CDR1, CDR2 and CDR3 in the heavy chain variable region of the mouse 13G5-57 antibody were SEQ ID NO: 55, SEQ ID NO: 56 and SEQ ID NO: 57, respectively.

The nucleic acid sequence of the light chain variable region of the obtained anti-PTPRS mouse 13G5-57 antibody is SEQ ID NO: 58, and the amino acid sequence is SEQ ID NO: 59. The amino acid sequences of the CDR1, CDR2 and CDR3 in the light chain variable region of the mouse 13G5-57 antibody are SEQ ID NO: 60, SEQ ID NO: 61 and SEQ ID NO: 62, respectively.

And the nucleic acid sequences of the heavy chain variable region and the light

chain variable region of obtained anti-PTPRS mouse 13G5-52 antibody were the same as those of 13G5-57 antibody, including the sequences of the CDR1, CDR2 and CDR3.

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PTPRS mouse 22H8-84 antibody was SEQ ID NO: 63, and the amino acid sequence was SEQ ID NO: 64. The amino acid sequences of the CDR1, CDR2 and CDR3 in the heavy chain variable region of the mouse 22H8-84 antibody were SEQ ID NO: 65, SEQ ID NO: 66 and SEQ ID NO: 67, respectively.

The nucleic acid sequence of the light chain variable region of the obtained anti-PTPRS mouse 22H8-84 antibody is SEQ ID NO: 68, and the amino acid sequence is SEQ ID NO: 69. The amino acid sequences of the CDR1, CDR2 and CDR3 in the light chain variable region of the mouse 22H8-84 antibody are SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PTPRS mouse 49F2-30 antibody was SEQ ID NO:25, and the amino acid sequence was SEQ ID NO:26. The amino acid sequences of the CDR1, CDR2 and CDR3 in the heavy chain variable region of the mouse 49F2-30 antibody are SEQ ID NO:27, SEQ ID NO:28 and SEQ ID NO:29, respectively.

The nucleic acid sequence of the light chain variable region of the obtained anti-PTPRS mouse 49F2-30 antibody is SEQ ID NO:30, and the amino acid sequence is SEQ ID NO:31. The amino acid sequences of the CDR1, CDR2 and CDR3 in the light chain variable region of the mouse 49F2-30 antibody are SEQ ID NO:32, SEQ ID NO:33 and SEQ ID NO:34, respectively.

The nucleic acid sequence (471bp) of the heavy chain variable region of the obtained anti-PTPRS mouse 9H5-4 antibody is shown below (SEQ ID NO: 43). The capital letters show the mouse 9H5-4 VH variable region and the small letters show the mouse IgG1 heavy chain constant region.

ATGGAGTTGGGACTGAGCTGGGTATTTCTTGTGGCTCTTTTGAATGGTGTCCAGTGTC
AGGTGCAGCTTGTAGAGACCGGGGGAGGCTTGGTGAGGCCTGGAAATTCTCTGAAA
CTCTCCTGTGTTACCTCGGGATTCACTTTCAGTAACTACCGGATGCACTGGCTTCGCC
AGCCTCCAGGGAAGAGGCTGGAGTGGATTGCTGTAATTACAGTCAAATCTGATAATTA
TGGAGCAAATTATGCAGAGTCTGTGAAAGGCAGATTCACTATTTCAAGAGATGATTCA
AAAAGCAGTGTCTACCTGCAGATGAACAGATTAAGAGAGGAAGACACTGCCACTTAT
TATTGTAGTAGATCGGTCTACTATGGTTACGTCCTAGCCTTTGACTACTGGGGCCAAGG
CACCACTCTCACAGTCTCCTCAgccaaaacgacacccccatctgtctatccactggcccctaagggc

The amino acid sequence (157 a.a) of the heavy chain variable region of the mouse 9H5-4 antibody is shown below (SEQ ID NO: 44). The capital letters show the sequence of VH variable region and the small letters show the mouse IgG1 heavy chain constant region. The underlined part means the signal sequence and the double-underlined part means the CDR region (CDR1, CDR2, CDR3).

MELGLSWVFLVALLNGVQCQVQLVETGGGLVRPGNSLKLSCVTSGFTFSNYRMHWLRQ PPGKRLEWIAVITVKSDNYGANYAESVKGRFTISRDDSKSSVYLQMNRLREEDTATYYCS RSVYYGYVLAFDYWGQGTTLTVSSakttppsvyplapkg

The CDR1 of the heavy chain variable region of the 9H5-4 antibody is NYRMH (SEQ ID NO: 45）, the CDR2 of the heavy chain variable region of the 9H5-4 antibody is VITVKSDNYGANYAESVKG (SEQ ID NO: 46）, and the CDR3 of the heavy chain variable region of the 9H5-4 antibody is SVYYGYVLAFDY (SEQ ID NO: 47）.

The nucleic acid sequence (402bp) of the light chain variable region of the obtained anti-PTPRS mouse 9H5-4 antibody is shown below (SEQ ID NO: 48). The capital letters show the mouse 9H5-4 VH variable region and the small letters show the mouse Ig κ light chain constant region.

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATG TGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTC ACCATCAGTTGCAGGGCAAGTCAGGACATTAGCAATTATTTAAACTGGTATCAGCAGA AACCAGATGGAACTGTTAAACTCCTGATCTACTACACATCAAGATTACACTCAGGAGT CCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAAC CTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTGGA CGTTCGGTGGAGGCACCAAGCTGGAAATCAAAcgggctgatgctgcaccaact

The amino acid sequence (134 a.a) of the light chain variable region of the mouse 9H5-4 antibody is shown below (SEQ ID NO: 49). The capital letters show the sequence of mouse 9H5-4 VH variable region and the small letters show the mouse Ig κ light chain constant region. The underlined part means the signal sequence and the double-underlined part means the CDR region (CDR1, CDR2, CDR3).

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQK PDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGG GTKLEIKradaapt

The CDR1 of the light chain variable region of the 9H5-4 antibody is

RASQDISNYLN (SEQ ID NO: 50) , the CDR2 of the light chain variable region of the 9H5-4 antibody is YTSRLHS (SEQ ID NO: 51) , and the CDR3 of the light chain variable region of the 9H5-4 antibody is QQGNTLP (SEQ ID NO: 52) .

The nucleic acid sequence (465bp) of the heavy chain variable region of the anti-PTPRS mouse 13G5-57 antibody is shown below (SEQ ID NO: 53). The capital letters show the mouse 13G5-57 VH variable region and the small letters show the mouse IgG2b heavy chain constant region.

ATGAACTTGGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTG
AAGTGAAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAACCTCTGGATTCACTTTCAGTGACTATTACATGTATTGGGTTCGCCA
GACTCCAGAGAAGAGGCTGGAGTGGGTCGCATACATTAGTAATGGTGGTGGTAGCAC
CTATTATCCAGACACTGTAAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAAC
ACCCTGTACCTGCAAATGAGCCGTCTGAAGTCTGAGGACACAGCCATGTATTACTGTG
CAAGACATGTTTACTACGGGAGGAACTATGCTATGGACTACTGGGGTCAAGGAACCT
CAGTCACCGTCTCCTCAgccaaaacaacacccccatcagtctatccactggcccctaagggc

The amino acid sequence (155 a.a) of the heavy chain variable region of the mouse 13G5-57 antibody is shown below (SEQ ID NO: 54). The capital letters show the sequence of VH variable region and the small letters show the mouse IgG2b heavy chain constant region. The underlined part means the signal sequence and the double-underlined part means the CDR region (CDR1, CDR2, CDR3).

MNLGLSLIFLVLVLKGVQCEVKLVESGGGLVQPGGSLKLSCATSGFTFSDYYMYWVRQT
PEKRLEWVAYISNGGGSTYYPDTVKGRFTISRDNAKNTLYLQMSRLKSEDTAMYYCAR
HVYYGRNYAMDYWGQGTSVTVSSakttppsvyplapkg

The CDR1 of the heavy chain variable region of the 13G5-57 antibody is DYYMY (SEQ ID NO: 55) , the CDR2 of the heavy chain variable region of the 13G5-57 antibody is YISNGGGSTYYPDTVKG (SEQ ID NO: 56) , and the CDR3 of the heavy chain variable region of the 13G5-57 antibody is HVYYGRNYAMDY (SEQ ID NO: 57) .

The nucleic acid sequence (465bp) of the light chain variable region of the obtained anti-PTPRS mouse 13G5-57 antibody is shown below (SEQ ID NO: 58). The capital letters show the mouse 13G5-57 VH variable region and the small letters show the mouse Ig κ light chain constant region.

ATGAACTTGGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTG
AAGTGAAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAA

CTCTCCTGTGCAACCTCTGGATTCACTTTCAGTGACTATTACATGTATTGGGTTCGCCA
GACTCCAGAGAAGAGGCTGGAGTGGGTCGCATACATTAGTAATGGTGGTGGTAGCAC
CTATTATCCAGACACTGTAAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAAC
ACCCTGTACCTGCAAATGAGCCGTCTGAAGTCTGAGGACACAGCCATGTATTACTGTG
CAAGACATGTTTACTACGGGAGGAACTATGCTATGGACTACTGGGGTCAAGGAACCT
CAGTCACCGTCTCCTCAgccaaaacaacacccccatcagtctatccactggcccctaagggc

The amino acid sequence (155 a.a) of the light chain variable region of the mouse 13G5-57 antibody is shown below (SEQ ID NO: 59). The capital letters show the sequence of mouse 13G5-57 VH variable region and the small letters show the mouse Ig κ light chain constant region. The underlined part means the signal sequence and the double-underlined part means the CDR region (CDR1, CDR2, CDR3).

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQK
PDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGG
GTKLEIKradaaptvsifppsseqltsggasvvcf

The CDR1 of the light chain variable region of the 13G5-57 antibody is RASQDISNYLN (SEQ ID NO: 60）, the CDR2 of the light chain variable region of the 13G5-57 antibody is YTSRLHS (SEQ ID NO: 61）, and the CDR3 of the light chain variable region of the 13G5-57 antibody is QQGNTLPY (SEQ ID NO: 62）.

The nucleic acid sequence (458bp) of the heavy chain variable region of the anti-PTPRS mouse 22H8-84 antibody is shown below (SEQ ID NO: 63). The capital letters show the mouse 22H8-84 VH variable region and the small letters show the mouse IgG1 heavy chain constant region.

ATGGAATGTAACTGGATACTTCCTTTTATTCTGTCAGTAACTTCAGGTGTCTACTCACA
GGTTCAGCTCCAGCAGTCTGGGGCTGAGCTGGCAAGACCTGGGGCTTCAGTGAAGT
TGTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACTGGATGCAGTGGGTAAAACA
GAGGCCTGGACAGGGTCTGGAATGGATTGGGGCTATTTATCCTGGAGATGGTGATACT
AGGTACACTCAGAAGTTCAAGGGCAAGGCCACATTGACTGCAGATAAATCCTCCAGC
ACAGCCTACATGCAACTCAGCAGCTTGGCATCTGAGGACTCTGCGGTCTATTACTGTG
CAAGAAGGATTTACTACGGCTATTACTATGCTATGGACTACTGGGGTCAAGGAACCTC
AGTCACCGTCTCCTCagccaaaacgacacccccatctgtctatccactggcccc

The amino acid sequence (152 a.a) of the heavy chain variable region of the mouse 22H8-84 antibody is shown below (SEQ ID NO: 64). The capital letters show the sequence of VH variable region and the small letters show the mouse IgG1 heavy chain constant region. The underlined part means the signal sequence and the double-underlined part means the

CDR region (CDR1, CDR2, CDR3).

MECNWILPFILSVTSGVYSQVQLQQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQ RPGQGLEWIGAIYPGDGDTRYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYCAR RIYYGYYYAMDYWGQGTSVTVSSakttppsvypla

The CDR1 of the heavy chain variable region of the 22H8-84 antibody is SYWMQ (SEQ ID NO: 65), the CDR2 of the heavy chain variable region of the 22H8-84 antibody is AIYPGDGDTRYTQKFKG (SEQ ID NO: 66), and the CDR3 of the heavy chain variable region of the 22H8-84 antibody is RIYYGYYYAMDY (SEQ ID NO: 67).

The nucleic acid sequence (430bp) of the light chain variable region of the obtained anti-PTPRS mouse 22H8-84 antibody is shown below (SEQ ID NO: 68). The capital letters show the mouse 22H8-84 VH variable region and the small letters show the mouse Ig κ light chain constant region.

ATGGAGACAGACACAATCCTGCTATGGGTGCTGCTGCTCTGGGTTCCAGGCTCCACT GGTGACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGG CCACCATCTCCTGCAAGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTATATGAA CTGGTACCAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATGCTGCATCCAA TCTAGAATCTGGGATCCCAGCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTTCAC CCTCAACATCCATCCTGTGGAGGAGGAGGATGCTGCAACCTATTACTGTCAGCAAAG TAATGAGGATCCTCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAAcgggctgatgctg caccaactgtatccatcaagggcg

The amino acid sequence (143 a.a) of the light chain variable region of the mouse 22H8-84 antibody is shown below (SEQ ID NO: 69). The capital letters show the sequence of mouse 22H8-84 VH variable region and the small letters show the mouse Ig κ light chain constant region. The underlined part means the signal sequence and the double-underlined part means the CDR region (CDR1, CDR2, CDR3).

METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNW YQQKPGQPPKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPL TFGAGTKLELKradaaptvsikg

The CDR1 of the light chain variable region of the 22H8-84 antibody is KASQSVDYDGDSYMN (SEQ ID NO: 70), the CDR2 of the light chain variable region of the 22H8-84 antibody is AASNLES (SEQ ID NO: 71), and the CDR3 of the light chain variable region of the 22H8-84 antibody is QQSNEDPL (SEQ ID NO: 72).

[0115]

The nucleic acid sequence of the heavy chain variable region of the anti-PTPRS mouse 49F2-30 antibody (469 bp) is shown below (SEQ ID NO:25). The capital letters show the variable region of the mouse 49F2-30 VH and the small letters show the mouse IgG1 heavy chain constant region.

ATGAACTTCGGGCTCAGGTTGATTTTCCTTGCCCTCATTTTAAAAGGTGTCCAGTGTG
AGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCATTTTCAGTAGCTATGGCATGTCTTGGGTTCGCC
AGACTCCAGACAAGAGGCTGGAGTGGGTCGCAACCATTAGTAGTGGTGGTAGTGAC
ACCTATTATCCAGACAGTGTGAAGGGGCGATTCACCATCTCCAGAGACAATGCCAA
CAACACCCTGTACCTGCAAATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTATT
ACTGTGCAAGACAGGTCTACTATGGTCTTTACTGGTATTTCGATGTCTGGGGCGCAG
GGACCACGGTCACCGTCTCCTCAgccaaaacgacacccccatctgtctatccactggcccctaagggcgaat

[0116]

The amino acid sequence of the heavy chain variable region of the mouse 49F2-30 antibody (156 a.a) is shown below (SEQ ID NO:26). The capital letters show the VH variable gene and the small letters show the mouse IgG1 heavy chain constant region. The underlined sequences show the signal sequences, and the double-underlined sequences show the CDR regions (CDR1, CDR2, CDR3).

MNFGLRLIFLALILKGVQCEVQLVESGGDLVKPGGSLKLSCAASGFIFSSYGMSWVRQTPDKRLEWVATISSGGSDTYYP
DSVKGRFTISRDNANNTLYLQMSSLKSEDTAMYYCARQVYYGLYWYFDVWGAGTTVTVSS akttppsvyplapkge

The CDR1 of the heavy chain variable region of the 49F2-30 antibody is SYGMS (SEQ ID NO:27), the CDR2 of the heavy chain variable region of the 49F2-30 antibody is TISSGGSDTYYPDSVKG (SEQ ID NO:28), and the CDR3 of the heavy chain variable region of the 49F2-30 antibody is QVYYGLYWYFDV (SEQ ID NO:29).

[0117]

The nucleic acid sequence of the light chain variable region of the obtained anti-PTPRS mouse 49F2-30 antibody (413 bp) is shown below (SEQ ID NO:30). The capital letters show the variable region of the mouse 49F2-30 VL and the small letters show the mouse Ig κ light chain constant region.

ATGGAGTCACAGATTCAGGTCTTTGTATTCGTGTTTCTCTGGTTGTCTGGTGTTGACG
GAGACATTGTGATGACCCAGTCTCACAAATTCATGTCCACATCAGTAGGAGACAGG
GTCAGCATCATTTGTAAGGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAA
CAGAAACCAGGACAATCTCCTAAATTACTGATTTACTCGGCATCCTACCGGTACACT
GGAGTCCCTGATCGCTTCACTGGCAGTGGATCTGGGACGGATTTCACTTTCACCATC
AGCAGTGTGCAGGCTGAAGACCTGGCAATTTATTACTGTCAGCAACATTATAGTACT
CCGTACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAAcgggctgatgctgcaccaactgtatcc
atcaa

[0118]

The amino acid sequence of the light chain variable region of the mouse 49F2-30 antibody (137 a.a) is shown below (SEQ ID NO:31). The capital letters show the mouse 49F2-30 VL variable region and the small letters show the mouse Ig κ light chain constant region. The underlined sequences show the signal sequences, and the double-underlined sequences show the CDR regions (CDR1, CDR2, CDR3).

MESQIQVFVFVFLWLSGVDGDIVMTQSHKFMSTSVGDRVSIICKASQDVNTAVAWYQQKPGQSPKLLIYSASYRYTGVPD
RFTGSGSGTDFTFTISSVQAEDLAIYYCQQHYSTPYTFGGGTKLEIK radaaptvsi

The CDR1 of the light chain variable region of the 49F2-30 antibody is KASQDVNTAVA (SEQ ID NO:32), the CDR2 of the light chain variable region of the 49F2-30 antibody is SASYRYT (SEQ ID NO:33), and the CDR3 of the light chain variable region of the 49F2-30 antibody is QQHYSTP (SEQ ID NO:34).

[0119]

3. Preparation of expression vector of chimerized antibody

3-1) Cloning of cDNA that codes for human Ig constant region

The cDNAs of a human IgG1 heavy chain constant region and a human Ig κ light chain constant region were cloned from the total RNA of human PBMC, and were each cloned to pCR4Blunt-TOPO vector and introduced in an E. coli competent cell using a commercially available kit "Zero Blunt TOPO PCR Cloning Kit" (Invitrogen, Catalog No.: 1325137) according to the instruction attached to the kit to give an E. coli transformant. The above-mentioned plasmid was obtained from this transformant and a plasmid DNA sample was sent to Operon Biotechnology Co.,Ltd.(Tokyo) for sequence analysis to confirm the cDNA base sequence in the plasmid.

[0120]

3-2) Preparation of expression vector of chimerized 9H5-4 (10F7-38, 14A8-85) antibody

In order to prepare a cDNA that coded for a heavy chain of a chimerized PTPRS antibody, the heavy chain variable region of the mouse 9H5-4 antibody obtained in 2-2 and the pEE6.4 vector (Lonza Biologics, Slough, UK) into which the human IgG heavy chain constant region was incorporated had been fused, and the heavy chain variable region of the mouse 9H5-4 antibody was amplified by the PCR method and the PCR product which had a length of about 450 bases was obtained. At that time, the primers were those as follows. The obtained PCR product was purified by a 1.5% low melting point agalose process.

The primer for expressing heavy chain in chimeric 9H5-4 antibody

1) forward primer : chi10F7VH-IF(Hind3)

Sequence : 5' ttt AAG CTT gcc gcc acc ATG GAG TTG GGA CTG AGC TGG 3' (39-mer) (SEQ ID NO: 73)

2) reverse primer : chi10F7VH-462R(ApaI)

Sequence : 5' cga tgg gcc ctt ggt gct agc TGA GGA GAC TGT GAG AGT GGT 3' (42-mer) (SEQ ID NO: 74)

"A PCR product that coded for the 9H5-4 heavy chain variable region" was obtained from the mouse 9H5-4 antibody heavy chain variable region obtained in 2-2 by the PCR process. The PCR product that coded for 9H5-4 heavy chain variable region was digested with Hind III and an Apa I restriction enzyme and purified by a 1.5% agalose gel process. This was dissolved by ddH_2O to give a solution of a cDNA fragment that coded for the heavy chain variable region.

The V_H coding region of the 9H5-4 of the obtained cDNA was amplified by PCR from a pCR4Blunt-TOPO plasmid clone including the V_H coding region of the 9H5-4, by using primers chi10F7VH-IF (Hind3) and chi10F7VH-462R (ApaI) to which preferable restriction sites for cloning into a pEE6.4 vector (Lonza Biologics, Slough, UK) (Hind III and ApaI) and an ideal Kozak sequence (GCCGCCACC) had been introduced using Hind III and ApaI as cloning sites. The chi9H5-4VH-pEE6.4 vector includes a heavy chain constant region of human IgG1. The V_H PCR fragment was inserted into the pEE6.4 vector by in-frame using Hind III and ApaI. The construct was investigated by a cDNA base sequence analysis and a plasmid DNA sample was sent to Operon Biotechnology Co.,Ltd.(Tokyo) for sequence analysis to confirm the cDNA base sequence in the plasmid.

In order to prepare a cDNA that coded for a light chain of a chimerized 9H5-4 antibody, the PCR product was amplified by a length of about 730 bases by a technique based on overlap extension PCR from the PCR fragment in which the mouse 9H5-4 antibody light chain variable region obtained in 2-3 and the human Ig κ light chain constant region obtained in 3-2 had been fused.

The PCR product that coded for the 9H5-4 light chain variable region was digested by Hind III and an EcoRI restriction enzyme, and purified by a 1.5% agalose gel process. This was dissolved in ddH_2O to give a solution of a cDNA fragment that codes for the light chain variable region.

The obtained V_L-coding cDNA of 9H5-4 was amplified by PCR from a pCR4Blunt-TOPO plasmid clone including the V_L region of the 9H5-4 using primers chi11G9VL-IF (Hind) and chi11G9VL-726R (RI) to which preferable restriction sites (Hind III

and EcoRI) for cloning into a pEE14.4 vector (Lonza Biologics) and an ideal Kozak sequence had been introduced. The Chi9H5-4VL-pEE14.4 vector includes a kappa light chain constant region. The V_L PCR fragment was inserted into the pEE14.4 vector by in-frame by using Hind III and EcoRI. The construct was investigated by a cDNA base sequence analysis.

The primer for expressing light chain in chimeric 9H5-4 antibody

1) Forward primer : chi11G9VL-IF(Hind)

Sequence : 5' acc AAG CTT gcc gcc acc ATG ATG TCC TCT GCT CAG TTC 3' (39-mer) (SEQ ID NO: 75)

2) reverse primer : chi11G9VL-408R

Sequence : 5' agc cac agt tcg TTT GAT TTC CAG CTT GGT GCC 3' (33-mer) (SEQ ID NO: 76)

3) Forward primer : chi11G9VL-385F

Sequence : 5' CTG GAA ATC AAA cga act gtg gct gca cca tct 3' (33-mer) (SEQ ID NO: 77)

4) reverse primer : chi11G9VL-726R(RI)

Sequence : 5' aaa GAA TTC cta gca ctc tcc cct gtt gaa 3' (30-mer) (SEQ ID NO: 78)

3-2) Preparation of expression vector of chimerized 13G5-57(13G5-52) antibody

In order to prepare a cDNA that codes for a heavy chain of a chimerized PTPRS antibody, the heavy chain variable region of the mouse 13G5-57 antibody obtained in 2-2 and the pEE6.4 vector (Lonza Biologics, Slough, UK) into which the human IgG heavy chain constant region was incorporated had been fused. In a similar method as the 9H5-4 antibody, the PCR product was obtained and purified. At that time, the primers were those as follows.

The primer for expressing heavy chain in chimeric 13G5-57 antibody

1) forward primer : chi13G5.57VH-IF(Hind3)

Sequence : 5' ttt AAG CTT gcc gcc acc ATG AAC TTG GGG CTC AGC TTG 3' (39-mer) (SEQ ID NO: 79)

2) reverse primer : chi13G5.57VH-456R(ApaI)

Sequence : 5' cga tgg gcc ctt ggt gct agc TGA GGA GAC GGT GAC TGA GGT 3' (42-mer) (SEQ ID NO: 80)

In order to prepare a cDNA that codes for a light chain of a chimerized 13G5-57 antibody, the PCR product was amplified by a length of about 730 bases by a technique based on overlap extension PCR from the PCR fragment in which the mouse 13G5-57 antibody light chain variable region obtained in 2-3 and the human Ig κ light chain constant region obtained in

3-2 had been fused.

The primer for expressing light chain in chimeric 13G5-57 antibody

1) Forward primer : chi11G9VL-IF(Hind)

Sequence : 5' acc AAG CTT gcc gcc acc ATG ATG TCC TCT GCT CAG TTC 3' (39-mer) (SEQ ID NO: 81)

2) reverse primer : chi11G9VL-408R

Sequence : 5' agc cac agt tcg TTT GAT TTC CAG CTT GGT GCC 3' (33-mer) (SEQ ID NO: 82)

3) Forward primer : chi11G9VL-385F

Sequence : 5' CTG GAA ATC AAA cga act gtg gct gca cca tct 3' (33-mer) (SEQ ID NO: 83)

4) reverse primer : chi11G9VL-726R(RI)

Sequence : 5' aaa GAA TTC cta gca ctc tcc cct gtt gaa 3' (30-mer) (SEQ ID NO: 84)

In a similar manner to preparation of expression vectors of chimerized 9H5-4 antibody, expression vectors for such a heavy chain and a light chain of chimerized 13G5-57 antibody were prepared.

3-2) Preparation of expression vector of chimerized 22H8-84 antibody

In order to prepare a cDNA that codes for a heavy chain of a chimerized PTPRS antibody, the heavy chain variable region of the mouse 22H8-84 antibody obtained in 2-2 and the pEE6.4 vector (Lonza Biologics, Slough, UK) into which the human IgG heavy chain constant region was incorporated had been fused. In a similar method as the 9H5-4 antibody, the PCR product was obtained and purified. At that time, the primers were those as follows.

The primer for expressing heavy chain in chimeric 22H8-84 antibody

1) forward primer : chi22H8VH-IF(Hind3)

Sequence : 5' ttt AAG CTT gcc gcc acc ATG GAA TGT AAC TGG ATA CTT 3' (39-mer) (SEQ ID NO: 85)

2) reverse primer : chi22H8VH -456R(ApaI)

Sequence : 5' cga tgg gcc ctt ggt gct agc TGA GGA GAC GGT GAC TGA GGT 3' (42-mer) (SEQ ID NO: 86)

In order to prepare a cDNA that codes for a light chain of a chimerized 22H8-84 antibody, the PCR product was amplified by a length of about 730 bases by a technique based on overlap extension PCR from the PCR fragment in which the mouse 22H8-84 antibody light chain variable region obtained in 2-3 and the human Ig κ light chain constant region obtained in

3-2 had been fused.

The primer for expressing light chain in chimeric 22H8-84 antibody

1) Forward primer : chi22H8VL-IF(Hind)

Sequence : 5' acc AAG CTT gcc gcc acc ATG GAG ACA GAC ACA ATC CTG 3' (39-mer) (SEQ ID NO: 87)

2) reverse primer : chi22H8VL-420R

Sequence : 5' agc cac agt tcg TTT CAG CTC CAG CTT GGT CCC 3' (33-mer) (SEQ ID NO: 88)

3) Forward primer : chi22H8VL-397F

Sequence : 5' CTG GAG CTG AAA cga act gtg gct gca cca tct 3' (33-mer) (SEQ ID NO: 89)

4) reverse primer : chi49F2VL-726R(RI)

Sequence : 5' aaa GAA TTC cta gca ctc tcc cct gtt gaa 3' (30-mer) (SEQ ID NO: 90)

In a similar manner to preparation of expression vectors of chimerized 9H5-4 antibody, expression vectors for such a heavy chain and a light chain of chimerized 22H8-84 antibody were prepared.

3-2) Preparation of cDNA that codes for heavy chain of chimerized PTPRS antibody

In order to prepare a cDNA that codes for a heavy chain of a chimerized PTPRS antibody, the two PCR fragments were altered by a procedure based on an overlap extension PCR process in a PCR fragment in which the heavy chain variable region of the mouse 49F2-30 antibody obtained in 2-2 and the human IgG heavy chain constant region obtained in 3-1 had been fused, and the PCR product was amplified by a length of 1434 bases by a method that allows partial formation of a double filament molecule as a result of a hybrid operation. At that time, the primers (SEQ ID NOS: 17 to 24) were those as shown in Table 1. The obtained PCR product was purified by a 1.5% low melting point agalose process.

[0121]

Table 1

Primer name		Sequence
Primer for expressing heavy chain in chimeric 49F2-30 antibody		
1)	chi49F2VH-IF(Hind3)	5' acc AAG CTT gcc gcc acc ATG AAC TTC GGG CTC AGG TTG 3' (39-mer)
2)	chi49F2VH-447R	5' ctt ggt gct agc TGA GGA GAC GGT GAC CGT GGT 3' (33- mer)
3)	chi49F2VH-424F	5' ACC GTC TCC TCA gct agc acc aag ggc cca tcg 3' (33-mer)
4)	chi49F2VH-1434R(RI)	5' ttt GAA TTC tca ttt acc cgg aga cag gga 3' (30-mer)
Primer for expressing light chain in chimeric 49F2-30 antibody		
5)	chi49F2VL-IF(Hind)	5' acc AAG CTT gcc gcc acc ATG GAG TCA CAG ATT CAG GTC 3' (33-mer)
6)	chi49F2VL-408R	5' agc cac agt tcg TTT TAT TTC CAG CTT GGT CCC 3' (33-mer)
7)	chi49F2VL-385F	5' CTG GAA ATA AAA cga act gtg gct gca cca tct 3' (33-mer)
8)	chi49F2VL-726R(RI)	5' aaa GAA TTC cta gca ctc tcc cct gtt gaa 3' (30-mer)

[0122]

There is a region in which cDNA overlaps the mouse 49F2-30 antibody heavy chain variable region obtained in 2-2 and the human IgG1 heavy chain constant region obtained in 3-1. Therefore, using this region, "a PCR product that codes for the 49F2-30 heavy chain variable region" was obtained by an overlap extension PCR process was obtained. The PCR product that codes for 49F2-30 heavy chain variable region was digested with Hind III and an EcoR I restriction enzyme and purified by a 1.5% agalose gel process. This was dissolved by ddH_2O to give a solution of a cDNA fragment that codes for the heavy chain variable region.

[0123]

The V_H coding region of the 49F2-30 of the obtained cDNA was amplified by PCR from a pCR4Blunt-TOPO plasmid clone including the V_H coding region of the 49F2-30, by using primers chi49F2VH-IF (Hind3) and chi49F2VH-1434R (RI) to which preferable restriction sites for cloning into a pEE6.4 vector (Lonza Biologics, Slough, UK) (Hind III and EcoRI) and an ideal Kozak sequence (GCCGCCACC) had been introduced using Hind III and EcoRI as cloning sites. The chi49F2VH-pEE6.4 vector includes a heavy chain constant region of human IgG1. The V_H PCR fragment was inserted into the pEE6.4 vector by in-frame using Hind III and EcoRI. The construct was investigated by a cDNA base sequence analysis.

[0124]

3-3) Preparation of cDNA that codes for light chain of chimerized PTPRS antibody

In order to prepare a cDNA that codes for a light chain of a chimerized PTPRS antibody, the PCR product was amplified by a length of 726 bases by a technique based on overlap extension PCR from the PCR fragment in which the mouse 49F2-30 antibody light chain variable region obtained in 2-3 and the human Ig κ light chain constant region obtained in 3-2 had been fused.

[0125]

The PCR product that codes for the 49F2-30 light chain variable region was digested by Hind III and an EcoRI restriction enzyme, and purified by a 1.5% agalose gel process. This was dissolved in ddH_2O to give a solution of a cDNA fragment that codes for the light chain variable region.

The obtained V_L-coding cDNA of 49F2-30 was amplified by PCR from a pCR4Blunt-TOPO plasmid clone including the V_L region of the 49F2-30 using primers chi49F2VL-IF (Hind) and chi 49F2VL-726R (RI) to which preferable restriction sites (Hind III and EcoRI) for cloning into a pEE14.4 vector (Lonza Biologics) and an ideal Kozak sequence had been introduced. The Chi49F2VL-pEE14.4 vector includes a kappa light chain constant region. The V_L PCR fragment was inserted into the pEE14.4 vector by in-frame by using Hind III and EcoRI. The construct was investigated by a cDNA base sequence analysis.

[0126]

3-4) Construction of chimerized PTPRS antibody double gene Lonza expression vector

A chimerized PTPRS antibody (double gene) Lonza expression vector in which the heavy chain expressing vector of the chimerized PTPRS antibody and the light chain expressing vector of the chimerized PTPRS antibody had been combined in one double gene vector was constructed by a standard cloning technology.

[0127]

4. Transient expression in HEK-293F cell

The following transient expression vector DNAs (80 μg) were used.

1) chi9H5-4VH/VL DG Lonza vector DNA
2) chi13G5-57VH/VL DG Lonza vector DNA
3) chi22H8-84VH/VL DG Lonza vector DNA
4) chi49F2-30VH/VL DG Lonza vector DNA

On the previous day of transfection, a 293F cell was adjusted to 80 mL at 8×10^5 cells/mL in a 250 mL Erlenmeyer flask (Corning#431144), and cultured by shaking under conditions of 37°C and a CO_2 concentration of 8% for 7 days.

After the culturing for 7 days, a culture liquid of a 293F cell that had undergone transfection was collected in a 50 mL tube and centrifuged under conditions of 2,070 g and 4°C for 5 minutes. The supernatant was filtered by a syringe filter (Catalog No.431220; CORNING) having a pore size of 0.45 μm, and the culture supernatants were gathered together.

[0128]

5. Purification of anti-PTPRS chimerized antibody

The chimerized 9H5-4, 13G5-57, 22H8-84 and 49F2-30 antibody were purified by protein A affinity chromatography. The crude antibody liquid obtained in 4. was each purified by a protein A affinity column (rProtein A Sepharose Fast Flow (Catalog No.17-1279-01; Lot. 311272; GE Healthcare). The column conditions are as follows. Affinity purification was conducted by using a binding buffer (20 mM Sodium phosphate, 0.15 M NaCl, pH 7.4) and an elution buffer (0.1 M Glycine-HCl, pH 2.7). The pH of the eluted fraction was adjusted to around 7.2 by adding a neutralizing buffer (1 M Tris-HCl pH 9.5). In order to substitute the buffer of the purified antibody with PBS, the buffer was replaced by using Slide-A-Lyzer MINI Dialysis unit 10kMWCO.

[0129]

The concentration of the purified antibody was calculated by measuring the absorbance at 280 nm and defining 1 mg/l as 1.38 OD.

The purified anti-PTPRS chimerized antibody (ch9H5-4Ab, ch13G5-57Ab, ch22H8-84Ab and ch49F2-30Ab) was analyzed by SDS-PAGE and a Flowcytometry process.

[0130]

The nucleic acid sequences and amino acid sequences of the heavy chain and light chain of the prepared chimera 9H5-4 antibody are represented respectively by the following sequence numbers.

Heavy chain	Light chain
SEQ ID NO: 91 (nucleic acid sequence)	SEQ ID NO: 93 (nucleic acid sequence)
SEQ ID NO: 92 (amino acid sequence)	SEQ ID NO: 94 (amino acid sequence)

The nucleic acid sequence of the heavy chain of the anti-PTPRS chimera 9H5-4 antibody (1419 bp) is shown below (SEQ ID NO: 91). The capital letters show the chimera

9H5-4 VH variable region, and the small letters show the human IgG1 heavy chain constant region.

ATGGAGTTGGGACTGAGCTGGGTATTTCTTGTGGCTCTTTTGAATGGTGTCCAGTGTC
AGGTGCAGCTTGTAGAGACCGGGGGAGGCTTGGTGAGGCCTGGAAATTCTCTGAAA
CTCTCCTGTGTTACCTCGGGATTCACTTTCAGTAACTACCGGATGCACTGGCTTCGCC
AGCCTCCAGGGAAGAGGCTGGAGTGGATTGCTGTAATTACAGTCAAATCTGATAATTA
TGGAGCAAATTATGCAGAGTCTGTGAAAGGCAGATTCACTATTTCAAGAGATGATTCA
AAAAGCAGTGTCTACCTGCAGATGAACAGATTAAGAGAGGAAGACACTGCCACTTAT
TATTGTAGTAGATCGGTCTACTATGGTTACGTCCTAGCCTTTGACTACTGGGGCCAAGG
CACCACTCTCACAGTCTCCTCAgctagcaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctc
tgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagc
ggcgtgcacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcaccc
agacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacat
gcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggac
ccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcata
atgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat
ggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccca
gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcctt
cttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatga

The amino acid sequence of the heavy chain of the anti-PTPRS chimera 9H5-4 antibody (472 a.a.) is shown below (SEQ ID NO: 92). The capital letters show the chimera 9H5-4 VH variable region, and the small letters show the human IgG1 heavy chain constant region.

MELGLSWVFLVALLNGVQCQVQLVETGGGLVRPGNSLKLSCVTSGFTFSNYRMHWLRQ
PPGKRLEWIAVITVKSDNYGANYAESVKGRFTISRDDSKSSVYLQMNRLREEDTATYYCS
RSVYYGYVLAFDYWGQGTTLTVSSastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtf
pavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevt
cvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqpre
pqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealh
nhytqkslslspgk

The nucleic acid sequence of the light chain of the anti-PTPRS chimera 9H5-4 antibody (705 bp) is shown below (SEQ ID NO: 93). The capital letters show the chimera 9H5-4 VL variable region, and the small letters show the human Ig κ light chain constant region.

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATG
TGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTC
ACCATCAGTTGCAGGGCAAGTCAGGACATTAGCAATTATTTAAACTGGTATCAGCAGA
AACCAGATGGAACTGTTAAACTCCTGATCTACTACACATCAAGATTACACTCAGGAGT
CCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAAC
CTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTGGA
CGTTCGGTGGAGGCACCAAGCTGGAAATCAAAcgaactgtggctgcaccatctgtcttcatcttcccgccatct
gatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataac
gccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctga
gcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacagg
ggagagtgctag

The amino acid sequence of the light chain of the anti-PTPRS chimera 9H5-4 antibody (234 a.a.) is shown below (SEQ ID NO: 94). The capital letters show the chimera 9H5-4 VL variable region, and the small letters show the human Ig κ light chain constant region.

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQK
PDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPWTFGG
GTKLEIKrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadye
khkvyacevthqglsspvtksfnrgec

<13G5-57>

The nucleic acid sequences and amino acid sequences of the heavy chain and light chain of the prepared chimera 13G5-57 antibody are represented respectively by the following sequence numbers.

Heavy chain	Light chain
SEQ ID NO: 95 (nucleic acid sequence)	SEQ ID NO: 97 (nucleic acid sequence)
SEQ ID NO: 96 (amino acid sequence)	SEQ ID NO: 98 (amino acid sequence)

The nucleic acid sequence of the heavy chain of the anti-PTPRS chimera 13G5-

57 antibody (1413 bp) is shown below (SEQ ID NO: 95). The capital letters show the chimera 13G5-57 VH variable region, and the small letters show the human IgG1 heavy chain constant region.

ATGAACTTGGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTG
AAGTGAAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAACCTCTGGATTCACTTTCAGTGACTATTACATGTATTGGGTTCGCCA
GACTCCAGAGAAGAGGCTGGAGTGGGTCGCATACATTAGTAATGGTGGTGGTAGCAC
CTATTATCCAGACACTGTAAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAAC
ACCCTGTACCTGCAAATGAGCCGTCTGAAGTCTGAGGACACAGCCATGTATTACTGTG
CAAGACATGTTTACTACGGGAGGAACTATGCTATGGACTACTGGGGTCAAGGAACCT
CAGTCACCGTCTCCTCAgctagcaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctctgggggc
acagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgca
caccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctaca
tctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgt
gcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggaccccctgaggt
cacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaga
caaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacag
gtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcg
ccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctaca
gcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgc
agaagagcctctccctgtctccgggtaaatga

The amino acid sequence of the heavy chain of the anti-PTPRS chimera 13G5-57 antibody (470 a.a.) is shown below (SEQ ID NO: 96). The capital letters show the chimera 13G5-57 VH variable region, and the small letters show the human IgG1 heavy chain constant region.

MNLGLSLIFLVLVLKGVQCEVKLVESGGGLVQPGGSLKLSCATSGFTFSDYYMYWVRQT
PEKRLEWVAYISNGGGSTYYPDTVKGRFTISRDNAKNTLYLQMSRLKSEDTAMYYCAR
HVYYGRNYAMDYWGQGTSVTVSSastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtf
pavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevt
cvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqpre
pqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealh
nhytqkslslspgk

The nucleic acid sequence of the light chain of the anti-PTPRS chimera 13G5-57 antibody (705 bp) is shown below (SEQ ID NO: 97). The capital letters show the chimera 13G5-57VL variable region, and the small letters show the human Ig κ light chain constant region.

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTACCAGATG
TGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTC
ACCATCAGTTGCAGGGCAAGTCAGGACATTAGCAATTATTTAAACTGGTATCAGCAGA
AACCAGATGGAACTGTTAAACTCCTGATCTACTACACATCAAGATTACACTCAGGAGT
CCCATCAAGGTTCAGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAAC
CTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTACA
CGTTCGGAGGGGGGACCAAGCTGGAAATAAAAcgaactgtggctgcaccatctgtcttcatcttcccgccatc
tgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataa
cgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgacgctg
agcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaagagcttcaacag
gggagagtgctag

The amino acid sequence of the light chain of the anti-PTPRS chimera 13G5-57 antibody (234 a.a.) is shown below (SEQ ID NO: 98). The capital letters show the chimera 13G5-57 VL variable region, and the small letters show the human Ig κ light chain constant region.

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQQK
PDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGG
GTKLEIKrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadye
khkvyacevthqglsspvtksfnrgec

The nucleic acid sequences and amino acid sequences of the heavy chain and light chain of the prepared chimera 22H8-84 antibody are represented respectively by the following sequence numbers.

Heavy chain	Light chain
SEQ ID NO: 99 (nucleic acid sequence)	SEQ ID NO: 101 (nucleic acid sequence)
SEQ ID NO: 100 (amino acid sequence)	SEQ ID NO: 102 (amino acid sequence)

The nucleic acid sequence of the heavy chain of the anti-PTPRS chimera 22H8-84 antibody (1413 bp) is shown below (SEQ ID NO: 99). The capital letters show the chimera 22H8-84 VH variable region, and the small letters show the human IgG1 heavy chain constant region.

ATGGAATGTAACTGGATACTTCCTTTTATTCTGTCAGTAACTTCAGGTGTCTACTCACA
GGTTCAGCTCCAGCAGTCTGGGGCTGAGCTGGCAAGACCTGGGGCTTCAGTGAAGT
TGTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACTGGATGCAGTGGGTAAAACA
GAGGCCTGGACAGGGTCTGGAATGGATTGGGGCTATTTATCCTGGAGATGGTGATACT
AGGTACACTCAGAAGTTCAAGGGCAAGGCCACATTGACTGCAGATAAATCCTCCAGC
ACAGCCTACATGCAACTCAGCAGCTTGGCATCTGAGGACTCTGCGGTCTATTACTGTG
CAAGAAGGATTTACTACGGCTATTACTATGCTATGGACTACTGGGGTCAAGGAACCTC
AGTCACCGTCTCCTCAgctagcaccaagggcccatcggtcttcccctggcaccctcctccaagagcacctctgggggcac
agcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcaca
ccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatc
tgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgtgc
ccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccctgaggtca
catgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagaca
aagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagta
caagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtg
tacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatcccagcgacatcgcc
gtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagc
aagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcag
aagagcctctccctgtctccgggtaaatga

The amino acid sequence of the heavy chain of the anti-PTPRS chimera 22H8-84 antibody (470 a.a.) is shown below (SEQ ID NO: 100). The capital letters show the chimera 22H8-84 VH variable region, and the small letters show the human IgG1 heavy chain constant region.

MECNWILPFILSVTSGVYSQVQLQQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQ
RPGQGLEWIGAIYPGDGDTRYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYCAR
RIYYGYYYAMDYWGQGTSVTVSSastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfp
avlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtc
vvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprep
qvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhn

hytqkslslspgk

The nucleic acid sequence of the light chain of the anti-PTPRS chimera 22H8-84 antibody (717 bp) is shown below (SEQ ID NO: 101). The capital letters show the chimera 22H8-84VL variable region, and the small letters show the human Ig κ light chain constant region.

ATGGAGACAGACACAATCCTGCTATGGGTGCTGCTGCTCTGGGTTCCAGGCTCCACT
GGTGACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGG
CCACCATCTCCTGCAAGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTATATGAA
CTGGTACCAACAGAAACCAGGACAGCCACCCAAACTCCTCATCTATGCTGCATCCAA
TCTAGAATCTGGGATCCCAGCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTTCAC
CCTCAACATCCATCCTGTGGAGGAGGAGGATGCTGCAACCTATTACTGTCAGCAAAG
TAATGAGGATCCTCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAAcgaactgtggctg
caccatctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagagg
ccaaagtacagtggaaggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcaccta
cagcctcagcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagct
cgcccgtcacaaagagcttcaacaggggagagtgctag

The amino acid sequence of the light chain of the anti-PTPRS chimera 22H8-84 antibody (238 a.a.) is shown below (SEQ ID NO: 102). The capital letters show the chimera 22H8-84 VL variable region, and the small letters show the human Ig κ light chain constant region.

METDTILLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISCKASQSVDYDGDSYMNW
YQQKPGQPPKLLIYAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYCQQSNEDPL
TFGAGTKLELKrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltl
skadyekhkvyacevthqglsspvtksfnrgec

The nucleic acid sequences and amino acid sequences of the heavy chain and light chain of the prepared chimera 49F2-30 antibody are represented respectively by the following sequence numbers.

Heavy chain	Light chain
SEQ ID NO:35 (nucleic acid sequence)	SEQ ID NO:37 (nucleic acid sequence)
SEQ ID NO:36 (amino acid sequence)	SEQ ID NO:38 (amino acid sequence)

The nucleic acid sequence of the heavy chain of the anti-PTPRS chimera 49F2-30 antibody (1413 bp) is shown below (SEQ ID NO:35). The capital letters show the chimera 49F2-30 VH variable region, and the small letters show the human IgG1 heavy chain constant region.

[0131]

ATGAACTTCGGGCTCAGGTTGATTTTCCTTGCCCTCATTTTAAAAGGTGTCCAGTGTG
AGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCATTTTCAGTAGCTATGGCATGTCTTGGGTTCGCC
AGACTCCAGACAAGAGGCTGGAGTGGGTCGCAACCATTAGTAGTGGTGGTAGTGAC
ACCTATTATCCAGACAGTGTGAAGGGGCGATTCACCATCTCCAGAGACAATGCCAA
CAACACCCTGTACCTGCAAATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTATT
ACTGTGCAAGACAGGTCTACTATGGTCTTTACTGGTATTTCGATGTCTGGGGCGCAG
GGACCACGGTCACCGTCTCCTCAgctagcaccaagggcccatcggtcttcccctggcaccctcctccaagagcacc
tctgggggcacagcggccctgggctgcctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccag
cggcgtgcacaccttcccggctgtcctacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacc
cagacctacatctgcaacgtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacat
gcccaccgtgcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggac
ccctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcata
atgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaat
ggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagccccgag
aaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctggtcaaaggcttctatccca
gcgacatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcctt
cttcctctacagcaagctcaccgtggacaagagcaggtggcagaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaac
cactacacgcagaagagcctctccctgtctccgggtaaatga

[0132]

The amino acid sequence of the heavy chain of the anti-PTPRS chimera 49F2-30 antibody (470 a.a.) is shown below (SEQ ID NO:36). The capital letters show the chimera 49F2-30 VH variable region, and the small letters show the human IgG1 heavy chain constant region.

MNFGLRLIFLALILKGVQCEVQLVESGGDLVKPGGSLKLSCAASGFIFSSYGMSWVRQT
PDKRLEWVATISSGGSDTYYPDSVKGRFTISRDNANNTLYLQMSSLKSEDTAMYYCAR
QVYYGLYWYFDVWGAGTTVTVSSastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtf
pavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevt
cvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqpre
pqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealh
nhytqkslslspgk

[0133]

The nucleic acid sequence of the light chain of the anti-PTPRS chimera 49F2-30 antibody (705 bp) is shown below (SEQ ID NO:37). The capital letters show the chimera 49F2-30 VL variable region, and the small letters show the human Ig κ light chain constant region.

ATGGAGTCACAGATTCAGGTCTTTGTATTCGTGTTTCTCTGGTTGTCTGGTGTTGACG
GAGACATTGTGATGACCCAGTCTCACAAATTCATGTCCACATCAGTAGGAGACAGG
GTCAGCATCATTTGTAAGGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAA
CAGAAACCAGGACAATCTCCTAAATTACTGATTTACTCGGCATCCTACCGGTACACT
GGAGTCCCTGATCGCTTCACTGGCAGTGGATCTGGGACGGATTTCACTTTCACCATC
AGCAGTGTGCAGGCTGAAGACCTGGCAATTTATTACTGTCAGCAACATTATAGTACT
CCGTACACGTTCGGAGGGGGGACCAAGCTGGAAATAAAAcgaactgtggctgcaccatctgtcttcat
cttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtgg
aaggtggataacgccctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagca
ccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaag
agcttcaacaggggagagtgctag

[0134]

The amino acid sequence of the light chain of the anti-PTPRS chimera 49F2-30 antibody (234 a.a.) is shown below (SEQ ID NO:38). The capital letters show the chimera 49F2-30 VL variable region, and the small letters show the human Ig κ light chain constant region.

MESQIQVFVFVFLWLSGVDGDIVMTQSHKFMSTSVGDRVSIICKASQDVNTAVAWYQQ
KPGQSPKLLIYSASYRYTGVPDRFTGSGSGTDFTFTISSVQAEDLAIYYCQQHYSTPYTFG
GGTKLEIKrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskady
ekhkvyacevthqglsspvtksfnrgec

[0135]

Example 6

Antibody-dependent cellular cytotoxicity of prepared anti-human PTPRS chimeric antibody (ch49F2-30,ch9H5-4,ch13G5-57 and ch22H8-84)

Antibody-dependent cellular cytotoxicity (ADCC activity) was measured. The activity was obtained by using the cellular cytotoxicity calculated from the measured value of lactase dehydrogenase (LDH) released from a cell as an index. Human peripheral blood mononuclear cells to be an effector cell was purified by specific gravity centrifugation using

HISTOPAQUE-1077. As a cell to be a target, a forcibly-transformed cell of an hPTPRS gene using CHO (Chinese hamster ovary cell strain) was used (2×10^4/well). The effector and target cells were mixed so that the ratio thereof became 10:1, 20:1, 40:1 and 80:1, 10 μg/ ml of prepared anti-human PTPRS chimeric antibody (ch49F2-30, ch9H5-4, ch13G5-57 and ch22H8-84) or a control antibody Synagis was added, and the mixture was cultured for 4 hours at 37°C to evaluate the cellular cytotoxicity effect of the antibody. As a result, the ch49F2-30, ch9H5-4, ch13G5-57 and ch22H8-84 of the anti-hPTPRS chimeric antibody lysed the hPTPRS/ CHO cell of the target in an effector cell number-dependent manner (FIG. 17A and FIG 17B). This result showed that the prepared anti-PTPRS chimeric antibody selectively showed the cytotoxicity to the cells expressing PTPRS.

[0136]

The effect of the anti-PTPRS antibody on pDC was studied. PBMCs were isolated from human peripheral blood, mixed with 10 μg/ml of an anti-human PTPRS chimeric antibody and cultured for 24 hours. Thereafter stimulation was conducted for 24 hours with CpG2216 that is a ligand of a Toll-like receptor 9 expressed in pDC to induce the production of IFNα. 24h after CpG stimulation, the production amount of IFNα was tested. In result, the production of IFNα was completely inhibited by the treatment of prepared anti-human PTPRS chimeric antibody (ch49F2-30, ch9H5-4, ch13G5-57 and ch22H8-84) (FIG. 18A). Furthermore, when the cell was collected 6 hours after the ch49F2-30, ch9H5-4, ch13G5-57 and ch22H8-84 treatment and the pDC was confirmed by double-staining with an anti-BDCA2 antibody and an anti-BDCA4 antibody, it was found that the pDC population was decreased more than the Synagis treatment of the control antibody (FIG. 18B and FIG 18C). These results showed that the treatment of anti-PTPRS chimeric antibody consequently abolished the production of IFNα by CpG2216 stimulation.

Industrial Applicability

[0137]

The present invention provides an antibody according to the claims that specifically recognizes human PTPRS, and a method for the production of the anti-human PTPRS antibody.

Accession Numbers

[0138]

FERM BP-11356

FERM BP-11357

FERM BP-11358

FERM BP-11359

FERM BP-11360

FERM BP-11361

FERM BP-11362

FERM BP-11363

Sequence listing free text

[0139]

SEQ ID NO: 3: forward primer

SEQ ID NO: 4: reverse primer

SEQ ID NO: 5: forward primer

SEQ ID NO: 6: reverse primer

SEQ ID NO: 7: forward primer

SEQ ID NO: 8: reverse primer

SEQ ID NO: 9: forward primer

SEQ ID NO: 10: reverse primer

SEQ ID NO: 11: antisense primer

SEQ ID NO: 12: anchor primer

SEQ ID NO: 12: n is deoxyinosine.

SEQ ID NO: 13: antisense primer

SEQ ID NO: 14: AUAP primer

SEQ ID NO: 15: antisense primer

SEQ ID NO: 16: antisense primer

SEQ ID NO: 17: primer

SEQ ID NO: 18: primer

SEQ ID NO: 19: primer

SEQ ID NO: 20: primer

SEQ ID NO: 21: primer

SEQ ID NO: 22: primer

SEQ ID NO: 23: primer

SEQ ID NO: 24: primer

SEQ ID NO: 35: anti-PTPRS chimera 49F2-30 antibody heavy chain nucleic acid sequence

SEQ ID NO: 36: anti-PTPRS chimera 49F2-30 antibody heavy chain amino acid sequence

SEQ ID NO: 37: anti-PTPRS chimera 49F2-30 antibody light chain nucleic acid sequence

SEQ ID NO: 38: anti-PTPRS chimera 49F2-30 antibody light chain amino acid sequence

SEQ ID NO: 39: primer

SEQ ID NO: 40: primer

SEQ ID NO: 41: primer

SEQ ID NO: 42: primer

SEQ ID NO: 73: forward primer

SEQ ID NO: 74: reverse primer

SEQ ID NO: 75: forward primer

SEQ ID NO: 76: reverse primer

SEQ ID NO: 77: forward primer

SEQ ID NO: 78: reverse primer

SEQ ID NO: 79: forward primer

SEQ ID NO: 80: reverse primer

SEQ ID NO: 81: forward primer

SEQ ID NO: 82: reverse primer

SEQ ID NO: 83: forward primer

SEQ ID NO: 84: reverse primer

SEQ ID NO: 85: forward primer

SEQ ID NO: 86: reverse primer

SEQ ID NO: 87: forward primer

SEQ ID NO: 88: reverse primer

SEQ ID NO: 89: forward primer

SEQ ID NO: 90: reverse primer

SEQ ID NO: 91: anti-PTPRS chimera 9H5-4 antibody heavy chain nucleic acid sequence

SEQ ID NO: 92: anti-PTPRS chimera 9H5-4 antibody heavy chain amino acid sequence

SEQ ID NO: 93: anti-PTPRS chimera 9H5-4 antibody light chain nucleic acid sequence

SEQ ID NO: 94: anti-PTPRS chimera 9H5-4 antibody light chain amino acid sequence

SEQ ID NO: 95: anti-PTPRS chimera 13G5-57 antibody heavy chain nucleic acid sequence

SEQ ID NO: 96: anti-PTPRS chimera 13G5-57 antibody heavy chain amino acid sequence

SEQ ID NO: 97: anti-PTPRS chimera 13G5-57 antibody light chain nucleic acid sequence

SEQ ID NO: 98: anti-PTPRS chimera 13G5-57 antibody light chain amino acid sequence

SEQ ID NO: 99: anti-PTPRS chimera 22H8-84 antibody heavy chain nucleic acid sequence

SEQ ID NO: 100: anti-PTPRS chimera 22H8-84 antibody heavy chain amino acid sequence

SEQ ID NO: 101: anti-PTPRS chimera 22H8-84 antibody light chain nucleic acid sequence

SEQ ID NO: 102: anti-PTPRS chimera 22H8-84 antibody light chain amino acid sequence