DESCRIPTION

ANTI-ILT7 ANTIBODY

Technical Field

[0001]

The present invention relates to an antibody which binds to human ILT7.

Background Art

[0002]

Interferon α (IFNα: hereinafter, "interferon" is abbreviated as IFN) and interferon β (IFNβ) are known as type 1 IFNs which possess antiviral activity or antitumor activity. On the other hand, it has also been revealed that IFNα is related to autoimmune disease. For example, abnormal production of IFNα has been reported in patients with the following autoimmune diseases. It has also been suggested that symptoms of the autoimmune diseases can be reduced by neutralization of IFNα.

Systemic lupus erythematosus (Shiozawa et al., Arthr. & Rheum. 35, 412, 1992)

Chronic rheumatism (Hopkins et al., Clin. Exp. Immunol. 73, 88, 1988)

Cases in which symptoms of the autoimmune diseases had been manifested or worsened by administration of recombinant IFNα2 or IFN were reported (Wada et al., Am. J. Gastroenterol. 90, 136, 1995; Perez et al., Am. J. Hematol. 49, 365, 1995; Wilson LE et al, Semin Arthritis. Rheum. 32, 163-173, 2002.).

[0003]

Further, it has also been revealed that IFNα induces differentiation of dendritic cells. The dendritic cell is also an antigen presenting cell. Therefore, it is considered that the differentiation induction of dendritic cells consists an important mechanism in autoimmune diseases. It has been suggested that there is a deep association between the differentiation induction of dendritic cells of IFNα and the onset of systemic lupus erythematosus (Blanco et al., Science, 16:294, 1540-1543, 2001). Thus, it has been

pointed out that IFNα is closely related to the antitumor activity as well as autoimmune diseases. In addition, IFNα is deeply involved in the onset of psoriasis (Nestle FO et al., J. Exp. Med. 202, 135-143, 2005).

[0004]

Interferon Producing cells (IPCs) were identified as cells which produce type 1 IFN in large quantities associated with virus infection. Few IPCs are presented in the blood. It is considered that peripheral blood lymphocytes account for 1% or less of IPCs. However, IPCs have a very high capacity to produce IFN. IFN producing capacity of IPCs reaches, for example, 3000 pg/mL/10^4 cells. That is, it may be said that most of the IFNα or IFNβ in the blood, which is produced at viral infection, is resulted from IPCs, although there are few cells.

[0005]

On the other hand, IPCs are undifferentiated lymphoid dendritic cells which are considered as precursor cells of dendritic cells. IPCs may be referred to as Plasmacytoid dendritic cells. IPCs are differentiated into dendritic cells by virus stimulation and induce the production of IFNγ or IL-10 by T cells. IPCs are also differentiated into dendritic cells by IL-3 stimulation. The differentiated dendritic cells by IL-3 stimulation induce the production of Th2 cytokine (IL-4, IL-5, and IL-10) by T cells. Thus, IPCs have properties which allow them to be differentiated into distinct dendritic cells by different stimulation.

[0006]

Accordingly, IPCs have two profiles: IFN producing cells and precursor cells of dendritic cells. Both cells play an important role in immune system. In other words, IPC is one of the important cells which support immune system in various aspects.

Non-patent document 1: Shiozawa et al., Arthr. & Rheum. 35, 412, 1992

Non-patent document 2: Hopkins et al., Clin. Exp. Immunol. 73, 88, 1988

Non-patent document 3: Wada et al., Am. J. Gastroenterol. 90, 136, 1995

Non-patent document 4: Parez et al., Am. J. Hematol. 49, 365,

1995

Non-patent document 5: Bianco et al., Science, 16:294, 1540-1543, 2001

Non-patent document 6: Ju et al., Gene. 2004 Apr 28; 331: 159-64.

Non-patent document 7: Colonna M et al., Seminars in Immunology 12: 121-127, 2000.

Non-patent document 8: Nakajima H. et al., J. Immunology 162: 5-8. 1999

Non-patent document 9: Wilson LE et al, Semin Arthritis. Rheum. 32, 163-173, 2002

Non-patent document 10: Nestle FO et al., J. Exp. Med. 202, 135-143, 2005

Patent-document 1: WO03/12061 (U.S. Patent Published Application No. 2003-148316)

Disclosure

[Problems to be Solved by the Disclosure]

[0007]

An objective of the present disclosure is to provide an antibody binding to Immunoglobulin-Like transcript-7 (ILT7), and to detect, identify, or isolate IPCs. Another objective of the present disclosure is to regulate the activity of IPCs.

[Means for Solving the Problems]

[0008]

In order to regulate activity of a humoral factor such as IFN, administration of antibodies, which recognize the factor, is effective. For example, the attempt to treat autoimmune diseases by antibodies against interleukin (IL)-1 or IL-4 have been realized (Guler et al., Arthritis Rheum., 44. S307, 2001). Further, it is assumed that neutralizing antibodies can serve as therapeutic agents of autoimmune diseases as with interferon (Stewart, TA. Cytokine Growth Factor Rev. 14; 139-154, 2003). It can be predicted that the same approach as described above is effective on IFN produced by IPCs. However, such an approach is based on the inhibition of effect of humoral factor after production of the factor. If the production of the desired humoral factor can be directly controlled, more substantial

therapeutic effects can be achieved.

[0009]

Antibodies, which recognize human IPC, have been reported. For example, anti-BDCA-2 monoclonal antibody is human IPC-specific monoclonal antibody (Dzionek A. et al. J. Immunol. 165: 6037-6046, 2000). It is found that anti-BDCA-2 monoclonal antibody is effective in inhibiting IFN production by human IPCs (J. Exp. Med. 194: 1823-1834, 2001.). In addition, it has also been reported that monoclonal antibodies, which recognize interferon-producing cells in mice, inhibit the production of interferon (Blood 2004 Jun 1; 103/11: 4201~4206.Epub 2003 Dec). It was reported that the reduced number of dendritic cells was due to monoclonal antibodies against plasmacytoid dendritic cells in mice (J. Immunol. 2003, 171: 6466~6477).

[0010]

Similarly, if antibodies which recognize human IPCs and can regulate the activity are provided, it will be useful. For example, the present inventors have already shown that an antibody, which recognizes Ly49Q, specifically binds to mouse IPCs. However, the antibody against Ly49Q did not interfere with the activity of mouse IPCs (Blood, 1 April 2005, Vol. 105, No. 7, and pp. 2787-2792.; WO2004/13325). On the other hand, ILT7 is known as a molecule whose specific expression is seen in Plasmacytoid dendritic cells (Ju XS et al. and Gene. 2004 Apr 28; 331: 159-64.; WO03/12061). However, any antibodies against ILT7 have not been obtained. Therefore, the effects of antibodies on IPCs are also unknown.

[0011]

ILT7 is a membrane protein containing an immunoglobulin-like motif. It has been reported as one of the molecules expressed in cells of the myeloid system or lymphatic system (Colonna M et al., Seminars in Immunology 12:121-127, 2000.). A plurality of molecules with structures analogous to ILT7 is referred to as ILT family. ILT family is also structurally similar to killer cell inhibitory receptors (KIR). ILT7 has four C-type immunoglobulin-like domains as with other molecules of ILT family. It is considered that ILT7 sends activation signals into the cell as with ILT1, ILT1-like protein, ILT8, and LIR6a.

It has been confirmed that a molecule belonging to ILT family is expressed in hemocyte system cells (Young et al., Immunogenetics 53: 270-278, 2001; "The KIR Gene Cluster. "Carrington, Mary and Norman, Paul. Bethesda (MD): National Library of Medicine (US), NCBI; 2003).

[0012]

Then, a high expression of ILT7 was detected in Plasmacytoid dendritic cells (PDC) and a low expression of ILT7 was detected in monocyte-derived dendritic cells (MDDC) by subtractive hybridization. ILT2 and ILT3 were expressed in not only PDC but also DC obtained from MDDC or CD34 positive cells. However, since mRNA in ILT7 was specifically expressed in PDC, it was found that the mRNA might serve as a marker of PDC. Additionally, it was found that at that time, the expression of ILT7 was reduced by stimulation of CpG (Ju XS et al. Gene. 2004 Apr 28; 331: 159-64.; WO03/12061).

[0013]

The present inventors confirmed that specific expression of ILT7 in IPC was facilitated through the study on human IPC. Then, the present inventors attempted to produce antibodies of ILT7 and to elucidate the effects. For example, molecules constituting ILT families such as ILT2 and ILT3 have high conservation, particularly in amino acid sequences of extracellular domains (Fig. 9). These ILT families exhibit characteristic expression profiles in various blood cells, respectively. Therefore, it is a very important subject to obtain an antibody which can immunologically distinguish between other ILT family molecules and ILT7. However, in fact, it was difficult to produce an antibody which binds specifically to human IPCs using ILT7 as an immunogen because of the obstacles described below.

[0014]

Generally, a protein produced by gene-recombination technology is used as an immunogen in order to obtain an antibody which recognizes a trace amount of proteins derived from living organisms. The present inventors tried to express human ILT7 on the basis of information of a base sequence of cDNA of human ILT7, which had already been found, and the amino acid sequence coded by the base sequence (GenBank Accession No. NM_012276). However, the present inventors could not produce human ILT7 as a recombinant under normal conditions.

[0015]

The partial amino acid sequence of natural protein is often tried to be used as an immunogen in order to obtain a protein antibody. However, there are few amino acid sequences specific to human ILT7 in proteins since homology to the amino acid sequences is extremely high in ILT family. In addition, it is necessary to select the region constituted of the portion that is recognized as an epitope by antibodies on the surface of cells for the purpose of allowing antibodies to recognize molecules on the surface of cells. Therefore, it has been considered that formation of an antibody which is specific to ILT7 by using a fragment amino acid sequence as an immunogen is not realistic.

[0016]

The present inventors showed that an antibody, which binds to IPCs, could be obtained by using a special immunogen under such conditions. Further, the present inventors found that the antibody thus obtained specifically recognized human IPCs and further had an effect of regulating the activity and thereby succeeded in completing the present disclosure. That is, the present disclosure relates to the following anti-ILT7 antibody, production method thereof, and use thereof.

[Effects of the Disclosure]

[0016a]

In one aspect, there is provided a monoclonal antibody which binds to human ILT7, binds to none of human ILT1, human ILT2 and human ILT3, and suppresses interferon-α (IFNα) production from ILT7-expressing cells, or a fragment comprising its antigen binding region.

[0017]

The present disclosure provides an immunogen useful in producing an antibody which recognizes human ILT7 and a production method of anti-human ILT7 antibody using the immunogen. ILT7 is a membrane protein belonging to ILT family. Particularly, the amino acid sequence of the extracellular region is highly conserved among ILT families. Therefore, it is extremely difficult to produce an antibody which distinguishes between ILT families by general immunization methods. The present inventors showed that the antibody, which recognizes human ILT7, can be easily obtained by using animal cells in which ILT7 is coexpressed with cell membrane protein. Anti-ILT7 antibody, which can be obtained by the present disclosure, has a high specificity which distinguishes cells expressing other ILT families from those expressing human IPCs.

[0018]

In one aspect, there is provided a method for detecting an interferon producing cell, comprising the steps of:
contacting the monoclonal antibody as defined above with a test cell; and detecting the monoclonal antibody or the fragment comprising its antigen binding region which is bound to the cell.

In a preferred embodiment, anti-human ILT7 antibody provided by the present disclosure binds to human IPCs. In addition, the antibody of the present disclosure specifically recognizes human IPCs. Therefore, it is useful in detecting and isolating IPCs. IPC is a cell which produces most of the type 1 interferon. Therefore, the detection and isolation are important in diagnosis and study of diseases that involve IPCs such as autoimmune diseases. Particularly, according to the findings of the present inventors, the expression of ILT7 in IPCs is not reduced under the presence of IFNα. IFNα expression is often facilitated in patients with autoimmune diseases. This means that anti-ILT7 antibody of the present disclosure can be used for the detection and isolation of IPCs as to the patients with autoimmune diseases in which the expression of IFNα is facilitated.
[0019]

Anti-ILT7 antibody provided by the present disclosure has an effect which regulates the activity of human IPCs in a preferred embodiment. Therefore, the anti-ILT7 antibody of the present disclosure can be used to inhibit the activity of IPCs. As described previously, the expression of ILT7 in IPCs is not reduced under the presence of IFNα. Therefore, if the inhibition of the activity of IPCs by the antibody of the present disclosure is used, a therapeutic effect on the patients with autoimmune diseases in which the expression of IFNα is facilitated may be expected.

[0020]

Scant IPCs produce a large amount of IFN. Antibodies as many as IFN molecules are necessary for neutralization of IFN. However, producing cell activation is directly inhibited in the present disclosure. As a result, a strong inhibitory effect on IFN can be expected even if smaller amount of antibodies are used compared with neutralization by anti-IFN antibody. Furthermore, in the case where IFN is continuously produced, it is predicted that neutralization by IFN antibodies is transient inhibition. In the present disclosure, since the activity of IPCs is inhibited, IFN production inhibiting effect can be expected over a long period of time.

[0020a]

In one aspect, there is provided an interferon a (IFNα)-suppressing agent comprising the monoclonal antibody as defined above as an active ingredient.

Brief Description of the Drawings

[0021]

Fig. 1a is a photograph in which the expression of mRNA of ILT7 gene is examined by RT-PCR method. It is a result of the analyzed expression of mRNA of ILT7 gene in human immunocytes.

Fig. 1b is a diagram in which the expression of mRNA of ILT7 gene in various human tissues and cells is compared and examined using quantitative PCR method. The horizontal axis shows the examined tissues and cells and the vertical axis shows the expression level of ILT7, which is standardized according to the expression level of GAPDH gene.

Fig. 2 is a diagram showing structures of ILT7 protein, where Fig. 2(a) shows an amino acid sequence of ILT7 protein and further shows the estimated secretion signal sequence and transmembrane domain in the drawing, and Fig. 2(b) shows a schematic diagram of ILT7 proteins that are encoded by constructed expression vectors.

Fig. 3 is a diagram showing a result that ILT7 expression vector and FcRγ expression vector were introduced into cells and the cell-surface expression of ILT7 molecules was examined by FCM. The horizontal axis shows the fluorescence intensity detected in anti-FLAG antibody, namely, the intensity of cell-surface expression of ILT7 molecules to which FLAG tag was attached and the vertical axis shows the number of cells.

Fig. 4 shows photographs in which ILT7 expression vector and FcRγ expression vector were introduced into cells and the association of molecules was analyzed by immunoprecipitation and Western blotting. The left side diagrams show results that ILT7 molecule was blotted with anti-FLAG antibody after immunoprecipitating FcRγ molecule with anti-myc antibody (the drawing above) and FcRγ molecule was blotted with anti-myc antibody (the drawing below). Similarly, the right side diagrams show results that ILT7 molecule was blotted with anti-FLAG antibody after immunoprecipitating FcRγ molecule with anti-FLAG antibody (above) and FcRγ molecule was blotted with anti-myc antibody (below).

Fig. 5 is a photograph in which glycosylation of ILT7 molecule was examined by introduction of ILT7 expression vector and FcRγ expression vector into the cell and N-glycosidase treatment. The left

side of the photograph shows the size of ILT7 in the case where ILT7 was not treated with N-glycosidase and the right side of the photograph shows the size of ILT7 in the case where N-glycosidase treatment was performed.

Fig. 6a is a diagram in which the responsiveness of the produced anti-ILT7 monoclonal antibody was examined by FCM analysis. (a) shows a result that binding of anti-ILT7 antibody to IPC fraction of BDCA-2 positive was analyzed by using human peripheral blood lymphocytes and double staining with the anti-ILT7 antibody and anti-BDCA-2 antibody. The vertical axis shows the responsiveness to BDCA-2 antibody and the horizontal axis shows the responsiveness to each of the produced anti-ILT7 antibodies.

Fig. 6b is a diagram in which the responsiveness of the produced anti-ILT7 monoclonal antibodies was examined by FCM analysis. (b) shows a result in which binding of anti-ILT7 antibody to ILT7 molecule was examined by using 293T cells into which ILT7 and FcRγ expression vectors had been introduced. The vertical axis shows the responsiveness of anti-FLAG antibody, namely, the intensity of expression of ILT7 molecules to which FLAG tag was attached and the horizontal axis shows the responsiveness of respective anti-ILT7 antibodies.

Fig. 7 is a diagram in which among the produced anti-ILT7 monoclonal antibodies, the responsiveness of two clones to human peripheral blood lymphocytes was examined by FCM analysis. Three graphs on the left shows the results of #11 and three graphs on the right shows the results of #17. In the left side diagrams, each axis with the mark of ILT7 shows the responsiveness of ILT7#11. Similarly, in the right side diagrams, each axis with the mark of ILT7 shows the responsiveness of ILT7#17.

Fig. 8 is a result in which binding activity of the produced anti-ILT7 monoclonal antibodies ILT7#11 and ILT7#17 to human lymphocytes was compared with that of anti-BDCA-2 antibody and examined. The vertical axis shows the responsiveness of anti-CD123 antibody and the horizontal axis shows the responsiveness of each antibody. That is, each antibody binds to a portion of CD123 positive cell. It is a diagram showing the results in which the responsiveness was

analyzed when lymphocyte cells were stimulated by two kinds of CpGs and IFNα.

Fig. 9a is a diagram showing amino acid sequences of family molecules with high homology to ILT7 molecules. Each amino acid sequence of the extracellular region is mainly shown as an alignment; Fig. 9b is a continuation of Fig. 9a; and Fig. 9c is a continuation of Fig. 9b.

Fig. 10 is a result in which the responsiveness of the produced anti-ILT7 monoclonal antibodies ILT7#11 and ILT7#17 to ILT1, ILT2, and ILT3 molecules was examined using cells into which their expression vectors were introduced. The upper diagram shows the results where the responsiveness to cells in which ILT7 molecules with a FLAG tag had been coexpressed with FcRγ was reaffirmed. The lower diagram shows the results where the responsiveness to cells into which ILT1, ILT2, ILT3, and FcRγ were introduced (left diagram: ILT7#11, right diagram: ILT7#17). The horizontal axis shows the responsiveness of each anti-ILT7 antibody.

Fig. 11 is a diagram showing the effect of the produced anti-ILT7 monoclonal antibodies ILT7#11 and ILT7#17 on the interferogenic capacity of human lymphocytes. In the diagram, the horizontal axis shows IFNα concentration in a culture supernatant when human lymphocytes were stimulated by influenza virus and the vertical axis shows the treated antibodies. The term "no infections" indicates the results of cells which were not stimulated by influenza virus.

Fig. 12 is a diagram showing CDC activity of the produced anti-ILT7 monoclonal antibodies ILT7#37, ILT7#28, and ILT7#33. Even when anti-ILT7 monoclonal antibodies obtained from any hybridoma were used, 80% or more of CDC activity was exhibited at the antibody concentration of 0.1 μg/ml or higher. In the case of antibodies other than anti-ILT7 monoclonal antibody, CDC activity to target cells was not observed.

Fig. 13 is a diagram showing internalization to target cells of the produced anti-ILT7 monoclonal antibodies ILT7#17, ILT7#26, ILT7#37, ILT7#28, and ILT7#33.

The fluorescence intensity of APC is an indicator of the amount of ILT7-anti-ILT7 antibody immune complex which was present on the surface of cells before incubation and it is detected regardless of

whether ILT7-anti-ILT7 antibody immune complex is present on the target cell surface or is incorporated into the cell after incubation. On the other hand, the fluorescence intensity of FITC is an indicator of the amount of ILT7-anti-ILT7 antibody immune complex which remains on the surface of cells after incubation. That is, the fluorescence intensity of FITC is decreased by internalization.

Best Mode for carrying out the Invention

[0022]

It has been reported that human ILT7 (immunoglobulin-like transcript-7) is a molecule which is specifically expressed in Plasmacytoid dendritic cells (Gene. 2004 Apr 28; 331:1 59-64.; WO03/12061). Alternatively, it is also known that human ILT7 can be used as a predictive indicator for prognosis of lymphoma (WO2005/24043). However, a method for producing an antibody capable of recognizing human ILT7 has not been established.

[0023]

Human ILT7 consists of 499 amino acid residues as shown in SEQ ID NO: 2 and it is a type 1 transmembrane protein comprising four immunoglobulin-like domains in the structure and one transmembrane region (445-466; from 429 to 450 in SEQ ID NO: 2). Among 444 amino acid residues including N-terminal, 16 amino acid residues (from -15 to -1, in SEQ ID NO: 2) are signal sequences and 17 to 444 amino acid residues (from 1 to 428, in SEQ ID NO: 2) constitute an extracellular domain. On the other hand, the C-terminal region is an intracellular domain. Most of portions of the human ILT7 are extracellular domains and 33 amino acid residues constitute an intracellular domain (from 467 to 499; from 451 to 483, in SEQ ID NO: 2). It is not predicted that a motif, which is involved in signalization, is present in an intracellular domain. A full length amino acid sequence of human ILT7 is shown in SEQ ID NO: 2 and a base sequence of cDNA encoding the amino acid sequence is shown in SEQ ID NO: 1. Here, the coding regions of the matured peptide (72)..(1520), shown in SEQ ID NO: 1, do not comprise the termination and initiation codons. That is, protein coding sequences which comprise the termination and initiation codons in SEQ ID NO: 1 are from 24 to 1523.

[0024]

It is considered that the ligand signal is transmitted to cells by association of human ILT7 with a signal-transducing molecule. For example, most of the Fc receptor γ-chains are present in cells. In addition, the intracellular domain contains an immunoreceptor tyrosine-based activation motif (ITAM) which is involved in signalization. ITAM is an amino acid sequence portion, which is commonly seen in adaptor molecules that are associated with immunoreceptors such as Fc receptors. A motif such as YxxL (SEQ ID NO: 76), which is a target of tyrosine phosphorylation, is comprised in ITAM and the signal is transmitted by the phosphorylation. Known examples of the signal-transducing molecule, which comprises ITAM in an intracellular domain, include CD3ζ and DAP12 in addition to Fc receptor γ-chain. Among these signal-transducing molecules, the molecule associated with human ILT7 is predicted to be the Fc receptor γ-chain. Currently, a ligand, which binds to human ILT7, has not been found.

[0025]

The present inventors confirmed that ILT7 was specifically expressed in human IPCs by gene expression analysis. The present inventors considered that it would be useful in the study of IPCs if an antibody capable of distinguishing human ILT7 from other molecules immunologically could be obtained. However, many molecules with similar structures exist in ILT family including ILT7. Molecules such as ILT1, ILT2, ILT3, ILT4, ILT5, ILT6, or LIR-8 comprise highly homologous amino acid sequences, particularly in their extracellular domains. Therefore, the present inventors considered that it was difficult to obtain an antibody capable of distinguishing between these molecules using a domain peptide comprising a partial amino acid sequence which constitute an extracellular domain as an immunogen. Then, the present inventors have tried to produce an antibody against human ILT7 using the cells expressing human ILT7 as immunogens.

[0026]

However, the use of general expression vectors did not cause the expression of cDNA of human ILT7 in animal cells. It has been reported that ILT1 molecule having a structure very similar to ILT7

associates with the Fc receptor γ-chain. That is, when cells in which the Fc receptor γ-chain was expressed such as RBL (rat basophilic leukemia) cells and P815 (mouse mastocytoma) cells were used as host cells, the expression of ILT1 on the cell surface was observed. However, if ILT1 was forced to be expressed in 293 cells in which Fc receptor γ-chain was not originally expressed, the cell-surface expression was not observed. On the other hand, it was shown that the cell-surface expression of ILT1 could be confirmed when ILT1 was coexpressed with the Fc receptor γ-chain (Nakajima H. et al., J. Immunology 162:5-8.1999). However, there is no information about an immunogen for producing ILT7 antibodies.

[0027]

For example, in the report, RBL cells into which ILT1 gene is introduced are used as immunogens to produce ILT1 antibodies. The present inventors tried to produce ILT7 antibodies using the combination of RBL cells with ILT7 gene in the same manner as described. However, even if ILT7 was forced to be expressed in RBL cells (P815), the cell-surface expression of ILT7 was not observed, and therefore it could not be used as an immunogen.

[0028]

The present inventors have conducted dedicated research in order to obtain the antibody capable of recognizing human ILT7. As a result, the present inventors found that the desired antibody could be produced by using a specific transformed cell as an immunogen and completed the present invention. That is, the present invention relates to a monoclonal antibody which binds to the extracellular domain of human ILT7, and relates to a fragment comprising its antigen binding region.

[0029]

In the present invention, human ILT7 can be defined as a natural molecule which is expressed in human IPCs or a molecule which is immunologically equivalent to ILT7 which is expressed in human IPCs. In the present invention, the binding of antibodies to human ILT7 can be confirmed, for example, as follows.

- Confirmation based on responsiveness to human cells:

According to the findings of the present inventors, specific expression of human ILT7 was observed in human IPCs. Originally, human

ILT7 was isolated as a gene whose expression is seen in Plasmacytoid dendritic cells (Blood. 2002 100; 3295-3303, Gene. 2004 Apr 28; 331:159-64.). In addition, it is also known that it can be used as a marker of Plasmacytoid dendritic cells (WO03/12061). It is assumed that Plasmacytoid dendritic cells and IPCs are mostly identical cell populations or their large portions are common. Therefore, there is no contradiction between these reports and the findings of the present inventors.

[0030]

Considering such expression profile of human ILT7, first, the binding activity of IPCs or Plasmacytoid dendritic cells to at least a certain subset is one of the important characteristics of the antibody which binds to human ILT in the present invention. Cell surface markers specific to respective cell populations can be used to determine whether a certain cell is IPC or Plasmacytoid dendritic cell. For example, binding to the desired cells can be confirmed by double staining with the antibody which binds to cell surface markers and the antibody whose binding activity should be checked. That is, IPCs in the present invention comprises, for example, cells which express BDCA2.

[0031]

- Confirmation based on responsiveness to transformed cells expressing human ILT7 gene:

The present inventors found that an immunological characteristic of ILT7 expressed in human IPCs was reconstructed when expression of human ILT7 gene was carried out under a specific condition. Therefore, the responsiveness to human ILT7 can also be confirmed based on the responsiveness of antibodies to cells into which a gene encoding ILT7 is artificially introduced. Namely, the present invention relates to a monoclonal antibody which comprises the amino acid sequence constituting an extracellular domain as the extracellular domain and binds to a molecule coexpressed with the signal-transducing molecule or relates to a fragment comprising its antigen binding region. Here, the extracellular domain is composed of an amino acid sequence which corresponds to the 17th to 444th position of the N terminal amino acid sequence shown in SEQ ID NO: 2 (from 1 to 428 in SEQ ID NO: 2).

For example, the immunological characteristic of ILT7 expressed in human IPCs is maintained in cells co-transfected with an expression vector comprising a DNA encoding human ILT7 and an expression vector comprising a DNA encoding the signal-transducing molecule. Therefore, a transformed cell, which coexpresses human ILT7 and the signal-transducing molecule, is preferable to confirm the binding affinity of antibodies to the extracellular domain of human ILT7 in the present invention. In the present invention, it is desirable to use a cell, which is not transformed as controls when the responsiveness of antibodies, is confirmed by using the transformed cell. Further, it is also important to confirm that the binding of antibodies is not detected using the same host cell which expresses only the signal-transducing molecule as a control.

[0032]

In the present invention, a molecule, which induces the expression of human ILT7 on the cell surface, can be used as the signal-transducing molecule for the coexpression. The signal-transducing molecule in the present invention can also be defined as a molecule which can impart the immunological characteristic of natural human ILT7 to at least the extracellular domain of ILT7 molecule in a cell which expresses ILT7. As used herein, the term "immunological characteristic" of natural human ILT7 means recognition by an antibody which binds to human IPCs.

Specifically, it is preferable to use Fc receptor γ-chain or DAP12 as a signal-transducing molecule. In the present invention, the Fc receptor γ-chain is particularly preferable as the signal-transducing molecule. The Fc receptor γ-chain is a molecule consisting of amino acid sequences shown in SEQ ID NO: 16. The signal-transducing molecule may be a fragment as long as human ILT7 to be coexpressed is localized at the cell surface. As long as human ILT7 to be coexpressed is localized at the cell surface, the mutation or addition of the amino acid sequence is permitted in the amino acid sequences shown in SEQ ID NO: 16. That is, the present invention provides methods for producing cells which produce a monoclonal antibody which binds to the extracellular domain of human ILT7, comprising the following steps of:

(1) administering a cell which exogenously expresses a protein comprising extracellular domain of human ILT7 and a molecule comprising amino acid sequences described in SEQ ID NO: 16 to immune animals; and

(2) selecting an antibody producing cell which produces the antibody which binds to human ILT7 from antibody producing cells of the immune animals.

[0033]

Subsequently, as the antibody which binds to human ILT7 in the present invention, it is preferable to use an antibody in which crossing with cell populations which are known to express ILT families other than ILT7 is not observed. Specifically, as the antibody which binds to human ILT7 in the present invention, it is preferable to use an antibody in which the binding to the cell populations which are known to express ILT families other than ILT7 cannot be observed under the same condition as the condition in which the binding to IPCs was confirmed. As already described, for example, ILT2 and ILT3 are expressed in not only PDC but also DC obtained from MDDC or CD34 positive cells (Gene. 2004 Ap 28; 331: 159-64.). On the other hand, the expression ILT7 cannot be detected due to the differentiation of IPCs into dendritic cells. Therefore, the antibody cannot detect the binding to DCs obtained from MDDC or CD34 positive cells under the condition in which the binding to IPCs can be confirmed is comprised in the antibody which binds to human ILT 7 in the present invention.

[0034]

The following expression patterns as to other ILT family molecules have been reported ("The KIR Gene Cluster" Carrington, Mary and Norman, Paul. Bethesda (MD): National Library of Medicine (US), NCBI; 2003, Gene. 2004 Apr 28; 331: 159-64.). Therefore, an antibody which binds to human IPCs or PDCs and whose binding to the following cells cannot be confirmed is included in an antibody having specificity to ILT7:

ILT1; myeloid lineage cells (monocytes, DCs derived from monocytes, macrophages);

ILT2; PDCs, B cells, CD34 positive cells, DCs derived from CD34 positive cells, and DCs derived from monocytes;

ILT3; PDCs and DCs;

ILT5; monocytes, DCs derived from CD34 positive cells, and DCs derived from monocytes; and

ILT8; monocyte lineage cells.

[0035]

That is, the monoclonal antibody, which binds to the extracellular domain of human ILT7 in the present invention preferably, comprises a monoclonal antibody which has the following immunological characteristics:

a) the monoclonal antibody binds to human IPCs; and

b) the binding of the monoclonal antibody to one or more cells selected from the group consisting of monocytes, macrophages, B cells, CD34 positive cells, and dendritic cells derived from these cells cannot be confirmed under the condition for binding to human IPCs.

As the monoclonal antibody of the present invention, it is preferable to use an antibody in which the binding to monocytes, macrophages, B cells, CD34 positive cells, and dendritic cells derived from these cells cannot be confirmed under the condition for binding, particularly to human IPCs.

[0036]

Alternatively, the monoclonal antibody, which binds to the extracellular domain of human ILT7 in the present invention, preferably comprises a monoclonal antibody which has the following immunological characteristics:

c) the monoclonal antibody binds to the transformed cell which is co-transfected with an expression vector expressively carrying the DNA encoding human ILT7 and an expression vector expressively carrying the DNA encoding the signal-transducing molecule;

d) the binding to the host cell prior to transformation cannot be confirmed under the condition for binding to the co-transfected cells as described in c); or

the monoclonal antibody of the present invention comprises a monoclonal antibody which has the following immunological characteristics:

e) the binding to the host cell which expresses only the signal-transducing molecule cannot be confirmed under the condition

for binding to the co-transfected cells as described in c).

[0037]

In the present invention, the fact that anti-ILT7 monoclonal antibody does not intersect with the ILT family of other molecules can be confirmed using cells in which each ILT family was forced to be expressed. That is, for forced expression, cDNA encoding each ILT family of amino acid sequences is introduced into an appropriate host cell. The anti-ILT7 monoclonal antibody whose crossing should be confirmed is made to contact with the obtained transformed cell. Then, it can be confirmed that if the binding of the antibody to the cell, which expresses ILT family molecules other than ILT7, is not observed, the antibody is able to immunologically distinguish between ILT7 and other ILT family molecules. For example, in examples described below, the fact that the anti-ILT7 monoclonal antibody obtained by the present invention does not intersect with ILT1, ILT2, and ILT3 is confirmed. Therefore, a preferable example of the monoclonal antibody in the present invention is the monoclonal antibody binding to ILT7 in which the binding to ILT1, ILT2, and ILT3 cannot be detected under the same condition.

[0038]

Particularly, ILT2 and ILT3 are genes whose expression in IPCs has been confirmed (Ju et al. Gene 331, 159-164, 2004). However, these molecules may show expression profiles unique to each cell type depending on the respective differentiation levels in IPCs or conditions such as the stimulation with viruses or other cytokines. The use of an antibody, which is able to immunologically distinguish these ILT family molecules from ILT7, allows for specifically detecting changes in the expression of ILT7.

[0039]

The binding of a monoclonal antibody whose binding activity should be confirmed to various kinds of cells can be confirmed based on, for example, the principle of flow cytometry. In order to confirm the responsiveness of antibodies based on the principle of flow cytometry, it is advantageous to label antibodies with molecule or atomic group which produces a detectable signal in advance. Generally, the fluorescent or luminescent labels are used.

Fluorescence-activated cell sorter (FACS) can be used to analyze the binding of the fluorescent-labeled antibodies to cells based on the principle of flow cytometry. The use of FACS allows for efficiently confirming the binding of a plurality of antibodies to a plurality of cells.

[0040]

Specifically, for example, antibody A which has been previously found to be able to identify IPCs and antibody B whose binding characteristics to IPCS should be analyzed are reacted with cell populations comprising IPCs at the same time. Antibody A and antibody B are labeled with a fluorescence signal which is mutually distinguished by these antibodies in advance. In the case where both signals are detected from the same cell populations, the binding of those antibodies to the same cell populations can be confirmed. In other words, it is found that both antibodies A and B have the same binding characteristics. In the case where they bind to different cell populations, it is clear that both antibodies have distinct binding characteristics.

[0041]

A preferable example of the monoclonal antibody in the present invention may comprise a monoclonal antibody which is produced by hybridoma ILT7#11 or ILT7#17. Hybridoma ILT7#11 and hybridoma ILT7#17 have been deposited with National Institute of Advanced Industrial Science and Technology, International Patent Organism Depositary under Accession Nos. FERM BP-10704 and FERM BP-10705 on Oct. 21, 2005.

The specified depository content is as follows:

(a) Appellation and address of depository institution

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

Address: AIST Tsukuba Central 6, 1-1-1, Higashi, Tsukuba-shi, Ibaraki, Japan (zip code 305-8566)

(b) Deposited date: October 21, 2005

(c) Accession number: FERM BP-10704 (hybridoma ILT7#11)

(c) Accession number: FERM BP-10705 (hybridoma ILT7#17)

[0042]

The monoclonal antibody of the present invention may be a fragment

comprising its antigen binding region. For example, an antibody fragment comprising the antigen binding region which is obtained by enzymatic digestion of IgG can be used as the antibody in the present invention. Specifically, antibody fragments such as Fab and $F(ab')_2$ can be obtained by digestion with papain or pepsin. It is well known that these antibody fragments can be used as antibody molecules which have affinity for antibodies. Alternatively, antibodies constructed by genetic recombination can also be used as long as satisfactory antigen-binding activity is maintained. Examples of the antibodies constructed by genetic recombination comprise chimeric antibodies, CDR-transplanted antibodies, single chain Fvs, diabodies, linear antibodies, and polyspecific antibodies formed of antibody fragments. It is common knowledge that these antibodies can be given by using monoclonal antibodies or antibody producing cells which produce the antibodies.

[0043]

The monoclonal antibody of the present invention can be obtained by using a specific transformed cell as an immunogen. That is, the present invention relates to a method for producing cells which produce a monoclonal antibody which binds to the extracellular domain of human ILT7, comprising the following steps of:

(1) administering a cell which expresses an exogenous protein comprising extracellular domain of human ILT7 and an exogenous molecule which is associated with human ILT7 to immune animals; and

(2) selecting an antibody producing cell which produces the antibody which binds to human ILT7 from antibody producing cells of the immune animals.

The antibody producing cells thus obtained or the immortalized antibody producing cells are cultured and the desired monoclonal antibodies can be recovered from the cultures. With reference to the method for immortalizing antibody producing cells, various methods are known.

[0044]

In the method for producing the monoclonal antibody of the present invention, usable examples of the molecule, which is associated with human ILT7 for producing a transformed cell to be used as an immunogen,

comprise cell membrane proteins. Among them, a signal-transducing molecule, which is localized in cell membranes, is preferable to use as a cell membrane protein in the present invention. The term "signal-transducing molecule" means a molecule which is associated with proteins and cells having receptor structures in the extracellular domain and transmits the stimulation of binding of ligands to receptors into cells. Examples of the signal-transducing molecule comprise Fc receptor γ-chain, DAP12, or the like. For example, Fc receptor γ-chain is preferable to use as a cell membrane protein in the present invention. Amino acid sequences of human DAP12 and Fc receptor γ-chain as well as a base sequence of cDNA, which encodes the sequences, are publicly known. A base sequence of human Fc receptor γ-chain and an amino acid sequence which is encoded by the base sequence are shown in SEQ ID NOs: 15 and 16, respectively.

[0045]

In the present invention, a transformed cell to be used as an immunogen can be obtained by preparing, for example, a cell expressively carrying the following (a) and (b):

(a) an exogenous polynucleotide encoding an amino acid sequence comprising an extracellular domain of human ILT7; and

(b) an exogenous polynucleotide encoding Fc receptor γ-chain.

In the present invention, an exogenous polynucleotide means a polynucleotide which is artificially introduced into a host cell. When human cells are used as cells, human genes are introduced into human cells. In such a combination, an artificially introduced polynucleotide means the exogenous polynucleotide. Therefore, ectopic expression of human ILT7 or human Fc receptor γ-chain is comprised in expression of the exogenous polynucleotide.

[0046]

As used herein, the term "extracellular domain of human ILT7" means the amino acid sequence from the 17th to 444th position of the amino acid sequence described in SEQ ID NO: 2 which corresponds to the extracellular domain of the amino acid sequence (from 1 to 428 in SEQ ID NO: 2). As an amino acid sequence comprising the extracellular domain of human ILT7 in the present invention, it is preferable to use the amino acid sequence which comprises each region, for example,

starting from the N terminal, in order of the following:
[Signal sequence + extracellular domain + transmembrane domain + intracellular region]

Alternatively, an amino acid sequence, which partially lacks an intracellular region as described below, is included in the amino acid sequence comprising the extracellular domain of human ILT7 in the present invention.
[Signal sequence + extracellular domain + transmembrane domain + a portion of intracellular region]

Furthermore, a structure, which lacks an intracellular region as mentioned below, is included in the amino acid sequence comprising the extracellular domain of human ILT7 in the present invention.
[Signal sequence + extracellular domain + transmembrane domain]
[0047]

In the structure, regions other than the extracellular domain may be amino acid sequences which are selected from the amino acid sequence shown in SEQ ID NO: 2, or may be combined with other amino acid sequences having homology with the regions. For example, the amino acid sequence constituting a signal sequence, a transmembrane domain, and an intracellular region may be an amino acid sequence of ILT family molecules other than ILT7. Or, it may be combined with the amino acid sequence of ILT family in species other than human. Further, the amino acid sequence, which constitutes regions other than the extracellular domain, may comprise a mutation in the range capable of maintaining the function of each region. Alternatively, other regions may intervene between each region. For example, an epitope tag such as FLAG can also be inserted between the signal sequence and the extracellular domain. Particularly, the signal sequence is removed by processing during its transfer to the cell membrane surface after being translated into protein. Therefore, arbitrary amino acid sequence, which induces transit of the translated protein to the cell membrane, can be used as the signal sequence. More specifically, it is preferable to use the amino acid sequence (SEQ ID NO: 2) of human ILT7 as the amino acid sequence comprising the extracellular domain of human ILT7.
[0048]

Therefore, in the present invention, an arbitrary base sequence which encodes the amino acid sequence constituting the above-mentioned structure [signal sequence + extracellular domain + transmembrane domain + intracellular region] can be used as the polynucleotide which constitutes the exogenous polynucleotide described in (a). For example, the amino acid sequence of SEQ ID NO: 2 is encoded by the base sequence described in SEQ ID NO: 1.

[0049]

In the present invention, an expression vector expressively carrying the above-mentioned polynucleotides (a) and (b) may be introduced to an appropriate host cell in order to obtain a transformed cell to be used as an immunogen. The Polynucleotides (a) and (b) can be carried on one vector or different vectors. When each polynucleotide is carried on different vectors, the host cells are co-transfected with two kinds of vectors.

[0050]

Preferable examples of the host cell in the present invention comprise mammalian cells. Specific examples of the host cell comprise cells derived from humans, monkeys, mice or rats. Particularly, the cells derived from humans are preferable as host cells. For example, it is preferable to use 293T cells derived from human as the host cell in the present invention. 293T cells can be obtained as ATCC CRL-11268. In addition, cells derived from immune animals can also be used as host cells. When cells derived from immune animals are used as immunogens, little immunological response to host cells is given. For that reason, an antibody against the extracellular domain of exogenously expressed ILT7 can be obtained efficiently. Therefore, for example, when mice are used as immune animals, cells derived from mice can also be used as host cells.

[0051]

The above-mentioned polynucleotides can be transformed into cells by carrying them on a vector capable of inducing expression in host cells. Commercially available vectors, which can induce the expression in mammalian cells, may be used. Expression vectors such as pCMV-Script(R) Vector, PSG5 Vector (manufactured by Stratagene), pcDNA3.1 (manufactured by Invitrogen) can be used for the present

invention.

[0052]

The transformed cells thus obtained are administered to immune animals together with additional components such as adjuvants, if necessary. Usable examples of the adjuvant include Freund's complete adjuvant, and the like. In case of using mice as immune animals, the transformed cells can be administered in the range of 10^4 to 10^9 cells, more specifically 10^4 to 10^6 cells. Generally, multiple doses of immunogen are given at regular intervals until the antibody titer is elevated. For example, in the case of a short-term immunization, the transformed cells are administered at 2 to 4 day intervals, more specifically at intervals of 3 days. After administering twice or three times, antibody producing cells can be recovered. Alternatively, they are administered once weekly and antibody producing cells can also be recovered after administering five or six times.

[0053]

In the present invention, the recovered antibody producing cells are cloned to give monoclonal antibodies. It is preferable that the antibody producing cells are immortalized for cloning. For example, the cell fusion method as typified by the hybridoma method or transformation by Epstein-Barr virus (EBV) can be used as the method of immortalization of antibody producing cells.

[0054]

As for antibody producing cells, one cell produces one kind of antibody. Therefore, the establishment of cell populations derived from one cell (i.e. cloning) allows for producing monoclonal antibodies. The hybridoma method involves the process in which antibody producing cells are fused with an appropriate cell line, which is immortalized and then subjected to cloning. The immortalized antibody producing cells can be cloned by a technique such as limiting dilution method. It is known that there are lots of cell lines useful for the hybridoma method. These cell lines are excellent in the immortalization efficiency of lymphocytic cells and have various genetic markers which are needed to select the successfully fused cells. Further, when the production of antibody producing cells is intended, a cell line lacking the ability to produce antibodies can also be used.

[0055]

For example, mouse myelomas P3x63Ag8.653 (ATCC CRL-1580) and P3x63Ag8U.1 (ATCCCRL-1597) are widely used as useful cell lines for the cell fusion method for mice or rats. In general, a hybridoma is produced by the fusion of homogeneous cells, while a monoclonal antibody can also be obtained from hetero hybridoma from a different species among closely related species.

[0056]

Specific protocols of the cell fusion have been publicly known. That is, antibody producing cells of immune animals are mixed with appropriate fusion partners to perform cell fusion. Usable examples of the antibody producing cell include splenic cells, lymphocyte cells collected from the lymph node, and peripheral blood B cells. As fusion partners, various cell lines described previously can be used. The polyethylene glycol method and electric fusion method can be used for cell fusion.

Next, on the basis of selective markers of fused cells, the successfully fused cells are selected. For example, when HAT sensitive cell line is used for cell fusion, the successfully fused cells are selected by selecting cells growing in HAT medium. Further, it is confirmed that the antibodies produced by the selected cells have the desired responsiveness.

[0057]

Each hybridoma is screened based on the responsiveness of antibodies. That is, the hybridoma producing antibodies which bind to human ILT7 is selected by the method as described previously. Preferably, when the selected hybridoma is subcloned and then the production of the desired antibody is finally confirmed, the confirmed antibody is selected as a hybridoma producing monoclonal antibody of the present invention.

[0058]

Specifically, the desired hybridoma can be selected based on the responsiveness to human cells or the responsiveness to the transformed cell which expresses human ILT7 gene. The antibodies, which bind to cells, can be detected based on the principle of immunoassay. For example, ELISA, which uses cells as antigens, can

be utilized for detection of the desired antibody. Specifically, a culture supernatant of hybridoma is made to contact with a support on which human IPC or the transformed cell used as an immunogen. In the case where the culture supernatant comprises the desired antibody, the antibody is trapped in the cell immobilized on the support. Then, the solid phase is separated from the culture supernatant, which is washed, if necessary. Thereafter, the antibody trapped in the solid phase can be detected. An antibody, which recognizes an antibody, can be used for the detection of antibodies. For example, an antibody of mouse can be detected by an anti-mouse immunoglobulin antibody. The detection is easy if the antibody, which recognizes an antibody, is labeled. Usable examples of the label include enzymes, fluorescent dyes, luminescent dyes, and the like.

On the other hand, particles and an inner wall of a microtiter plate can be used as the support on which cells are immobilized. Cells can be immobilized on particles made of plastic or the surface of a container by physical adsorption. Usable examples of the support for immobilizing cells include beads made of polystyrene and reaction vessels.

[0059]

In the selection of hybridomas, the production of the antibody against not ILT7 but the host cell of the transformed cell used as an immunogen may be predicted. For example, as illustrated in Examples, in the case where a human cell is used as an immunogen and a mouse is used as an immune animal, the human cell is recognized as a foreign substance. Thus, the production of an antibody, which binds to the foreign substance, is predicted. In the present invention, it is intended to obtain an antibody capable of recognizing human ILT7. Therefore, it is not necessary to obtain an antibody which recognizes human cell antigens other than human ILT7. In order to remove hybridomas, which produce such an antibody in screening, undesired antibodies, can be absorbed prior to the confirmation of the antibody responsiveness.

[0060]

Undesired antibodies can be absorbed by an antigen to which an antibody presumed to exist binds. Specifically, for example, an

antibody against human cell antigens other than human ILT7 can be absorbed by a cell which cannot detect the expression of human ILT7. In the present invention, it is preferable to use the host cell used for the immunogen as an antigen for absorbing the undesired antibodies. Alternatively, a host cell which does not express the extracellular domain of human ILT7, but expresses molecule which associates with ILT7 can be used as the antigen for absorbing the antibodies.

[0061]

As for the monoclonal antibody whose binding activity to antigen is confirmed, its actual effect on the IPC activity is confirmed, if necessary. The effect on IPC can be confirmed by methods such as the methods described below.

[0062]

As for the monoclonal antibody of the present invention, a hybridoma producing the monoclonal antibody is cultured and the monoclonal antibody of the present invention is recovered from the resulting culture. The hybridoma can be cultured in vitro or in vivo. In the case of in vitro, the hybridoma can be cultured by using a known culture medium such as RPMI1640. The immunoglobulin secreted by the hybridoma is accumulated in the culture supernatant. Therefore, the monoclonal antibody of the present invention can be obtained by collecting the culture supernatant and purifying it, if necessary. It is easier to purify the immunoglobulin when serum is not added to the culture medium. However, for the purpose of more rapid proliferation of the hybridoma and facilitation of antibody production, 10% fetal bovine serum can also be added to the culture medium.

[0063]

The hybridoma can also be cultured in vivo. Specifically, the intraperitoneal cultivation of the hybridoma can be made by inoculating the hybridoma into the abdominal cavity of nude mice. Monoclonal antibodies are accumulated in the ascites. Therefore, if the ascites is obtained and purified as needed, the required monoclonal antibody can be produced. The obtained monoclonal antibodies can be appropriately modified or processed in accordance with the intended use.

[0064]

The monoclonal antibody of the present invention can be expressed by obtaining cDNA which encodes the antigen binding region of antibody from the hybridoma and inserting into an appropriate expression vector. The technique, in which cDNA which encodes a variable region of antibody is obtained and then it is expressed in an appropriate host cell, is known. In addition, the method in which a chimeric antibody is made by ligating a variable region comprising the antigen binding region into a constant region is also known.

[0065]

Preferable examples of the monoclonal antibody in the present invention comprise monoclonal antibodies produced by hybridoma #11 (Accession number: FERM BP-10704), hybridoma #17 (Accession number: FERM BP-10705), or hybridoma #37. Amino acid sequences which constitute variable regions of these monoclonal antibodies as well as base sequences of cDNA encoding thereof are described below. Therefore, for example, chimeric antibodies to be obtained by conjugating these variable regions to constant regions of other immunoglobulins are preferable in the present invention. In amino acid sequences described in the Sequence Listing, the amino acid sequence from 1 to C terminus constitutes a mature protein. That is, the consecutive amino acid sequence from 1 to C terminus for each amino acid sequence is a mature sequence of each amino acid sequence. On the other hand, the amino acid sequence represented by a numerical value from N terminus to -1 is a signal sequence.

	Heavy chain variable region	Light chain variable region
#11	SEQ ID NO: 38	SEQ ID NO: 40
	(base sequence)	(base sequence)
	SEQ ID NO: 39	SEQ ID NO: 41
	(amino acid sequence)	(amino acid sequence)
#17	SEQ ID NO: 42	SEQ ID NO: 44
	(base sequence)	(base sequence)
	SEQ ID NO: 43	SEQ ID NO: 45
	(amino acid sequence)	(amino acid sequence)
#37	SEQ ID NO: 46	SEQ ID NO: 48
	(base sequence)	(base sequence)
	SEQ ID NO: 47	SEQ ID NO: 49
	(amino acid sequence)	(amino acid sequence)

For example, a mouse (variable region)-human (constant region) chimeric antibody can be made by ligating these variable region genes

into a human IgG1 heavy chain constant region and a gene encoding human Ig kappa light chain constant region, respectively. Amino acid sequences of such a chimeric antibody and base sequences encoding thereof are respectively described below. Chimeric antibodies specified by these sequences show the construction of a preferred embodiment of anti-ILT7 monoclonal antibody in the present invention. In the following amino acid sequences of chimeric antibodies, the amino acid sequence from N terminus to -1 corresponds to the signal sequence and the amino acid sequence from 1 to C terminus corresponds to the mature protein. That is, a chimeric antibody comprised of heavy and light chains, which consist of the amino acid sequence from 1 to C terminus for each amino acid sequence, is preferable in the present invention.

	Heavy chain	Light chain
#11	SEQ ID NO: 50 (base sequence)	SEQ ID NO: 52 (base sequence)
	SEQ ID NO: 51 (amino acid sequence)	SEQ ID NO: 53 (amino acid sequence)
#17	SEQ ID NO: 54 (base sequence)	SEQ ID NO: 56 (base sequence)
	SEQ ID NO: 55 (amino acid sequence)	SEQ ID NO: 57 (amino acid sequence)

[0066]

Further, the antigen-binding activity of monoclonal antibody can also be grafted to other immunoglobulins. The variable region of immunoglobulin is comprised of a complementarity-determining region (CDR) and a frame region. The antigen-binding property of each immunoglobulin is determined by CDR and the frame maintains the structure of antigen binding region. The amino acid sequence of CDR is extremely rich in diversity, while the amino acid sequence of the portion of the frame is highly conserved. It is known that the amino acid sequence constituting CDR is incorporated into the frame region of other immunoglobulin molecules, which allows for grafting of the antigen-binding activity. The method in which the antigen-binding property of different immunoglobulins is grafted to human immunoglobulin by using this process has been established. As used herein, the term "antigen binding region" can comprise the CDR which is grafted to the frame. Therefore, the term "fragment comprising

the antigen binding region" of a certain monoclonal antibody" comprises a fragment of human immunoglobulin comprising the variable region to which CDR of the monoclonal antibody is grafted. For example, each of the amino acid sequences of the above-mentioned variable regions comprises the following amino acid sequences (SEQ ID NOs) as CDRs.

	CDR1	CDR2	CDR3
#11 heavy chain	SDYAWN (58)	YISYSGSTSYNPSLKSR (59)	SPPYYAMDY (60)
#11 light chain	KASQDVGTAVA (61)	WASTRHT (62)	QQYSSYPLT (63)
#17 heavy chain	SYWIH (64)	RIYPGTGSTYYNEKFKG (65)	YPTYDWYFDV (66)
#17 light chain	RASQSISNYLH (67)	YASQSIS (68)	QQSNSWPLT (69)
#37 heavy chain	SDYAWN (70)	YISYSGSTSYNPSLKSR (71)	ALPLPWFAY (72)
#37 light chain	KASQDVGTAVA (73)	WASTRHT (74)	QQYSSYPYT (75)

Based on the information of the base sequence which encodes the above-mentioned amino acid sequences and the information of the base sequence which encodes the frame (FR) of human immunoglobulin, a primer can be designed and cDNA having a base sequence obtained by conjugating both of the base sequences can be amplified. The operation is repeated for each frame and a variable region in which CDR1, CDR2, and CDR3 of mice are connected by human FR can be constructed. Further, when the base sequence, which encodes a constant region of human immunoglobulin, is conjugated as needed, a humanized antibody with the constant region can be obtained.

As the chimeric antibody comprising the above-mentioned variable regions or a humanized antibody to which CDR constituting a variable region is grafted, an antibody with a constant region derived from IgG or IgM is comprised in a preferable antibody in the present invention. The present inventors confirmed that the monoclonal antibody against ILT7 showed CDC action on ILT7 expressing cells. Therefore, the antibody having a constant region derived from IgG or IgM exhibits cytotoxicity against ILT7 expressing cells due to CDC effect. Such antibodies are useful in inhibiting the number of ILT7 expressing cells such as IPCs.

[0067]

The chimeric antibody capable of recognizing ILT7 or humanized antibody, which is provided by the present invention, can be produced by genetic engineering using polynucleotides encoding these antibodies. For example, a polynucleotide which is the base sequence described in the following SEQ ID NOs and encodes the amino acid sequence constituting a mature protein for each amino acid sequence can be used as a polynucleotide encoding the variable region #11 or #17. The consecutive amino acid sequence from 1 to C terminus for each amino acid sequence corresponds to a mature protein. In the case where each mature protein is expressed as a separate protein, it is preferable to place the secretion signal at the N terminus of each amino acid sequence. For example, in the amino acid sequences shown in these SEQ ID NOs, the amino acid sequence from N terminus to -1 can be used as a signal sequence when such proteins are expressed in animal cells. Alternatively, these variable regions can be secreted as mature proteins by using an arbitrary signal sequence which enables the secretion of immunoglobulin.

#11 SEQ ID NO: 50 (base sequence) SEQ ID NO: 52 (base sequence)
#17 SEQ ID NO: 54 (base sequence) SEQ ID NO: 56 (base sequence)

[0068]

In the same manner as described above, as for the polynucleotide encoding the humanized antibody, a polynucleotide which expresses the humanized antibody can be made by using the base sequence which encodes a protein having the signal sequence to be added to the N terminus. When heavy and light chains are carried on separate vectors, both vectors are co-transfected into the same host cell. The heavy and light chains expressed from each vector are used to construct an immunoglobulin molecule with both chains. Or, a polynucleotide encoding a heavy chain and a polynucleotide encoding a light chain can also be carried on the same vector. The host cell into which a vector carrying both polynucleotides is co-transfected expresses heavy and light chains and produces an immunoglobulin having both chains.

These polynucleotides can be expressed as antibodies using a host-vector system capable of expressing an antibody gene. Furthermore, in the case where they are expressed as a single protein molecule by connecting a heavy chain variable region with a light

chain variable region, a signal sequence can be placed at the N terminus of the protein molecule. A known example of such an antibody molecule includes scFv molecule in which a heavy chain variable region and a light chain variable region are connected by a linker.

[0069]

Each of the monoclonal antibodies thus produced is comprised in the monoclonal antibody of the present invention. In other words, a monoclonal antibody which consists of an immunoglobulin comprising the antigen binding region encoded by a polynucleotide derived from cDNA encoding the antigen binding region of the above-mentioned monoclonal antibodies is comprised in the monoclonal antibody in the present invention.

[0070]

As described previously, RBL cells in which ILT1 gene was forced to be expressed could be used as an immunogen for obtaining ILT1 antibodies. However, the expression of ILT7 on the surface of RBL cells (P815) could not be confirmed and thus it could not be used as the immunogen. The present inventors found out that the expression of human ILT7 on the cell surface could be induced by the coexpression of human ILT7 and other cell membrane proteins which associate with human ILT7. Then, the present inventors found that the antibody, which binds to human IPCs, can be obtained by using the transformed cell whose expression is thus induced as an immunogen and completed the present invention.

That is, the present invention provides the immunogen for producing the antibody which binds to the extracellular domain of human ILT7, and comprises animal cells in which (a) a polynucleotide which encodes the amino acid sequence comprising the extracellular domain of human ILT7; and (b) a polynucleotide which encodes Fc receptor γ-chain are maintained so as to be exogenously expressed or cell membrane fractions thereof.

[0071]

Six years or more have already passed since the structure of human ILT7 was found in 1998. However, the antibody capable of specifically recognizing ILT7 has still not been obtained. The antibody capable of recognizing human ILT7 was provided by using the

immunogen of the present invention for the first time. That is, the present invention provided the antibody capable of recognizing human ILT7 which can be obtained by the following steps of:

(1) administering a cell which exogenously expresses a protein comprising extracellular domain of human ILT7 and a molecule which is associated with human ILT7 to immune animals;

(2) selecting an antibody producing cell which produces the antibody which binds to human ILT7 from antibody producing cells of the immune animals; and

(3) culturing the antibody producing cells selected by step (2) and recovering an antibody capable of recognizing human ILT7 from the cultures.

[0072]

It is found that human ILT7 is specifically expressed in human IPC. In the analysis of gene expression by SAGE which was performed by the present inventors, the specific expression of human ILT7 in human IPC was also confirmed. However, in the past reports, the expression levels of ILT7 of both cases were analyzed based on mRNA. Since the antibody capable of detecting human ILT7 was not provided, the expression state of protein was not analyzed conventionally. The analysis of human ILT7 protein was realized by the provision of the antibody which binds to the extracellular domain of human ILT7 in the present invention.

[0073]

The present inventors actually confirmed that the monoclonal antibody which binds to the extracellular domain of human ILT7 based on the present invention specifically detected human IPCs. That is, the present invention relates to a method for detecting interferon producing cells which comprise the steps of: contacting a monoclonal antibody which binds to the extracellular domain of human ILT7 or a fragment comprising the antigen binding region with a test cell; and detecting the monoclonal antibody which is bound to cells or a fragment comprising its antigen binding region.

[0074]

The detection of human ILT7 based on the present invention allows for determining whether a certain cell is IPC. That is, the present

invention provides a method for identifying IPCs using human ILT7 as an indicator. Or, human IPCs can be separated by separating the cells in which human ILT7 was detected based on the present invention. That is, the present invention provides a method for separating IPCs using human ILT7 as an indicator.

[0075]

Based on the analysis by human ILT7 antibody, it was confirmed that the expression level of ILT7 in IPCs whose differentiation was induced by CpG, and the like was reduced. That is, the IPCs before their differentiation is induced can be specifically detected by using ILT7 as an indicator. In other words, the monoclonal antibody of the present invention is useful, particularly in detecting IPCs before their differentiation into dendritic cells. As used herein, the term "IPCs before their differentiation" can be defined as cell populations which maintain the capacity to produce interferon.

[0076]

In the present invention, the monoclonal antibody which binds to the extracellular domain of human ILT7 or the fragment comprising its antigen binding region can be labeled in advance. For example, antibodies can be easily detected by labeling with luminescent dyes or fluorescent dyes. More specifically, the fluorescent-dye labeled antibody is made to contact with a cell population which may comprise IPCs and then cells to which the antibody of the present invention bound can be detected by using the fluorescent dye as an indicator. Further, IPCs can be separated by separating the cells in which the fluorescent dye is detected. A series of the steps can be easily performed based on the principle of FACS.

[0077]

Alternatively, the antibody of the present invention can be bound to a solid phase support such as magnetic particles in advance. The antibody bound to the solid phase support recognizes human ILT7 and then IPCs are trapped in the solid phase support. As a result, IPCs can be detected or separated.

[0078]

The antibody necessary for the method for detecting IPCs based on the present invention can be provided as a reagent for detecting

IPCs. That is, the present invention provides a reagent for detecting interferon producing cells, comprising the monoclonal antibody which binds to the extracellular domain of human ILT7 or the fragment comprising its antigen binding region. The reagent for detecting IPCs of the present invention can be used in combination with a positive control or a negative control in addition to antibodies. For example, the transformed cells which express the extracellular domain of human ILT7 and are used for the immunogen as well as the IPCs obtained from human can be used as the positive controls. Usually, only a few human IPCs can be obtained from the peripheral blood. Therefore, it is preferable to use, particularly a transformed cell as the positive control in the reagent of the present invention. On the other hand, an arbitrary cell, which does not express human ILT7, can be used as the negative control.

[0079]

That is, the present invention provides a kit for detecting human IPCs which comprises:

(a) the monoclonal antibody which binds to the extracellular domain of human ILT7 or the fragment comprising its antigen binding region; and

(b) the cell which expresses an exogenous protein comprising extracellular domain of human ILT7 and an exogenous molecule which is associated with human ILT7.

[0080]

The present inventors analyzed the effect of the antibody which binds to the extracellular domain of human ILT7 on IPCs. As a result, it is confirmed that the antibody, which binds to the extracellular domain of human ILT7, inhibits the activity of IPCs. That is, the present invention relates to a method for inhibiting the activity of interferon producing cells, comprising a step of contacting any of the following components with interferon producing cells:

(a) a monoclonal antibody which binds to human ILT7 and inhibits the activity of interferon producing cells or a fragment comprising its antigen binding region; and

(b) an immunoglobulin to which a complementarity-determining region of the monoclonal antibody described in (a) is grafted or a

fragment comprising its antigen binding region.

[0081]

Or, the present invention relates to a method for inhibiting the activity of interferon producing cells in living organisms, comprising a step of administering any of the following components to the living organisms:

(a) the monoclonal antibody which binds to human ILT7 and inhibits the activity of interferon producing cells or a fragment comprising its antigen binding region;

(b) a fragment comprising the immunoglobulin to which a complementarity-determining region of the monoclonal antibody described in (a) is grafted or a fragment comprising its antigen binding region; and

(c) a polynucleotide which encodes the components described in (a) or (b).

[0082]

As used herein, the term "Interferon Producing cells (IPCs)" means cells which have the ability to produce IFN and express ILT7 on the cell surface. Hereinafter, unless otherwise noted, the term "IPCs" encompasses not only cells which are precursor cells of dendritic cells but also the cells which have the ability to produce IFN and express ILT7 on the cell surface. Methods for identifying such IPCs are commonly known. IPCs can be distinguished from other blood cells using some cell surface markers as indicators. Specifically, a profile of cell surface markers of human IPCs is described below (Shortman, K. and Liu, YJ. Nature Reviews 2: 151-161, 2002). In recent years, a certain report has also suggested that BDCA-2 positive cell is defined as IPC (Dzionek, A. et al. J. Immunol. 165: 6037-6046, 2000.).

[Profile of cell surface antigens of human IPCs]

CD4 positive, CD123 positive,

Lineage (CD3, CD14, CD16, CD19, CD20, CD56) negative, and CD11c negative

Therefore, it can also be said that IPCs are cells which have the expression profile of these known markers and have the ability to produce IFN. Further, cells in living organisms with the ability to produce IFN are comprised in IPCs, even if the cells are a cell population with profiles different from the expression pattern of

the expression profile of these markers. Further, examples of the characteristics, which are commonly seen in human IPCs, are as follows:

[Morphological characteristic of cells]

- Similar to plasma cells
- Round cells with a smooth cell surface
- The nucleus is relatively large

[Functional characteristic of cells]

- During virus infection, a large amount of Type-1 interferons are produced in a short period of time.
- Differentiated into dendritic cells after virus infection.

[0083]

As used herein, the term "inhibition of the activity of IPCs" means the inhibition of at least one of the functions of IPCs. Examples of the function of IPCs include the production of IFN and the cell survival. The cell survival can also be translated into the number of cells. Therefore, in the case of inhibiting both or either of these functions, it is said that the activity of IPCs is inhibited. It is found that type 1 IFN produced by IPCs leads to various diseases. Therefore, the inhibition of the number of IPCs and IFN production is useful for a medical treatment strategy of those diseases.

For example, the relationship between the pathological condition of autoimmune diseases and IFNα has been pointed out. Most of the IFNα is produced by IPCs. Therefore, pathological conditions caused by IFNα can be alleviated by inhibiting the production of IFNα. As used herein, the term "inhibition of IFN production by IPCs" means the inhibition of the production of at least one of the IFN produced by IPCs. Preferable IFN in the present invention is the type 1 IFN. Among them, IFNα is important.

[0084]

That is, the present invention relates to an inhibitor of the production of IFN which comprises an antibody which binds to the extracellular domain of ILT7 as an active ingredient. Or, the present invention provides a method for inhibiting the production of IFN comprising a step of administering the antibody which binds to the extracellular domain of ILT7. Further, the present invention relates to the use of the antibody which binds to the extracellular domain

of ILT7 in the production of a medicinal composition for inhibiting the production of IFN.

[0085]

Cells in which a large amount of IFN is produced by a small number of cells are included in IPCs. For example, precursor cells of dendritic cells stimulated by viruses and the like produce most of the IFN produced by the living body. The inhibition of the number of IPCs which produce a lot of IFN results in suppressing the IFN production. Therefore, pathological conditions caused by IFNα can be reduced by inhibiting the number of IPCs. It was confirmed that anti-ILT7 monoclonal antibody bound to ILT7 expressing cells and then the effect of cytotoxicity was given by Complement Dependent Cytotoxicity (CDC) in a preferable embodiment of the present invention. CDC effect is one of the important mechanisms of antibody drug. The anti-ILT7 monoclonal antibody of the present invention also has potent cytotoxicity against ILT7 expressing cells such as IPCs due to CDC effect thereof. That is, as for the anti-ILT7 monoclonal antibody, the IFN production inhibiting effect can be expected by cytotoxicity against IPCs, in addition to the inhibition mechanism of IFN production in a preferable embodiment.

[0086]

The antibody, which recognizes the extracellular domain of human ILT7 to be used for the present invention, can be obtained based on the method described previously. The antibody in the present invention may be of any class. Organism species from which the antibody is derived are not limited, either. Further, a fragment comprising the antigen binding region of antibody can be used as an antibody. For example, an antibody fragment comprising the antigen binding region which is obtained by enzymatic digestion of IgG can be used as the antibody in the present invention. Specifically, antibody fragments such as Fab and $F(ab')_2$ can be obtained by digestion with papain or pepsin. It is well known that these antibody fragments can be used as antibody molecules which have affinity for antibodies. Alternatively, antibodies constructed by genetic recombination can also be used as long as satisfactory antigen-binding activity is maintained. Examples of the antibodies constructed by genetic recombination

include chimeric antibodies, CDR-transplanted antibodies, single chain Fvs, diabodies, linear antibodies, and polyspecific antibodies formed of antibody fragments. It is common knowledge that these antibodies can be given by using monoclonal antibodies.

[0087]

In the present invention, antibodies can be modified, if necessary. According to the present invention, the antibody, which recognizes the extracellular domain of human ILT7, has an inhibiting effect on the activity of IPCs. That is, it is contemplated that the antibody itself has cytotoxicity against IPCs. Subclasses of antibodies which exhibit potent effector activity are known. Alternatively, the inhibiting effect on the IPC activity can be further enhanced by modifying antibodies with a cytotoxic agent. Examples of the cytotoxic agent are described below.

Toxins: Pseudomonas Endotoxin (PE), diphtheria toxin, ricin
Radioisotopes: Tc^{99m}, Sr^{89}, I^{131}, Y^{90}
Anticancer agents: calicheamicin, mitomycin, paclitaxel

Toxins consisting of proteins can be conjugated to antibodies or their fragments with a bifunctional reagent. Alternatively, a gene encoding toxin is connected to a gene encoding antibody and fusion proteins of both genes can also be obtained. The method for conjugating antibodies with radioisotopes is also known. For example, the method for labeling antibodies with radioisotopes using a chelating agent is known. Furthermore, the anticancer agents can be conjugated to antibodies using sugar chains or the bifunctional reagent.

[0088]

The present inventors have confirmed a phenomenon in which a monoclonal antibody which is bound to ILT7 expressed on a cell membrane is incorporated into cells after binding (internalization). Therefore, the cytotoxic agents can be delivered into cells by contacting antibodies conjugated with these cytotoxic agents of the present invention with ILT7 expressing cells. That is, the present invention provides an active inhibitor of ILT7 expressing cells which comprises anti-ILT7 monoclonal antibody to which the cytotoxic agent is conjugated as an active ingredient. Or, the present invention relates to the use of anti-ILT7 monoclonal antibody to which the

cytotoxic agent is conjugated in the production of the active inhibitor of ILT7 expressing cells. Further, the present invention provides a method for inhibiting the activity of ILT7 expressing cells comprising a step of administering anti-ILT7 monoclonal antibody to which the cytotoxic agent is conjugated.

[0089]

In the present invention, an antibody whose structure is artificially modified can also be used as an active ingredient. For example, various modification methods are known in order to improve the cytotoxicity and stability of antibodies. Specifically, an immunoglobulin in which sugar chains of heavy chains are modified is known (Shinkawa, T. et al. J. Biol. Chem. 278:3466-3473. 2003.). Antibody Dependent Cell-mediated Cytotoxicity (ADCC) activity of immunoglobulin was enhanced by the modification of sugar chains. Or, an immunoglobulin in which the amino acid sequence of Fc region is modified is also known. That is, ADCC activity was enhanced by artificially increasing the binding activity of immunoglobulin to Fc receptor (Shield, RL. et al. J. Biol. Chem. 276; 6591-6604, 2001.).

[0090]

IgG, which is bound to Fc receptor, is incorporated in cells once. Then, IgG binds to Fc receptor which is expressed in endosome and it is released into blood again. This phenomenon has been revealed. IgG with a high binding activity with Fc receptor has a better chance of being released into blood again after its incorporation into cells. As a result, the retention time of IgG in blood is extended (Hinton, PR. et al. J Biol Chem. 279: 6213-6216. 2004). In addition to this, it is said that modification of amino acid sequence of Fc region causes a change of complement dependent cytotoxicity (CDC) activity. These modified antibodies can be used as the antibody in the present invention.

[0091]

When the antibody, which binds to the extracellular domain of human ILT7, is contacted to IPCs, the activity of IPCs is inhibited. Therefore, these antibodies can be used for an inhibitor or method for inhibiting the activity of IPCs. That is, the present invention provides an active inhibitor of IPCs which comprises at least one component selected from the group consisting of the following (a)

to (c) as an active ingredient. Or, the present invention relates to a method for inhibiting the activity of IPCs comprising a step of administering at least one component selected from the group consisting of the following (a) to (c). Further, the present invention relates to the use of at least one component selected from the group consisting of the following (a) to (c) in the production of active inhibitor of IPCs:

(a) the monoclonal antibody which binds to human ILT7 or a fragment comprising its antigen binding region;

(b) the immunoglobulin to which a complementarity-determining region of the antibody described in (a) is grafted or a fragment comprising its antigen binding region; and

(c) a polynucleotide which encodes components described in (a) or (b).

In the present invention, the monoclonal antibody, which recognizes the extracellular domain of human ILT7, can be used as the monoclonal antibody which inhibits the activity of IPCs. In the present invention, one or more monoclonal antibodies can be used. For example, one or more monoclonal antibodies, which recognize the extracellular domain of human ILT7, are blended to use in the present invention.

[0092]

It can be confirmed that antibodies have an inhibiting effect on IFN production by IPCs in the manner as described below. IPCs produce a large amount of IFN due to virus stimulation. Antibodies are given to IPCs before, after, or at the same time as the stimulation of IPCs with viruses. The capacity to produce IFN each for the resulting IPCs is compared to that of each control to which antibodies are not given. The ability to produce IFN can be evaluated by measuring IFNα or IFNβ contained in culture supernatant of IPCs. As a result of the comparison, it can be confirmed that the tested antibodies are effective in inhibiting the ability to produce IFN when the amount of IFN in the supernatant is significantly decreased by the addition of antibodies. These methods for measuring IFN are known. IPCs produce most of the IFN in the living body. Therefore, IFN producing state in the living body can be regulated by inhibiting the ability to produce IFN of

IPCs.

[0093]

In the present invention, the activity of IPCs encompasses the maintenance of the number of IPCs. Therefore, the inhibition of the activity of IPCs in the present invention comprises the inhibition of the number of IPCs. When it is confirmed that the number of IPCs is inhibited under the presence of antibodies, it can be found that the antibodies are inhibiting the activity of IPCs. As with IFN production, an inert immunoglobulin derived from the same animal species as the antibody whose activity should be confirmed can be used as a comparative control. The number of IPCs can be quantitatively compared by counting cells. The number of cells can be counted with FACS or a microscope.

[0094]

Further, it is said that IPCs are differentiated into cells which induce Th2 referred to as dendritic cell 2 (DC2) as a result of infection with virus or the like. If IFN production of IPCs by virus stimulation can be inhibited, their differentiation into Th2 may also be inhibited. Therefore, it can be expected that the monoclonal antibody of the present invention, which inhibits IFN production, may also have a therapeutic effect on various allergic diseases.

[0095]

When the antibody, which recognizes the extracellular domain of human ILT7, is administered to a host different from organism species from which the antibody is derived, it is desirable to process the antibody into a shape which is hard to be recognized as a foreign substance by the host. For example, immunoglobulin cannot be easily recognized as the foreign substance by processing the antibody into the following molecules. The technique for processing immunoglobulin molecules as described below is known. Fragment comprising the 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)

-Chimeric antibody composed of the antigen binding region of monoclonal antibody and the constant region of immunoglobulin of the host ("Gene Expression Experiment Manual", Isao Ishida, Tamie Ando,

eds., Kodansha, 1994)

-CDR-substituted antibody in which complementarity-determining region (CDR) of immunoglobulin of the host is substituted to CDR of monoclonal antibody ("Gene Expression Experiment Manual", Isao Ishida, Tamie Ando, eds., Kodansha, 1994)

[0096]

Alternatively, a human antibody can be obtained by using non-human animals into which a human antibody gene is incorporated as immune animals, while the non-human animals are used. For example, transgenic mice with human antibody genes have been put to practical use in order to produce human antibodies as immune animals (Ishida et al., Cloning and Stem Cells, 4:85-95, 2002). The use of such animals allows for obtaining the human antibody which recognizes ILT7 using immunogens as described previously. It is preferable to administer human antibody to humans.

[0097]

Alternatively, a human immunoglobulin variable region gene can also be obtained by a 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 human immunoglobulin variable region is incorporated into a phage gene. A phage library can also be produced by using various immunoglobulin genes as sauces. A phage expresses a variable region as a fusion protein of the protein composed of the phage. The variable region expressed by the phage on the phage surface maintains the binding activity with an antigen. Therefore, phages, which bind to antigens or cells in which antigens are expressed, are selected, thereby allowing for screening a phage in which a variable region having the desired binding activity is expressed from the phage library. Further, a gene encoding a variable region, which has the desired binding activity, is retained in the phage particles thus selected. That is, in the phage display method, a gene encoding a variable region with the desired binding activity can be obtained by using the binding activity of the variable region as an indicator.

[0098]

In the active inhibitor of IPCs or the method for inhibiting

the activity of IPCs in the present invention, the antibody which recognizes the extracellular domain of human ILT7 or the antibody fragment which comprises at least the antigen binding region of the antibody can be administered as a protein or a polynucleotide encoding the protein. In administration of polynucleotides, it is desirable to use a vector in which a polynucleotide encoding the desired protein is placed under the control of an appropriate promoter so as to express the desired protein. An enhancer and a terminator can also be placed in the vector. A vector which carries a gene of heavy and light chains which constitutes an immunoglobulin and is able to express an immunoglobulin molecule is known.

The vector capable of expressing the immunoglobulin can be administered by introducing into cells. In the administration to living organisms, a vector, which can be infected with cells by administering to the living organisms, can be administered directly. Once lymphocytes are separated from the living organisms, then the vector is introduced into the lymphocytes, which can be returned to the living organisms again (ex vivo).

[0099]

In the active inhibitor of IPCs or the method for inhibiting the activity of IPCs based on the present invention, as for the amount of monoclonal antibody to be administered to the living organisms, immunoglobulin is administered usually in the range of 0.5 mg to 100 mg, for example, 1 mg to 50 mg, preferably 2 mg to 10 mg per kg of body weight. Intervals of administration of the antibody to living organisms can be properly adjusted so as to maintain an effective concentration of immunoglobulin in the living organisms during the period of treatment. Specifically, for example, the antibody can be administered at intervals of 1 to 2 weeks. The administration route is optional. Those skilled in the art can properly select an effective administration route in treatments. Specific examples thereof include oral or parenteral administration. Antibodies are administered systemically or topically for example, by intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection, or the like. Examples of an appropriate formulation for parenteral administration in the present invention

include injectable solutions, suppositories, and sprays. When the antibody is given to cells, immunoglobulin is added to culture medium usually in the range of 1 μg/ML, preferably 10 μg/ML, more preferably 50 μg/ML, further preferably 0.5 mg/ML.

[0100]

In the active inhibitor of IPCs or method for inhibiting the activity of IPCs of the present invention, the monoclonal antibody can be administered to living organisms by optional methods. Usually, the monoclonal antibody is blended with a pharmaceutically acceptable support. If necessary, the monoclonal antibody can be blended with additive agents such as thickeners, stabilizers, preservatives, and solubilizers. Examples of such a support or additive agent include lactose, citric acid, stearic acid, magnesium stearate, sucrose, starch, talc, gelatin, agar, vegetable oil, and ethylene glycol. The term "pharmaceutically acceptable" means approved by a regulatory agency in each government or listed in the Pharmacopeia in each country or other generally recognized pharmacopeia for use in animals, in mammalians, and more particularly, in humans. The active inhibitor of IPCs of the present invention can also be provided in the form of single or multiple doses of lyophilized powders or tablets. Further, the lyophilized powders or tablets can be used in combination with sterilized water for injection, physiological salt solution or buffer solution for dissolving the compositions so as to be a desired concentration before administration.

[0101]

Further, when the monoclonal antibody is administered as a vector, which expresses immunoglobulin, heavy and light chains are co-transfected to another plasmid and each plasmid can be administered in the range of 0.1 to 10 mg, for example, 1 to 5 mg per kg of body weight. In order to introduce the plasmids into cells in vitro, the content of the vectors for use is 1 to 5 $\mu g/10^6$ cells. Herein below, the present invention will be specifically described with reference to Examples.

All prior art documents cited herein are incorporated by reference in their entirety.

EXAMPLES

[0102]

Example 1

A. Analysis of expression of ILT7

A-1) Analysis using SAGE library

The expression of genes in human monocytes, IPCs, and HSV-treated IPCs was compared and analyzed by Serial Analysis of Gene Expression (Trade name; SAGE) method. The analysis method is as follows. Monocytes were separated as BDCA-4 positive cells and IPCs were separated as CD14 positive cells from human peripheral blood using a cell sorter. Further, IPCs were cultured for 12 hours under the presence of Herpes Simplex Virus (HSV) and then the differentiated IPCs were prepared. RNAs were obtained from respective cells, followed by producing a SAGE library using I-SAGE (Trade name) kit (manufactured by Invitrogen). Data on the obtained base sequences of about 100,000 tags was analyzed using SAGE Analysis Software (manufactured by Invitrogen). As a result, a gene whose score value of monocyte/IPC/IPC+HSV is 0/16/0, namely, ILT7 (Gen Bank Acc#NM_012276) known as a gene, which shows IPC specific expression, was found. ILT7 is a membrane protein with immunoglobulin-like domains encoded by a base sequence shown in SEQ ID NO: 1 (Fig. 2 (a)). It has been reported that mRNA of ILT7 is expressed in IPCs (Blood 100, 3295-3303 (2002)).

[0103]

A-2) RT-PCR

The expression of ILT7 in hemocyte cells was examined in more detail. Each cell was preparatively isolated from human peripheral blood by the cell sorter. RNAs were extracted from each of the isolated cell populations, from which cDNA was synthesized. Quantitative PCR was performed in accordance with an ordinary method using the resulting cDNA as a template and the expression level of mRNA of ILT7 was analyzed. The used conditions for the base sequences of primers and PCR are as follows:

Forward primer: 5′ CTC CAA CCC CTA CCT GCT GTC 3′ (SEQ ID NO: 3)

Reverse primer: 5′ TTC CCA AGG CTC CAC CAC TCT 3′ (SEQ ID NO: 4)

1 cycle of PCR (at 94°C for 3 minutes)

25 cycles of PCR [at 94°C for 30 seconds, at 58°C for 30 seconds, and

at 72°C for 1 minute]

1 cycle of PCR (at 72°C for 6 minutes)

When IPCs stimulated by monocytes, IPCs, HSVs, and CD19 positive cells (i.e. B cells), CD3 positive cells (i.e. T cells), T cells stimulated by PMAs, and CD56 positive cells (i.e. NK cells) were examined, it was found that ILT7 was expressed specifically in IPCs (Fig. 1 (a)).

[0104]

A-3) Quantitative RT-PCR

Further, the expression in other organs and tissues was examined by quantitative PCR using ABI PRISM 7000 (manufactured by Applied Biosystem). As cDNA panel, BD (Trade name) MTC multiple tissue cDNA panel (*Human I*; Cat.No.636742, *Human immune*; Cat.No.636748, Human blood fractions; Cat.No.636750; all of them are manufactured by Becton Dickinson) and the same cDNA derived from hemocyte cells as described in 2) were used.

The used base sequences of primers are as follows:

Forward primer for ILT7: 5′ CCT CAA TCC AGC ACA AAA GAA GT 3′ (SEQ ID NO: 5)

Reverse primer for ILT7: 5′ CGG ATG AGA TTC TCC ACT GTG TAA 3′ (SEQ ID NO: 6)

Forward primer for GAPDH: 5′ CCA CCC ATG GCA AAT TCC 3′ (SEQ ID NO: 7)

Reverse primer for GAPDH: 5′ TGG GAT TTC CAT TGA TGA CAA G 3′ (SEQ ID NO: 8)

PCR was performed by using ABI PRISM 7000 (manufactured by Applied Biosystem) and SYBR green PCR master mix kit (manufactured by the same company). Sequence Detection System Software (manufactured by the same company) was used for analysis.

The reaction conditions are as follows:

Step 1: 1 cycle of PCR (at 50°C for 2 minutes)

Step 2: 1 cycle of PCR (at 95°C for 10 minutes)

Step 3: 40 cycles of PCR (at 95°C for 15 seconds, at 60°C for 1 minute)

The expression of ILT7 gene was compared between each tissue by standardizing at the level of expression of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, which is known

to be expressed constitutively. As a result, it was observed that ILT7 was not expressed in any organs other than lymphoid tissues and expressed specifically in IPCs.

[0105]

B. Production of ILT7 and FcRγ expression vectors

Subsequently, cloning of genes and production of expression vectors were carried out in order to express ILT7 proteins.

B-1) Cloning of ILT7 genes

Poly(A)$^+$RNA separated from human peripheral blood was extracted from IPCs, from which cDNA was synthesized using oligo dT primer and Super Script Choice System for cDNA Synthesis kit. An EcoRI adapter was ligated into the synthesized cDNA, which was ligated into pME18S vector cleaved by EcoRI, resulting in production of human IPC cDNA library. ILT7 gene was amplified by PCR method using the produced cDNA library as a template as well as using primers with the following base sequences. 1 unit of KOD Plus DNA polymerase (manufactured by TOYOBO CO., LTD.) was used for PCR reaction. Reaction conditions were set to 25 cycles of PCR [at 94°C for 15 seconds, at 55°C for 30 seconds, and at 68°C for 2 minutes] after 1 cycle of PCR at 94°C for 2 minutes.

Forward primer: 5′ CAG GGC CAG GAG GAG GAG ATG 3′ (SEQ ID NO: 9)

Reverse primer: 5′ TCA GCA GAC ACT TCC CCA ACT 3′ (SEQ ID NO: 10)

The 2-kb ILT7cDNA fragment amplified was separated and recovered by electrophoresis using 1% agarose gel, which was cloned to pCR4 Blunt-TOPO plasmid vector (manufactured by Invitrogen) using Zero Blunt TOPO PCR Cloning kit (manufactured by Invitrogen). The base sequences of the genes obtained was analyzed, and it was found that the desired ILT7 gene shown in SEQ ID NO: 1 was obtained.

[0106]

B-2) Production of FLAG-tagged ILT7 expression vectors

A plasmid expressing a protein in which FLAG tags were fused to N- and C- termini of ILT7, respectively was constructed. ILT7 was fused with a tag, which allowed for confirming the expression of ILT7 protein by detection of the tag. The desired sequence was amplified by PCR method using the ILT7 gene produced as described in 1) as a template as well as using primers with the following base sequences. 1 unit of KOD Plus DNA polymerase (manufactured by TOYOBO CO., LTD.)

was used for PCR reaction. Reaction conditions were set to 25 cycles of PCR [at 94°C for 15 seconds, at 55°C for 30 seconds, and at 68°C for 2 minutes] after 1 cycle of PCR at 94°C for 2 minutes.

For N-FLAG ILT7

Forward primer (SEQ ID NO: 11): 5′ CCG ctc gag ATG ACC CTC ATT CTC ACA AGC CTG CTC TTC TTT GGG CTG AGC CTG GGC [GAT TAC AAG GAT GAC GAC GAT AAG] CCC AGG ACC CGG GTG CAG GCA GAA 3′

Reverse primer (SEQ ID NO: 12): 5′ C TAG act agt TCA GAT CTG TTC CCA AGG CTC 3′

For C-FLAGILT7

Forward primer (SEQ ID NO: 13): 5′ CCG ctc gag ATG ACC CTC ATT CTC ACA AGC 3′

Reverse primer (SEQ ID NO: 14): 5′ C TAG act agt TCA [CTT ATC GTC GTC ATC CTT GTA ATC] GAT CTG TTC CCA AGG CTC 3′

In the above-mentioned base sequences, each underlined portion in parentheses shows a base sequence encoding the attached FLAG tag and each lowercase letter shows the cleavage site for the restriction enzyme XhoI or SpeI. DNA fragments amplified by PCR were cleaved by XhoI and SpeI, which were then separated by Gel electrophoresis. 2 kb DNA fragments were recovered, which were ligated into pME18X vector cleaved by XhoI and SpeI in the same manner as described above. Then, two types of plasmids capable of expressing the desired fusion protein, i.e. pME18X-N-FLAG ILT7 and pME18 X-C-FLAG ILT7 were constructed, respectively.

[0107]

B-3) Cloning of FcRγ genes

FcRγ protein was considered as a protein capable of associating with ILT7 protein. The present molecule is a gene with base sequences and amino acid sequences of SEQ ID NO: s 15 and 16 (GenbankAcc#NM_004106, J. Biol. Chem. 265, 6448-6452 (1990)). The molecule is a molecule (γ chain) which constitutes Fc ε RI, i.e. a high affinity IgE receptor. Although it is also named as Fc ε RI γ, it will be referred to as FcRγ hereinafter. In this regard, the present molecule has also been known as a component of FcγR or FcαR. The present gene was cloned by PCR method as shown below to produce expression vectors. FcRγ gene was amplified by PCR method using the human IPC cDNA library produced

as described in 1) as a template as well as using primers with the following base sequences. 1 unit of KOD Plus DNA polymerase (manufactured by TOYOBO CO., LTD.) was used for PCR reaction. Reaction conditions were set to 25 cycles of PCR [at 94°C for 15 seconds, at 55°C for 30 seconds, and at 68°C for 1 minute] after 1 cycle of PCR at 94°C for 2 minutes.

Forward primer: 5′ CCC AAG ATG ATT CCA GCA GTG 3′ (SEQ ID NO: 17)

Reverse primer: 5′ GGA AGA ACC AGA AGC CAA AGA 3′ (SEQ ID NO: 18)

The 0.3-kb FcRγcDNA fragment amplified was separated and recovered by electrophoresis using 2% agarose gel, which was cloned to pCR4 Blunt-TOPO plasmid vector (manufactured by Invitrogen) using Zero Blunt TOPO PCR Cloning kit (manufactured by Invitrogen). The base sequences of the genes obtained were analyzed, and it was confirmed that the desired FcRγ gene shown in SEQ ID NO: 15 was cloned.

[0108]

B-4) Production of Myc-tagged FcRγ expression vectors

A plasmid expressing a protein in which Myc tag was attached to C terminus was constructed so that the expression of FcRγ protein could be confirmed. The desired sequence was amplified by PCR method using the FcRγ gene produced as described in 3) as a template as well as using primers with the following base sequences. 1 unit of KOD Plus DNA polymerase (manufactured by TOYOBO CO., LTD.) was used for PCR reaction. The conditions were set to 25 cycles of PCR [at 94°C for 15 seconds, at 55°C for 30 seconds, and at 68°C for 1 minute] after 1 cycle of PCR at 94°C for 2 minutes.

Forward primer (SEQ ID NO: 19): 5′ CCG ctc gag ATG ATT CCA GCA GTG GTC TTG 3′

Reverse primer (SEQ ID NO: 20): 5′ CTA Gac tag tCT A[CA GAT CCT CTT CAG AGA TGA GTT TCT GCT C]CT GTG GTG GTT TCT CAT G 3′

Of the above-mentioned primer sequences, the underlined portion in parentheses shows a base sequence encoding the attached Myc tag and each lowercase letter shows the cleavage site for the restriction enzyme XhoI or SpeI. DNA fragments amplified by PCR were cleaved by XhoI and SpeI, which were then separated by Gel electrophoresis. About 0.3-kb DNA fragments were recovered, which were ligated into pME18X vector cleaved by XhoI and SpeI in the same manner as described above.

Then, a plasmid capable of expressing the desired fusion protein, i.e. pME18X-Myc-FcRγ was constructed.

[0109]

C. Expression of ILT7 in animal cells

The expression of ILT7 in animal cells was examined using expression vectors produced as described above.

C-1) Expression in 293T cells

DNAs consisting of the following five combinations were introduced into 293T cells (7×10^5 cells) using effectene transfection kit (manufactured by Qiagen). Two days after the introduction, flow cytometry analysis (FCM analysis) was carried out.

(1) pME18X-N-FLAG ILT7 2 μg

(2) pME18X-C-FLAG ILT7 2 μg

(3) pME18X-N-FLAG ILT7 1 μg + pME18X-Myc-FcRγ 1 μg

(4) pME18X-C-FLAG ILT7 1 μg + pME18X-Myc-FcRγ 1 μg

(5) pME18X-Myc-FcRγ 2 μg

The method of FCM analysis was performed in the same manner as described in A-4 of the following Example 2. Cy3 conjugated anti-Flag antibody (manufactured by Sigma) was used for the reaction and FACScan (manufactured by Becton Dickinson) was used for the analysis. As a result, it was found that only a few ILT7 was expressed on the cell surface when reacted alone, while ILT7 was expressed extracellularly and robustly when coexisted with FcRγ (Fig. 3). It is known that mouse FcRγ has high homology with human FcRγ. However, when p815 cells (mouse mastocytoma) which express mouse FcRγ were used as hosts, the expression of ILT7 could not be observed.

[0110]

C-2) Analysis by immunoprecipitation and western blotting method

ILT7 was expressed with accompanying FcRγ on the cell surface, which was confirmed as follows. After immunoprecipitation, various antibodies for each 293T cell which was coexpressed with both genes in respective combinations described in (1) to (5) were analyzed. DNAs were introduced into 293T cells (7×10^5 cells), from which 293T cells were recovered two days after the introduction in the same manner as described in 1). Cell fractionations were dissolved in lysis buffer (0.5% Triton, 150 mM Nacl), which was left on ice for 20 minutes.

Thereafter, aspiration using a needle (27G) was repeated several times, followed by centrifuging at 15 Krpm for 20 minutes. Anti-myc antibody (2 μg, manufactured by Santa cruz biotechnology) or anti-Flag antibody (2 μg, manufactured by Sigma) was added to 200 μg of lysate of the resulting products, which was further stirred by rotation at 4°C for 4 hours. Then, Protein A/G Sepharose 4 Fast Flow mix (manufactured by Amersham bioscience) was added thereto, which was stirred by rotation at 4°C for 1 hour. Then, the resulting precipitated fractions were washed with lysis buffer with the following composition 3 times.

lysis buffer:
0.5% TritonX-100,
50 mM HEPES (pH 7.6),
150 mM NaCl,
1 mM EDTA,
10% glycerol,
1 mM DTT,
2 mM PMSF,
1 μg/ml Aprotinin,
1 μg/ml Leupeptin,
1 μg/ml Pepstatin A,
0.1 μg/ml Chymostatin,
1 mM Na_3VO_4,
0.1 mM β-glycerophosphate

[0111]

A sample buffer for SDS-PAGE was added to the washed precipitates, which was boiled for 5 minutes and centrifuged, followed by performing electrophoresis with 10% SDS gel. Samples were transferred from gels after electrophoresis to PVDF membrane (Immobilon-p-transfer membrane: manufactured by Millipore) in accordance with an ordinary method. Blotting was performed with anti-Flag antibody and anti-myc antibody. It was confirmed that the ILT7 associated with FcRγ was present in 293T cells because their presence in each immune precipitate was observed (Fig. 4).

[0112]

C-3) Analysis of sugar chain

Since several bands of ILT7 were observed in the Western analysis,

the possibility that ILT7 was glycosylated was examined. 200 μg of lysate of 293T cells which express N-FLAG ILT7 and Myc-FcRγ was immunoprecipitated with anti-Flag antibody in the manner as described in 1) and 2). Thereafter, the precipitated fractions were suspended in 60 μL of N-glycosidase buffer with the following composition and 30 μL of each resulting solution was aliquoted into two tubes.

N-glycosidase buffer:

10 mM EDTA,

0.2% SDS,

0.5% TritonX100,

1% 2-mercaptoethanol in PBS (phosphate buffer)

[0113]

Then, 3 units of 3 μL of N-glycosidase (#1365177, manufactured by Roche) were added to one tube, which was reacted at 37°C for 15 hours. Further, 7μL of sample buffer was added thereto, which was heated at 100°C for 5 minutes, followed by performing electrophoresis with 10% SDS gel. After electrophoresis, gel was transferred to PVDF membrane, to which 1 μg of anti-ILT7 polyclonal-antibody as described in 4) was added and reacted at 4°C overnight. The resulting product was washed with TBS-T buffer and reacted with 100,000-fold diluted HRP-labeled anti-rabbit antibody (manufactured by Jackson) at room temperature. Then, it was colored with ECL Western Blotting Detection System (manufactured by Amersham bioscience). As a result, the apparent molecular weight was decreased by performing N-glycosidase treatment. Thus, it was expected that sugar chains were added to ILT7 (Fig. 5).

[0114]

C-4) Production of anti-ILT7 polyclonal antibody

The used anti-ILT7 polyclonal antibody as described in 3) was produced as follows. Peptide of 23 amino acids corresponding to C terminus of ILT7 (CSQEANSRKDNAPFRVVEPWEQI; SEQ ID NO: 21) was chemically synthesized and bound to KLH protein which is a career, and the resulting product was used as an immunogen. Rabbits were intradermally immunized with immunogen mixed with Freund complete adjuvant. After six immunizations in all (once per week), the increased antibody titer in serum was confirmed and then whole blood was collected.

Then, some serum was affinity purified using peptide column of the same sequence. The resulting product was determined as anti-ILT7 polyclonal antibody.

[0115]

Example 2

A. Production of anti-ILT7 monoclonal antibody

A-1) Production of immunogen

Cells to be used as immunogens were prepared by introducing genes to 293T cells as described below. 46.4 μg of transgene (pME18 X-C-FLAG ILT7 23.2 μg and pME18X-Myc-FcRγ 23.2 μg) was added to the bottom of 100 mm/Collagen Coated Dish (IWAKI) coated with 3 mL of opti-MEM (GIBCO) and mixed. Subsequently, aside from the transgene solution, 58 μL of Lipofectamine (Trade name) 2000 (Invitrogen) was diluted with 3 mL of opti-MEM, which was allowed to stand at room temperature for 5 minutes and Lipofectamine solution was prepared. Thereafter, Lipofectamine solution was gently added to the dish containing the transgene solution and mixed. After standing at room temperature for 20 minutes, 10 mL of 293T-cells, diluted to 1 x 10^6 cells/ML using DMEM culture medium (SIGMA) containing 10% FBS (fetal bovine serum), was gently added to the dish. The resulting medium was subjected to static culture in an incubator at 37°C under CO_2 for 48 hours, from which cells were recovered by pipetting. The obtained cells were used as transfectants for immunogens.

[0116]

A-2) Production of hybridomas

On the day before cells were immunized, 50 μL of emulsion obtained by mixing 200 μL of PBS with 200 μL of complete adjuvant (FREUND) (RM606-1, manufactured by Mitsubishi Kagaku Iatron, Inc.) was injected to the bottoms of both feet of four Balb/c female mice (four-week-old) for immunization. On the following day, 50 μL of 2 x 10^7 cells suspended in 400 μL of PBS was immunized. The second and third immunizations were performed every four days. Three days following the third immunization, cell fusion was performed as follows. Cells were collected from lymph nodes of mice feet immunized. Mouse myeloma cells P3-X63-Ag8-U1 cultured in RPMI1640 culture medium (SIGMA) containing 10% FBS were mixed with the cells derived from lymph nodes and myeloma

so that the ratio of the mouse myeloma cells to the cells derived from lymph nodes and myeloma should be 2:1 to 10:1, from which cells were recovered by centrifugation. PEG4000 (MERCK) equivalently diluted with RPMI1640 culture medium was added to the obtained cell fractions, which was subjected to cell fusion. After washing cells, the resulting product was suspended in 160 mL of 15% FBS-HAT-medium containing a supplement and then inoculated into sixteen 96-well plates at 200 μL/well. The culture medium was exchanged after three days. One to two weeks after observation of the colony formation, primary screening was performed.

[0117]

A-3) Screening of hybridoma by Cell ELISA method

Hybridoma, which produces target antibody, was screened by the following Cell ELISA. The produced cells as described in 1) were used at 1 x 10^7 cells per 96-well plate, which were suspended in 0.5% BSA/2 mM EDTA/PBS and then aliquoted to a plate for Cell ELISA (NUNC 249570 96V NW PS) at 100 μL/well. Centrifugation was carried out at 2,000 rpm at 4°C and then the supernatant was discarded. Sampled culture supernatant was added at 50 μL/well, which was reacted at room temperature for 30 minutes. Washing operation that involves adding 0.5% BSA/2 mM and EDTA/PBS to each well, centrifuging at 2,000 rpm at 4°C for 2 minutes, and then discarding the supernatant was carried out twice. 50 μL/well of 10,000-fold diluted peroxidase-labeled goat anti-mouse IgG antibody (IM0819; Beckman coulter) was added to each well after washing, which was reacted for 30 minutes. The washing operation using 0.5% BSA/2 mM-EDTA/PBS was carried out twice, followed by adding a coloring solution. The prepared antibody solution was substituted with PBS (-) by a dialysis membrane (10,000 cuts manufactured by PIERCE) to give purified anti-ILT7 chimeric antibodies.

[0118]

A-4) Examination of antibody responsiveness by flow cytometry (FCM) analysis

Hybridoma culture supernatant was analyzed by flow cytometry (FCM) analysis. The produced cells as described in 1) was suspended in 0.5% BSA/2 mM EDTA/PBS, which was transferred into a centrifugal

tube at 1 x 10^5 per one sample, followed by adding 40 μL of each culture and reacting at room temperature for 30 minutes. Washing operation that involves adding 1 ml of 0.5% BSA/2 mM and EDTA/PBS to each tube, centrifuging at 1200 rpm at 4°C for 3 minutes, and discarding the supernatant was carried out twice. 40 μL of 100-fold diluted FITC-labeled goat anti-mouse IgG antibody (IM0819; Beckman coulter) was added to each well after washing, which was reacted at room temperature for 30 minutes. The washing operation using 0.5% BSA/2 mM-EDTA/PBS was carried out twice, followed by analyzing using flow cytometry FC500 (Beckman coulter). A hybridoma producing an antibody which did not respond to only host cell and responded specifically to the cell into which gene had been introduced was selected. The selected hybridoma was cloned by the limiting dilution method and hybridomas #11 and #17 which produce monoclonal antibodies were obtained.

[0119]

B. Examination of responsiveness of anti-ILT7 antibody

ILT7 in which FLAG tag was attached to N terminus was coexpressed with *FcRγ molecule in 293T cells in the same manner as described in* C-1) of Example 1. Then, the responsiveness of the antibody obtained in Example 2 was confirmed by FCM analysis using FACScan (Becton Dickinson). As a result, it was confirmed that all antibodies produced by hybridomas #11 and #17 which were obtained as described in A responded to the cells into which ILT7 gene was introduced and which expressed ILT7 (Fig. 6 (b)). Further, lymphocytes were separated from human peripheral blood using Ficoll and then double staining with the produced anti-ILT7 antibody and PE-labeled anti-BDCA-2 antibody (Miltenyi) was performed. Then, the responsiveness to the lymphocytes was examined. As a result, the binding of monoclonal antibody produced by hybridomas #11 and #17 to BDCA-2 positive cell was detected. That is, it was confirmed that both monoclonal antibodies recognized ILT7 molecules expressed on human IPCs (Fig. 6 (a)). These monoclonal antibodies were designated as anti-ILT7 antibody #11 and anti-ILT7 antibody #17, respectively. More detailed analysis was performed.

[0120]

Multiple-staining analysis for human peripheral blood

lymphocytes was carried out using the produced anti-ILT7 antibody, anti-Lineage-1 antibody (anti-CD3, CD14, CD16, CD19, CD56 antibodies; Becton Dickinson), anti-CD123 antibody (Becton Dickinson), and anti-BDCA-2 antibody (Miltenyi). As for ILT7 antibody-positive fractions, Lineage Marker was negative, CD123 was positive, and BDCA-2 was positive. From the results, it was confirmed that IPCs were stained by only ILT7#11 and ILT7#17 (Fig. 7).

[0121]

Further, the expression of various molecules was examined by FCM analysis when human peripheral blood lymphocytes were stimulated by CpG or IFNα for 24 hours. CpGODN2216 was used as CpGA, which induces the production of IFN from IPCs and CpGODN2006, was used as CpGB which facilitates the maturation of dendritic cells (Moseman et al. J. Immunology. 173, 4433-4442, 2004). A gate was set to Lineage Marker negative fraction. When the responsiveness of anti-BDCA-2 antibody and anti-ILT7 antibody to CD123 positive cell population was analyzed, most of the ILT7 positive fractions were disappeared even after 24-hours CpG stimulation. On the other hand, some cells of BDCA-2 showed positive after 24-hours CpG stimulation (Fig. 8). It has been considered that IPCs are differentiated into different cells immediately after the CpG stimulation. It was indicated that the anti-ILT7 antibody of the present invention was useful as a stage specific antibody to IPCs. Further, it was confirmed that IPCs in peripheral blood lymphocytes were not differentiated under the presence of IFNα, in this case where the survival ratio was high, the expression of ILT7 was maintained on IPCs, further ILT7 was stably present on IPCs in autoimmune diseases with the possibility that IFN in serum was at a high level.

[0122]

C. Examination of specificity of anti-ILT7 antibody

ILT7 belongs to ILT/LIR family and there is a plurality of molecules with high homology, particularly with high homology in the extracellular region (Fig. 9). It has been reported that mRNAs of molecules, especially such as ILT2 and ILT3 are expressed in IPCs (Ju et al. Gene 331, 159-164, 2004). Therefore, the responsiveness of these molecules was confirmed using transgenic cells.

C-1) Cloning of ILT1 molecule and production of expression vectors

cDNA was synthesized from RNA derived from human tonsil using oligo dT primer and SuperScript Choice System for cDNA Synthesis kit. Next, a NotI adapter was ligated into pME18S vector cleaved by NotI, resulting in production of human tonsil cDNA library.

[0123]

ILT1 gene with a FLAG tag at the C terminus was amplified by PCR method using the produced cDNA library as a template as well as using primers with the following base sequences. 1 unit of KOD Plus DNA polymerase (manufactured by TOYOBO CO., LTD.) was used for PCR reaction. Reaction conditions were set to 25 cycles of PCR [at 94°C for 15 seconds, at 55°C for 30 seconds, and at 68°C for 2 minutes] after 1 cycle of PCR at 94°C for 2 minutes.

Forward primer (SEQ ID NO: 22): 5′ CCG ctc gag ATG ACC CCC ATC CTC ACG GTC C 3′

Reverse primer (SEQ ID NO: 23): 5′ CTA Gac tag tTC A[CT TAT CGT CGT CAT CCT TGT AAT C]CC TCC CGG CTG CAT CTT G 3′

In the above-mentioned primer sequences, the underlined portion in parentheses shows a base sequence encoding the attached FLAG tag and each lowercase letter shows the cleavage site for the restriction enzyme XhoI or SpeI. DNA fragments amplified by PCR were cleaved by XhoI and SpeI, which were then separated by Gel electrophoresis. About 2-kb DNA fragments were recovered, which were ligated into pME18X vector cleaved by XhoI and SpeI in the same manner as described above. Then, a plasmid capable of expressing the desired fusion protein, i.e. pME18X-C-FLAGILT1 was constructed. The base sequence and amino acid sequence are shown in SEQ ID NO: s: 24 and 25.

[0124]

C-2) Production of expressing cells and examination of antibody responsiveness

As for ILT2 (SEQ ID NO: 26) and ILT3 (SEQ ID NO: 28), expression vectors in which respective genes were cloned to XbaI or XhoI sites of pcDNA4.1 (manufactured by Invitrogen) were used. DNAs of the following combinations were introduced into 293T cells (7×10^5 cells) in the same manner as described in C-1). Two days after the introduction, FCM analysis was carried out and then anti-ILT7 antibody was analyzed.

(1) pME18X-N-FLAG ILT7 1 μg + pME18X-Myc-FcRγ 1 μg

(2) pME18X-C-FLAG ILT1 0.5 μg + pME18X-Myc-FcRγ 0.5 μg + pcDNA4.1-ILT2 0.5 μg + pcDNA4.1-ILT3 0.5 μg

As a result, any antibodies did not respond to the cells in which ILT1 was expressed. For this reason, it was suggested that these anti-ILT7 antibodies specifically recognized ILT7 molecules on IPCs (Fig. 10).

[0125]

Example 3

Effect of anti-ILT7 antibody on ability to produce human IFN

Human peripheral blood lymphocytes were inoculated into 96 well plate at 2 x 10^5 cells/well, which were reacted with 5 μg/mL of various antibodies at 37°C. After 1-hour culture, influenza virus PR8 was added thereto. After 24-hours culture, IFNα in the culture supernatant was measured by ELISA kit (Bender Med System). As a result, the production of IFN was inhibited by the addition of anti-ILT7 antibody (Fig. 11). Namely, it was found out that the IFN production by IPCs was affected by the anti-ILT7 antibody of the present invention.

[0126]

Example 4

CDC activity of anti-ILT7 antibody

A. Production of anti-ILT7 monoclonal antibody

A clone which produces a monoclonal antibody was obtained in the same manner as described in A-1) to A-4) of Example 2. The responsiveness was examined in the same manner as described in B of Example 2 and the specificity was examined in the same manner as described in C of Example 2. As a result, hybridomas #37, #28, and #33, which produced anti-ILT7 monoclonal antibodies with good responsiveness and specificity, were obtained. CDC activity was measured as described below using anti-ILT7 monoclonal antibody in which three kinds of these hybridomas were produced.

[0127]

B. Determination of CDC activity

B-1) On the previous day of production of target Production of target cell line (ILT7-CHO cell line), the following DNA was introduced into CHO-k1 cells, which were inoculated so as to be 6 x 10^5 cells per

one dish (6 cmϕ) using Effectene Transfection Reagent (manufactured by QLAGEN) and then resistant strains were selected using 800 μg/ml of Zeocin (manufactured by Invitrogen).

Introduced DNA: pcDNA3.1-C-FLAG ILT7 1 μg + pME18X-Myc FcRγ 2 μg

Thereafter, a cell line, which highly expressed ILT7, was obtained using the cell sorter (BD FACSAria, manufactured by Becton Dickinson). It was confirmed that the selected cell line highly expressed ILT7 by FCM analysis. Operation of FCM analysis was carried out in accordance with the method as described in A-4) of Example 2except that BD FACSCaliber (manufactured by BD) was used for FCM. The following antibodies were used for a primary antibody and a secondary antibody, respectively.

Primary antibody: 5 μg/ml mouse anti-ILT7 antibody (#37),

Secondary antibody: R-phycoerythrin (R-PE)-conjugated goat anti-mouse immunoglobulin specific polyclonal antibody (BD)

[0128]

B-2) Response of target cells to anti-ILT7 antibodies

The obtained target cells as described in B-1) (ILT7-CHO cell) were recovered using 5 mM EDTA/PBS solution, which were suspended in CDC medium with the following composition so as to be a concentration of 4 x 10^5 cells/ml. The suspension was aliquoted into each 96-well plate at 50 μl/well.

CDC medium:

RPMI1640

0.1% BSA

100 units/ml Penicillin

100 μg/ml Streptomycin

10 mM Hepes (pH 7.6)

2 mM L-Glutamin

50 μl of anti-ILT7 antibody solution prepared by CDC medium was added to each well and mixed so that the final concentration of antibodies should be 0.1 μg/ml, 0.5 μg/ml, 1 μg/ml, and 5 μg/ml. Further, 50 μl of CDC medium containing a complement with the following composition was added thereto and mixed so that the final complement concentration should be 6%, followed by culturing at 37°C for 2 hours.

CDC medium containing a complement:

1 ml of complement of juvenile rabbit (Catalog No.: CL3441, manufactured by CEDARLANE)

CDC medium (vide supra)

Then, the suspension was centrifuged (centrifugal condition: at 250 G for 4 minutes) and the supernatant was recovered while paying attention not to be contaminated with cells. LDH in the supernatant was measured by an ordinary method, which was determined as "The amount of LDH leaked from the target cell by the complement activity" (Experimental Sample).

[0129]

The following parameters were also prepared in order to determine CDC activity.

-Target Cell Spontaneous LDH Release: only target cells were cultured in the same volume as the sample and prepared.

-Target Cell Maximum LDH Release: only target cells were cultured in the same volume as the sample, and then TritonX-100 solution included with the kit was added thereto 60 minutes before recovery of the supernatant so that the final concentration should be 0.8% and prepared.

-Volume Correction Control: the same amount of TritonX-100 as that added when Target Cell Maximum LDH Release was prepared was added to the culture medium of the same volume as the sample and prepared.

-Culture Medium Background: the culture medium of the same volume as the sample and the solution to which complement containing CDC medium was added to the culture medium so as to be the same volume as the sample were prepared.

[0130]

The same volume of culture medium as the sample was subtracted from the absorbance of Target Maximum and Target Spontaneous. The solution to which complement containing CDC medium was added to the culture medium so as to be the same volume as the sample was subtracted from the absorbance of Experimental Sample and corrected. The CDC activity was calculated by the following equation. The results are shown in Table 1 and Fig. 12. Even in the case where anti-ILT7 monoclonal antibodies obtained from any hybridoma were used, 80% or more of CDC activity was exhibited when the antibody concentration was 0.5 μg/ml or more.

$$\text{CDC activity (\%)} = \frac{\text{Experimental Sample} - \text{Target Spontaneous}}{\text{Target Maximum} - \text{Volume Control} - \text{Target Spontaneous}} \times 100$$

[0131]

[Table 1]

	Antibody concentration (μg/ml)	Cytotoxicity (Aver)	Cytotoxicity (STD)
#37	0.1	14.78	3.16
	0.5	85.50	0.60
	1	86.13	2.93
	5	90.26	1.87
#28	0.1	18.52	0.60
	0.5	80.97	1.62
	1	83.64	1.99
	5	88.17	3.32
#33	0.1	4.42	1.58
	0.5	82.16	3.35
	1	85.39	2.78
	5	86.18	1.71
Mouse IgG2a	0.1	1.53	0.60
	0.5	1.47	2.50
	1	3.68	2.90
	5	3.06	1.72
no Ab	0	2.10	0.49

[0132]

Comparative example 1

Exactly the same operation was performed in the same manner as described in B and C of Example 4 except that mouse IgG2a was used in place of anti-ILT7 antibody. The results are shown in Example 4 as well as Table 5 and Fig. The CDC activity to the target cells was not observed in antibodies other than anti-ILT7 monoclonal antibody.

[0133]

Example 5

Internalization of anti-ILT7 antibody to target cells

A. Anti-ILT7 monoclonal antibody

The following anti-ILT7 monoclonal antibodies were used.

Anti-ILT7 monoclonal antibodies: #17, #26, #37, #28, and #33

[0134]

B. Observation of internalization

B-1) Production of target cell line (ILT7-CHO cell line)

The target cell line (ILT7-CHO cell line) was produced in the same manner as described in B-1 of Example 4.

[0135]

B-2) Response of target cells to anti-ILT7 antibody

The recovered ILT7-CHO cells were suspended in ice-cold buffer (T(-) + 10% FBS) with the following composition at 1 x 10^6 Cells/mL using 5 mM of EDTA/PBS solution.

T (-) medium:

RPMI1640

100 units/ml Penicillin

100 μg/ml Streptomycin

10 mM Hepes (pH 7.6)

2 mM L-Glutamin

1 mM sodium pyruvate

50 μM 2-mercaptoethanol

10% heat inactivated Fetal Bobine Serum

1 mL of suspension as described above was placed into a 15 mL centrifugal tube, which was centrifuged (centrifugal condition: at 1200 rpm, at 4°C, for 5 minutes) and then the supernatant was discarded. 200 μL of anti-ILT7 monoclonal antibody suspension of (10 μg/mL) was added to cell pellets, which was mixed and incubated at 4°C for 30 minutes, followed by washing with ice-cold T (-) medium twice (the amount of the medium used: 10 mL per washing, centrifugal condition: at 1200 rpm, at 4°C, for 5 minutes).

[0136]

B-3) Modification of ILT7-anti-ILT7 antibody immune complex present on the surface of target cells

Subsequently, ILT7-anti-ILT7 antibody immune complex present on the surface of cells was modified with a secondary antibody, which was labeled with fluorescence for detection. Specific method is described below. APC-labeled goat anti-mouse IgG polyclonal antibody (Catalog number: 550826BD, manufactured by Biosciences) containing ice-cold T (-) medium was added to cell pellets obtained as described in B-2), which was incubated with shading at 4°C for 20 minutes, followed

by washing with ice-cold T (-) medium twice (the amount of the medium used: 10 mL per washing, centrifugal condition: at 1200 rpm, at 4°C, for 5 minutes). Then, ice-cold T (-) medium was added thereto, which was used as 1 x 10^6 Cells/mL of suspension.

[0137]

B-4) Induction of internalization by incubation at 37°C

The suspension obtained as described in B-3 was equally divided into two tubes (i.e. tubes (a) and (b)). The tubes (a) and (b) were incubated at 37 and 4°C, respectively, under shading condition for 60 minutes. After the incubation, 1% FBS/PBS (ice-cold) was added thereto in order to stop internalization. The resulting solution was centrifuged (centrifugal condition: at 1200 rpm, at 4°C, for 5 minutes) and then the supernatant was discarded, followed by washing with 1% of FBS/PBS (ice-cold) twice (the amount of the solution: 10 mL per washing, centrifugal condition: at 1200 rpm, at 4°C, for 5 minutes).

[0138]

B-5) Modification of ILT7-anti-ILT7 antibody immune complex remained on the surface of target cells after incubation

ILT7-anti-ILT7 antibody immune complex remained on the cell surface after incubation was modified with a tertiary antibody in order to detect, by fluorescence. Specific method is described below. 20 µL of suspension containing tertiary antibodies (FITC-labeled donkey anti-goat IgG antibody (Catalog number: sc-2024, manufactured by Santa cruz biotechnology)) was added to cell pellets obtained as described in B-4), which was mixed and allowed to stand at 4°C for 15 minutes under shading condition. The resulting solution was washed with (the amount of the solution: 10 mL per washing, centrifugal condition: at 1200 rpm, at 4°C, for 5 minutes).

[0139]

B-6) Analysis of anti-ILT7 antibody present in target cells

Subsequently, 150 µL of 1% FBS/PBS was added to cell pellets obtained as described in B-5), which was suspended and collected into a 1.2 ml microtiter tube, followed by performing FCM analysis. In analysis, the mean fluorescence intensity (MPI) of each cell was analyzed separately in FITC and APC. Further, the fluorescence intensity ratio (%) was calculated by the following equation.

$$\text{Fluorescence intensity ratio (\%)} = \frac{\text{Mean fluorescence intensity of cells incubated at 37 °C for 60 minutes}}{\text{Mean fluorescence intensity of cells incubated at 4 °C for 60 minutes}} \times 100$$

The results are shown in Table 2, Table 3, and Fig. 13.

[0140]

[Table 2]

	FITC			APC		
	Mean fluorescence intensity		Fluorescence intensity ratio (%)	Mean fluorescence intensity		Fluorescence intensity ratio (%)
	Temperature of incubation (°C)			Temperature of incubation (°C)		
	4	37		4	37	
#17	35.7	15.9	44.5	1384	1320	95.4
#26	29.8	16.5	55.4	844	816	96.7
#37	51.0	28.5	55.9	2194	2155	98.2
#28	40.6	19.3	47.5	1746	1709	97.9
#33	47.7	22.6	47.4	1882	1845	98.0
IgG2a	3.7	4.2	116.2	3	3.64	121.3

[0141]

[Table 3]

	Species of primary antibodies	Fluorescence intensity ratio (%)	
		APC	FITC
Example 5	Anti-ILT7 antibody #17	95.4	44.5
	Anti-ILT7 antibody #26	96.7	55.4
	Anti-ILT7 antibody #37	98.2	55.9
	Anti-ILT7 antibody #28	97.9	47.5
	Anti-ILT7 antibody #33	98.0	47.4
Comparative example 2	Mouse IgG2a	121.3	116.2

[0142]

The fluorescence intensity of FITC is an indicator of the amount of ILT7-anti-ILT7 antibody immune complex remained on the cell surface after incubation. The mean fluorescence intensity of FITC as to the cells incubated at 37°C for 60 minutes fell to about 50% as compared

with the cells incubated at 4°C.

On the other hand, APC fluorescence intensity is an indicator of the amount of ILT7-anti-ILT7 antibody immune complex presented on the cell surface before incubation. ILT7-anti-ILT7 antibody immune complex is detected regardless of whether it is present on the cell surface or incorporated into cells after incubation. In Example 5, APC fluorescence intensity after the incubation in the case of incubation at 37°C was equivalent to that in the case of incubation at 4°C. It shows that ILT7-anti-ILT7 antibody immune complex may be present in any site of target cells even when the incubation is performed at either temperature. As mentioned above, it was found that the anti-ILT7 monoclonal antibody evoked the internalization of ILT7 by the incubation at 37°C.

[0143]

Comparative example 2

Exactly the same operation was performed in the same manner as described in Example 5 except that mouse IgG2a was used in place of anti-ILT7 antibody. The results are shown in Example 5 as well as Table 2, Table 3, and Fig. 13. In the case where the mouse IgG2a was used, any changes in the fluorescence intensity of FITC and APC were not observed, thus it was found that the mouse IgG2a did not evoke the internalization of ILT7.

[0144]

Example 6

Concerning the structure of mouse anti-human ILT7 monoclonal antibody

[Sequences of variable regions]

A. Cloning of cDNA encoding variable region of mouse anti-ILT7 antibody

A-1) Concerning hybridomas which produce mouse anti-ILT7 antibodies

The following hybridomas were used as hybridomas which produce mouse anti-ILT7 antibodies.

-Hybridoma #11 (Accession number: FERM BP-10704)

-Hybridoma #17 (Accession number: FERM BP-10705)

[0145]

A-2) Isolation of the total RNAs

The total RNAs were isolated from hybridomas described in A-1) using a commercially available kit "RNeasy Mini Kit" (Catalog number:

74106, manufactured by Qiagen) in accordance with the instruction attached to the kit. In both cases, about 200 μg of the total RNAs was obtained from 1 x 10^7 hybridomas.

[0146]

A-3) Amplification and fragmentation of cDNA encoding a mouse heavy chain variable region

cDNA encoding a mouse heavy chain variable region was amplified by 5'RACE method using 5 μg of the total RNAs isolated as described in A-2. As for amplification, commercially available kit "5'RACE System for Rapid Amplification of cDNA ENDs, Version 2.0 Kit" (Catalog number: 18374-058, manufactured by Invitrogen) was used. It will be specifically described as follows. First, a first strand cDNA was synthesized from the total RNAs obtained as described in A-2) by reverse transcriptase. The base sequences of antisense primers (GSP1) used at the time are shown in Table 4.

[0147]

[Table 4]

Primers used for amplification of a gene encoding a mouse heavy chain variable region

Used hybridomas	Names of primers	SEQ ID No.	Sequence
#11	Mu IgG3VH5RACE-GSP1	30	5' CCA TAG TTC CAT TTT ACA GTT ACC 3' (24-mer)
	Mu IgG3VH5RACE-GSP2	31	5' GGG ACC AAG GGA TAG ACA GA 3' (20-mer)
#17	Mu IgG2aVH5RACE-GSP1	32	5' TCC AGA GTT CCA GGT CAA GGT CAC 3' (24-mer)
	Mu IgG2aVH5RACE-GSP2	33	5' GCC AGT GGA TAG ACC GAT GG 3' (20-mer)

[0148]

Subsequently, the total RNAs were degraded by RNaseH and the first strand cDNA remained as a single strand was purified by low-melting point agarose method (1.5%). Further, dC (i.e. nucleotide homopolymer) was attached to the 3'-terminus of the first chain cDNA using terminal deoxynucleotidyl transferase (TdT). cDNA was amplified by PCR method using an anchor primers (SEQ ID NO: 34) having a nucleotide polymer complementary to dC (anchor sequence) at 3'-terminus and antisense primers (GSP2) shown in Table 4. Further, the obtained PCR products were used as templates. cDNA was amplified

by Nested PCR method using AUAP primer (SEQ ID NO: 35) and antisense primers (GSP2) shown in Table 4. Further, the PCR products were purified by low-melting point agarose method (1.5%).

Anchor primer for 5'RACE (SEQ ID NO: 34)

5'-GGC CAC GCG TCG ACT AGT ACG GGI IGG GII GGG IIG-3' (36-mer)

AUAP primer gor 5'RACE (SEQ ID NO: 35)

5'-GGC CAC GCG TCG ACT AGT AC-3' (20-mer)

[0149]

A-4) Amplification and fragmentation of cDNA encoding mouse light chain variable region

cDNA encoding a mouse light chain variable region was amplified from the total RNAs isolated as described in A-2) in the same manner as described in A-3). The base sequences of primers used at the time is shown in Table 5. The obtained PCR products were purified by low-melting point agarose method (1.5%).

[0150]

[Table 5]

Primers used for amplification of a gene encoding a mouse light chain variable region

Used hybridomas	Names of primers	SEQ ID No.	Sequence
#11、#17	Mu IgVL5RACE-GSP1	36	5' TTC ACT GCC ATC AAT CTT CCA CTT 3' (24-mer)
	Mu IgVL5RACE-GSP2	37	5' GAT GGA TAC AGT TGG TGC AGC 3' (21-mer)

[0151]

A-5) Confirmation of base sequence of cDNA and determination of CDR region

A heavy chain variable region obtained as described in A-3) and cDNA fragment of light chain variable region obtained as described in A-4) were cloned to pCR4 Blunt-TOPO vector using a commercially available kit "Zero Blunt TOPO PCR Cloning Kit" (Catalog number: 1325137, manufactured by Invitrogen) in accordance with the instruction attached to the kit, which was then introduced into Escherichia coli competent cells to give Escherichia coli transformant. The above-mentioned plasmid was obtained from the transformant, then cDNA base sequence in the plasmid was confirmed using an automatic DNA

sequencer "PCR-based ABI PRISM 3100 Genetic Analyzer" (manufactured by Applied Biosystems). Correct sequences were extracted by excluding transcripts obtained from an inactive RNA due to frame shift and nonsense mutations around the complementarity-determining region (hereinafter referred to as "CDR region"). Further, the homology of cDNA base sequence comprised in the plasmid was compared with Kabat database and sequences of the CDR region and the variable region in respective variable regions were determined. Also, as for the hybridoma #37 produced in Example 4, sequences of the CDR region and the variable region in variable regions were determined in the same procedure as described in A-1) to A-5) of Example 6 using hybridoma #17. cDNA base sequences of the heavy chain variable regions and light chain variable regions of the anti-ILT7 monoclonal antibodies produced by each hybridoma and amino acid sequences encoded by the sequences are shown in the following SEQ ID NOs.

	Heavy chain variable region	Light chain variable region
#11	SEQ ID NO: 38 (base sequence)	SEQ ID NO: 40 (base sequence)
	SEQ ID NO: 39 (amino acid sequence)	SEQ ID NO: 41 (amino acid sequence)
#17	SEQ ID NO: 42 (base sequence)	SEQ ID NO: 44 (base sequence)
	SEQ ID NO: 43 (amino acid sequence)	SEQ ID NO: 45 (amino acid sequence)
#37	SEQ ID NO: 46 (base sequence)	SEQ ID NO: 48 (base sequence)
	SEQ ID NO: 47 (amino acid sequence)	SEQ ID NO: 49 (amino acid sequence)

[0152]

[Confirmation of isotype of constant region]

As for the hybridoma culture supernatant, the isotype of the constant region of the produced monoclonal antibody was confirmed using a commercially available mouse monoclonal antibody isotyping kit (Catalog number: MMT1, manufactured by Serotec Product). The heavy chain constant region of mouse anti-human ILT7 antibody #11 was Igγ3 and the light chain constant region was Igκ. Further, each of the heavy chain constant regions of mouse anti-human ILT7 antibody #17 and mouse anti-human ILT7 antibody #37 was Igγ2a and each of the light chain constant region was Igκ.

[0153]

Example 7

Production of chimeric antibodies

A. Cloning of cDNA encoding human IgG constant region

Human IgG1 heavy chain constant region and human Ig kappa light chain constant region were selected from cDNA library of human IPCs. Then, the selected regions were cloned to pCR4 Blunt-TOPO vector using a commercially available kit "Zero Blunt TOPO PCR Cloning Kit" (Catalog number: 1325137, manufactured by Invitrogen) in accordance with the instruction attached to the kit, which was then introduced into Escherichia coli competent cells to give Escherichia coli transformant. The above-mentioned plasmid was obtained from the transformant, then cDNA base sequence in the plasmid was confirmed using an automatic DNA sequencer "PCR-based ABI PRISM 3100 Genetic Analyzer" (manufactured by Applied Biosystems).

[0154]

B. Ligation of variable region with constant region and cloning

The cDNA encoding the heavy chain constant region obtained as described in A and the cDNA encoding the heavy chain variable region obtained as described in A-5 of Example 6 was used, respectively. Both DNAs have a region in which a base sequence of DNA is overlapped. Then, double-stranded DNA was obtained by the overlap extension method using the region. Specific process is as follows.

[0155]

C-1) Preparation of cDNA encoding heavy chain of chimeric ILT7 antibody

The "plasmid with cDNA encoding heavy chain variable regions of #11 and #17" which was obtained as described in A-5) was digested with restriction enzymes NotI and XbaI, which was purified by the agarose gel method (1.5%). The resulting products were dissolved in each TE buffer with the following composition so as to be 100 pmol/μL to prepare a solution of the cDNA fragment encoding the heavy chain variable region.

TE buffer:

10 mM Tris-HCl

1 mM EDTA

pH 7.5 to 8.0

Further, the "plasmid with cDNA encoding the heavy chain constant region" obtained as described in B was treated in the same manner as described above to prepare 100 pmol/μL of solution. Subsequently, both solutions were mixed, and then both of the overlap regions were hybridized by first keeping them at 70°C for 10 minutes and next keeping them at 37°C for 5 minutes. Thereafter, cDNA was amplified by PCR method and the obtained cDNA was digested with restriction enzymes NotI and XbaI, which was purified by the low-melting point agarose gel method (1.5%).

[0156]

C-2) Preparation of cDNA encoding light chain of chimeric ILT7 antibody

The cDNA encoding the light chain constant region obtained as described in A and the cDNA encoding the light chain variable region obtained as described in A-5 of Example 6 was used, respectively. cDNA encoding the light chain of chimeric ILT7 antibody was obtained in the same manner as described in C-1) using these cDNAs.

[0157]

C-3) Cloning

cDNA obtained as described in C-1) was cloned to plasmid vector pcDNA3.1-Zeocin (manufactured by Invitrogen) using NotI and XbaI as cloning sites to produce a chimeric ILT7 antibody heavy chain expression vector. Further, cDNA obtained as described in C-2) was cloned to plasmid vector pcDNA3.1-hygromycin (manufactured by Invitrogen) using NotI and XbaI as cloning sites to produce a chimeric ILT7 antibody light chain expression vector. Names of each vector are shown in Table 6.

[0158]

[Table 6]

Names of plasmid vectors

	Chimeric ILT7 antibody heavy chain for expression	Chimeric ILT7 antibody light chain for expression
#11	pcDNA-#11VH	pcDNA-#11VL
#17	pcDNA-#17VH	pcDNA-#17VL

[0159]

D. Expression of chimeric ILT7 antibody

D-1) Transient transformation

1 μg of chimeric ILT7 antibody heavy chain expression vector and 1 μg of chimeric ILT7 antibody light chain expression vector, which were obtained as described in C-3), were co-transfected to 293T cells using effectine transfection kit (Catalog number: 301427, manufactured by Qiagen). Thereafter, the resulting products were cultured at 37°C using 2% Low IgG FBS-added DMEM culture medium with the following composition.

2% Low IgG FBS-added DMEM culture medium:

DMEM culture medium (Catalog number: D5796, manufactured by Sigma)

2% Low IgG FBS (Catalog number: SH30151.03, manufactured by HyClone)

2 mM L-Glutamin

100 U/ml Penicillin

100 μg/ml Streptomycin

pH 7.2 to pH 7.4

After introduction of vectors, the resulting medium was cultured for 96 hours and the culture supernatant was collected. Then, the cell fragments were removed by centrifugation to give a crude antibody solution.

[0160]

D-2) Homeostasis transformation

1 μg of chimeric ILT7 antibody heavy chain expression vector and 1 μg of chimeric ILT7 antibody light chain expression vector, which were obtained as described in C-3), were co-transfected to YB 2/0 cells (cells derived from rat myeloma, ATCC#CRL-1622) using effectine transfection kit (Catalog number: 301427, manufactured by Qiagen). Among the used plasmid vectors, the vector for the heavy chain expression is a marker for Zeocin resistance and the vector for the light chain expression is a marker for hygromycin resistance. Therefore, cells into which both vectors were introduced can be grown in a culture medium to which Zeocin and hygromycin are added at the same time. Then, the cells were cultured in RPMI culture medium to which Zeocin and hygromycin were added and a resistant strain was selected.

Zeocin-hygromycin-added RPMI culture medium:

RPMI1640 culture medium (Catalog number: R8758, manufactured by Sigma)

10% FBS
0.01 M HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)
1 mM Sodium pyruvate
2 mM L-Glutamine
100 U/ml Penicillin
100 μg/ml Streptomycin
55 μM 2-mercaptoethanol
0.5 mg/ml Zeocin
0.5 mg/ml Hygromycin
pH 7.2 to pH 7.4

Three days after that, the amount of antibody production in the culture supernatant was determined by the ELISA method. ILT7 chimeric-antibody producing cell line with a high expression level and cells sufficiently increased was selected. Furthermore, a single cloning of the selected cell lines was performed by the cell sorter method to obtain the following cell lines.

#11 ILT7 chimeric-antibody-producing cell line: #11-5 cell line and #11-16 cell line

#17 ILT7 chimeric-antibody-producing cell line: #17-24 cell line

The above-mentioned cell lines (#11-5 cell line, #11-16 cell line, and #17-24 cell line) were respectively cultured in 5% FBS-added RPMI culture medium with the following composition. The incubation temperature and incubation time were set to 37°C and 96 hours, respectively.

5% FBS-added RPMI culture medium:
RPMI1640 culture medium (Catalog number: R8758S, manufactured by Sigma)
5% FBS
0.01 M HEPES
1 mM Sodium pyruvate
2 mM L-Glutamin
100 U/ml Penicillin
100 μg/ml Streptomycin
55 μM 2-mercaptoethanol
pH 7.2 to pH 7.4

The culture supernatant was collected and then the cell fragments

were removed by centrifugation to give a crude antibody solution.

[0161]

E. Purification of antibodies

Each of the crude antibody solutions obtained as described in D-1 and D-2 was purified by protein A affinity column (rProtein A Sepharose FF, Catalog number: 17-1279-01, manufactured by Amershram Pharmacia). Purification conditions are as follows. Affinity purification was carried out using PBS (-) buffer with the following composition as an adsorption buffer and 0.1M sodium citrate buffer (pH 3) as an elution buffer in accordance with the attached instruction manual. 1 M Tris-HCl (pH 8.0) was added to the eluted fractions to adjust the pH to around 7.2. The ODs at 450 to 620 nm were measured and then wells showing positive reaction were selected. With reference to the concentration of purified antibodies, the absorbance at 280 nm was determined and calculated based on 1.38 OD/mg/ml. Relationships among chimeric ILT7 antibodies obtained, hybridomas from which the variable region gene was derived, and host cells were summarized in Table 7.

PBS (-) buffer:

0.2 g/L Monopotassium dihydrogen phosphate

0.2 g/L Potassium chloride

8 g/L Sodium chloride

1.15g/L Disodium monohydrogen phosphate anhydrous

[0162]

[Table 7]

Produced chimeric antibodies

Names of produced chimeric antibodies	Used hybridomas	Form of transformation	Introduced cells
#11 ILT7 chimeric antibody	#11	Transient manner	293T
#17 ILT7 chimeric antibody	#17		
#11-5 ILT7 chimeric antibody	#11	Homeostasis	YB 2/0
#11-16 ILT7 chimeric antibody	#11		
#17-24 ILT7 chimeric antibody	#17		

[0163]

cDNA base sequences and amino acid sequences of the heavy and light chains of the produced chimeric antibodies are shown below,

respectively. In each amino acid sequence, the amino acid sequence from N-terminus to -1 is a signal sequence and the amino acid sequence from 1 to C terminus is an amino acid sequence of a mature protein. That is, heavy and light chains, which constitute these chimeric antibodies, consist of the amino acid sequence from 1 to C terminus of each of the following amino acid sequences.

	Heavy chain	Light chain
#11	SEQ ID NO: 50 (base sequence) SEQ ID NO: 51 (amino acid sequence)	SEQ ID NO: 52 (base sequence) SEQ ID NO: 53 (amino acid sequence)
#17	SEQ ID NO: 54 (base sequence) SEQ ID NO: 55 (amino acid sequence)	SEQ ID NO: 56 (base sequence) SEQ ID NO: 57 (amino acid sequence)

Industrial applicability

[0164]

The present invention provided the immunogen useful in producing the antibody specifically recognizing human ILT7 and the method for producing anti-ILT7 antibody using the immunogen. The antibody specifically recognizing human ILT7 of the present invention specifically recognizes ILT7 under the presence of ILT family. Therefore, the antibody of the present invention can be used for the detection and isolation of human ILT7. For example, the localization of ILT7 can also be analyzed using the antibody of the present invention. It is considered that ILT7 is a molecule closely related to the differentiation and function of IPCs or dendritic cells. Therefore, the antibody, which recognizes ILT7 with high specificity, is useful for the analysis of function of IPCs or dendritic cells. IPC-like (having the characteristic in which BDCA-2 is expressed) cancer cells are known (Chaper of L et al. Eur. J. Immunol. 34; 418-426, 2004, Maeda T et al., Int. J. Hematol. 81; 148-154, 2005). Confirmation of the expression of ILT7 in these cells may allow for the diagnosis of cancer and a therapeutic agent.

In the case of autoimmune diseases, for example, the deep relationship between IFNα produced by IPCs and the development of psoriasis, which is a skin disease, is pointed out (Nestle FO et al.,

J. Exp. Med. 202, 135-143, 2005). Therefore, the severity of psoriasis can be examined by identifying IPCs in the skin tissue of psoriasis patients, i.e. in biopsy specimens using the anti-ILT7 antibody.

It is known that the development of AIDS in HIV-infected patients is correlated with the number of IPCs. Namely, lots of IPCs have been observed in patients who do not show symptoms and the reduction in IPCs has been observed in the onset (Soumells V. et al., Blood 98; 906-912, 2001). Therefore, it is effective in predicting the prognosis of virus infection, such as HIV.

[0165]

For example, ILT7 is a molecule which is expressed specifically in human IPCs. Therefore, the anti-ILT7 antibody of the present invention can be used to detect, identify, or isolate IPCs. IPCs are cells which produce most of the type 1 interferon. Therefore, the detection, identification, or isolation is an important objective in diagnosis and study of diseases that involve type 1 interferon. As such diseases, various autoimmune diseases and infections that interferon is involved in the formation of the pathological condition may be illustrated.

[0166]

Additionally, the anti-ILT7 antibody of the present invention has the inhibitory effect on the activity of IPCs. Therefore, the activity of IPCs can be inhibited by using the anti-ILT7 antibody of the present invention. Furthermore, the diseases that involve type 1 interferon can be treated by inhibiting the activity of IPCs. Specifically, the anti-ILT7 antibody of the present invention is useful for various autoimmune diseases and infections that interferon is involved in the formation of the pathological condition. Particularly, since the anti-ILT7 antibody has a high specificity, it can remove IPCs efficiently.

Claims

1. A monoclonal antibody which binds to human ILT7, binds to none of human ILT1, human ILT2 and human ILT3, and suppresses interferon-α (IFNα) production from ILT7-expressing cells, or a fragment comprising its antigen binding region.

2. The monoclonal antibody according to claim 1, wherein the ILT7 expressing cell is an interferon producing cell (IPC).

3. The monoclonal antibody according to claim 1 or 2, wherein the cell is CD123 positive or BDCA-2 positive.

4. The monoclonal antibody according to any one of claims 1 to 3, wherein the cell is CD3 negative, CD14 negative, CD16 negative, CD19 negative, CD20 negative, and CD56 negative.

5. The monoclonal antibody according to any one of claims 1 to 4, wherein the monoclonal antibody is produced by a method comprising the following steps of:

   (1) administering to a non-human animal a cell retaining the following (a) and (b) in an expressible manner:

      (a) an exogenous polynucleotide encoding an amino acid sequence comprising an extracellular domain of human ILT7; and

      (b) an exogenous polynucleotide encoding Fc receptor γ-chain (FcRγ);

   (2) producing and selecting an antibody producing cell which produces an antibody which binds to human ILT7 from the antibody producing cell of the non-human animal; and

   (3) culturing the cell so selected and recovering a monoclonal antibody from the culture.

6. The monoclonal antibody according to claim 5, wherein the cell is an animal cell; and optionally wherein the cell is a human-derived cell; and further optionally wherein the human-derived cell is a 293T cell.

7. The monoclonal antibody according to any one of claims 1 to 4, wherein the fragment comprising its antigen binding region is Fab, F(ab')2, single chain Fv, or diabody.

8. A method for detecting an interferon producing cell, comprising the steps of:

   contacting the monoclonal antibody of any one of claims 1 to 7 with a test cell; and detecting the monoclonal antibody or the fragment comprising its antigen binding region which is bound to the cell.

9. An interferon a (IFNα)-suppressing agent comprising the monoclonal antibody according to any one of claims 1 to 7 as an active ingredient.

ANTI-ILT7 ANTIBODY

Abstract

An antibody binding to IPC was obtained by using an animal cell in which a cell membrane protein associatable with ILT7 was co-expressed as an immunogen. The antibody of the invention has a high specificity which allows immunological distinction between other ILT family molecules and ILT7. The anti-ILT7 antibody of the invention bound to IPC and inhibited the activity thereof. With the anti-ILT7 antibody of the invention, the IPC activity can be inhibited and an interferon-related disease can be treated or prevented. ILT7 expression is maintained even in IPC in the presence of IFNα. Therefore, an inhibitory action of IPC activity by the anti-ILT7 antibody can be expected even in an autoimmune disease patient with an increased production of IFNα.

No suitable figure