DESCRIPTION

[Title of Invention]

TITLE OF INVENTION: MEDICAMENT COMPRISING ANTI-PHOSPHOLIPASE D4 ANTIBODY

[Technical Field]

[0001]

The present invention relates to a use of an antibody binding to phospholipase D4. Hereinafter, "phospholipase D" may be abbreviated as PLD and "phospholipase D4" and the like may be abbreviated as PLD4 and the like.

[Background Art]

[0002]

PLD is an enzyme which catalyzes a reaction to produce phosphatidic acid and choline by hydrolyzing phosphatidyl choline and causes various intracellular signaling. It has been believed that the produced phosphatidic acid functions as a lipid signal molecule.

PLD1 and PLD2 have been known as two types of mammal PLD, which have been previously known, and contain a phosphatidyl inositide-binding Phox homology domain (PX domain) and a phosphatidyl inositide-binding pleckstrin homology domain (PH domain) in the N-terminal region thereof. Both domains are involved in membrane localization of PLD.

PLD1 and PLD2 further contain two His-x-Lys-x-x-x-x-Asp sequences (HKD motifs). The HKD motifs are essential domains for PLD activity.

Phosphatidic acid produced by PLD1 and PLD2 has been

suggested to be involved in cytoskeleton reconstruction, exocytosis, phagocytosis, canceration, cell adhesion, chemotaxis and the like, and mainly acts on nervous systems, immune systems and the like.

[0003]

Human Hu-K4 and mouse SAM9, which are now officially named PLD3, lack the PX and PH domains and do not show PLD activity despite having two HKD motifs. Although there are further three PLD family members, PLD4, PLD5 and PLD6, little has been known about these non-classical PLDs.

[0004]

As a result of searching a gene expression pattern in mouse cerebellar development in Cerebellar Development Transcriptome Database (CDT-DB), a transcription product, PLD4, controlled during the development was identified (see Non Patent Literature 1). Basic characteristics of PLD4 have not been reported. Enzymatic activity of PLD4 with or without glycosylation needs to be determined.

[0005]

PLD4 has a 506 amino acid sequence shown in SEQ ID NO: 1 and is encoded by a cDNA base sequence of SEQ ID NO: 44 (Non Patent Literatures 1 and 2). The PLD4 protein has two tentative PDE regions (phosphodiesterase motifs) constituted of two HKD motifs (His-x-Lys-x-x-x-x-Asp amino acid sequence, x represents other amino acids) conserved in the C-terminal region, and a putative phosphorylation site (Thr 472). The structure of the PLD4 protein is estimated as a type II

transmembrane protein. In addition, PLD4 does not have PX and PH domains, which PLD1 and PLD2 in a classical PLD family have them, in the N-terminal region.

On the other hand, PLD4 belongs to the PLD family because of having two HKD motifs, but lacks the PX domain and the PH domain and has a putative transmembrane domain instead (Non Patent Literature 3).

[0006]

The expression of PLD4 mRNA has been found at low to medium levels in small cell clusters preferentially localized around white matter regions including corpus callosum and cerebellar white matter of 1 week old mice. These PLD4 mRNA-expressing cells have been identified as Iba1-positive microglia (Non Patent Literature 3). However, the PLD4-positive cells in mouse cerebellum is dispersed 10-day-old mice. It suggested that PLD4 expression is temporarily restricted during early postnatal development in mouse cerebellum.

Myelin formation in mouse begins in the corpora callosa and the cerebellar white matter at one week after birth. At this time, PLD4 is highly expressed in amoeboid (an activated state) microglia existing in the white matter, and thus it has been also believed that there is a possibility that PLD4-expressing cells in the white matter in this time are involved in myelin formation. In particular, it has also been revealed that PLD4 accumulates in food vacuoles, and it has been suggested that there is a possibility that PLD4 is involved in phagocytosis. In amoeboid microglia which is in an activated

state, various cytokines and growth factors are secreted and simultaneously phagocytosis is activated. It has been believed that in the brain white matter of mouse in a developmental period, surplus oligodendrocytes (central nervous system glial cells, which form myelin by wrapping around axons) undergo apoptosis. There is a possibility that the oligodendrocytes are decomposed and removed in amoeboid microglia to secrete signal molecules and thereby adjust a myelin-forming environment in the white matter. It has been suggested that PLD4 is involved in these processes including the myelin formation.

[0007]

Expression of mouse PLD4 mRNA is also observed in non-neuronal tissues and mainly distributed in the spleen. Strong expression of PLD4 protein is detected around a marginal zone of the splenic red pulp, and splenic PLD4 protein collected from subcellular membrane fractions is highly N-glycosylated. When PLD4 was expressed in a heterologous cell system, PLD4 was localized in the endoplasmic reticulum and Golgi apparatus. The heterologously expressed PLD4 did not show PLD enzyme activity (Non Patent Literature 3).

From the expression pattern of PLD4, which is spatiotemporally restricted, it has been suggested that PLD4 may play a role in common functions among the microglia and splenic marginal zone cells during early postnatal brain development.

[0008]

On the other hand, the present inventors have found that PLD4 is specifically highly expressed in pDC (plasmacytoid Dendritic Cell) in a resting period (resting pDC) (Patent Literature 1). The present inventors further have reported that a PLD4-specific antibody can be utilized for suppression of pDC activity.

[0009]

Further, PLD4 has been reported as one of novel susceptibility genes of Systemic Sclerosis in Japanese (Non Patent Literature 4). As a result of the same analysis in Europe, however, significant correlation with PLD4 has not been found and strong results showing a relationship between PLD4 and autoimmune diseases such as Systemic Sclerosis have not been obtained.

[0010]

An immune mechanism is roughly classified into two groups. One is "natural immunity (innate immunity)" which detects foreign substances such as pathogens and carries out an initial attack, and the other is "acquired immunity" through information exchange which is presentation of antigen peptides and the like derived from foreign substances. Neutrophils, macrophage, dendritic cells (DC), NK (Natural Killer) cells and the like are mainly involved in the "natural immunity", and T cells and B cells to which information of antigen peptides and the like presented by the above dendritic cells and the like is transmitted are involved in the "acquired immunity". T cells activated by transmission of information of antigen peptides

are capable of specifically recognizing and attacking pathogens in a direct manner as the cell-mediated immunity, and B cells activated in the same manner as above are capable of specific recognition and attack against pathogens in an indirect manner by producing antibodies (hormonal immunity).

[0011]

In the "natural immunity", pathogen-associated molecular patterns (PAMPs) universally existing in pathogens (LPS, CpG DNA, lipoproteins, RNA etc.) are recognized through Toll-like receptors (TLR), and secretion of inflammatory cytokines is promoted via NF-kB, or secretion of interferon (IFN) is promoted via IRF (Interferon regulatory factor). TLR is roughly classified into two groups by subcellular localization sites: a group expressed on cell surfaces and a group expressed in endosomes and endoplasmic reticula (ER). In pDC, IRF7 is activated via TLR7 and TLR9 localized in endosomes and endoplasmic reticula to induce IFN-α production. The reason why these TLRs are not expressed on cell surfaces but in cells has been suggested to decrease a risk of onset of autoimmune diseases. TLR7 and TLR9 recognize single-stranded RNA and DNA respectively as a ligand. Not only foreign pathogenic bacteria but also hosts hold these nucleic acids, and thus it has been suggested that receptors, which recognize nucleic acids and activate immune cells, always induce the autoimmune diseases.

[0012]

On the other hand, B cells (B lymphocytes) showing an important role in the "acquired immunity" are lymphocytes which

express immunoglobulin Ig receptors on the surface thereof. B cells are produced from hematopoietic stem cells in the bone marrow, and are differentiated into pre-B cells and immature B cells, and then mature into naive B cells (mature, unprimed B cells). The naive B cells are activated by not only the stimulation through the above T cells but also the direct antigen stimulation, and further become antibody-producing cells by differentiation and proliferation to produce and secrete antibodies such as IgM, IgD, IgA, IgE, IgG (including subclasses such as IgG1, IgG2, IgG2b, IgG3 and the like). It has been known that in addition to B cell receptors (BCR) recognizing specific foreign antigens, the above TLRs are expressed in B cells. It has been previously known, for example, that LPS which has been known to cause the proliferation and antibody production of B cells is a ligand of TLR4 and the above TLR7 and TLR9 are also expressed in B cells. Such B cells have been suggested to have a possibility to induce not only the above autoimmune diseases but also allergic diseases due to the overreaction of the antibody-producing ability thereof.

IgG, immunoglobulin G, is an antibody isotype consisting of four peptide chains—two identical heavy chains and two identical light chains. IgG is produced by B cells and plays a critical role for adaptive immunity. Naive B cells which do not produce IgG, differentiate into plasmablasts, and eventually into plasma cells. Plasmablasts and plasma cells can produce a large amount of antibodies. Conventionally, myeloid dendritic cells (DCs) have been shown to trigger B cell growth

and differentiation by stimulating with IL-12 and IL-6 and/or membrane molecules such as BAFF/APRIL (Non Patent Literatures 5, 6 and 7). In addition, plasmacytoid DCs (pDCs) induce maturation and differentiation of naive B cells into antibody-secreting plasmablasts and plasma cells producing IFN-α and IL-6 (Non Patent Literature 8). The variable region of IgG captures various pathogens such as viruses, bacteria, and fungi, resulting in protection of the body from such infections.

SLE is regarded as a classic immune complex-mediated autoimmune disease. Immune complexes (ICs) are formed in circulation or in situ as a result of produced auto-antibodies against nucleic acids and their associated proteins, such as dsDNA, ribonucleoprotein, and histone. Such ICs cause inflammation with disease-characteristic clinical symptoms such as nephritis, arthritis, skin rashes, and vasculitis. Blood from SLE patient is characterized by reduction of naive B cells and increased memory B cells, plasmablasts and plasma cells (Non Patent Literatures 9, 10 and 11). Therefore, suppression of differentiation into plasma cells and antibody production through manipulation of auto-reactive antibody-secreting plasmablasts would result in a promising strategy to cure autoimmune diseases.

In PBMCs, there are various subsets of B cells, such as naive B cells, memory B cells, and plasmablasts. Most of B cell subset in PBMCs is naive B cells. Naive B cells are the one who are not exposed by foreign antigen. Memory B cells are the one

who are formed by primary infection and are critical in quick antibody-mediated immune response by differentiation into plasmablasts. Plasmablasts are the one who secrete a large amount of antibody and marked by CD19+CD27+IgD-CD38+.

Once exposed by foreign antigen, naive B cells become activated B cells. The activated B cells are further differentiated in to memory B cell and/ or also plasmablasts that secrete antibodies. This change is called "maturation".

B cell maturation occurs in multiple phases. The initial, antigen-independent phase induces mature B cells that can bind to a unique antigen. This stage of maturation happens in the bone marrow and the spleen in living body. The antigen-dependent phase of B cell maturation happens following B cell activation by antigen binding and co-stimulation. These signals promote B cell maturation into either memory B cells or antibody-secreting plasmablasts. The antigen-dependent phase of B cell maturation involves activated B cell proliferation, antibody affinity maturation, and antibody class switching. Those maturations occur in the germinal centers of secondary lymphoid tissues.

It has been reported that, in vitro experimental condition, pDCs induce the maturation of activated B cells into Ig-secreting plasmablasts through release of IFN-α and IL-6. CpG2216 activates pDCs to induce IFN-α production and B cells to initiate maturation. IFN-α from pDCs further supports maturation of activated B cells into plasmablasts in the presence of IL-6.

[Citation List]

[Patent Literature]

[0013]

[PTL 1] PCT/JP2013/052781

[Non Patent Literature]

[0014]

[NPL 1] Tao et al., Nat. Methods 2(8), pp 591-598 (2005)

[NPL 2] Clark et al., Genome Res. 13(10), pp 2265-2270 (2003)

[NPL 3] Plos ONE www.plosone.org, November 2010, Volume 5, Issue 11, e13932

[NPL 4] ARTHRITIS & RHEUMATISM Vol. 65, No. 2, February 2013, pp 472-480

[NPL 5] Balazs et al., 2002, Immunity, 17, 341-352

[NPL 6] Litinskiy et al., 2002, Nat Immunol, 3, 822-829

[NPL 7] MacLennan and Vinuesa, 2002, Immunity, 17, 235-238

[NPL 8] Jego et al, 2003, Immunity, 19, 225-234

[NPL 9] Odendahl et al., 2000, JI, 165, 5970-5979

[NPL 10] Arce et al., 2001, JI, 167, 2361-2369

[NPL 11] Wei et al., 2007, JI, 178, 6624-6633

[Summary]

[Technical Problem]

[0015]

A problem to be solved by the present invention is to regulate activated B cells using an antibody binding to PLD4 and to improve symptoms of diseases caused thereby.

[Solution to Problem]

[0016]

Through research on PLD4, the present inventors verified that in addition to pDC cells in a resting period which have been previously reported, PLD4 expression was also induced in activated B cells. The present inventors therefore examined influence of PLD4 antibodies on activated B cells. A method for producing and purifying anti-PLD4 antibodies is carried out by a method in Patent Literature 1.

[0017]

That is, the present invention relates to a second use using anti-PLD4 antibodies described below.

(1) A pharmaceutical composition for suppressing activated B cells, wherein the pharmaceutical composition comprises a monoclonal antibody binding to a phospholipase D4 (PLD4) protein, or a fragment containing an antigen-binding region thereof as an active ingredient.

(2) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SYWMH (SEQ ID NO: 2) as CDR1, a sequence DIYPGSDSTNYNEKFKS (SEQ ID NO: 3) as CDR2 and GGWLDAMDY (SEQ ID NO: 4) as a sequence CDR3 in a variable region of a heavy chain.

(3) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RASQDISNYLN (SEQ ID NO: 5) as CDR1, a sequence YTSRLHS (SEQ ID NO: 6) as CDR2 and a sequence QQGNTLPW (SEQ ID NO: 7) as CDR3 in a variable

region of a light chain.

(4) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SYWMH as CDR1, the sequence DIYPGSDSTNYNEKFKS as CDR2 and the sequence GGWLDAMDY as CDR3 in the variable region of the heavy chain, and has the sequence RASQDISNYLN as CDR1, the sequence YTSRLH as CDR2 and the sequence QQGNTLPW as CDR3 in the variable region of the light chain.

(5) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence TYWMH (SEQ ID NO: 8) as CDR1, a sequence AIYPGNSETSYNQKFKG (SEQ ID NO: 9) as CDR2 and GYSDFDY (SEQ ID NO: 10) as a sequence CDR3 in the variable region of the heavy chain.

(6) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence HASQGIRSNIG (SEQ ID NO: 11) as CDR1, a sequence HGTNLED (SEQ ID NO: 12) as CDR2 and a sequence VQYVQFP (SEQ ID NO: 13) as CDR3 in the variable region of the light chain.

(7) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence TYWMH as CDR1, the sequence AIYPGNSETSYNQKFKG as CDR2 and the sequence GYSDFDY as CDR3 in the variable region of the heavy chain, and has the sequence HASQGIRSNIG as CDR1, the sequence HGTNLED as CDR2 and

the sequence VQYVQFP as CDR3 in the variable region of the light chain.

(8) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence DYNLH (SEQ ID NO: 14) as CDR1, a sequence YIYPYNGNTGYNQKFKR (SEQ ID NO: 15) as CDR2 and GGIYDDYYDYAIDY (SEQ ID NO: 16) as a sequence CDR3 in the variable region of the heavy chain.

(9) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RASENIYSHIA (SEQ ID NO: 17) as CDR1, a sequence GATNLAH (SEQ ID NO: 18) as CDR2 and a sequence QHFWGTP (SEQ ID NO: 19) as CDR3 in the variable region of the light chain.

(10) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence DYNLH as CDR1, the sequence YIYPYNGNTGYNQKFKR as CDR2 and the sequence GGIYDDYYDYAIDY as CDR3 in the variable region of the heavy chain, and has the sequence RASENIYSHIA as CDR1, the sequence GATNLAH as CDR2 and the sequence QHFWGTP as CDR3 in the variable region of the light chain.

(11) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SYYLY (SEQ ID NO: 20) as CDR1, a sequence LINPTNSDTIFNEKFKS (SEQ ID NO: 21) as CDR2 and EGGYGYGPFAY (SEQ ID NO: 22) as a sequence CDR3 in the

variable region of the heavy chain.

(12) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence TSSQTLVHSNGNTYLH (SEQ ID NO: 23) as CDR1, a sequence KVSNRFS (SEQ ID NO: 24) as CDR2 and a sequence HSTHVP (SEQ ID NO: 25) as CDR3 in the variable region of the light chain.

(13) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SYYLY as CDR1, the sequence LINPTNSDTIFNEKFKS as CDR2 and the sequence EGGYGYGPFAY as CDR3 in the variable region of the heavy chain, and has the sequence TSSQTLVHSNGNTYLH as CDR1, the sequence KVSNRFS as CDR2 and the sequence HSTHVP as CDR3 in the variable region of the light chain.

(14) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SYGMS (SEQ ID NO: 26) as CDR1, a sequence TISSGGSYIYYPESVKG (SEQ ID NO: 27) as CDR2 and LYGGRRGYGLDY (SEQ ID NO: 28) as a sequence CDR3 in the variable region of the heavy chain.

(15) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RSSKSLLHSDGITYLY (SEQ ID NO: 29) as CDR1, a sequence QMSNLAS (SEQ ID NO: 30) as CDR2 and a sequence AQNLEL (SEQ ID NO: 31) as CDR3 in the variable region of the light chain.

(16) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SYGMS as CDR1, the sequence TISSGGSYIYYPESVKG as CDR2 and the sequence LYGGRRGYGLDY as CDR3 in the variable region of the heavy chain, and has the sequence RSSKSLLHSDGITYLY as CDR1, the sequence QMSNLAS as CDR2 and the sequence AQNLEL as CDR3 in the variable region of the light chain.

(17) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SHYYWT (SEQ ID NO: 32) as CDR1, a sequence YISYDGSNNYNPSLKN (SEQ ID NO: 33) as CDR2 and EGPLYYGNPYWYFDV (SEQ ID NO: 34) as a sequence CDR3 in the variable region of the heavy chain.

(18) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RASQDIDNYLN (SEQ ID NO: 35) as CDR1, a sequence YTSRLHS (SEQ ID NO: 36) as CDR2 and a sequence QQFNTLP (SEQ ID NO: 37) as CDR3 in the variable region of the light chain.

(19) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SHYYWT as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2 and the sequence EGPLYYGNPYWYFDV as CDR3 in the variable region of the heavy chain, and has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2 and the sequence QQFNTLP as CDR3 in the variable

region of the light chain.

(20) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SHYYWS (SEQ ID NO: 38) as CDR1, a sequence YISYDGSNNYNPSLKN (SEQ ID NO: 39) as CDR2 and EGPLYYGNPYWYFDV (SEQ ID NO: 40) as a sequence CDR3 in the variable region of the heavy chain.

(21) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RASQDIDNYLN (SEQ ID NO: 41) as CDR1, a sequence YTSRLHS (SEQ ID NO: 42) as CDR2 and a sequence QQFNTLP (SEQ ID NO: 43) as CDR3 in the variable region of the light chain.

(22) The pharmaceutical composition according to (1) above, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SHYYWS as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2 and the sequence EGPLYYGNPYWYFDV as CDR3 in the variable region of the heavy chain, and has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2 and the sequence QQFNTLP as CDR3 in the variable region of the light chain.

(23) A pharmaceutical composition for suppressing activated B cells, wherein the pharmaceutical composition comprises a monoclonal antibody produced by any of hybridomas mp5B7, mp7B4, mp13D4 and mp13H11 of Deposit Nos. NITE BP-1211, NITE BP-1212, NITE BP-1213 and NITE BP-1214, or a fragment containing an antigen-binding region thereof as an active ingredient.

[0018]

(24) The pharmaceutical composition according to any one of (1) to (23) above, further for preventing or treating autoimmune diseases.

(25) The pharmaceutical composition according to any one of (1) to (23) above, further for preventing or treating allergic diseases.

[0019]

(26) A method for detecting activated B cells, the method including a step of bringing a monoclonal antibody binding to an extracellular domain of PLD4 or a fragment containing an antigen-binding region thereof into contact with cells to be tested and detecting the monoclonal antibody or the fragment containing the antigen-binding region thereof which binds to the cells.

(27) A reagent for detecting activated B cells, wherein the reagent comprises a monoclonal antibody binding to an extracellular domain of PLD4 or a fragment containing an antibody-binding region thereof.

(28) A method for suppressing activated B cells, the method including a step of bringing either of the following components into contact with activated B cells:

(a) a monoclonal antibody which binds to PLD4 and suppresses activated B cells, or a fragment containing an antigen-binding region thereof, and

(b) immunoglobulin into which a complementarity-determining region of the monoclonal antibody

in (a) is grafted, or a fragment containing an antigen-binding region thereof.

(29) A method for suppressing activated B cells in a living body, the method including a step of administering either of the following components to the living body:

(a) a monoclonal antibody which binds to PLD4 and suppresses an activity of activated B cells, or a fragment containing an antigen-binding region thereof, and

(b) immunoglobulin into which a complementarity-determining region of the monoclonal antibody in (a) is grafted, or a fragment containing an antigen-binding region thereof.

(30) The method according to (28) above or (29) above, wherein the activity of the activated B cells is an antibody-producing activity.

(31) An agent for suppressing activated B cells, wherein the agent comprises either of the following components as an active component:

(a) a monoclonal antibody which binds to PLD4 and suppresses activated B cells, or a fragment containing an antigen-binding region thereof, and

(b) immunoglobulin into which a complementarity-determining region of the monoclonal antibody in (a) is grafted, or a fragment containing an antigen-binding region thereof.

(32) The agent for suppressing activated B cells according to (31) above, wherein an activity of the activated B cells is an

antibody-producing activity.

[0020]

The "activated B cells" may include B cells possessing the activity of proliferation and antibody production and secretion by not only direct stimulation through BCR and TLR but also stimulation through T cells.

[0021]

The "fragment containing an antigen-binding region" may include Fab, Fab', $F(ab')_2$ fragments and the like obtained by partial digestion with papain or pepsin, but is not limited thereto. In addition, the fragment containing an antigen-binding region also may include a fragment of immunoglobulin containing a variable region into which CDR (complementarily-determining region) of a monoclonal antibody is grafted. It is well known that these antibody fragments can be used as antibody molecules having binding affinity to antigens. Alternatively, insofar as required antigen-binding activity is maintained, antibodies constructed by gene recombination can be used. Examples of antibodies constructed by gene recombination can include chimeric antibodies, CDR-grafted antibodies, single chain Fv (scFv), diabody (diabodies), linear antibodies, and polyspecific antibodies formed from antibody fragments and the like. A method for obtaining these antibodies based on monoclonal antibodies or antibody-producing cells producing the monoclonal antibodies is known.

[0022]

The "autoimmune diseases" are diseases which are caused by attacks of immune functions by misunderstanding one's own body tissues as foreign substances. Organ-specific autoimmune diseases include Guillain-Barre syndrome, myasthenia gravis, chronic gastritis (chronic atrophic gastritis), autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune pancreatitis, aortitis syndrome, Goodpasture syndrome, rapidly progressive glomerulonephritis, megaloblastic anemia, autoimmune hemolytic anemia, autoimmune neutropenia, idiopathic thrombocytopenic purpura, Basedow disease, Hashimoto thyroiditis, primary hypothyroidism, idiopathic Addison's disease, type 1 diabetes, ulcerative colitis, Crohn's disease, celiac disease and the like; and systemic autoimmune diseases include articular rheumatism, systemic lupus erythematosus, anti-phospholipid antibody syndrome, polymyositis, scleroderma, Sjogren's syndrome, vasculitis syndrome, autoimmune lymphoproliferative syndrome (ALPS) and the like, but are not limited thereto.

[0023]

The "allergic diseases" are diseases caused by abnormal immune reactions against foreign substances, and include atopic dermatitis, bronchial asthma, pollinosis, allergic rhinitis, urticaria, infantile asthma, allergic gastroenteritis, contact dermatitis, serum sickness, vascular purpura and the like but are not limited thereto.

[Advantageous Effects of Invention]

[0024]

The present invention provides a therapeutic method attributable to suppression of activated B cells using an antibody specifically recognizing PLD4 and a fragment thereof, and a medicament having its therapeutic effect.

[0025]

The present invention can be further expected to have preventive and therapeutic effects on patients with autoimmune diseases or allergic diseases by using the activated B cell-suppressing activity.

[Brief Description of Drawings]

[0026]

[Fig. 1]

Fig. 1 is a FACS analysis diagram which shows staining of human B cells (CD19+) with anti-PLD4 antibodies. PLD4 protein was induced on CD19+ B cells by stimulation with TLR9 ligand, CpG2006. Induction of PLD4 in activated B cells (CD19+) could be detected by a TLR9 ligand (CpG2006). Monoclonal antibodies 11G9.6 and 5B7 were used to detect PLD4. Mouse IgG2b, κ was used as a negative control.

[Fig. 2]

Fig. 2 is a FACS analysis diagram which shows staining of human PBMC with an anti-PLD4 antibody and an anti-CD19 antibody. PLD4+ cells were increased in activated B cells (CD19+) by stimulation with TLR9 ligand. Mouse IgG1, κ was used as a negative control.

[Fig. 3]

Fig. 3 is a FACS analysis diagram which shows staining

of human PBMC with anti-PLD4 antibodies and an anti-CD19 antibody in the presence or absence of TLR9 ligand stimulation. A significant increase of PLD4+ TLR9 ligand-stimulated B cells (CD19+) could be detected with anti-PLD4 antibodies (5B7, 13D4, 13H11 and 11G9.6). Mouse IgG2b, κ was used as a negative control.

[Fig. 4]

Fig. 4 is a FACS analysis diagram which shows reduction of PLD4+ activated B cells by the indicated each anti-PLD4 chimeric antibody. Co-culture of PBMCs with the anti-PLD4 chimeric antibodies (ch3B4, ch13D4, ch13H11, ch5B7 and ch11G9.6) reduced PLD4+ activated B cells in the presence of TLR9 ligand. In a case in which an antibody was not added (NoAb) and a case in which a non-specific antibody was used (Control Ig), however, the activation of B cells by adding CpG2006 could not be suppressed.

[Fig. 5]

Fig. 5 is a diagram in which suppressive effect in Fig. 4 is expressed in numbers. An activated B cell group which expresses PLD4 and was treated with control Ig is considered as 100% and changes in an activated B cell group which expresses PLD4 and was treated with each anti-PLD4 chimeric antibody are shown.

[Fig. 6]

Fig.6 is a result of flow cytometry. PBMCs were cultured with the indicated chimeric PLD4 antibodies in the presence of TLR9 ligand and recombinant human IL-6. Plasmablast population

(CD19+CD27+IgD-CD38+) was reduced by the treatment with ch3B4, ch5B7, ch13D4, ch13H11, or ch11G9.6 compared with control Ig treatment.

[Fig. 7]

Fig.7 is a result of ELISA assay of the culture supernatant of Fig. 6. Human IgG production from plasmablasts was reduced by the treatment with ch3B4, ch5B7, ch13D4, ch13H11, or ch11G9.6 compared with control Ig treatment.

[Description of Embodiments]

[0027]

The present inventors newly found that PLD4 was a molecule whose expression is induced with activation of B cells.

[0028]

The present inventors have previously reported expression, subcellular localization, structure and function of human PLD4 (Patent Literature 1). In the present invention, it further turned out that the expression of PLD4 is induced in not only pDC but also activated B cells. It was further newly found that anti-PLD4 antibodies suppressed activated B cells. Such findings not only strengthen a possibility that anti-PLD4 antibodies have a therapeutic effect on autoimmune diseases by suppression of pDC activity, which has been previously reported, but also B cell activity.

[0029]

Proteins such as CD19, CD20, CD22 and BAFF-R are expressed on the surface of B cells. CD19 is expressed on B cells from an early stage such as pro-B cells to antibody-secreting plasma

cells, and functions as an auxiliary receptor controlling activation in mature B cells. CD20 is expressed from pre B cells to activated B cells, CD22 is expressed on the cell surface of mature B cells, and the expression of BAFF-R is observed in the extensive differentiation stage of B cells. Therefore, there is concern that antibodies recognizing these proteins suppress not only activated B cells but also unprimed naive B cells. The anti-PLD4 antibodies of the present invention are however characterized by suppressing activated B cells without influence on naive B cells.

[0030]

The anti-PLD4 antibodies used in the present invention are the same as those reported previously (Patent Literature 1). In short, using as an immunogen a recombinant PLD4-Ig fusion protein encoding an amino acid sequence containing an extracellular domain of PLD4 (the amino acid sequence corresponding to from position 54 to 506 in the amino acid sequence shown in SEQ ID NO: 1), an antibody against PLD4 was obtained as follows.

[0031]

<Creation of anti-human PLD4 monoclonal antibodies>

1) Immunization

As an immunogen, the above recombinant PLD4-Ig fusion protein was used. The PLD4-Ig fusion protein was administered to the dorsal hypodermis of three BALB/c mice. As adjuvants, Freund's Adjuvants, Complete and Incomplete (SIGMA), were used. The volume of first administration was 200 μg/mouse, and the

volume of second to fourth administration was 50 μg/mouse.

2) Confirmation of anti-serum titer

Blood was collected after third and fourth immunization and anti-serum titer was evaluated by ELISA.

The PLD4-Ig fusion protein was transformed into a solid phase on a 96 well microtiter plate. An antiserum was serially diluted in 3-fold increments from 1000-fold and a dilution series up to 729000-fold was prepared. To the antigen-coated plate, each 50 μl of each sample was added and a first-order reaction was carried out. After washing, a second-order reaction was carried out with the HRP-labeled anti-mouse IgG (κ, λ) antibody and color development was detected with OPD (orthophenylene diamine) (490 nm).

3) Cell fusion

Splenic cells were extracted from mice in which an increase in anti-serum titer was observed. The extracted splenic cells and mouse myeloma cells (P3U1) were fused by the PEG method and the fused splenic cells were selectively cultured in an HAT medium.

[0032]

<FACS screening of hybridomas using CAL-1 cells>

An antibody produced from each clone of the fused splenic cells obtained by HAT selective culture was evaluated by FACS. Consequently, 3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, 14C1 and 11G9.6 in hybridoma culture supernatant well reacted to human PLD4.

[0033]

In each monoclonal antibody produced from the above hybridomas, CDR regions (CDRs; CDR1, CDR2 and CDR3) and FW regions (Frame work regions) in a variable region and a sequence of the variable region were determined according to an analytical method of 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).

[0034]

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 11G9.6 antibody is SEQ ID NO: 74, and the amino acid sequence is SEQ ID NO: 75. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 11G9.6 antibody are SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 3B4 antibody is SEQ ID NO: 76, and the amino acid sequence is SEQ ID NO: 77. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 3B4 antibody are SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 5B7 antibody is SEQ ID NO: 78, and the amino acid sequence is SEQ ID NO: 79. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 5B7 antibody are SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 7B4 antibody is SEQ ID NO: 80, and the amino acid sequence is SEQ ID NO: 81. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 7B4 antibody are SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, respectively. The 7B4 antibody is an antibody which has the same CDR sequences in the variable regions of the heavy and light chains as of the 5B7 antibody.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 8C11 antibody is SEQ ID NO: 82, and the amino acid sequence is SEQ ID NO: 83. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 8C11 antibody are SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 10C3 antibody is SEQ ID NO: 84, and the amino acid sequence is SEQ ID NO: 85. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 10C3 antibody are SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 11D10 antibody is SEQ ID NO: 86, and the amino acid sequence is SEQ ID NO: 87. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 11D10 antibody are SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, respectively. The 11D10 antibody is an antibody which has the same CDR sequences in the variable

regions of the heavy and light chains as of the 10C3 antibody. Their heavy chain isotypes are, however, different (10C3 has the constant region of mouse IgG2a and 11D10 has the constant region of mouse IgG2b).

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 13D4 antibody is SEQ ID NO: 88, and the amino acid sequence is SEQ ID NO: 89. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 13D4 antibody are SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 13H11 antibody is SEQ ID NO: 90, and the amino acid sequence is SEQ ID NO: 91. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 13H11 antibody are SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, respectively.

The nucleic acid sequence of the heavy chain variable region of the obtained mouse 14C1 antibody is SEQ ID NO: 92, and the amino acid sequence is SEQ ID NO: 93. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 14C1 antibody are SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, respectively. The 14C1 antibody is an antibody which has the same CDR sequences in the variable regions of the heavy and light chains as of the 13H11 antibody. Their heavy chain isotypes are, however, different (13H11 has the constant region of mouse IgG2b and 14C1 has the constant region of mouse IgG1).

[0035]

The nucleic acid sequence of the light chain variable region of the mouse 11G9.6 antibody is SEQ ID NO: 94, and the amino acid sequence is SEQ ID NO: 95. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 11G9.6 antibody are SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 3B4 antibody is SEQ ID NO: 96, and the amino acid sequence is SEQ ID NO: 97. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 3B4 antibody are SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 5B7 antibody is SEQ ID NO: 98, and the amino acid sequence is SEQ ID NO: 99. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 5B7 antibody are SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 7B4 antibody is SEQ ID NO: 100, and the amino acid sequence is SEQ ID NO: 101. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 7B4 antibody are SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 8C11 antibody is SEQ ID NO: 102, and the

amino acid sequence is SEQ ID NO: 103. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 8C11 antibody are SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 10C3 antibody is SEQ ID NO: 104, and the amino acid sequence is SEQ ID NO: 105. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 10C3 antibody are SEQ ID NO: 29, SEQ ID NO: 30 and SEQ ID NO: 31, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 11D10 antibody is SEQ ID NO: 106, and the amino acid sequence is SEQ ID NO: 107. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 11D10 antibody are SEQ ID NO: 29, SEQ ID NO: 30 and SEQ ID NO: 31, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 13D4 antibody is SEQ ID NO: 108, and the amino acid sequence is SEQ ID NO: 109. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 13D4 antibody are SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 13H11 antibody is SEQ ID NO: 100, and the amino acid sequence is SEQ ID NO: 111. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 13H11 antibody are SEQ ID NO: 41, SEQ ID NO: 42

and SEQ ID NO: 43, respectively.

The nucleic acid sequence of the light chain variable region of the mouse 14C1 antibody is SEQ ID NO: 112, and the amino acid sequence is SEQ ID NO: 113. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 14C1 antibody are SEQ ID NO: 41, SEQ ID NO: 42 and SEQ ID NO: 43, respectively.

[0036]

Examples of more preferred monoclonal antibodies in the present invention can include monoclonal antibodies produced by

hybridomas mp5B7, mp7B4, mp13D4 and mp13H11.

Hybridomas mp5B7, mp7B4, mp13D4 and mp13H11 were accepted by National Institute of Technology and Evaluation, International Patent Organism Depositary,

under accession No. NITE ABP-1211, NITE ABP-1212, NITE ABP-1213 and NITE ABP-1214

as of January 27, 2012. The details specifying the deposition will be described as follows.

(1) Name and Address of Depositary Authority

Name: National Institute of Technology and Evaluation, Advanced Industrial Science and Technology, International Patent Organism Depositary

Address: 2-5-8 Kazusa Kamatari Kisarazu-shi, Chiba Ibaraki, 292-0818, Japan

(2) Deposit Date: January 27, 2012

(3) Deposit number NITE BP-1211 (hybridoma mp5B7)

NITE BP-1212 (hybridoma mp7B4)

NITE BP-1213 (hybridoma mp13D4)

NITE BP-1214 (hybridoma mp13H11)

[0037]

In particular, more preferred antibodies are an antibody having a combination of

the heavy chain CDR1: DYNLH, CDR2: YIYPYNGNTGYNQKFKR, and CDR3: GGIYDDYYDYAIDY, and

the light chain CDR1: RASENIYSHIA, CDR2: GATNLAH, and CDR3: QHFWGTP

as the sequences of CDRs constituting its variable regions;

an antibody having a combination of

the heavy chain CDR1: SHYYWT, CDR2: YISYDGSNNYNPSLKN, and CDR3: EGPLYYGNPYWYFDV, and

the light chain CDR1: RASQDIDNYLN, CDR2: YTSRLHS, and CDR3: QQFNTLP

as the sequences of CDRs constituting its variable regions; and

an antibody having a combination of

the heavy chain CDR1: SHYYWS, CDR2: YISYDGSNNYNPSLKN, and CDR3: EGPLYYGNPYWYFDV, and

the light chain CDR1: RASQDIDNYLN, CDR2: YTSRLHS, and CDR3: QQFNTLP,

as the sequences of CDRs constituting its variable regions.

[0038]

A chimeric antibody or a humanized antibody recognizing PLD4 can be produced by genetic engineering using a polynucleotide encoding it. As described in Patent Document

1, for example, each active chimeric antibody (ch3B4Ab, ch5B7Ab, ch7B4Ab, ch8C11Ab, ch10C3Ab, ch11D10Ab, ch13D4Ab, ch13H11Ab, ch14C1Ab, ch11G9.6Ab etc.) can be easily produced using each CDR region of the above mouse monoclonal antibodies (3B4, 5B7, 7B4, 8C11, 10C3, 11D10, 13D4, 13H11, 14C1, 11G9.6 etc.) by those of skill in the art.

[0039]

The present inventors have verified that monoclonal antibodies against PLD4 have CDC (Complement Dependent Cytotoxicity) activity and ADCC (Antibody-dependent cellular cytotoxicity) activity against the PLD4-expressing cells. Therefore, the anti-PLD4 monoclonal antibodies according to the present invention have cytotoxicity action against PLD4-expressing cells.

[0040]

That is, the present invention relates to an agent for suppressing activated B cells, wherein the agent comprises an antibody binding to an extracellular domain of PLD4 as an active component. Alternatively, the present invention provides a method for suppressing antibody production, the method including a step of administering an antibody binding to an extracellular domain of PLD4. The present invention further relates to use of an antibody binding to an extracellular domain of PLD4 in production of a pharmaceutical composition for suppressing activated B cells.

[0041]

In the present invention, an antibody modified as needed

can be used. According to the present invention, an antibody recognizing the extracellular domain of PLD4 has the activated B cell-suppressing action. That is, it has been believed that there is a possibility that an antibody itself have cytotoxicity action against activated B cells. The subclass of an antibody showing intense effector action is known. Alternatively, suppressive effect on activated B cells can be further increased by modifying an antibody with a cytotoxic agent. As the cytotoxic agents, the following substances can be mentioned.
Toxins: Pseudomonas Endotoxin (PE), diphtheria toxin, lysine
Radioisotopes: Tc99m, Sr89, I131, Y90
Anticancer agents: calicheamicin, mitomycin, paclitaxel

The toxins containing proteins can be bound to an antibody or a fragment thereof or the like by a bifunctional reagent. Alternatively, by conjugating a gene encoding an antibody with a gene encoding a toxin, a fusion protein of the two can be also obtained. A method for binding a radioisotope to an antibody is also known. A method for labeling an antibody with a radioisotope, for example, using a chelating agent is known. Further, an anticancer agent can be bound to an antibody, using glycan or a bifunctional reagent or the like.

[0042]

In the present invention, an antibody whose structure is artificially modified can be used as an active component. For example, various modification methods for improving the cytotoxicity action and stability of antibodies are known. Concretely, immunoglobulin in which the glycan of its heavy

chain is modified is known (Shinkawa, T. et al. J. Biol. Chem. 278:3466-3473. 2003). By modification of glycan, the ADCC (Antibody Dependent Cell-mediated Cytotoxicity) activity of immunoglobulin was increased.

[0043]

In the present invention, one or more monoclonal antibodies can be used. For example, several types of monoclonal antibodies recognizing the extracellular domain of PLD4 can be combined and used for the present invention.

[0044]

As described below, it can be verified that anti-PLD4 antibodies have suppressive action on the acquired immune antibody-producing activity of activated B cells. B cells produce a large amount of antibodies by stimulation of a BCR ligand or a TLR ligand (preferably TLR4 ligand, TLR7 ligand or TLR9 ligand). An anti-PLD4 antibody is provided before and after stimulation of the above ligand on B cells or simultaneously with stimulation of the ligand, and using B cells for which an anti-PLD4 antibody is not provided as a control, ability to produce acquired immune antibodies derived from B cells is compared. The antibody-producing ability can be evaluated by measuring secretory immunoglobulin contained in a culture supernatant of B cells. As a result of the comparison, when the amount of the acquired immune antibody derived from B cells in the supernatant significantly declines by adding an anti-PLD4 antibody, it can be verified that the tested anti-PLD4 antibody has suppressive action on the antibody-producing

ability of B cells. A method for measuring the antibodies is known. B cells are cells which produce hormonal immunity (secretory antibody) in a living body. Therefore, hormonal immunity can be adjusted by suppressing the antibody-producing ability of B cells.

[0045]

When an antibody recognizing the extracellular domain of PLD4 is administered to a host different from an organism species from which the antibody is derived, it is desired to process into a form which is difficult to be recognized as a foreign substance by such a host. By processing into molecules described below, for example, it can be difficult that immunoglobulin is recognized as a foreign substance. Techniques for processing immunoglobulin molecules as described below are known:

- a fragment containing an antigen-binding region which 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 constituted of an antigen-binding region of a monoclonal antibody and a constant region of host immunoglobulin (Experimental manual for genetic expression, Kodansha Ltd. 1994 (edited by Isao Ishida and Tamie Ando)); and
- a CDR-substituted antibody in which a complementarity-determining region (CDR) in host immunoglobulin is substituted by the CDR of a monoclonal antibody (Experimental manual for genetic expression, Kodansha

Ltd. 1994 (edited by Isao Ishida and Tamie Ando)).

[0046]

Alternatively, a variable region gene of human immunoglobulin can be also obtained by the phage display method (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 method, a gene encoding a variable region of human immunoglobulin is incorporated into a phage gene. Using various types of immunoglobulin genes as sources, a phage library can be also created. A phage expresses such a variable region as a fusion protein of a protein constructing the phage itself. The variable region expressed by the phage on the phage surface maintains binding activity to antigens. Therefore, by selecting a phage binding to an antigen or cells expressing the antigen or the like, a phage expressing a variable region having target binding activity can be screened from a phage library. Further, a gene encoding a variable region having target binding activity is maintained in the phage particle selected as above. That is, in the phage display method, using the binding activity of a variable region as an index, a gene encoding a variable region having target binding activity can be obtained.

[0047]

In the agent for suppressing B cell activity or the method for suppressing B cell activity according to the present invention, an antibody recognizing the extracellular domain of PLD4 or an antibody fragment containing at least the antigen-binding region thereof can be administered as a protein

or a polynucleotide encoding it. In order to administer a polynucleotide, it is desired that a vector in which a polynucleotide encoding a target protein is arranged be used under control of a proper promoter so that the target protein can be expressed. In a vector, an enhancer and a terminator can be also arranged. Vectors which maintain the genes of heavy and light chains constituting immunoglobulin and in which an immunoglobulin molecule can be expressed are known. A vector in which immunoglobulin can be expressed can be administered by introduction into cells. For administration to a living body, a vector which can infect cells by administration to the living body can be directly administered. Alternatively, a vector is introduced into a lymphocyte separated from a living body and then the vector can be returned into the living body (ex vivo).

[0048]

In the agent for suppressing B cell activity or the method for suppressing B cell activity based on the present invention, the amount of monoclonal antibody to be administered to a living body is normally 0.5 mg to 10 mg, for example 1 mg to 50 mg, preferably 2 mg to 10 mg as immunoglobulin per kg of body weight. An interval of administration of an antibody to a living body can be properly adjusted in order that an effective concentration of immunoglobulin in the living body during treatment period can be maintained. Concretely, for example, an antibody can be administered at intervals of 1 to 2 weeks. Any administration route can be used. Those of skill in the art can properly select an effective administration route for

treatment. Concretely, oral or parenteral administration can be mentioned. By an intravenous injection, an intramuscular injection, an intraperitoneal injection or a subcutaneous injection or the like, for example, an antibody can be systemically or locally administered. The formulations suitable for parenteral administration in the present invention include injections, suppositories, sprays and the like. In addition, when provided to cells, immunoglobulin is provided in a culture fluid in an amount of normally 1 μg/ml, preferably 10 μg/mL or more, more preferably 50 μg/mL or more, and further preferably 0.5 mg/mL or more.

[0049]

In the agent for suppressing B cell activity or the method for suppressing B cell activity based on the present invention, a monoclonal antibody can be administered to a living body by any method. A monoclonal antibody is normally combined with a pharmaceutically acceptable carrier. A monoclonal antibody can be combined with additives as needed, such as a thickener, a stabilizer, an antiseptic and a solubilizing agent. Such carriers or additives include lactose, a citric acid, a stearic acid, magnesium stearate, sucrose, starch, talc, gelatin, agar, plant oil, ethylene glycol and the like. The term "pharmaceutically acceptable" means to be approved by government authorities of various countries, or that its use for animals, mammals and, in particular, human is listed in pharmacopoeias of various countries or pharmacopoeias commonly acknowledged. The agent for suppressing B cell activity in the

present invention can be also supplied in the form of freeze-drying powders or tablets at one or more doses. Further, sterilized water for injections, a physiological salt solution or a buffer solution, which are used for dissolution, can be combined with freeze-drying powders or tablets in order that the composition will obtain a desired concentration before administration.

[0050]

Further, for administration as a vector expressing immunoglobulin, a heavy chain and a light chain are cotransfected as different plasmids and each plasmid can be administered at 0.1 to 10 mg, for example 1 to 5 mg per kg of body weight. In addition, 1 to 5 μg vectors/10^6 cells are used to introduce into cells in vitro. The present invention will be now described in more detail by way of examples.

[0051]

All of the related art literatures cited in the present description are incorporated by reference herein.

[0052]

The present invention will be now described in more detail by way of examples. It should be noted, however, that the present invention is not limited to the examples.

[Examples]

[0053]

Example 1

Human PBMC (1×10^7 cells/ml) was stimulated by CpG2006, a ligand of TLR9, (a final concentration of 1 μM) and incubated

in a 24 well plate in a CO_2 incubator (37°C, 5% CO_2) for about 20 hours. In parallel, human PBMC (1×10^7 cells/ml) which was not stimulated was also cultured in a CO_2 incubator (37°C, 5% CO_2) for about 20 hours.

Human PBMC was treated with FcR Blocking Reagent (Miltenyi), which was diluted 5-fold with FACS buffer (1% FBS/PBS), at 4°C for 20 minutes. After washing, staining was carried out with 5B7, 11G9.6 or mouse IgG2b, κ, a primary antibody, (each 10 μg/ml) at 4°C for 15 minutes. A secondary antibody and subsequent antibodies were diluted with FACS buffer so that FcR Blocking Reagent would be diluted 25-fold. PE-labeled anti-mouse Ig (BD), a secondary antibody, was diluted 100-fold and the solution was added thereto and mixed. Besides, to fractionate B cells on FACS, an APC-labeled anti-human CD 19 antibody (Biolegend) was diluted 30-fold with FACS buffer containing FcR Blocking Reagent and staining was carried out at 4°C for 15 minutes. Using FACS Calibur (BD), data was incorporated. Living cells were gated on a dot plot of the X axis: FSC and the Y axis: SSC. Data was incorporated until the number of cells in the living cell gate became 100,000 counts. B cells: anti-marker molecule antibody-positive cells were gated. The gated cells were analyzed on the histogram with the X axis: PLD4, and the results of staining with mouse IgG2b, κ were overlaid thereon. Consequently, anti-PLD4 antibodies were hardly bound to non-stimulated, but were selectively bound to activated B cells by stimulation with TLR9 ligand (Fig. 1). This shows that PLD4 is expressed on activated B cells.

[0054]

Example 2

<Binding test to B cells by each monoclonal antibody>

Human PBMC was stimulated with CpG2006 with a final concentration of 1 μM for about 20 hours. Cells were collected and treated with FcR Blocking Reagent at 4°C for 20 minutes. After washing, staining was carried out with each 10 μg/ml of 3B4, 5B7, 13D4, 13H11, 11G9.6, mouse IgG1, κ or mouse IgG2b, κ, a primary antibody, at 4°C for 15 minutes. Staining was carried out with PE-labeled anti-mouse Ig, a secondary antibody, at 4°C for 15 minutes. For gating of a B cell group, double staining was carried out with an APC-labeled anti-human CD19 antibody at 4°C for 15 minutes. A living cell group on the dot plot of the X axis: FSC and the Y axis: SSC was analyzed by binding of anti-PLD4 antibody to CD19+ B cells (Fig. 2 and Fig.3). Consequently, all of the tested anti-PLD4 monoclonal antibodies were bound to B cells stimulated by TLR9. That is, it was verified that by all anti-PLD4 monoclonal antibodies, expression of PLD4 was induced in B cells in an activation-dependent manner.

[0055]

Example 3

<Cytotoxic activity of anti-PLD4 chimeric antibodies against activated B cells>

Frequency of PLD4+ activated B cells induced by stimulation with TLR9 ligand (1 μM) was used as an index. Human PBMC was cultured with CpG2006 and each anti-PLD4 chimeric

antibody or control Ig for about 16 hours. As a medium, RPM11640 (SIGMA) was used (including 10% FBS (Equitech-bio), 5 ml of 200 mM L-Glutamine (GIBCO), 5 ml of Pen-Strep (GIBCO), 5 ml of Sodium Pyruvate (GIBCO), and 0.5 ml of 50 mM 2-ME (SIGMA)). The cells were collected and treated with FcR Blocking Reagent at 4°C for 20 minutes. After washing, the cells were further stained by 5B7 or 13D4, 3B4 or mouse IgG2b, κ, a primary antibody, at 4°C for 15 minutes (each 10 μg/ml). A sample in which PBMC was treated with a chimeric 3B4 antibody (ch3B4), a chimeric 3D4 antibody (ch3D4), or a chimeric 13H11 antibody (ch13H11) was stained with 5B7, and a sample in which PBMC was treated with a chimeric 5B7 antibody (ch5B7) or a chimeric 11G9.6 antibody (ch11G9.6) was stained with 13D4. It has been verified that an anti-PLD4 antibody clone treated for ADCC and an anti-PLD4 antibody clone used for staining do not compete with each other. The binding of the anti-PLD4 was found by PE-labeled anti-mouse Ig, a secondary antibody, at 4°C for 15 minutes. For gating of B cells, double staining was carried out with an APC-labeled anti-human CD19 antibody at 4°C for 15 minutes (Fig. 4). The population of PLD4 + activated B cells treated with each chimeric anti-PLD4 antibody was compared with that of PLD4 + activated B cells treated with the control antibody (Fig. 5). Consequently, all of the chimeric anti-PLD4 antibodies reduced activated PLD4 + B cells compared to the treatment with control Ig (when a case of treating with control Ig was considered as 100%, ch3B4: 70.2%, ch13D4: 56.0%, ch13H11: 55.3%, ch5B7: 25.8%, ch11G9.6: 66.4%).

[0056]

Example 4

<Inhibitory effects of the chimeric anti-PLD4 antibodies against activated B cells>

To determine the effect of anti-human PLD4 antibody on B cells maturation and Ig production through B cell activation, whole human PBMCs were treated with ch3B4, ch5B7, ch13D4, ch13H11, ch11G9.6, or control Ig for 24 h. Then, the PBMCs were further cultured in the presence of CpG2216 (1 μM) and recombinant human IL-6 to induce B cell activation, resulting in B cell maturation. In the result of culture of activated B cells for 7 days, Plasmablasts, CD19+CD27+IgD-CD38+, in the activated B cells was analyzed by flow cytometry with a PE-labeled anti-human CD19 antibody. In order to measure human IgG production, the cultured activated B cells were re-stimulated with 50 ng/ ml of PMA (Phorbol myristate acetate) after washed with PBS 2 times. Two days later, human IgG production was measured in the culture supernatants by ELISA. Plasmablasts in the activated B cells were reduced by the treatment with ch3B4, ch5B7, ch13D4, ch13H11, or ch11G9.6 compared with control Ig treatment (Figure 6). Also, human IgG production was reduced by the treatment with ch3B4, ch5B7, ch13D4, ch13H11, or ch11G9.6 compared to control Ig treatment (Figure 7). These results indicated that the treatment with the chimeric anti-human PLD4 Abs reduced Ab-secreting activated human B cells.

[Industrial Applicability]

[0057]

As shown in the above examples, anti-PLD4 antibodies recognize and suppress activated B cells. Therefore, the antibodies are useful for prevention and treatment of diseases involved in immune function (autoimmune diseases and allergic diseases).

[0058]

<Explanation of sequence information of anti-PLD4 monoclonal antibodies according to the present invention>

1. Anti-PLD4 mouse 11G9.6 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 11G9.6 antibody is SEQ ID NO: 74, and the amino acid sequence is SEQ ID NO: 75. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 11G9.6 antibody are SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 11G9.6 antibody (504 bp) [capital letters: mouse 11G9.6 VH variable region, small letters: mouse IgG2b heavy chain constant region] (SEQ ID NO: 74)

ATGAGATCACAGTTCTCTATACAGTTACTGAGCACACAGAACCTCACCTTGG
GATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTGTCCACTC
CCAGGTCCAACTGCAGCAGCCTGGGGCTGAACTGGTGAAGCCTGGGACTTC
AGTGAAAATGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTGGATG
CACTGGGTGAAGCAGAGGCCGGGACAAGGCCTTGAGTGGATTGGAGATATT
TATCCTGGTAGTGATAGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCC
ACACTGACTGTAGACACATCCTCCAGCACAGCCTACATGCAACTCAGCAGC
CTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGAGGAGGGTGGTTG
GATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAgccaaa
acaacacccccatcagtctatccactggcccctaagggc

The amino acid sequence of the heavy chain variable region of the mouse 11G9.6 antibody (168 a. a.) [capital letters: mouse 11G9.6 VH variable region, small letters: mouse IgG2b heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3) (SEQ ID NO: 75).

MRSQFSIQLLSTQNLTLGWSCIILFLVATATGVIISQVQLQQPGAELVKPGTSVKMSCK
ASGYTFTSYWMHWVKQRPGQGLEWIGDIYPGSDSTNYNEKFKSKATLTVDTSSSTA
YMQLSSLTSEDSAVYYCARGGWLDAMDYWGQGTSVTVSSakttppsvyplapkg

CDR1 in the heavy chain variable region of the 11G9.6 antibody (SEQ ID NO: 2)

CDR2 in the heavy chain variable region of the 11G9.6 antibody (SEQ ID NO: 3)

CDR3 in the heavy chain variable region of the 11G9.6 antibody (SEQ ID NO: 4)

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 11G9.6 antibody is SEQ ID NO: 38, and the amino acid sequence is SEQ ID NO: 39. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 11G9.6 antibody are SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 11G9.6 antibody (421 bp) [capital letters: mouse 11G9.6 VL variable region, small letters: mouse Ig κ light chain constant region] (SEQ ID NO: 94)

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTAC
CAGATGTGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTG

GGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACATTAGCAATTAT
TTAAACTGGTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACT
ACACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGT
CTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTG
CCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTGGACGTTCGGTGGAGG
CACCAAGCTGGAAATCAAAcgggctgatgctgcaccaactgtatccatcaagggcgaat

The amino acid sequence of the light chain variable region of the mouse 11G9.6 antibody (140 a. a.) [capital letters: mouse 11G9.6 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3) (SEQ ID NO: 95).

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGNTLP
WTFGGGTKLEIKradaaptvsikge

CDR1 in the light chain variable region of the 11G9.6 antibody (SEQ ID NO: 5)

CDR2 in the light chain variable region of the 11G9.6 antibody (SEQ ID NO: 6)

CDR3 in the light chain variable region of the 11G9.6 antibody (SEQ ID NO: 7)

[0059]

2. Anti-PLD4 mouse 3B4 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 3B4 antibody is SEQ ID NO: 76, and the amino acid sequence is SEQ ID NO: 77. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 3B4 antibody are SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 3B4 antibody (437 bp) [capital letters: mouse 3B4 VH variable region, small letters: mouse IgG1 heavy chain constant region]

ATGGAATGTAACTGGATACTTCCTTTTATTCTGTCGGTAATTTCAGGGGTCT
CCTCAGAGGTTCAGCTCCAGCAGTCTGGGACTGTGCTGTCAAGGCCTGGGG
CTTCCGTGACGATGTCCTGCAAGGCTTCTGGCGACAGCTTTACCACCTACTG
GATGCACTGGGTAAAACAGAGGCCTGGACAGGGTCTAGAATGGATTGGTGC
TATCTATCCTGGAAATAGTGAAACTAGCTACAACCAGAAGTTCAAGGGCAA
GGCCAAACTGACTGCAGTCACATCCGCCAGCACTGCCTATATGGAGTTCACT
AGCCTGACAAATGAGGACTCTGCGGTCTATTACTGTACGGGGGGTTATTCCG
ACTTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAgccaaaacgaca
cccccatctgtctatccact

The amino acid sequence of the heavy chain variable region of the mouse 3B4 antibody (145 a. a.) [capital letters: mouse 3B4 VH variable region, small letters: mouse IgG1 heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MECNWILPFILSVISGVSSEVQLQQSGTVLSRPGASVTMSCKASGDSFTTYWMHWVK
QRPGQGLEWIGAIYPGNSETSYNQKFKGKAKLTAVTSASTAYMEFTSLTNEDSAVYY
CTGGYSDFDYWGQGTTLTVSSakttppsvyp

CDR1 in the heavy chain variable region of the 3B4 antibody

TYWMH

CDR2 in the heavy chain variable region of the 3B4 antibody

AIYPGNSETSYNQKFKG

CDR3 in the heavy chain variable region of the 3B4 antibody

GYSDFDY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 3B4 antibody is SEQ ID

NO: 96, and the amino acid sequence is SEQ ID NO: 97. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 3B4 antibody are SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 3B4 antibody (459 bp) [capital letters: mouse 3B4 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGATGGTCCTTGCTCAGTTTCTTGCATTCTTGTTGCTTTGGTTTCCAGGTGC
AGGATGTGACATCCTGATGACCCAATCTCCATCCTCCATGTCTGTATCTCTG
GGAGACACAGTCAGCATCACTTGCCATGCAAGTCAGGGCATTAGAAGTAAT
ATAGGGTGGTTGCAGCAGAAACCAGGGAAATCATTTAAGGGCCTGATCTTT
CATGGAACCAACTTGGAAGATGGAGTTCCATCAAGGTTCAGTGGCAGAGGA
TCTGGAGCAGATTATTCTCTCACCATCAACAGCCTGGAATCTGAAGATTTTG
CAGACTATTACTGTGTACAGTATGTTCAGTTTCCTCCAACGTTCGGCTCGGG
GACAAAGTTGGAAATAAGAcgggctgatgctgcaccaactgtatccatcttcccaccatccagtgagcagtt
aacatctggaggtgcctcagtcgtg

The amino acid sequence of the light chain variable region of the mouse 3B4 antibody (153 a. a.) [capital letters: mouse 3B4 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MMVLAQFLAFLLLWFPGAGCDILMTQSPSSMSVSLGDTVSITCHASQGIRSNIGWLQ
QKPGKSFKGLIFHGTNLEDGVPSRFSGRGSGADYSLTINSLESEDFADYYCVQYVQFP
PTFGSGTKLEIRradaaptvsifppsseqltsggasvv

CDR1 in the light chain variable region of the 3B4 antibody
HASQGIRSNIG

CDR2 in the light chain variable region of the 3B4 antibody
HGTNLED

CDR3 in the light chain variable region of the 3B4 antibody VQYVQFP

[0060]

3. Anti-PLD4 mouse 5B7 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 5B7 antibody is SEQ ID NO: 78, and the amino acid sequence is SEQ ID NO: 79. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 5B7 antibody are SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 5B7 antibody (475 bp) [capital letters: mouse 5B7 VH variable region, small letters: mouse IgG2b heavy chain constant region]

ATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCAGGCGTCC
ACTCTGAGGTCCAGCTTCAGCAGTCAGGACCTGAACTGGTGAAACCTGGGG
CCTCAGTGAAGATATCCTGCAAGGCTTCTGGATACACATTCACTGACTACAA
CTTGCACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGATA
TATTTATCCTTACAATGGTAATACTGGCTACAACCAGAAGTTCAAGAGGAA
GGCCACATTGACTGTAGACAATTCCTCCGGCACAGTCTACATGGAGCTCCGC
AGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGAGGAGGGATC
TATGATGATTACTACGACTATGCTATCGACTATTGGGGTCAAGGAACCTCAG
TCACCGTCTCCTCAgccaaaacaacacccccatcagtctatccactggcccctaagggcgaat

The amino acid sequence of the heavy chain variable region of the mouse 5B7 antibody (158 a. a.) [capital letters: mouse 5B7 VH variable region, small letters: mouse IgG2b heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MGWSWIFLFLLSGTAGVHSEVQLQQSGPELVKPGASVKISCKASGYTFTDYNLHWV
KQSHGKSLEWIGYIYPYNGNTGYNQKFKRKATLTVDNSSGTVYMELRSLTSEDSAV
YYCARGGIYDDYYDYAIDYWGQGTSVTVSSakttppsvyplapkge

CDR1 in the heavy chain variable region of the 5B7 antibody

DYNLH

CDR2 in the heavy chain variable region of the 5B7 antibody

YIYPYNGNTGYNQKFKR

CDR3 in the heavy chain variable region of the 5B7 antibody

GGIYDDYYDYAIDY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 5B7 antibody is SEQ ID NO: 98, and the amino acid sequence is SEQ ID NO: 99. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 5B7 antibody are SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 5B7 antibody (467 bp) [capital letters: mouse 5B7 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGATG
CCAGATGTGACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGTATCTGT
GGGAGAAACTGTCGCCATCACATGTCGAGCAAGTGAGAATATTTACAGTCA
TATAGCATGGTATCAGCAGAAAGAGGGAAAATCTCCTCAGCGCCTGGTCTA
TGGTGCAACAAACTTAGCACATGGTGTGCCATCAAGGTTCAGTGGCAGTGG
ATCAGGCACACAGTATTCCCTCAAGATCAACAGCCTTCAGTCTGAAGATTTT
GGGAGTTATTACTGTCAACATTTTTGGGGTACTCCGTGGACGTTCGGTGGAG
GCACCAAGCTGGAAATCAAAcgggctgatgctgcaccaactgtatccatcttcccaccatccagtgagcag
ttaacatctggaggtgcctcagtcgtgtgcttctt

The amino acid sequence of the light chain variable region of the mouse 5B7 antibody (155 a. a.) [capital letters: mouse

5B7 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MSVPTQVLGLLLLWLTDARCDIQMTQSPASLSVSVGETVAITCRASENIYSHIAWYQ
QKEGKSPQRLVYGATNLAHGVPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHFWGT
PWTFGGGTKLEIKradaaptvsifppsseqltsggasvvcf

CDR1 in the light chain variable region of the 5B7 antibody
RASENIYSHIA
CDR2 in the light chain variable region of the 5B7 antibody
GATNLAH
CDR3 in the light chain variable region of the 5B7 antibody
QHFWGTP

[0061]

4. Anti-PLD4 mouse 7B4 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 7B4 antibody is SEQ ID NO: 80, and the amino acid sequence is SEQ ID NO: 81. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 7B4 antibody are SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 7B4 antibody (470 bp) [capital letters: mouse 7B4 VH variable region, small letters: mouse IgG2b heavy chain constant region]

ATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCAGGCGTCC
ACTCTGAGGTCCAGCTTCAGCAGTCAGGACCTGAACTGGTGAAACCTGGGG
CCTCAGTGAAGATATCCTGCAAGGCTTCTGGATACACATTCACTGACTACAA
CTTGCACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGATTGGATA

TATTTATCCTTACAATGGTAATACTGGCTACAACCAGAAGTTCAAGAGGAA
GGCCACATTGACTGTAGACAATTCCTCCGGCACAGTCTACATGGAGCTCCGC
AGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGAGGAGGGATC
TATGATGATTACTACGACTATGCTATCGACTATTGGGGTCAAGGAACCTCAG
TCACCGTCTCCTCAgccaaaacaacacccccatcagtctatccactggcccctaaggg

The amino acid sequence of the heavy chain variable region of the mouse 7B4 antibody (156 a. a.) [capital letters: mouse 7B4 VH variable region, small letters: mouse IgG2b heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MGWSWIFLFLLSGTAGVHSEVQLQQSGPELVKPGASVKISCKASGYTFTDYNLHWV
KQSHGKSLEWIGYIYPYNGNTGYNQKFKRKATLTVDNSSGTVYMELRSLTSEDSAV
YYCARGGIYDDYYDYAIDYWGQGTSVTVSSakttppsvyplapk

CDR1 in the heavy chain variable region of the 7B4 antibody
DYNLH
CDR2 in the heavy chain variable region of the 7B4 antibody
YIYPYNGNTGYNQKFKR
CDR3 in the heavy chain variable region of the 7B4 antibody
GGIYDDYYDYAIDY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 7B4 antibody is SEQ ID NO: 100, and the amino acid sequence is SEQ ID NO: 101. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 7B4 antibody are SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 7B4 antibody (454 bp) [capital

letters: mouse 7B4 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGATG
CCAGATGTGACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGTATCTGT
GGGAGAAACTGTCGCCATCACATGTCGAGCAAGTGAGAATATTTACAGTCA
TATAGCATGGTATCAGCAGAAAGAGGGAAAATCTCCTCAGCGCCTGGTCTA
TGGTGCAACAAACTTAGCACATGGTGTGCCATCAAGGTTCAGTGGCAGTGG
ATCAGGCACACAGTATTCCCTCAAGATCAACAGCCTTCAGTCTGAAGATTTT
GGGAGTTATTACTGTCAACATTTTTGGGGTACTCCGTGGACGTTCGGTGGAG
GCACCAAGCTGGAAATCAAAcgggctgatgctgcaccaactgtatccatcttcccaccatccagtgagcag
ttaacatctggaggtgcctcag

The amino acid sequence of the light chain variable region of the mouse 7B4 antibody (151 a. a.) [capital letters: mouse 7B4 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MSVPTQVLGLLLLWLTDARCDIQMTQSPASLSVSVGETVAITCRASENIYSHIAWYQ
QKEGKSPQRLVYGATNLAHGVPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHFWGT
PWTFGGGTKLEIKradaaptvsifppsseqltsggas

CDR1 in the light chain variable region of the 7B4 antibody

RASENIYSHIA

CDR2 in the light chain variable region of the 7B4 antibody

GATNLAH

CDR3 in the light chain variable region of the 7B4 antibody

QHFWGTP

[0062]

5. Anti-PLD4 mouse 8C11 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 8C11 antibody is SEQ ID

NO: 82, and the amino acid sequence is SEQ ID NO: 83. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 8C11 antibody are SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 8C11 antibody (462 bp) [capital letters: mouse 8C11 VH variable region, small letters: mouse IgG2b heavy chain constant region]

ATGGGATGGAGCTATATCATCCTCTTTTTGGTAGCAACAGCAACAGGGGTCC
ACTCCCAGGTCCAACTGCAGCAGTCGGGGGCTGAACTGGTGAAGCCTGGGG
CTTCAGTGAAGTTGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTA
TTTGTACTGGGTGAGGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGACT
GATTAATCCTACCAATAGTGATACTATCTTCAATGAGAAGTTCAAGAGCAA
GGCCACACTGACTGTAGACAAATCCTCCAGCACAGCATACATGCAACTCAG
CAGCCTGACATCTGAGGACTCTGCGGTCTATTACTGTACACGAGAGGGGGG
ATATGGTTACGGCCCGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTC
TCTGCAgccaaaacaacacccccatcagtctatccactggcccctaagggc

The amino acid sequence of the heavy chain variable region of the mouse 8C11 antibody (154 a. a.) [capital letters: mouse 8C11 VH variable region, small letters: mouse IgG2b heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MGWSYIILFLVATATGVHSQVQLQQSGAELVKPGASVKLSCKASGYTFTSYYLYWV
RQRPGQGLEWIGLINPTNSDTIFNEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYY
CTREGGYGYGPFAYWGQGTLVTVSAakttppsvyplapkg

CDR1 in the heavy chain variable region of the 8C11 antibody

SYYLY

CDR2 in the heavy chain variable region of the 8C11 antibody

LINPTNSDTIFNEKFKS

CDR3 in the heavy chain variable region of the 8C11 antibody EGGYGYGPFAY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 8C11 antibody is SEQ ID NO: 102, and the amino acid sequence is SEQ ID NO: 103. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 8C11 antibody are SEQ ID NO: 23, SEQ ID NO: 24 and SEQ ID NO: 25, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 8C11 antibody (457 bp) [capital letters: mouse 8C11 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCA
GCAGTGATGTTGTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGG
AGATCAAGCCTCCATCTCTTGCACATCTAGTCAGACCCTTGTACACAGTAAT
GGAAACACCTATTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAG
CTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCA
GTGGCAGTGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGG
CTGAGGATCTGGGAGTTTATTTCTGCTCTCACAGTACACATGTTCCATTCAC
GTTCGGCTCGGGGACAAAGTTGGAAATAAAAcgggctgatgctgcaccaactgtatccatcttc
ccaccatccagtgagcagttaacatctggag

The amino acid sequence of the light chain variable region of the mouse 8C11 antibody (152 a. a.) [capital letters: mouse 8C11 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MKLPVRLLVLMFWIPASSSDVVMTQTPLSLPVSLGDQASISCTSSQTLVHSNGNTYLH
WYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSHST
HVPFTFGSGTKLEIKradaaptvsifppsseqltsg

CDR1 in the light chain variable region of the 8C11 antibody

TSSQTLVHSNGNTYLH

CDR2 in the light chain variable region of the 8C11 antibody

KVSNRFS

CDR3 in the light chain variable region of the 8C11 antibody

HSTHVP

[0063]

6. Anti-PLD4 mouse 10C3 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 10C3 antibody is SEQ ID NO: 84, and the amino acid sequence is SEQ ID NO: 85. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 10C3 antibody are SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 10C3 antibody (450 bp) [capital letters: mouse 10C3 VH variable region, small letters: mouse IgG2a heavy chain constant region]

ATGAACTTCGGGCTCAGCTTGATTTTCCTTGCCCTCATTTTAAAAGGTGTCC
AGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAGGCCTGGAG
GGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTCAGTTTCAGTAGCTATGG
CATGTCTTGGTTTCGCCAGACTCCAGACAAGAGGCTGGAGTGGGTCGCAAC
CATTAGTAGTGGTGGTAGTTACATCTACTATCCAGAAAGTGTGAAGGGGCG
ATTCACCATCTCCAGAGACAATGCCAGGAACATCCTGTACCTGCAAATGAG
CAGTCTGAAGTCTGAGGACACAGCCATGTATTATTGTGTAAGACTCTACGGT
GGTAGGAGAGGCTATGGTTTGGACTACTGGGGTCAAGGAACCTCAGTCACC
GTCTCCTCAgccaaaacaacagccccatcggtctatcca

The amino acid sequence of the heavy chain variable region of the mouse 10C3 antibody (150 a. a.) [capital letters: mouse 10C3 VH variable region, small letters: mouse IgG2a heavy chain

constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MNFGLSLIFLALILKGVQCEVQLVESGGDLVRPGGSLKLSCAASGFSFSSYGMSWFRQ
TPDKRLEWVATISSGGSYIYYPESVKGRFTISRDNARNILYLQMSSLKSEDTAMYYCV
RLYGGRRGYGLDYWGQGTSVTVSSakttapsvyp

CDR1 in the heavy chain variable region of the 10C3 antibody

SYGMS

CDR2 in the heavy chain variable region of the 10C3 antibody

TISSGGSYIYYPESVKG

CDR3 in the heavy chain variable region of the 10C3 antibody

LYGGRRGYGLDY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 10C3 antibody is SEQ ID NO: 104, and the amino acid sequence is SEQ ID NO: 105. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 10C3 antibody are SEQ ID NO: 29, SEQ ID NO: 30 and SEQ ID NO: 31, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 10C3 antibody (423 bp) [capital letters: mouse 10C3 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGAGGTTCTCTGCTCAGCTTCTGGGGCTGCTTGTGCTCTGGATCCCTGGAT
CCACTGCGGAAATTGTGATGACGCAGGCTGCATTCTCCAATCCAGTCACTCT
TGGAACATCAGCTTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTACATAGT
GATGGCATCACTTATTTGTATTGGTATCTGCAGAAGCCAGGCCAGTCTCCTC
AGCTCCTGATTTATCAGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTT
CAGTAGCAGTGGGTCAGGAACTGATTTCACACTGAGAATCAGCAGAGTGGA
GGCTGAGGATGTGGGTGTTTATTACTGTGCTCAAAATCTAGAACTTTACACG
TTCGGAGGGGGGACCAAGCTGGAAATAAAAcgggctgatgctgcaccaactgtatccatc

The amino acid sequence of the light chain variable region of the mouse 10C3 antibody (141 a. a.) [capital letters: mouse 10C3 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MRFSAQLLGLLVLWIPGSTAEIVMTQAAFSNPVTLGTSASISCRSSKSLLHSDGITYLY
WYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQN
LELYTFGGGTKLEIKradaaptvsi

CDR1 in the light chain variable region of the 10C3 antibody
RSSKSLLHSDGITYLY
CDR2 in the light chain variable region of the 10C3 antibody
QMSNLAS
CDR3 in the light chain variable region of the 10C3 antibody
AQNLEL

[0064]

7. Anti-PLD4 mouse 11D10 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 11D10 antibody is SEQ ID NO: 86, and the amino acid sequence is SEQ ID NO: 87. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 11D10 antibody are SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 11D10 antibody (450 bp) [capital letters: mouse 11D10 VH variable region, small letters: mouse

IgG2b heavy chain constant region]
ATGAACTTCGGGCTCAGCTTGATTTTCCTTGCCCTCATTTTAAAAGGTGTCC
AGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAGGCCTGGAG
GGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATTCAGTTTCAGTAGCTATGG
CATGTCTTGGTTTCGCCAGACTCCAGACAAGAGGCTGGAGTGGGTCGCAAC
CATTAGTAGTGGTGGTAGTTACATCTACTATCCAGAAAGTGTGAAGGGGCG
ATTCACCATCTCCAGAGACAATGCCAGGAACATCCTGTACCTGCAAATGAG
CAGTCTGAAGTCTGAGGACACAGCCATGTATTATTGTGTAAGACTCTACGGT
GGTAGGAGAGGCTATGGTTTGGACTACTGGGGTCAAGGAACCTCAGTCACC
GTCTCCTCAgccaaaacaacacccccatcagtctatcca

The amino acid sequence of the heavy chain variable region of the mouse 11D10 antibody (150 a. a.) [capital letters: mouse 11D10 VH variable region, small letters: mouse IgG2b heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MNFGLSLIFLALILKGVQCEVQLVESGGDLVRPGGSLKLSCAASGFSFSSYGMSWFRQ
TPDKRLEWVATISSGGSYIYYPESVKGRFTISRDNARNILYLQMSSLKSEDTAMYYCV
RLYGGRRGYGLDYWGQGTSVTVSS akttppsvyp

CDR1 in the heavy chain variable region of the 11D10 antibody
SYGMS
CDR2 in the heavy chain variable region of the 11D10 antibody
TISSGGSYIYYPESVKG
CDR3 in the heavy chain variable region of the 11D10 antibody
LYGGRRGYGLDY

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 11D10 antibody is SEQ ID NO: 106, and the amino acid sequence is SEQ ID NO: 107. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 11D10 antibody are SEQ ID

NO: 29, SEQ ID NO: 30 and SEQ ID NO: 31, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 11D10 antibody (423 bp) [capital letters: mouse 11D10 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGAGGTTCTCTGCTCAGCTTCTGGGGCTGCTTGTGCTCTGGATCCCTGGAT
CCACTGCGGAAATTGTGATGACGCAGGCTGCATTCTCCAATCCAGTCACTCT
TGGAACATCAGCTTCCATCTCCTGCAGGTCTAGTAAGAGTCTCCTACATAGT
GATGGCATCACTTATTTGTATTGGTATCTGCAGAAGCCAGGCCAGTCTCCTC
AGCTCCTGATTTATCAGATGTCCAACCTTGCCTCAGGAGTCCCAGACAGGTT
CAGTAGCAGTGGGTCAGGAACTGATTTCACACTGAGAATCAGCAGAGTGGA
GGCTGAGGATGTGGGTGTTTATTACTGTGCTCAAAATCTAGAACTTTACACG
TTCGGAGGGGGGACCAAGCTGGAAATAAAAcgggctgatgctgcaccaactgtatccatc

The amino acid sequence of the light chain variable region of the mouse 11D10 antibody (141 a. a.) [capital letters: mouse 11D10 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MRFSAQLLGLLVLWIPGSTAEIVMTQAAFSNPVTLGTSASISCRSSKSLLHSDGITYLY
WYLQKPGQSPQLLIYQMSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQN
LELYTFGGGTKLEIKradaaptvsi

CDR1 in the light chain variable region of the 11D10 antibody

RSSKSLLHSDGITYLY

CDR2 in the light chain variable region of the 11D10 antibody

QMSNLAS

CDR3 in the light chain variable region of the 11D10 antibody

AQNLEL

[0065]

8. Anti-PLD4 mouse 13D4 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 13D4 antibody is SEQ ID NO: 88, and the amino acid sequence is SEQ ID NO: 89. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 13D4 antibody are SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 13D4 antibody (472 bp) [capital letters: mouse 13D4 VH variable region, small letters: mouse IgG2b heavy chain constant region]

ATGAAAGTGTTGAGTCTGTTGTACCTGTTGACAGCCATTCCTGGTATCCTGT
CTGATGTACAGCTTCAGGAGTCAGGACCTGGCCTCGTGAAACCTTCTCAATC
TCTGTCTCTCACCTGCTCTGTCACTGGCTACTCCATCACCAGTCATTATTACT
GGACCTGGATCCGGCAGTTTCCAGGAAACAAACTGGAATGGATGGGCTACA
TAAGCTACGACGGTAGCAATAACTACAACCCATCTCTCAAAAATCGAATCT
CCATCACTCGTGACACATCTAAGAACCAGTTTTTCCTGAAGTTGAATTCTGT
GACTACTGAGGACACAGCTACATATAACTGTGCAAGAGAGGGCCCGCTCTA
CTATGGTAACCCCTACTGGTATTTCGATGTCTGGGGCGCAGGGACCACGGTC
ACCGTCTCCTCAgccaaaacaacacccccatcagtctatccactggcccctaagggcg

The amino acid sequence of the heavy chain variable region of the mouse 13D4 antibody (157 a. a.) [capital letters: mouse 13D4 VH variable region, small letters: mouse IgG2b heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MKVLSLLYLLTAIPGILSDVQLQESGPGLVKPSQSLSLTCSVTGYSITSHYYWTWIRQF
PGNKLEWMGYISYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYNCAR
EGPLYYGNPYWYFDVWGAGTTVTVSSakttppsvyplapkg

CDR1 in the heavy chain variable region of the 13D4 antibody

SHYYWT

CDR2 in the heavy chain variable region of the 13D4 antibody

YISYDGSNNYNPSLKN

CDR3 in the heavy chain variable region of the 13D4 antibody

EGPLYYGNPYWYFDV

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 13D4 antibody is SEQ ID NO: 108, and the amino acid sequence is SEQ ID NO: 109. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 13D4 antibody are SEQ ID NO: 35, SEQ ID NO: 36 and SEQ ID NO: 37, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 13D4 antibody (404 bp) [capital letters: mouse 13D4 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTAC
CAGATGTGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTG
GGGGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACATTGACAATTAT
TTAAACTGGTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACT
ACACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGT
CTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATGTTG
CCACTTACTTTTGCCAGCAGTTTAATACGCTTCCTCGGACGTTCGGTGGAGG
CACCAAACTGGAAATCAAAcgggctgatgctgcaccaactgt

The amino acid sequence of the light chain variable region of the mouse 13D4 antibody (134 a. a.) [capital letters: mouse 13D4 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDIDNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDVATYFCQQFNTLPR
TFGGGTKLEIKradaapt

CDR1 in the light chain variable region of the 13D4 antibody

RASQDIDNYLN

CDR2 in the light chain variable region of the 13D4 antibody

YTSRLHS

CDR3 in the light chain variable region of the 13D4 antibody

QQFNTLP

[0066]

9. Anti-PLD4 mouse 13H11 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 13H11 antibody is SEQ ID NO: 90, and the amino acid sequence is SEQ ID NO: 91. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 13H11 antibody are SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, respectively.

The nucleic acid sequence of the heavy chain variable region of the anti-PLD4 mouse 13H11 antibody (471 bp) [capital letters: mouse 13H11 VH variable region, small letters: mouse IgG2b heavy chain constant region]

ATGAAAGTGTTGAGTCTGTTGTACCTGTTGACAGCCATTCCTGGTATCCTGT
CTGATGTACAGCTTCAGGAGTCAGGACCTGGCCTCGTGAAACCTTCTCAGTC
TCTGTCTCTCACCTGCTCTGTCACTGGCTACTCCATCTCCAGTCATTATTACT
GGAGTTGGATCCGGCAGTTTCCAGGAAACAGACTGGAATGGATGGGCTACA
TAAGCTACGACGGTAGCAATAACTACAACCCATCTCTCAAAAATCGAATCT
CCATCACTCGTGACACATCTAAGAACCAGTTTTTCCTGAAGTTGAATTCTGT
GACTACTGAGGACACAGCTACATATAACTGTGCAAGAGAGGGCCCGCTCTA
CTATGGTAACCCCTACTGGTATTTCGATGTCTGGGGCGCAGGGACCACGGTC
ACCGTCTCCTCAgccaaaacaacacccccatcagtctatccactggcccctaagggc

The amino acid sequence of the heavy chain variable region of the mouse 13H11 antibody (157 a. a.) [capital letters: mouse 13H11 VH variable region, small letters: mouse IgG2b heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MKVLSLLYLLTAIPGILSDVQLQESGPGLVKPSQSLSLTCSVTGYSISSHYYWSWIRQF
PGNRLEWMGYISYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYNCARE
GPLYYGNPYWYFDVWGAGTTVTVSSakttppsvyplapkg

CDR1 in the heavy chain variable region of the 13H11 antibody
SHYYWS
CDR2 in the heavy chain variable region of the 13H11 antibody
YISYDGSNNYNPSLKN
CDR3 in the heavy chain variable region of the 13H11 antibody
EGPLYYGNPYWYFDV

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 13H11 antibody is SEQ ID NO: 110, and the amino acid sequence is SEQ ID NO: 111. The amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 13H11 antibody are SEQ ID NO: 41, SEQ ID NO: 42 and SEQ ID NO: 43, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 13H11 antibody (414 bp) [capital letters: mouse 13H11 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTAC
CAGATGTGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTG
GGGGGCAGCGTCACCATCAGTTGCAGGGCAAGTCAGGACATTGACAATTAT
TTAAACTGGTATCAGCAAAAACCAGATGGAACTGTTAAACTCCTGATCTACT

ACACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGT
CTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAACAAGAAGATATTG
CCACTTACTTTTGCCAACAGTTTAATACGCTTCCTCGGACGTTCGGTGGAGG
CACCAAGCTGGAAATCAAAcgggctgatgctgcaccaactgtatccatcttc

The amino acid sequence of the light chain variable region of the mouse 13H11 antibody (138 a. a.) [capital letters: mouse 13H11 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGGSVTISCRASQDIDNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQFNTLPR
TFGGGTKLEIKradaaptvsif

CDR1 in the light chain variable region of the 13H11 antibody

RASQDIDNYLN

CDR2 in the light chain variable region of the 13H11 antibody

YTSRLHS

CDR3 in the light chain variable region of the 13H11 antibody

QQFNTLP

[0067]

10. Anti-PLD4 mouse 14C1 antibody

The nucleic acid sequence of the heavy chain variable region of the obtained anti-PLD4 mouse 14C1 antibody is SEQ ID NO: 92, and the amino acid sequence is SEQ ID NO: 93. The amino acid sequences of CDR1, CDR2 and CDR3 within the heavy chain variable region of the mouse 14C1 antibody are SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, respectively.

The nucleic acid sequence of the heavy chain variable

region of the anti-PLD4 mouse 14C1 antibody (470 bp) [capital letters: mouse 14C1 VH variable region, small letters: mouse IgG1 heavy chain constant region]

ATGAAAGTGTTGAGTCTGTTGTACCTGTTGACAGCCATTCCTGGTATCCTGT
CTGATGTACAGCTTCAGGAGTCAGGACCTGGCCTCGTGAAACCTTCTCAGTC
TCTGTCTCTCACCTGCTCTGTCACTGGCTACTCCATCTCCAGTCATTATTACT
GGAGTTGGATCCGGCAGTTTCCAGGAAACAGACTGGAATGGATGGGCTACA
TAAGCTACGACGGTAGCAATAACTACAACCCATCTCTCAAAAATCGAATCT
CCATCACTCGTGACACATCTAAGAACCAGTTTTTCCTGAAGTTGAATTCTGT
GACTACTGAGGACACAGCTACATATAACTGTGCAAGAGAGGGCCCGCTCTA
CTATGGTAACCCCTACTGGTATTTCGATGTCTGGGGCGCAGGGACCACGGTC
ACCGTCTCCTCAgccaaaacgacacccccatctgtctatccactggcccctaaggg

The amino acid sequence of the heavy chain variable region of the mouse 14C1 antibody (156 a. a.) [capital letters: mouse 14C1 VH variable region, small letters: mouse IgG1 heavy chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MKVLSLLYLLTAIPGILSDVQLQESGPGLVKPSQSLSLTCSVTGYSISSHYYWSWIRQF
PGNRLEWMGYISYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTTEDTATYNCARE
GPLYYGNPYWYFDVWGAGTTVTVSS akttppsvyplapk

CDR1 in the heavy chain variable region of the 14C1 antibody
SHYYWS
CDR2 in the heavy chain variable region of the 14C1 antibody
YISYDGSNNYNPSLKN
CDR3 in the heavy chain variable region of the 14C1 antibody
EGPLYYGNPYWYFDV

The nucleic acid sequence of the light chain variable region of the obtained anti-PLD4 mouse 14C1 antibody is SEQ ID NO: 112, and the amino acid sequence is SEQ ID NO: 113. The

amino acid sequences of CDR1, CDR2 and CDR3 within the light chain variable region of the mouse 14C1 antibody are SEQ ID NO: 41, SEQ ID NO: 42 and SEQ ID NO: 43, respectively.

The nucleic acid sequence of the light chain variable region of the anti-PLD4 mouse 14C1 antibody (465 bp) [capital letters: mouse 14C1 VL variable region, small letters: mouse Ig κ light chain constant region]

ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTAC
CAGATGTGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTG
GGGGGCAGCGTCACCATCAGTTGCAGGGCAAGTCAGGACATTGACAATTAT
TTAAACTGGTATCAGCAAAAACCAGATGGAACTGTTAAACTCCTGATCTACT
ACACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGT
CTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAACAAGAAGATATTG
CCACTTACTTTTGCCAACAGTTTAATACGCTTCCTCGGACGTTCGGTGGAGG
CACCAAGCTGGAAATCAAAcgggctgatgctgcaccaactgtatccatcttcccaccatccagtgagcagtt
aacatctggaggtgcctcagtcgtgtgcttc

The amino acid sequence of the light chain variable region of the mouse 14C1 antibody (155 a. a.) [capital letters: mouse 14C1 VL variable region, small letters: mouse Ig κ light chain constant region] The underlined sequence shows its signal sequence and the double underline shows its CDR regions (CDR1, CDR2 and CDR3).

MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGGSVTISCRASQDIDNYLNWYQ
QKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQFNTLPR
TFGGGTKLEIKradaaptvsifppsseqltsggasvvcf

CDR1 in the light chain variable region of the 14C1 antibody

RASQDIDNYLN

CDR2 in the light chain variable region of the 14C1 antibody

YTSRLHS

CDR3 in the light chain variable region of the 14C1 antibody

QQFNTLP

[0068]

The base sequences and the amino acid sequences of the heavy chain and the light chain of the created chimeric 11G9.6 antibody are as the sequence numbers given below.

Heavy chain

SEQ ID NO: 120 (base sequence)

SEQ ID NO: 121 (amino acid sequence)

Light chain

SEQ ID NO: 122 (base sequence)

SEQ ID NO: 123 (amino acid sequence)

[0069]

11. The nucleic acid sequence of the heavy chain of the anti-PLD4 chimeric 11G9.6 antibody (1401 bp) [capital letters: chimeric 11G9 VH variable region, small letters: human IgG1 heavy chain constant region] (SEQ ID NO: 120)

ATGAAAGTGTTGAGTCTGTTGTACCTGTTGACAGCCATTCCTGGTATCCTGT
CTcagGTCCAACTGCAGCAGCCTGGGGCTGAACTGGTGAAGCCTGGGACTTC
AGTGAAAATGTCCTGCAAGGCTTCTGGCTACACCTTCACCAGCTACTGGATG
CACTGGGTGAAGCAGAGGCCGGGACAAGGCCTTGAGTGGATTGGAGATATT
TATCCTGGTAGTGATAGTACTAACTACAATGAGAAGTTCAAGAGCAAGGCC
ACACTGACTGTAGACACATCCTCCAGCACAGCCTACATGCAACTCAGCAGC
CTGACATCTGAGGACTCTGCGGTCTATTACTGTGCAAGAGGAGGGTGGTTG
GATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAgctagc
accaagggcccatcggtcttcccctggcaccctcctccaagagcacctctgggggcacagcggccctgggctgcctggtc
aaggactacttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcggcgtgcacaccttcccggctgtcc
tacagtcctcaggactctactccctcagcagcgtggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaac
gtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaatcttgtgacaaaactcacacatgcccaccgt
gcccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacc
cctgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggag
gtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcac
caggactggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctcc
aaagccaaagggcagccccgagaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtca
gcctgacctgcctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcagccggagaacaact
acaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaagctcaccgtggacaagagcaggtggca
gcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgg

gtaaatga

[0070]

12. The amino acid sequence of the heavy chain of the anti-PLD4 chimeric 11G9.6 antibody (466 a. a.) [capital letters: chimeric 11G9 VH variable region, small letters: human IgG1 heavy chain constant region] (SEQ ID NO: 121)
MKVLSLLYLLTAIPGILSQVQLQQPGAELVKPGTSVKMSCKASGYTFTSYWMH
WVKQRPGQGLEWIGDIYPGSDSTNYNEKFKSKATLTVDTSSSTAYMQLSSLTSE
DSAVYYCARGGWLDAMDYWGQGTSVTVSSastkgpsvfplapsskstsggtaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppcpape
llggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqd
wlngkeykckvsnkalpapiektiskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpenny
kttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk

[0071]

13. The nucleic acid sequence of the light chain of the anti-PLD4 chimeric 11G9.6 antibody (705 bp) [capital letters: chimeric 11G9 VL variable region, small letters: human Ig κ light chain constant region] (SEQ ID NO: 122)
ATGATGTCCTCTGCTCAGTTCCTTGGTCTCCTGTTGCTCTGTTTTCAAGGTAC
CAGATGTGATATCCAGATGACACAGACTACATCCTCCCTGTCTGCCTCTCTG
GGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACATTAGCAATTAT
TTAAACTGGTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACT
ACACATCAAGATTACACTCAGGAGTCCCATCAAGGTTCAGTGGCAGTGGGT
CTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTG
CCACTTACTTTTGCCAACAGGGTAATACGCTTCCGTGGACGTTCGGTGGAGG
CACCAAGCTGGAAATCAAAcgaactgtggctgcaccatctgtcttcatcttcccgccatctgatgagcagttg
aaatctggaactgcctctgttgtgtgcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgcc
ctccaatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcaccctgac
gctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggcctgagctcgcccgtcacaaa
gagcttcaacaggggagagtgctag

[0072]

14. The amino acid sequence of the light chain of the anti-PLD4 chimeric 11G9.6 antibody (234 a. a.) [capital letters: chimeric 11G9 VL variable region, small letters: human Ig κ light chain constant region] (SEQ ID NO: 123)
MMSSAQFLGLLLLCFQGTRCDIQMTQTTSSLSASLGDRVTISCRASQDISNYLN
WYQQKPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQ

QGNTLPWTFGGGTKLEIKrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsq
esvteqdskdstyslsstltlskadyekhkvyacevthqglsspvtksfnrgec

[0073]

<cDNA and protein sequences of PLD4-related molecules>

> Human PLD4 cDNA (1521 bp) (SEQ ID NO: 44)
ATGCTGAAGCCTCTTTGGAAAGCAGCAGTGGCCCCCACATGGCCATGCTCC
ATGCCGCCCCGCCGCCCGTGGGACAGAGAGGCTGGCACGTTGCAGGTCCTG
GGAGCGCTGGCTGTGCTGTGGCTGGGCTCCGTGGCTCTTATCTGCCTCCTGT
GGCAAGTGCCCCGTCCTCCCACCTGGGGCCAGGTGCAGCCCAAGGACGTGC
CCAGGTCCTGGGAGCATGGCTCCAGCCCAGCTTGGGAGCCCCTGGAAGCAG
AGGCCAGGCAGCAGAGGGACTCCTGCCAGCTTGTCCTTGTGGAAAGCATCC
CCCAGGACCTGCCATCTGCAGCCGGCAGCCCCTCTGCCCAGCCTCTGGGCCA
GGCCTGGCTGCAGCTGCTGGACACTGCCCAGGAGAGCGTCCACGTGGCTTC
ATACTACTGGTCCCTCACAGGGCCTGACATCGGGGTCAACGACTCGTCTTCC
CAGCTGGGAGAGGCTCTTCTGCAGAAGCTGCAGCAGCTGCTGGGCAGGAAC
ATTTCCCTGGCTGTGGCCACCAGCAGCCCGACACTGGCCAGGACATCCACC
GACCTGCAGGTTCTGGCTGCCCGAGGTGCCCATGTACGACAGGTGCCCATG
GGGCGGCTCACCAGGGGTGTTTTGCACTCCAAATTCTGGGTTGTGGATGGAC
GGCACATATACATGGGCAGTGCCAACATGGACTGGCGGTCTCTGACGCAGG
TGAAGGAGCTTGGCGCTGTCATCTATAACTGCAGCCACCTGGCCCAAGACC
TGGAGAAGACCTTCCAGACCTACTGGGTACTGGGGGTGCCCAAGGCTGTCC
TCCCCAAAACCTGGCCTCAGAACTTCTCATCTCACTTCAACCGTTTCCAGCC
CTTCCACGGCCTCTTTGATGGGGTGCCCACCACTGCCTACTTCTCAGCGTCG
CCACCAGCACTCTGTCCCCAGGGCCGCACCCGGGACCTGGAGGCGCTGCTG
GCGGTGATGGGGAGCGCCCAGGAGTTCATCTATGCCTCCGTGATGGAGTAT
TTCCCCACCACGCGCTTCAGCCACCCCCCGAGGTACTGGCCGGTGCTGGACA
ACGCGCTGCGGGCGGCAGCCTTCGGCAAGGGCGTGCGCGTGCGCCTGCTGG
TCGGCTGCGGACTCAACACGGACCCCACCATGTTCCCCTACCTGCGGTCCCT
GCAGGCGCTCAGCAACCCCGCGGCCAACGTCTCTGTGGACGTGAAAGTCTT
CATCGTGCCGGTGGGGAACCATTCCAACATCCCATTCAGCAGGGTGAACCA
CAGCAAGTTCATGGTCACGGAGAAGGCAGCCTACATAGGCACCTCCAACTG
GTCGGAGGATTACTTCAGCAGCACGGCGGGGGTGGGCTTGGTGGTCACCCA
GAGCCCTGGCGCGCAGCCCGCGGGGGCCACGGTGCAGGAGCAGCTGCGGC
AGCTCTTTGAGCGGGACTGGAGTTCGCGCTACGCCGTCGGCCTGGACGGAC
AGGCTCCGGGCCAGGACTGCGTTTGGCAGGGCTGA

> Human PLD4 protein (506 amino acids) (SEQ ID NO: 1)
MLKPLWKAAVAPTWPCSMPPRRPWDREAGTLQVLGALAVLWLGSVALICLL
WQVPRPPTWGQVQPKDVPRSWEHGSSPAWEPLEAEARQQRDSCQLVLVESIPQ
DLPSAAGSPSAQPLGQAWLQLLDTAQESVHVASYYWSLTGPDIGVNDSSSQLG
EALLQKLQQLLGRNISLAVATSSPTLARTSTDLQVLAARGAHVRQVPMGRLTR
GVLHSKFWVVDGRHIYMGSANMDWRSLTQVKELGAVIYNCSHLAQDLEKTFQ
TYWVLGVPKAVLPKTWPQNFSSHFNRFQPFHGLFDGVPTTAYFSASPPALCPQ
GRTRDLEALLAVMGSAQEFIYASVMEYFPTTRFSHPPRYWPVLDNALRAAAFG
KGVRVRLLVGCGLNTDPTMFPYLRSLQALSNPAANVSVDVKVFIVPVGNHSNI

PFSRVNHSKFMVTEKAAYIGTSNWSEDYFSSTAGVGLVVTQSPGAQPAGATVQ
EQLRQLFERDWSSRYAVGLDGQAPGQDCVWQG

> Cynomolgus monkey PLD4 cDNA (1521 bp) (SEQ ID NO: 63)
ATGCTGAAGCCTCTTCGGAGAGCgGCAGTGACCCCCATGTGGCCGTGCTCCA
TGCTGCCCCGCCGCCTGTGGGACAGAGAGGCTGGCACGTTGCAGGTCCTGG
GAGTGCTGGCTATGCTGTGGCTGGGCTCCATGGCTCTTACCTACCTCCTGTG
GCAAGTGCGCCGTCCTCCCACCTGGGGCCAGGTGCAGCCCAAGGACGTGCC
CAGGTCCTGGGGGGCATGGTTCCAGCCCAGCTCTGGAGCCCCTGGAAGCGGA
GGTCAGGAAGCAGAGGGACTCCTGCCAGCTTGTCCTTGTGGAAAGCATCCC
CCAGGACCTGCCATTTGCAGCCGGCAGCCTCTCCGCCCAGCCTCTGGGCCAG
GCCTGGCTGCAGCTGCTGGACACTGCCCAGGAGAGCGTCCACGTGGCTTCA
TACTACTGGTCCCTCACAGGGCCCGACATTGGGGTCAACGACTCATCTTCCC
AGCTGGGAGAGGCCCTTCTGCAGAAGCTGCAGCAGCTGCTGGGCAGGAACA
TTTCCTTGGCTGTGGCCACCAGCAGTCCAACACTGGCCAGGAAGTCCACCG
ACCTGCAGGTCCTGGCTGCCCGAGGTGCCCAGGTACGACGGGTGCCCATGG
GGCGGCTCACCAGGGGCGTTTTGCACTCCAAATTCTGGGTTGTGGATGGACg
GCACATATACATGGGCAGTGCcAACATGGACTGGCGGTCCCTGACGCAGGT
GAAGGAGCTTGGCGCTGTCATCTATAACTGCAGCCACCTGGCCCAAGACCT
GGAGAAGACCTTCCAGACCTACTGGGTGCTGGGGGGTGCCCAAGGCTGTCCT
CCCCAAAACCTGGCCTCAGAACTTCTCATCTCACATCAACCGTTTCCAGCCC
TTCCAGGGCCTCTTTGATGGGGTGCCCACCACTGCCTACTTCTCAGCATCGC
CACCcGCACTCTGTCCCCAGGGCCGCACCCCTGACCTGGAGGCGCTGTTGGC
GGTGATGGGGAGCGCCCAGGAGTTCATCTATGCCTCCGTGATGGAGTATTTC
CCTACCACgCGCTTCAGCCACCCCCGCAGGTACTGGCCGGTGCTGGACAACG
CGCTGCGGGCGGCAGCCTTCAGCAAGGGTGTGCGCGTGCGCCTGCTGGTCA
GCTGCGGACTCAACACGGACCCCACCATGTTCCCCTATCTGCGGTCCCTGCA
GGCGCTCAGCAACCCCGCGGCCAACGTCTCTGTGGACGTGAAAGTCTTCAT
CGTGCCGGTGGGGAATCATTCCAACATCCCGTTCAGCAGGGTGAACCACAG
CAAGTTCATGGTCACGGAGAAGGCAGCCTACATAGGCACCTCCAACTGGTC
GGAGGATTACTTCAGCAGCACGACGGGGGTGGGCCTGGTGGTCACCCAGAG
CCCCGGCGCGCAGCCCGCGGGGGCCACGGTACAGGAGCAGCTGCGGCAGCT
CTTTGAGCGGGACTGGAGTTCGCGCTACGCCGTCGGCCTGGACGGACAGGC
TCCGGGCCAGGACTGCGTTTGGCAGGGCTGA

> Cynomolgus monkey PLD4 protein (506 amino acids) (SEQ ID NO: 129)
MLKPLRRAAVTPMWPCSMLPRRLWDREAGTLQVLGVLAMLWLGSMALTYLL
WQVRRPPTWGQVQPKDVPRSWGHGSSPALEPLEAEVRKQRDSCQLVLVESIPQ
DLPFAAGSLSAQPLGQAWLQLLDTAQESVHVASYYWSLTGPDIGVNDSSSQLG
EALLQKLQQLLGRNISLAVATSSPTLARKSTDLQVLAARGAQVRRVPMGRLTR
GVLHSKFWVVDGRHIYMGSANMDWRSLTQVKELGAVIYNCSHLAQDLEKTFQ
TYWVLGVPKAVLPKTWPQNFSSHINRFQPFQGLFDGVPTTAYFSASPPALCPQG
RTPDLEALLAVMGSAQEFIYASVMEYFPTTRFSHPRRYWPVLDNALRAAAFSK
GVRVRLLVSCGLNTDPTMFPYLRSLQALSNPAANVSVDVKVFIVPVGNHSNIPF

SRVNHSKFMVTEKAAYIGTSNWSEDYFSSTTGVGLVVTQSPGAQPAGATVQEQ
LRQLFERDWSSRYAVGLDGQAPGQDCVWQG

> Rhesus monkey PLD4 cDNA (1521 bp) (SEQ ID NO: 124)
ATGCTGAAGCCTCTTCGGAGAGCGGCAGTGACCCCCATGTGGCCGTGCTCC
ATGCTGCCCCGCCGCCTGTGGGACAGAGAGGCTGGCACGTTGCAGGTCCTG
GGAGTGCTGGCTATGCTGTGGCTGGGCTCCATGGCTCTTACCTACCTCCTGT
GGCAAGTGCGCTGTCCTCCCACCTGGGGCCAGGTGCAGCCCAGGGACGTGC
CCAGGTCCTGGGGGGCATGGTTCCAGCCTAGCTCTGGAGCCCCTGGAAGCGG
AGGTCAGGAAGCAGAGGGACTCCTGCCAGCTTGTCCTTGTGGAAAGCATCC
CCCAGGACCTGCCATTTGCAGCCGGCAGCCTCTCCGCCCAGCCTCTGGGCCA
GGCCTGGCTGCAGCTGCTGGACACTGCCCAGGAGAGCGTCCACGTGGCTTC
ATACTACTGGTCCCTCACAGGGCCCGACATTGGGGTCAACGACTCATCTTCC
CAGCTGGGAGAGGCCCTTCTGCAGAAGCTGCAGCAGCTGCTGGGCAGGAAC
ATTTCCTTGGCTGTGGCCACCAGCAGTCCAACACTGGCCAGGAAGTCCACC
GACCTGCAGGTCCTGGCTGCCCGAGGTGCCCAGGTACGACGGGTGCCCATG
GGGCGGCTCACCAGGGGCGTTTTGCACTCCAAATTCTGGGTTGTGGATGGA
CGGCACATATACATGGGCAGTGCCAACATGGACTGGCGGTCCCTGACGCAG
GTGAAGGAGCTTGGCGCTGTCATCTATAACTGCAGCCACCTGGCCCAAGAC
CTGGAGAAGACCTTCCAGACCTACTGGGTGCTGGGGGGTGCCCAAGGCTGTC
CTCCCCAAAACCTGGCCTCAGAACTTCTCATCTCACATCAACCGTTTCCAGC
CCTTCCAGGGCCTCTTTGATGGGGTGCCCACCACTGCCTACTTCTCAGCATC
GCCACCCGCACTCTGTCCCCAGGGCCGCACCCCTGACCTGGAGGCGCTGTTG
GCGGTGATGGGGAGCGCCCAGGAGTTCATCTATGCCTCCGTGATGGAGTAT
TTCCCTACCACGCGCTTCAGCCACCCCCGCAGGTACTGGCCGGTGCTGGACA
ACGCGCTGCGGGCGGCAGCCTTCAGCAAGGGTGTGCGCGTGCGCCTGCTGG
TCAGCTGCGGACTCAACACGGACCCCACCATGTTCCCCTATCTGCGGTCCCT
GCAGGCGCTCAGCAACCCCGCGGCCAACGTCTCTGTGGACGTGAAAGTCTT
CATCGTGCCGGTGGGGAATCATTCCAACATCCCGTTCAGCAGGGTGAACCA
CAGCAAGTTCATGGTCACGGAGAAGGCAGCCTACATAGGCACCTCCAACTG
GTCGGAGGATTACTTCAGCAGCACGACGGGGGTGGGCCTGGTGGTCACCCA
GAGCCCCGGCGCGCAGCCCGCGGGGGCCACGGTACAGGAGCAGCTGCGGC
AGCTCTTTGAGCGGGACTGGAGTTCGCGCTACGCCGTCGGCCTGGACGGAC
AGGCTCCGGGCCAGGACTGCGTTTGGCAGGGCTGA

> Rhesus monkey PLD4 protein (506 amino acids) (SEQ ID NO: 130)
MLKPLRRAAVTPMWPCSMLPRRLWDREAGTLQVLGVLAMLWLGSMALTYLL
WQVRCPPTWGQVQPRDVPRSWGHGSSLALEPLEAEVRKQRDSCQLVLVESIPQ
DLPFAAGSLSAQPLGQAWLQLLDTAQESVHVASYYWSLTGPDIGVNDSSSQLG
EALLQKLQQLLGRNISLAVATSSPTLARKSTDLQVLAARGAQVRRVPMGRLTR
GVLHSKFWVVDGRHIYMGSANMDWRSLTQVKELGAVIYNCSHLAQDLEKTFQ
TYWVLGVPKAVLPKTWPQNFSSHINRFQPFQGLFDGVPTTAYFSASPPALCPQG
RTPDLEALLAVMGSAQEFIYASVMEYFPTTRFSHPRRYWPVLDNALRAAAFSK
GVRVRLLVSCGLNTDPTMFPYLRSLQALSNPAANVSVDVKVFIVPVGNHSNIPF
SRVNHSKFMVTEKAAYIGTSNWSEDYFSSTTGVGLVVTQSPGAQPAGATVQEQ

LRQLFERDWSSRYAVGLDGQAPGQDCVWQG

> Mouse PLD4 cDNA (1512 base pairs) (SEQ ID NO: 131)

ATGGACAAGAAGAAAGAGCACCCAGAGATGCGGATACCACTCCAGACAGC
AGTGGAGGTCTCTGATTGGCCCTGCTCCACATCTCATGATCCACATAGCGGA
CTTGGCATGGTACTGGGGATGCTAGCTGTACTGGGACTCAGCTCTGTGACTC
TCATCTTGTTCCTGTGGCAAGGGGCCACTTCTTTCACCAGTCATCGGATGTT
CCCTGAGGAAGTGCCCTCCTGGTCCTGGGAGACCCTGAAAGGAGACGCTGA
GCAGCAGAATAACTCCTGTCAGCTCATCCTTGTGGAAAGCATCCCCGAGGA
CTTGCCATTTGCAGCTGGCAGCCCCACTGCCCAGCCCCTGGCCCAGGCTTGG
CTGCAGCTTCTTGACACTGCTCGGGAGAGCGTCCACATTGCCTCGTACTACT
GGTCCCTCACTGGACTGGACATTGGAGTCAATGACTCGTCTTCTCGGCAGGG
AGAGGCCCTTCTACAGAAGTTCCAACAGCTTCTTCTCAGGAACATCTCTGTG
GTGGTGGCCACCCACAGCCCAACATTGGCCAAGACATCCACTGACCTCCAG
GTCTTGGCTGCCCATGGTGCCCAGATACGACAAGTGCCCATGAAACAGCTT
ACTGGGGGTGTTCTACACTCCAAATTCTGGGTTGTGGATGGGCGACACGTCT
ACGTGGGCAGCGCCAACATGGACTGGCGGTCCCTGACTCAGGTGAAGGAAC
TTGGTGCAATCATCTACAACTGCAGCAACCTGGCTCAAGACCTTGAGAAAA
CATTCCAGACCTACTGGGTGCTAGGGACTCCCCAAGCTGTTCTCCCTAAAAC
CTGGCCTCGGAACTTCTCATCCCACATCAACCGCTTCCATCCCTTGCGGGGT
CCCTTTGATGGGGTTCCCACCACGGCCTATTTCTCGGCCTCCCCTCCCTCCCT
CTGCCCGCATGGCCGGACCCGGGATCTGGACGCAGTGTTGGGAGTGATGGA
GGGTGCTCGCCAGTTCATCTATGTCTCGGTGATGGAGTATTTCCCTACCACG
CGCTTCACCCACCATGCCAGGTACTGGCCCGTGCTGGACAATGCGCTACGG
GCAGCGGCCCTCAATAAGGGTGTGCATGTGCGCTTACTGGTCAGCTGCTGGT
TCAACACAGACCCCACCATGTTCGCTTATCTGAGGTCCCTGCAGGCTTTCAG
TAACCCCTCGGCTGGCATCTCAGTGGATGTGAAAGTCTTCATCGTGCCTGTG
GGAAATCATTCCAACATCCCGTTCAGCCGCGTGAACCACAGCAAGTTCATG
GTCACAGACAAGACAGCCTATGTAGGCACCTCTAACTGGTCAGAAGACTAC
TTCAGCCACACCGCTGGTGTGGGCCTGATTGTCAGCCAGAAGACCCCCAGA
GCCCAGCCAGGCGCAACCACCGTGCAGGAGCAGCTGAGGCAACTCTTTGAA
CGAGACTGGAGTTCCCACTATGCTATGGACCTAGACAGACAAGTCCCGAGC
CAGGACTGTGTCTGGTAG

> Mouse PLD4 protein (503 amino acids) (SEQ ID NO: 132)

MDKKKEHPEMRIPLQTAVEVSDWPCSTSHDPHSGLGMVLGMLAVLGLSSVTLI
LFLWQGATSFTSHRMFPEEVPSWSWETLKGDAEQQNNSCQLILVESIPEDLPFA
AGSPTAQPLAQAWLQLLDTARESVHIASYYWSLTGLDIGVNDSSSRQGEALLQ
KFQQLLLRNISVVVATHSPTLAKTSTDLQVLAAHGAQIRQVPMKQLTGGVLHS
KFWVVDGRHVYVGSANMDWRSLTQVKELGAIIYNCSNLAQDLEKTFQTYWV
LGTPQAVLPKTWPRNFSSHINRFHPLRGPFDGVPTTAYFSASPPSLCPHGRTRDL
DAVLGVMEGARQFIYVSVMEYFPTTRFTHHARYWPVLDNALRAAALNKGVH
VRLLVSCWFNTDPTMFAYLRSLQAFSNPSAGISVDVKVFIVPVGNHSNIPFSRVN
HSKFMVTDKTAYVGTSNWSEDYFSHTAGVGLIVSQKTPRAQPGATTVQEQLR
QLFERDWSSHYAMDLDRQVPSQDCVW

> Human PLD3 cDNA sequence (SEQ ID NO: 55)

ATGAAGCCTAAACTGATGTACCAGGAGCTGAAGGTGCCTGCAGAGGAGCCC
GCCAATGAGCTGCCCATGAATGAGATTGAGGCGTGGAAGGCTGCGGAAAA
GAAAGCCCGCTGGGTCCTGCTGGTCCTCATTCTGGCGGTTGTGGGCTTCGGA
GCCCTGATGACTCAGCTGTTTCTATGGGAATACGGCGACTTGCATCTCTTTG
GGCCCAACCAGCGCCCAGCCCCCTGCTATGACCCTTGCGAAGCAGTGCTGG
TGGAAAGCATTCCTGAGGGCCTGGACTTCCCCAATGCCTCCACGGGGAACC
CTTCCACCAGCCAGGCCTGGCTGGGCCTGCTCGCCGGTGCGCACAGCAGCC
TGGACATCGCCTCCTTCTACTGGACCCTCACCAACAATGACACCCACACGCA
GGAGCCCTCTGCCCAGCAGGGTGAGGAGGTCCTCCGGCAGCTGCAGACCCT
GGCACCAAAGGGCGTGAACGTCCGCATCGCTGTGAGCAAGCCCAGCGGGCC
CCAGCCACAGGCGGACCTGCAGGCTCTGCTGCAGAGCGGTGCCCAGGTCCG
CATGGTGGACATGCAGAAGCTGACCCATGGCGTCCTGCATACCAAGTTCTG
GGTGGTGGACCAGACCCACTTCTACCTGGGCAGTGCCAACATGGACTGGCG
TTCACTGACCCAGGTCAAGGAGCTGGGCGTGGTCATGTACAACTGCAGCTG
CCTGGCTCGAGACCTGACCAAGATCTTTGAGGCCTACTGGTTCCTGGGCCAG
GCAGGCAGCTCCATCCCATCAACTTGGCCCCGGTTCTATGACACCCGCTACA
ACCAAGAGACACCAATGGAGATCTGCCTCAATGGAACCCCTGCTCTGGCCT
ACCTGGCGAGTGCGCCCCCACCCCTGTGTCCAAGTGGCCGCACTCCAGACCT
GAAGGCTCTACTCAACGTGGTGGACAATGCCCGGAGTTTCATCTACGTCGCT
GTCATGAACTACCTGCCCACTCTGGAGTTCTCCCACCCTCACAGGTTCTGGC
CTGCCATTGACGATGGGCTGCGGCGGGCCACCTACGAGCGTGGCGTCAAGG
TGCGCCTGCTCATCAGCTGCTGGGGACACTCGGAGCCATCCATGCGGGCCTT
CCTGCTCTCTCTGGCTGCCCTGCGTGACAACCATACCCACTCTGACATCCAG
GTGAAACTCTTTGTGGTCCCCGCGGATGAGGCCCAGGCTCGAATCCCATATG
CCCGTGTCAACCACAACAAGTACATGGTGACTGAACGCGCCACCTACATCG
GAACCTCCAACTGGTCTGGCAACTACTTCACGGAGACGGCGGGCACCTCGC
TGCTGGTGACGCAGAATGGGAGGGGCGGCCTGCGGAGCCAGCTGGAGGCC
ATTTTCCTGAGGGACTGGGACTCCCCTTACAGCCATGACCTTGACACCTCAG
CTGACAGCGTGGGCAACGCCTGCCGCCTGCTCTGA

> Human PLD3 protein (490 amino acids) (SEQ ID NO: 127)

MKPKLMYQELKVPAEEPANELPMNEIEAWKAAEKKARWVLLVLILAVVGFGA
LMTQLFLWEYGDLHLFGPNQRPAPCYDPCEAVLVESIPEGLDFPNASTGNPSTS
QAWLGLLAGAHSSLDIASFYWTLTNNDTHTQEPSAQQGEEVLRQLQTLAPKGV
NVRIAVSKPSGPQPQADLQALLQSGAQVRMVDMQKLTHGVLHTKFWVVDQT
HFYLGSANMDWRSLTQVKELGVVMYNCSCLARDLTKIFEAYWFLGQAGSSIPS
TWPRFYDTRYNQETPMEICLNGTPALAYLASAPPPLCPSGRTPDLKALLNVVDN
ARSFIYVAVMNYLPTLEFSHPHRFWPAIDDGLRRATYERGVKVRLLISCWGHSE
PSMRAFLLSLAALRDNHTHSDIQVKLFVVPADEAQARIPYARVNHNKYMVTER
ATYIGTSNWSGNYFTETAGTSLLVTQNGRGGLRSQLEAIFLRDWDSPYSHDLDT
SADSVGNACRLL

> Human PLD5 cDNA (1338 base pairs) (SEQ ID NO: 56)

ATGGGAGAGGATGAGGATGGACTCTCAGAAAAAAATTGCCAAAATAAATG
TCGAATTGCCCTGGTGGAAAATATTCCTGAAGGCCTTAACTATTCAGAAAAT
GCACCATTTCACTTATCACTTTTCCAAGGCTGGATGAATTTACTCAACATGG
CCAAAAAGTCTGTTGACATAGTGTCTTCCCATTGGGATCTCAACCACACTCA
TCCATCAGCATGTCAGGGTCAACGTCTTTTTGAAAAGTTGCTCCAGCTGACT
TCGCAAAATATTGAAATCAAGCTAGTGAGTGATGTAACAGCTGATTCAAAG
GTATTAGAAGCCTTGAAATTAAAGGGAGCCGAGGTGACGTACATGAACATG
ACCGCTTACAACAAGGGCCGGCTGCAGTCCTCCTTCTGGATCGTGGACAAA
CAGCACGTGTATATCGGCAGTGCCGGTTTGGACTGGCAATCCCTGGGACAG
ATGAAAGAACTCGGTGTCATCTTCTACAACTGCAGCTGCCTGGTCCTAGATT
TACAAAGGATATTTGCTCTATATAGTTCATTAAAATTCAAAAGCAGAGTGCC
TCAAACCTGGTCCAAAAGACTCTATGGAGTCTATGACAATGAAAAGAAATT
GCAACTTCAGTTGAATGAAACCAAATCTCAAGCATTTGTATCGAATTCTCCA
AAACTCTTTTGCCCTAAAAACAGAAGTTTTGACATAGATGCCATCTACAGTG
TGATAGATGATGCCAAGCAGTATGTGTACATCGCTGTCATGGACTACCTGCC
TATCTCCAGCACAAGCACCAAAAGGACTTACTGGCCAGACTTGGATGCAAA
AATAAGAGAAGCATTAGTTTTACGAAGCGTTAGAGTTCGACTCCTTTTAAGC
TTCTGGAAGGAAACTGATCCCCTTACGTTTAACTTTATTTCATCTCTTAAAGC
GATTTGCACTGAAATAGCCAACTGCAGTTTGAAAGTTAAATTTTTTGATCTG
GAAAGAGAGAATGCTTGTGCTACAAAAGAACAAAAGAATCACACCTTTCCT
AGGTTAAATCGCAACAAGTACATGGTGACAGATGGAGCAGCTTATATTGGA
AATTTTGATTGGGTAGGGAATGATTTCACTCAGAATGCTGGCACGGGCCTTG
TTATCAACCAGGCAGATGTGAGGAACAACAGAAGCATCATTAAGCAACTTA
AAGATGTGTTTGAAAGGGACTGGTATTCACCGTATGCCAAAACCTTACAGC
CAACCAAACAGCCGAACTGCTCAAGCCTGTTCAAACTCAAACCCCTCTCCA
ACAAAACTGCCACAGACGACACAGGCGGAAAGGATCCCCGGAACGTATGA

> Human PLD5 protein (445 amino acids) (SEQ ID NO: 128)

MGEDEDGLSEKNCQNKCRIALVENIPEGLNYSENAPFHLSLFQGWMNLLNMA
KKSVDIVSSHWDLNHTHPSACQGQRLFEKLLQLTSQNIEIKLVSDVTADSKVLE
ALKLKGAEVTYMNMTAYNKGRLQSSFWIVDKQHVYIGSAGLDWQSLGQMKE
LGVIFYNCSCLVLDLQRIFALYSSLKFKSRVPQTWSKRLYGVYDNEKKLQLQLN
ETKSQAFVSNSPKLFCPKNRSFDIDAIYSVIDDAKQYVYIAVMDYLPISSTSTKRT
YWPDLDAKIREALVLRSVRVRLLLSFWKETDPLTFNFISSLKAICTEIANCSLKV
KFFDLERENACATKEQKNHTFPRLNRNKYMVTDGAAYIGNFDWVGNDFTQNA
GTGLVINQADVRNNRSIIKQLKDVFERDWYSPYAKTLQPTKQPNCSSLFKLKPL
SNKTATDDTGGKDPRNV

> Human PLD4-Ig fusion protein cDNA (2142 bp) (SEQ ID NO: 125)

ATGGAGTTTCAGACCCAGGTCTTTGTATTCGTGTTGCTCTGGTTGTCTGGTGT
TGATGGAgattacaaggatgacgacgataaaGGATCCcccagagggcccacaatcaagccctgtcctccatgca
aatgcccagcacctaacctcttgggtggaccatccgtcttcatcttccctccaaagatcaaggatgtactcatgatctccctgagc
cccatagtcacatgtgtggtggtggatgtgagcgaggatgacccagatgtccagatcagctggtttgtgaacaacgtggaagt
acacacagctcagacacaaacccatagagaggattacaacagtactctccgggtggtcagtgccctccccatccagcaccag

gactggatgagtggcaaggagttcaaatgcaaggtcaacaacaaagacctcccagcgcccatcgagagaaccatctcaaaa
cccaaagggtcagtaagagctccacaggtatatgtcttgcctccaccagaagaagagatgactaagaaacaggtcactctgac
ctgcatggtcacagacttcatgcctgaagacatttacgtggagtggaccaacaacgggaaaacagagctaaactacaagaac
actgaaccagtcctggactctgatggttcttacttcatgtacagcaagctgagagtggaaaagaagaactgggtggaaagaaat
agctactcctgttcagtggtccacgagggtctgcacaatcaccacacgactaagagcttctcccggactccgggtaaaCGT
CCTCCCACCTGGGGCCAGGTGCAGCCCAAGGACGTGCCCAGGTCCTGGGAG
CATGGCTCCAGCCCAGCTTGGGAGCCCCTGGAAGCAGAGGCCAGGCAGCAG
AGGGACTCCTGCCAGCTTGTCCTTGTGGAAAGCATCCCCCAGGACCTGCCAT
CTGCAGCCGGCAGCCCCTCTGCCCAGCCTCTGGGCCAGGCCTGGCTGCAGCT
GCTGGACACTGCCCAGGAGAGCGTCCACGTGGCTTCATACTACTGGTCCCTC
ACAGGGCCTGACATCGGGGTCAACGACTCGTCTTCCCAGCTGGGAGAGGCT
CTTCTGCAGAAGCTGCAGCAGCTGCTGGGCAGGAACATTTCCCTGGCTGTG
GCCACCAGCAGCCCGACACTGGCCAGGACATCCACCGACCTGCAGGTTCTG
GCTGCCCGAGGTGCCCATGTACGACAGGTGCCCATGGGGCGGCTCACCAGG
GGTGTTTTGCACTCCAAATTCTGGGTTGTGGATGGACGGCACATATACATGG
GCAGTGCCAACATGGACTGGCGGTCTCTGACGCAGGTGAAGGAGCTTGGCG
CTGTCATCTATAACTGCAGCCACCTGGCCCAAGACCTGGAGAAGACCTTCC
AGACCTACTGGGTACTGGGGGTGCCCAAGGCTGTCCTCCCCAAAACCTGGC
CTCAGAACTTCTCATCTCACTTCAACCGTTTCCAGCCCTTCCACGGCCTCTTT
GATGGGGTGCCCACCACTGCCTACTTCTCAGCGTCGCCACCAGCACTCTGTC
CCCAGGGCCGCACCCGGGACCTGGAGGCGCTGCTGGCGGTGATGGGGAGCG
CCCAGGAGTTCATCTATGCCTCCGTGATGGAGTATTTCCCCACCACGCGCTT
CAGCCACCCCCCGAGGTACTGGCCGGTGCTGGACAACGCGCTGCGGGCGGC
AGCCTTCGGCAAGGGCGTGCGCGTGCGCCTGCTGGTCGGCTGCGGACTCAA
CACGGACCCCACCATGTTCCCCTACCTGCGGTCCCTGCAGGCGCTCAGCAAC
CCCGCGGCCAACGTCTCTGTGGACGTGAAAGTCTTCATCGTGCCGGTGGGG
AACCATTCCAACATCCCATTCAGCAGGGTGAACCACAGCAAGTTCATGGTC
ACGGAGAAGGCAGCCTACATAGGCACCTCCAACTGGTCGGAGGATTACTTC
AGCAGCACGGCGGGGGTGGGCTTGGTGGTCACCCAGAGCCCTGGCGCGCAG
CCCGCGGGGGCCACGGTGCAGGAGCAGCTGCGGCAGCTCTTTGAGCGGGAC
TGGAGTTCGCGCTACGCCGTCGGCCTGGACGGACAGGCTCCGGGCCAGGAC
TGCGTTTGGCAGGGCTGA

> Human PLD4-Ig fusion protein (713 amino acids) (SEQ ID NO: 126)

MEFQTQVFVFVLLWLSGVDGDYKDDDDKGSPRGPTIKPCPPCKCPAPNLLGGP
SVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVEVHTAQTQTHR
EDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQ
VYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDS
DGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGKRPPTW
GQVQPKDVPRSWEHGSSPAWEPLEAEARQQRDSCQLVLVESIPQDLPSAAGSPS
AQPLGQAWLQLLDTAQESVHVASYYWSLTGPDIGVNDSSSQLGEALLQKLQQ
LLGRNISLAVATSSPTLARTSTDLQVLAARGAHVRQVPMGRLTRGVLHSKFWV
VDGRHIYMGSANMDWRSLTQVKELGAVIYNCSHLAQDLEKTFQTYWVLGVP
KAVLPKTWPQNFSSHFNRFQPFHGLFDGVPTTAYFSASPPALCPQGRTRDLEAL
LAVMGSAQEFIYASVMEYFPTTRFSHPPRYWPVLDNALRAAAFGKGVRVRLLV

GCGLNTDPTMFPYLRSLQALSNPAANVSVDVKVFIVPVGNHSNIPFSRVNHSKF
MVTEKAAYIGTSNWSEDYFSSTAGVGLVVTQSPGAQPAGATVQEQLRQLFERD
WSSRYAVGLDGQAPGQDCVWQG

ACCESSION NUMBERS

[0074]

NITE ABP-1211

NITE ABP-1212

NITE ABP-1213

NITE ABP-1214

SEQUENCE LISTING FREE TEXT

[0075]

SEQ ID NO: 45: Forward primer

SEQ ID NO: 46: Reverse primer

SEQ ID NO: 47: Forward primer

SEQ ID NO: 48: Reverse primer

SEQ ID NO: 49: Forward primer

SEQ ID NO: 50: Reverse primer

SEQ ID NO: 51: Forward primer

SEQ ID NO: 52: Reverse primer

SEQ ID NO: 53: Forward primer

SEQ ID NO: 54: Reverse primer

SEQ ID NO: 70: Anchor primer

SEQ ID NO: 70: n is deoxyinosine

SEQ ID NO: 71: AUAP primer

SEQ ID NO: 72: Primer

SEQ ID NO: 73: Primer

SEQ ID NO: 114: Primer

SEQ ID NO: 115: Primer

SEQ ID NO: 116: Primer

SEQ ID NO: 117: Primer

SEQ ID NO: 118: Primer

SEQ ID NO: 119: Primer

[Sequence Listing]

Claims

[Claim 1]

A pharmaceutical composition for suppressing activated B cells, wherein the pharmaceutical composition comprises a monoclonal antibody binding to phospholipase D4 (PLD4) protein, or a fragment containing an antigen-binding region thereof as an active ingredient.

[Claim 2]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SYWMH as CDR1, a sequence DIYPGSDSTNYNEKFKS as CDR2 and GGWLDAMDY as a sequence CDR3 in a variable region of heavy chain.

[Claim 3]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RASQDISNYLN as CDR1, a sequence YTSRLHS as CDR2 and a sequence QQGNTLPW as CDR3 in a variable region of light chain.

[Claim 4]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SYWMH as CDR1, the sequence DIYPGSDSTNYNEKFKS as CDR2 and the sequence GGWLDAMDY as CDR3 in the variable region of the heavy chain, and has the sequence RASQDISNYLN as CDR1, the sequence YTSRLH as CDR2 and the sequence QQGNTLPW as CDR3 in the variable region

of the light chain.

[Claim 5]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence TYWMH as CDR1, a sequence AIYPGNSETSYNQKFKG as CDR2 and GYSDFDY as a sequence CDR3 in the variable region of the heavy chain.

[Claim 6]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence HASQGIRSNIG as CDR1, a sequence HGTNLED as CDR2 and a sequence VQYVQFP as CDR3 in the variable region of the light chain.

[Claim 7]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence TYWMH as CDR1, the sequence AIYPGNSETSYNQKFKG as CDR2 and the sequence GYSDFDY as CDR3 in the variable region of the heavy chain, and has the sequence HASQGIRSNIG as CDR1, the sequence HGTNLED as CDR2 and the sequence VQYVQFP as CDR3 in the variable region of the light chain.

[Claim 8]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence DYNLH as CDR1, a sequence YIYPYNGNTGYNQKFKR as CDR2 and GGIYDDYYDYAIDY as a

sequence CDR3 in the variable region of the heavy chain.

[Claim 9]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RASENIYSHIA as CDR1, a sequence GATNLAH as CDR2 and a sequence QHFWGTP as CDR3 in the variable region of the light chain.

[Claim 10]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence DYNLH as CDR1, the sequence YIYPYNGNTGYNQKFKR as CDR2 and the sequence GGIYDDYYDYAIDY as CDR3 in the variable region of the heavy chain, and has the sequence RASENIYSHIA as CDR1, the sequence GATNLAH as CDR2 and the sequence QHFWGTP as CDR3 in the variable region of the light chain.

[Claim 11]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SYYLY as CDR1, a sequence LINPTNSDTIFNEKFKS as CDR2 and EGGYGYGPFAY as a sequence CDR3 in the variable region of the heavy chain.

[Claim 12]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence TSSQTLVHSNGNTYLH as CDR1, a sequence KVSNRFS as CDR2 and a sequence HSTHVP as

CDR3 in the variable region of the light chain.

[Claim 13]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SYYLY as CDR1, the sequence LINPTNSDTIFNEKFKS as CDR2 and the sequence EGGYGYGPFAY as CDR3 in the variable region of the heavy chain, and has the sequence TSSQTLVHSNGNTYLH as CDR1, the sequence KVSNRFS as CDR2 and the sequence HSTHVP as CDR3 in the variable region of the light chain.

[Claim 14]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SYGMS as CDR1, a sequence TISSGGSYIYYPESVKG as CDR2 and LYGGRRGYGLDY as a sequence CDR3 in the variable region of the heavy chain.

[Claim 15]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RSSKSLLHSDGITYLY as CDR1, a sequence QMSNLAS as CDR2 and a sequence AQNLEL as CDR3 in the variable region of the light chain.

[Claim 16]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SYGMS as CDR1, the sequence TISSGGSYIYYPESVKG as CDR2 and the sequence

LYGGRRGYGLDY as CDR3 in the variable region of the heavy chain, and has the sequence RSSKSLLHSDGITYLY as CDR1, the sequence QMSNLAS as CDR2 and the sequence AQNLEL as CDR3 in the variable region of the light chain.

[Claim 17]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SHYYWT as CDR1, a sequence YISYDGSNNYNPSLKN as CDR2 and EGPLYYGNPYWYFDV as a sequence CDR3 in the variable region of the heavy chain.

[Claim 18]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RASQDIDNYLN as CDR1, a sequence YTSRLHS as CDR2 and a sequence QQFNTLP as CDR3 in the variable region of the light chain.

[Claim 19]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SHYYWT as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2 and the sequence EGPLYYGNPYWYFDV as CDR3 in the variable region of the heavy chain, and has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2 and the sequence QQFNTLP as CDR3 in the variable region of the light chain.

[Claim 20]

The pharmaceutical composition according to claim 1,

wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence SHYYWS as CDR1, a sequence YISYDGSNNYNPSLKN as CDR2 and EGPLYYGNPYWYFDV as a sequence CDR3 in the variable region of the heavy chain.

[Claim 21]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has a sequence RASQDIDNYLN as CDR1, a sequence YTSRLHS as CDR2 and a sequence QQFNTLP as CDR3 in the variable region of the light chain.

[Claim 22]

The pharmaceutical composition according to claim 1, wherein the monoclonal antibody or the fragment containing the antigen-binding region thereof has the sequence SHYYWS as CDR1, the sequence YISYDGSNNYNPSLKN as CDR2 and the sequence EGPLYYGNPYWYFDV as CDR3 in the variable region of the heavy chain, and has the sequence RASQDIDNYLN as CDR1, the sequence YTSRLHS as CDR2 and the sequence QQFNTLP as CDR3 in the variable region of the light chain.

[Claim 23]

A pharmaceutical composition for suppressing activated B cells, wherein the pharmaceutical composition comprises a monoclonal antibody produced by any of hybridomas mp5B7, mp7B4, mp13D4 and mp13H11 of Deposit Nos. NITE BP-1211, NITE BP-1212, NITE BP-1213 and NITE BP-1214, or a fragment containing an antigen-binding region thereof as an active ingredient.

[Claim 24]

The pharmaceutical composition according to any one of claim 1 to claim 23, further for preventing or treating autoimmune diseases.

[Claim 25]

The pharmaceutical composition according to any one of claim 1 to claim 23, further for preventing or treating allergic diseases.

[Claim 26]

A method for detecting activated B cells, the method including a step of bringing a monoclonal antibody binding to an extracellular domain of PLD4 or a fragment containing an antigen-binding region thereof into contact with cells to be tested and detecting the monoclonal antibody or the fragment containing the antigen-binding region thereof which binds to the cells.

[Claim 27]

A reagent for detecting activated B cells, wherein the reagent comprises a monoclonal antibody binding to an extracellular domain of PLD4 or a fragment containing an antibody-binding region thereof.

[Claim 28]

A method for suppressing activated B cells, the method including a step of bringing either of the following components into contact with activated B cells:

(a) a monoclonal antibody which binds to PLD4 and suppresses activated B cells, or a fragment containing an antigen-binding region thereof, and

(b) immunoglobulin into which a complementarity-determining region of the monoclonal antibody in (a) is grafted, or a fragment containing an antigen-binding region thereof.

[Claim 29]

A method for suppressing activated B cells in a living body, the method including a step of administering either of the following components to the living body:

(a) a monoclonal antibody which binds to PLD4 and suppresses an activity of activated B cells, or a fragment containing an antigen-binding region thereof, and

(b) immunoglobulin into which a complementarity-determining region of the monoclonal antibody in (a) is grafted, or a fragment containing an antigen-binding region thereof.

[Claim 30]

The method according to claim 28 or claim 29, wherein the activity of the activated B cells is an antibody-producing activity.

[Claim 31]

An agent for suppressing activated B cells, wherein the agent comprises either of the following components as an active component:

(a) a monoclonal antibody which binds to PLD4 and suppresses activated B cells, or a fragment containing an antigen-binding region thereof, and

(b) immunoglobulin into which a

complementarity-determining region of the monoclonal antibody in (a) is grafted, or a fragment containing an antigen-binding region thereof.

[Claim 32]

The agent for suppressing activated B cells according to claim 31, wherein an activity of the activated B cells is an antibody-producing activity.