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(54)Title: CELL SURFACE MOLECULE MEDIATING CELL ADHESION AND SIGNAL TRANSMISSION

(54)発明の名称 細胞間接着及びシグナル伝達を媒介する細胞表面分子

(57) Abstract

A novel cell surface molecule recognized by a monoclonal antibody, which is expressed specifically in thymocytes, lymphocytes activated by ConA-stimulation and peripheral blood lymphocytes and has been found out from among monoclonal antibodies against cell surface molecules in lymphocytic cells having important roles in autoimmune diseases and allergic diseases, is isolated and identified. Further, functions of this molecule are analyzed. Moreover, it is found that an antibody against this molecule significantly ameliorates conditions of autoimmune diseases and allergic diseases.

Cell Surface Molecule Mediating Cell Adhesion and Signal Transmission

Technical Field

The present invention relates to novel cell surface molecules of mammals; polypeptides and their fragments constituting the molecules; fusion polypeptides comprising the polypeptide fragments and immunoglobulin fragments; genes encoding the polypeptides and the fragments; vectors comprising the genes; transformants into which the vectors are introduced; antibodies having reactivity to the polypeptides or cell surface molecules comprising the polypeptides; hybridomas producing the antibodies; pharmaceutical compositions comprising the polypeptide fragments or the fusion polypeptides; pharmaceutical compositions comprising the antibodies; transgenic mice; and knockout mice.

Background Art

A living body of mammals has immune response systems that excludes pathogenic microorganisms (viruses, bacteria, parasites, etc.) or foreign bodies (both are called "antigen" in the following) that have invaded the living body. One of them is called natural immune response system, another acquired immune response system. The former is an exclusion mechanism comprising phagocytosis by phagocytes (polymorphonuclear leucocytes, monocytes, macrophages, etc.), attack by natural killer (NK) cells, and non-specific recognition such as opsonisation of antigen by complements. The latter, acquired immune response system, is an exclusion mechanism by lymphocytes (mainly, T cells and B cells) that acquired the specificity to the antigen (namely, activated lymphocytes). B cells that acquired antigen specificity attack the antigen existing outside of the cells through production of antibodies specific to the antigen. T cells that acquired antigen specificity (namely, activated T cells) are classified into helper T cells and cytotoxic T cells (cytotoxic lymphocyte, CTL). The helper T cells regulate a differentiation of B cells and a production of antibodies, and destroy the antigen cooperating with phagocytes. The latter, CTLs attack virus-infected cells and so on by themselves (Experimental Medicine: SUPPLEMENT, "Bio Science Term Library, Immunity", Yodosha, pp.14-17 (1995)).

This acquisition of antigen specificity by T cells (namely, activation of T cells) is initiated through recognition by T cells the antigen presented by antigen-presenting cells (APC) such as macrophage, B cells, or dendritic cells. Antigen-presenting cells process the antigens so incorporated and present these processed antigens through binding them to major histocompatibility complex (MHC). T cells receive primary signal for activation of the cells (or acquisition of specificity) by recognising the processed antigens presented by antigen-presenting cells through a complex between T cell receptor (TcR) and CD3 antigen existing on the surface of the cell membrane (TcR/CD3 complex).

However, the TcR/CD3 complex-mediated primary signal alone cannot activate T cells sufficiently and leads to unresponsiveness or clonal anergy, so that the cells can not react with any stimulation received thereafter. The autocrine of interleukin 2 (IL-2) is necessary for T cells to be activated, to be differentiated into antigen specific T cell clones, and to be proliferated. In clonal anergy, T cells are inactivated due to no production of IL-2 and no cell division. Namely, the activation of T cells accompanied by production of cytokines such as IL-2 requires the secondary signal

following the first signal through TcR/CD3 complex. This secondary signal is called costimulatory signal.

T cells receive this secondary signal and transmit it into the cells by interacting (cell adhesion) with molecules other than MHC on antigen-presenting cells through molecules other than TcR/CD3 complex on the T cell surface. This secondary signal avoids cell anergy (clonal anergy) and activates the cells.

Although some part of the mechanism of the secondary signal transmission between antigen-presenting cells and lymphocytes such as T cells have not yet been elucidated in detail, studies so far have revealed that an important factor for the secondary signal transmission is the interaction of CD28 (also named Tp44,T44, or 9.3 antigen), which is a cell surface molecule expressed mainly on T cells and thymus cells, with CD80 (also named B7-1, B7, BB1, or B7/BB1), which is a cell surface molecule expressed on antigen-presenting cells (macrophages, monocytes, dendritic cells, etc.) and with CD86 (also named B7-2 or B70), which is also a cell surface molecule on antigen-presenting cells (namely, cell adhesion through the binding between these molecules). Moreover, it has been experimentally elucidated that the interaction of Cytolytic T lymphocyte associated antigen 4 (CTLA-4), whose expression is thought to be enhanced depending on the secondary signal, with the CD80 (B7-1) and CD86 (B7-2) (namely, cell adhesion through the binding between these molecules) also plays an important role in the regulation of T cell activation by the secondary signal. In other words, the regulation of T cell activation by the transmission of the secondary signal involves at least the interaction between CD28 and CD80/CD86, the enhancement of CTLA-4 expression, which is thought to depend on the interaction, and the interaction between CTLA-4 and CD80/CD86.

CD28 is known to be a costimulator molecule transmitting the secondary signal (costimulatory signal) required for the activation of T cells and for the avoidance of anergy. The secondary signal transmitted by binding this molecule to costimulator molecules, CD80 (B7-1) and CD86 (B7-2), on antigen-presenting cells (cell adhesion through the binding between these molecules), stabilises mRNA of Th1-type cytokines and consequently promotes production by T cells of a large amount of Th1-type cytokines such as Il-2, IFNγ, and TNFα. The expression of CTLA-4 is induced by the primary signal transmitted through TcR/CD3, and the expression is also enhanced by the secondary signal transmitted by the binding between CD28 and CD80. It is being revealed that CTLA-4 receives these signals to work to inhibit T cell function, which is contrary to the activation of T cells by the secondary signal transmitted by CD28.

Human CD28 and CTLA-4 are I-type glycoproteins whose molecular weights are 44kD and 41 to 43kD, respectively. Both have an immunoglobulin-like domain, belong to the immunoglobulin superfamily, and have both function as a cell adhesion molecule and function as a signal transmission molecule.

Human CD28 forms a homodimer with a disulfide bond while CTLA-4 exists as a monomer. Both CD28 and CTLA-4 genes are located at "2q33" on human chromosome and "1C" on mouse chromosome, and are composed of four (4) exons. Human CD28 and CTLA-4 are composed of 220 and 223 amino acids, respectively, including the leader sequences, and amino acid homology between them is 20 to 30%.

The ligands for CD28 and CTLA-4 are CD80 (B7-1) and CD86 (B7-2) in human and mice. CTLA-4 has about 20 times as high affinity to both ligands as CD28. It has been elucidated that the amino acid sequence structures "MYPPPY (Met-Tyr-Pro-Pro-Pro-Tyr)" conserved through animal species is important for the binding of CD28 and CTLA-4 to CD80 (B7-1). It has also been reported that, when CD28 is stimulated, PI3 kinase (phosphoinositide 3 kinase, PI3K) associates with the phosphorylated tyrosine residue in a partial sequence "YMNM (Tyr-Met-Asn-Met)" of CD28 and that CD28 plays an important role in intracellular signal transmission through this "YxxM" structure. Furthermore, it has been reported that CTLA-4 also has a sequence represented by "YxxM," namely "YVKM (Tyr-Val-Lys-Met)" in its cytoplasmic region and that, after being stimulated, SYP associates with this sequence.

CD28 is expressed specifically in thymocytes and peripheral blood T cells, and CTLA-4 is expressed specifically in activated T cells (Cell Engineering: SUPPLEMENT, "Handbook of Adhesion Molecule", Shujunsha, pp.93-102 (1994); ibid. pp.120-136; Experimental Medicine: SUPPLEMENT, "BIO SCIENCE Term Library, Immunity", Yodosha, pp.94-98 (1995); Experimental Medicine: SUPPLEMENT, "BIO SCIENCE Term Library, Intracellular Signal Transduction", Yodosha, pp.58-59 (1997); Nihon Rinsho, Vol.55, No.6, pp.215-220 (1997)).

In the regulation of T cell function (the activation and the inhibition of function of T cells), the importance of interactions among multiple molecules such as costimulator molecules (CD28, CD80 (B7-1), CD86 (B7-2), etc.) and CTLA-4, which cooperates with them, (in other words, cell adhesion through the binding between these molecules) has thus been recognised, and this has been drawn attention to the relationship between these molecules and diseases, and the treatment of diseases by regulating the function of these molecules.

As described above, although a living body activates its acquired immune response system against antigens that are foreign bodies to the living body (self), it also has immunological tolerance so as to show no immune response against its own component (autoantigen). If immunological tolerance breaks down by some reason, immune response to the autoantigen occurs, autoantigen-reactive T cells are induced by the same mechanism as mentioned above to fall into abnormal state of immunity, and various autoimmune diseases are caused.

In other words, since non-stimulated antigen presenting cells (APC) in normal tissues do not express costimulatory molecules when the immune system of a living body is normal, T cells are in the unresponsiveness state to maintain immunological tolerance even if autoantigen-reactive T cells, which reacts with autoantigen, exist. It has been suggested that in abnormal state of immunity, more autoantigen-reactive T cells are activated due to abnormal excess and continuous expression of costimulatory molecules to thereby cause autoimmune diseases.

From such viewpoints recently, many attempts to treat various autoimmune diseases by modulating the transmission of costimulatory signals, for example, the above-mentioned signal transmission between CD28/CTLA-4 and CD80/CD86, are proposed.

It has been observed CD80, a costimulatory molecule as the ligand of CD28 and CTLA-4, is abnormally expressed in the antigen presenting cells at the nidus of autoimmune disease such as rheumatoid arthritis, multiple sclerosis, autoimmune thyroiditis, allergic contact-type dermatitis, and

chronic inflammatory dermatosis such as lichen planus, and psoriasis. From such observation, many attempts to treat various autoimmune diseases by modulating the function of CD80 have been made.

It has been proposed to block the function of CD80, by methods using an antibody against CD80, solubilised protein of CD28 that is a ligand of CD80, and solubilised protein of CTLA-4 that is also a ligand of CD80. Particularly, based on the fact that the binding affinity of CTLA-4 to CD80 is 20 or more times higher than that of CD28, therapeutic attempts using "solubilised CTLA-4," specifically, the fusion protein (CTLA-4-IgFc) comprising the extracellular domain of "CTLA-4" and the Fc region of human immunoglobulin IgG1, were performed in animal model and clinical tests (Nihon Rinsho, Vol.55, No.6, pp.215-220 (1997)).

As shown in 1 to 5 below, therapeutic effects of CTLA-4-IgFc in model animals of autoimmune diseases has been reported.

1. In a (NZB/NZW)F1 mouse, that is a model for human systemic lupus erythematosus (SLE), the production of autoantibodies and the onset of lupus nephritis were suppressed by administration of CTLA-4-IgFc before the onset, and the pathologic conditions were improved by administration of the drug even after the onset (Science, Vol.125, p.1225-1227 (1994)).

2. In experimental allergic encephalomyelitis (EAE), that is a model for multiple sclerosis (MS), the onset was prevented by short-term administration of CTLA-4-IgFc immediately after immunisation (J. Clin. Invest., Vol.95, pp.2783-2789 (1995)).

3. In an NOD (non-obese diabetes) mouse, which is a model for insulin dependent diabetes mellitus (IDDM), the onset rate was remarkably decreased by administering CTLA-4-IgFc to the 2- or 3-week-old female mouse for two weeks (J. Exp. Med., Vol.181, pp.1145-1155 (1995)).

4. In rat nephritis by renal glomerulus basement membrane immunity, Goodpasture's nephritis model, the improvement of the symptom has been improved by the administration of CTLA-4-IgFc (Eur. J. Immunol., Vol.24, No.6, pp.1249-1254 (1994)).

5. In type II collagen-induced arthritis (CIA) using a DBA/1 mouse, that is a model for human rheumatoid arthritis, the onset of arthritis was suppressed by administering the test drug at the time of the immunisation and the production of autoantibodies (IgG1 and IgG2) against collagen was inhibited (Eur. J. Immunol., Vol.26, pp.2320-2328 (1996)).

The results of the experiments as mentioned above have not yet clarified in detail the mechanism of the T cell activation by interaction between costimulatory molecules and the related molecules (in other words, cell adhesion through the binding between these molecules). Other unknown molecules may be involved in this mechanism.

Disclosure of the Invention

Pharmaceuticals useful for treating or preventing various diseases such as the above-mentioned autoimmune diseases, allergic diseases, and inflammatory diseases can be developed if the mechanism of the activation of lymphocytes such as T cells by cell adhesion through the binding between molecules involved in the transmission of the secondary signal essential for the activation of lymphocytes such as T cells mentioned above and the mechanism of the regulation of lymphocyte function are clarified, and known or unknown molecules capable of mediating cell adhesion involved in the mechanism and of transmitting signals are identified and characterised.

An objective of the present invention is to identify novel cell surface molecules having both functions of mediating such cell adhesion and signal transmission, and to clarify its structural and biological characteristics. Another objective of the present invention is to provide pharmaceuticals useful for treating or preventing various autoimmune diseases and inflammatory diseases by using the novel molecules or antibodies against the molecules.

In order to identify such useful molecules, the present inventors focused on the fact that lymphocytes such as T cells play an important role in autoimmune diseases and allergic diseases, and the fact that cell adhesion are essential for the signal transmission of the secondary signal (costimulatory signal) from antigen presenting cells into lymphocytes, and planned to isolate and identify cell surface molecules that are expressed specifically on lymphocytic cells and that mediate cell adhesion.

The present inventors obtained monoclonal antibodies against various cell surface molecules expressed on the surface of lymphocytic cells by immunising animals against the lymphocytic cells, and isolated and identified desired cell surface molecules that mediate cell adhesion using the monoclonal antibodies so obtained. The methods used are described in detail below.

The present inventors first administered rat lymphocytic cell line as an antigen to mice and prepared various monoclonal antibodies. Then, the monoclonal antibodies obtained were reacted with rat lymphocytic cells used as an antigen and tested the effect of the monoclonal antibodies given to the cells. As a result, one of the monoclonal antibodies was found to agglutinate the rat lymphocytic cells strongly (this monoclonal antibody was designated "JTT-1 antibody"). Moreover, other one of the monoclonal antibodies was found to strongly inhibit the agglutination of rat lymphocytic cells induced by the "JTT-1 antibody" (this monoclonal antibody was designated "JTT.2 antibody").

Since the agglutination of rat lymphocytic cells by "JTT-1 antibody" was not inhibited by antibodies against Intercellular adhesion molecule-1 (ICAM-1) or Lymphocyte function-associated antigen-1 (LFA-1), which are the most representative known cell adhesion molecules expressed on the cells, the present inventors thought that this agglutination was caused by cell adhesion through unknown adhesion molecules that mediate cell adhesion.

Cell surface molecules (designated "JTT-1 antigen" and "JTT.2 antigen") recognised by each of these two monoclonal antibodies were then identified, isolated, and characterised.

First, the analysis of the expression patterns of "JTT-1 antigen" and "JTT.2 antigen" in various cells were analysed by flow cytometry based on fluorescent antibody technique using "JTT-1 antibody" and "JTT-2 antibody." While both "JTT-1 antigen" and "JTT.2 antigen" were strongly expressed in activated lymphoblast cells (activated T lymphoblast cells, activated B lymphoblast cells, etc.) activated by stimulating thymocytes and spleen cells with Concanavalin A (ConA), a mitogen, in particular, in the activated lymphoblast cells, the expression was hardly found in spleen cells not stimulated at all (these cells are sometimes called "resting lymphocytes" herein). The expression patterns of molecules recognised by each of "JTT-1 antibody" and "JTT-2 antibody" were almost the same.

Using an affinity column prepared by binding "JTT-1 antibody" to adsorbents, molecules trapped by the "JTT-1 antibody", namely, "JTT-1 antigens" were purified from the mixture of soluble cell surface molecules prepared from the above-described rat lymphocytic cells. The molecular weights of these purified "JTT-1 antigens" were analysed by immunoprecipitation using "JTT-1 antibody" and "JTT-2 antibody" and by SDS-PAGE. As a result, it was found that molecules immunoprecipitated by each of "JTT-1 antibody" and "JTT-2 antibody" were the same, and that each molecule was a homodimer having different sugar chains. Specifically, when N-linked sugar chains were not digested, the molecules were identified as one molecule with about 47kD under non-reduction condition, and as two molecules with about 24kD and about 28kD under reduction condition; when N-linked sugar chains were digested, the molecules were identified as one molecule with about 36kD under non-reduction condition and as one molecule with about 20kD under reduction condition.

The adhesion of rat thymocytes to the plate coated by the purified "JTT-1 antigen" was then analysed. As a result, thymocytes significantly adhered to the plate (namely, to "JTT-1 antigen") only in the presence of "JTT-1 antibody" and the adhesion was significantly inhibited in the co-presence of "JTT.2 antibody", indicating that "JTT-1 antigen" was the cell surface molecule mediating cell adhesion.

Next, the present inventors cloned genes encoding "JTT-1 antigen" from rat, human, and mouse, and analysed their structures.

First, the cDNA encoding the full length of "rat JTT-1 antigen" was isolated from the cDNA library made from the lymphoblasts derived from ConA-stimulated rat spleen by expression cloning method utilising panning method using "JTT-1 antibody" and a completely novel rat gene was isolated and identified by determining its nucleotide sequence by dideoxy method. The cDNA encoding the full length of "human JTT-1 antigen" was also isolated from the cDNA library made from ConA-stimulated human peripheral blood lymphoblasts by plaque hybridisation using the cDNA encoding "rat JTT-1 antigen" so obtained as a probe and a completely novel human gene was isolated and identified by determining its nucleotide sequence by dideoxy method. Similarly, the cDNA encoding the full length of "mouse JTT-1 antigen" was isolated from the cDNA library made from the lymphoblasts derived from ConA-stimulated mouse spleen and a completely novel mouse gene was isolated and identified by determining its nucleotide sequence by dideoxy method. Furthermore, the cDNA encoding the full length of alternative splicing variant of "rat JTT-1 antigen" mentioned above was isolated similarly from the cDNA library made from the rat thymoma cell line and another completely novel rat gene was isolated and identified by determining its nucleotide sequence by dideoxy method.

"JTT-1 antigen" was found to be a transmembrane protein (cell surface molecule) composed of a signal sequence, an extracellular region having the glycosylation site(s), a transmembrane region, and an intracellular region by hydropathy plot analysis of the amino acid sequence encoded by the isolated cDNA of "human JTT-1 antigen". Homology search with known molecules revealed that "JTT-1 antigens" from rat, human, and mouse had no significant homology to any known molecules including cell adhesion molecules, indicating that they are novel cell surface molecules that mediates cell adhesion.

As the result of motif search based on the amino acid sequence of "human JTT-1 antigen", it was found that "human JTT-1 antigen" had structural similarity with the above-mentioned "CD28", a cell surface molecule on lymphocytes such as T cells, which transmits costimulatory signal important for T cell activation through cell adhesion, and with "CTLA-4", a cell surface molecule on lymphocytes such as T cells, which regulates the functions of activated lymphocytes such as activated T cells, cooperating with the signal.

The structural similarity is as follows.

1. 20 or more amino acid residues including cysteine residues are highly conserved.

2. Proline repeating sequence "Pro-Pro-Pro (PPP)" essential as the ligand binding region, is conserved in the extracellular region.

3. A sequence "Tyr-Xaa-Xaa-Met (YxxM)" (Xaa and x represents any amino acid) sequence essential as the signal transmitting region is conserved in the cytoplasmic region.

The locus of the gene encoding "mouse JTT-1 antigen" on mouse chromosome was found to be "1C3", which is the same location as that of mouse "CD28" and "CTLA-4" using fluorescence *in situ* hybridisation (FISH) method.

Next, the effectiveness of therapy of autoimmune diseases and allergic diseases by regulating the function of "JTT-1 antigen" was examined by experiments in which "JTT-2 antibody" mentioned above was administered to model rats for experimental allergic encephalomyelitis (EAE) and glomerulus basement membrane (GBM) nephritis. It was found that the pathological states were significantly suppressed in both disease model animals, and that autoimmune diseases or allergic diseases can be treated by regulating the functions of "JTT-1 antigen".

It was also found that the monoclonal antibody against "human JTT-1 antigen" significantly proliferated human peripheral blood lymphocytes, and that the proliferation was further enhanced in the co-presence of a monoclonal antibody against CD3 constituting a TcR/CD3 complex on T cells, which receives the primary signal essential for T cell activation from antigen presenting cells, indicating that "JTT-1 antigen" was a cell surface molecule involved in signal transmission into lymphocytes.

Furthermore, the present inventors succeeded in producing a fusion polypeptide comprising of the extracellular region of "human JTT-1 antigen" and Fc region of human immunoglobulin. The fusion polypeptide is useful as pharmaceuticals for treating autoimmune diseases, allergic diseases, and inflammatory diseases by regulating the functions of "JTT-1 antigen" and/or its ligand.

Moreover, the present inventors succeeded in preparing a transgenic mouse into which a gene encoding "JTT-1 antigen" of other animal species was introduced. The transgenic mouse is useful for analysing detailed functions of "JTT-1 antigen" and for developing pharmaceuticals for treating autoimmune diseases, allergic diseases, and inflammatory diseases. The inventors also produced a knockout mouse in which the endogenous gene encoding "mouse JTT-1 antigen" was inactivated. This knockout mouse is also useful for the above-mentioned purpose.

The present inventions relate to polypeptides, genes, antibodies, vectors, transformants, pharmaceutical compositions, transgenic mice, knockout mice and so on, which are relevant to a

novel mammalian "JTT-1 antigen" isolated and identified as mentioned above. Specifically, the present invention are as described in (1) to (36) below.

(1) An isolated polypeptide constituting a cell surface molecule having the following characteristics:

(a) said cell surface molecule is expressed in at least thymocytes and mitogen-stimulated lymphoblast cells;

(b) a specific antibody reactive to said cell surface molecule induces adhesion between mitogen-stimulated lymphoblast cells;

(c) a specific antibody reactive to said cell surface molecule induces proliferation of peripheral blood lymphocytes in the presence of an antibody against CD3;

(d) said cell surface molecule has a partial amino acid sequence represented by Phe-Asp-Pro-Pro-Pro-Phe in its extracellular region; and

(e) said cell surface molecule has a partial amino acid sequence represented by Tyr-Met-Phe-Met in its cytoplasmic region.

(2) The polypeptide of (1) comprising the amino acid sequence of SEQ ID NO: 2 or the amino acid sequence of SEQ ID NO: 2 in which one or more amino acids are substituted, deleted, or added.

(3) The polypeptide of (1), which is encoded by a DNA hybridising with a DNA having the nucleotide sequence of SEQ ID NO: 1 under stringent conditions.

(4) The polypeptide of (1) comprising an amino acid sequence having 60% or more homology with an amino acid sequence of SEQ ID NO: 2.

(5) The polypeptide of any one of (1) to (4) wherein said cell surface molecule is derived from human.

(6) A gene encoding the polypeptide of any one of (1) to (5).

(7) The gene of (6) wherein said gene is a cDNA.

(8) The gene of (7) wherein said cDNA has a nucleotide sequence of SEQ ID NO: 1.

(9) The gene of (7) wherein said cDNA comprises a nucleotide sequence corresponding to nucleotide residues 26 to 625 of SEQ ID NO: 3, nucleotide residues 35 to 637 of SEQ ID NO: 4, nucleotide residues 1 to 603 of SEQ ID NO: 5, or nucleotide residues 35 to 685 of SEQ ID NO: 6.

(10) A vector comprising the gene of any one of (6) to (9).

(11) A transformant into which the vector of (10) has been introduced.

(12) A transformant identified by an international deposit accession No. FERM BP-5725.

(13) A polypeptide fragment comprising an extracellular region of the polypeptide of any one of (1) to (5).

(14) The polypeptide fragment of (13) wherein said polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

(15) A gene encoding the polypeptide fragment of (13) or (14).

(16) A homodimer molecule comprising two polypeptide fragments, wherein each of the fragments comprises an extracellular region of the polypeptide of any one of (1) to (5) and said two polypeptide fragments bridged through disulfide bonds to each other.

(17) The homodimer molecule of (16) wherein said polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

(18) A pharmaceutical composition comprising either of the polypeptide fragment of (14) or the homodimer molecule of (17), or both of them, and a pharmaceutically acceptable carrier.

(19) A fusion polypeptide comprising an extracellular region of the polypeptide of any one of (1) to (5) and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region.

(20) The fusion polypeptide of (19) wherein the immunoglobulin is IgG.

(21) The fusion polypeptide of (19) wherein the portion of the constant region comprises a hinge region, C2 domain, and C3 domain of IgG.

(22) The fusion polypeptide of any one of (19) to (21) wherein said polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

(23) A homodimer molecule comprising two fusion polypeptide of any one of (19) to (22) wherein the two polypeptides bridged through disulfide bonds to each other.

(24) A homodimer molecule comprising two fusion polypeptides of (22) wherein the two polypeptides bridged through disulfide bonds to each other.

(25) A pharmaceutical composition comprising either of the fusion polypeptide of (22) or the homodimer molecule of (24), or both of them, and a pharmaceutically acceptable carrier.

(26) The pharmaceutical composition of (25) wherein said pharmaceutical composition is utilised for treating autoimmune diseases or allergic diseases, or for preventing said disease symptom.

(27) An antibody or a portion thereof reactive to the polypeptide of any one of (1) to (5), the polypeptide fragment of (13) or (14), or the cell surface molecule comprising said polypeptide.

(28) The antibody of (27) or a portion of it wherein said antibody is a monoclonal antibody.

(29) A monoclonal antibody or a portion thereof reactive to the polypeptide having an amino acid sequence of SEQ ID NO: 2, the polypeptide fragment of (14), or the human-derived cell surface molecule comprising said polypeptide.

(30) A monoclonal antibody or a portion thereof reactive to the polypeptide of any one of (1) to (5) or the cell surface molecule comprising said polypeptide, wherein the effect of said monoclonal antibody on mitogen-stimulated lymphoblast cells is substantially the same as the effect of a monoclonal antibody produced by a hybridoma identified by an international deposit accession No. FERM BP-5707 on mitogen-stimulated rat lymphoblast cells.

(31) A monoclonal antibody or a portion thereof reactive to the polypeptide of any one of (1) to (5) or the cell surface molecule comprising said polypeptide, wherein the effect of said monoclonal antibody on mitogen-stimulated lymphoblast cells is substantially the same as the effect of a monoclonal antibody produced by a hybridoma identified by an international deposit accession No. FERM BP-5708 on mitogen-stimulated rat lymphoblast cells.

(32) A pharmaceutical composition comprising the monoclonal antibody of (29) or a portion thereof and a pharmaceutically acceptable carrier.

(33) The pharmaceutical composition of (32) wherein said pharmaceutical composition is utilised for treating autoimmune diseases or allergic diseases, or for preventing said disease symptom.

(34) A hybridoma producing the monoclonal antibody of any one of (28) to (31).

(35) A transgenic mouse in which a gene encoding the polypeptide of (1) which is a human-derived gene comprising a nucleotide sequence of SEQ ID NO: 1 or a rat-derived gene comprising a nucleotide sequence corresponding to nucleotide residues 35 to 637 of SEQ ID NO: 4, which is integrated into its endogenous gene.

(36) A knockout mouse in which its endogenous gene encoding the mouse polypeptide of claim 1 comprising the amino acid sequence encoded by the gene of SEQ ID NO: 5 is inactivated so that said mouse polypeptide is not produced.

As described above, the cell surface molecule of the present invention ("JTT-1 antigen") is involved in cell adhesion, signal transmission into lymphocytes such as T cells, and regulation of function of activated lymphocytes. General knowledge of lymphocytic cells, cell adhesion molecules, and the relationship between them and diseases are described below just for general understanding of these biological events, but the following general knowledge is not for interpreting the present invention limitedly.

Lymphocytes are roughly classified into two kinds, T cells and B cells. After differentiation from multipotent stem cells in bone marrow to lymphoid stem cells, some of them flow into blood to reach thymus. Lymphocytes differentiated and matured in thymus, which are called T cells (Thymus-derived T cells), get into blood again, and circulate through the whole body. Matured T cells have a molecule called CD3 on their surface. The existence of CD3 molecule is an marker to determine whether the cells are matured T cells or not. CD3 is a convincing T cell marker. In addition, T cells express CD4 or CD8. T cells are classified into helper T cells (Th cells) assisting the antibody production by B lymphocytes, cytotoxic T cells (Tc cells, CTL) or killer T cells that are bound to target cells to destroy them directly, suppressor T cells that suppress the antibody production by B lymphocytes, and effector T cells that secrete effector substances such as lymphokines to cause delayed allergy.

B cells are derived from the lymphoid stem cells differentiated and matured in bone marrow. B cells are antibody-producing precursor cells since they produce antibodies with an appropriate stimulus. B cells have immunoglobulins on their cell surface, which were produced in a cell. Such immunoglobulins function as receptors for antigens. Matured B cells have both IgM and IgD on their surface. If B cells are differentiated with antigen stimulation and signals from T cells, the production of IgM increases and their C-terminal cell membrane binding regions are changed to be secreted. With sufficient stimulation, not only the surface immunoglobulins change into IgG, IgE, and IgA, but also the immunoglobulins of each class are secreted. The immunoglobulin on the B cell surface is sometimes represented as sIg, abbreviation of surface Ig, or mIg, abbreviation of membrane Ig. All Igs on the surface of the same B cell have the same antigen binding sites.

There are lymphocytes called large granular lymphocytes (LGL) or null cells, which are neither T cells nor B cells. These cells can destroy tumour cells and virus-infected cells without pre-stimulation with antigen, which is comparative to the case of cytotoxic T cells. So, they are also called natural killer cells (NK cells).

Among the T cells mentioned above, CD4-positive T cells secrete various cytokines, newly express receptors for these cytokines, enlarge their own size, start cell dividing, and proliferate, when

they react with antigens presented by antigen-presenting cells. Prior to these reactions at the cell level, the complex of the antigen peptides on antigen presenting cells and MHC class II molecules binds to the corresponding T cell antigen receptor (TCR). This causes various biochemical changes in the cells, and the signal is transmitted into nuclei to start the transcription of specific DNAs and to produce respective proteins. As a result, reactions at the cell level are raised. For example, cells infected with a virus produce virus proteins and they are degraded into peptides by proteasomes in the cytoplasm. A part of the peptides enters endoplasmic reticulum through TAP, forms stable complex with MHC class I molecules just produced, and transfers to the cell surface. The peptide transferred to the cell surface is recognised specifically by CD8-positive T cells, but the T cells can not yet destroy the infected cells at this stage. These T cells reacted with the antigen expresses IL-2 receptor (IL-2R), are differentiated into CTL cellular cytotoxicity upon IL-2 action, and destroy their target cells to kill them in the next time when they meet the same antigen peptide/MHC class I complex. Cytokines required for the differentiation into CTL are not only IL-2 but also IFNγ or other cytokines, which are thought to have similar actions. Thus, lymphokines secreted by T cells are necessary for the differentiation into CTL. The lymphokines are produced as the result that CD4-positive Th1 cells (CD4-positive T cells secreting IL-2 or IFNγ) recognise the antigen peptides derived from the same virus with class II molecules. In some cases, without the help of CD4-positive T cells, CD8-positive T cells react with antigens and produce IL-2 and other cytokines. When CD8-positive T cells are differentiated into CTL, granules increase in the cytoplasm. These granules comprise various high molecular weight proteins, represented by perforin. Perforin resembles a membrane attack complex (MAC) composed of the fifth to ninth components of complement, and makes holes in the cell membrane of target cells. In addition, the granules comprise serine proteases, LT, proteoglycan, etc. Moreover, if CD8-positive cells differentiated into CTL receive antigen stimulation, they also secrete lymphokines such as IFNγ, LT, TNF, or IL-2. Moreover, T cells show blast transformation phenomenon, when they react with hemagglutinin (phytohemagglutinin, PHA) or ConA.

Matured T cells not stimulated at all are called resting T cells, and have smaller cell size and shorter lifetime, a few days. When they receive stimulation, the cells enlarge as already mentioned above, and are apt to react with various kinds of stimulation. Such T cells are called activated T cells. A part of the activated T cells become memory T cells, which bring secondary immunoreaction if they receive the same antigen stimulation. Memory T cells are thought to be kept in circulating around the body for a few years or decades.

B cells not stimulated at all are called resting B cells like in the case of T cells, and proliferating B cells stimulated with multivalent antigens or CD40L, are called activated B cells. Since resting B cells have no costimulator molecules, which stimulate T cells with signals through TCR, such as B7-1 (CD80) or B7-2 (CD86), presenting antigens to resting T cells are thought only to stimulate TCR and to be unable to express CD40 ligands (CD40L) or produce lymphokines. Therefore, it is thought that activated helper T cells stimulated with antigen presented by other antigen-presenting cells react with the antigen presented by resting B cells. Namely, if an antigen invades, first, dendritic cells (cells having extremely dendritic projections) expressing B7 molecules or macrophages activated by reacting with microorganisms present the antigen and stimulate resting helper T cells to activate them

so as to express CD40L. The activated helper T cells then bind to resting B cells presenting the same antigen and stimulate their CD40. Once B cells are activated by stimulation with multivalent antigens or CD40L, they also express B7 molecules, activate helper T cells by stimulating CD28 on their surface with TCR, and allow the helper T cells to express CD40L or produce lymphokines. B cells that show changes such as the expansion of the cell size with stimulation but not show antibody secretion are called activated B cells. If B cells so matured meet antigens, the IgM production increases together with the stimulation from T cells and the IgM molecules so produced are secreted by turning from the membrane type into secretory type. Moreover, they produce isotypic antibodies other than IgM, such as IgG upon the humoral factors from T cells. This is called isotype switching or class switching. B cells secreting antibodies are called antibody-secreting cells. A part of them becomes morphologically characteristic cells and is called a plasma cell (Knowledge of Immunology, Ohmsha, (1996)).

Incidentally, in various reactions of immune system, the subpopulation of white blood cells, namely, T lymphocytes, B lymphocytes, NK, neutrophils, etc. often show dynamics different from one another. Even the same lymphocytes as mentioned above show dynamics different from one another depending on whether the cells are activated or resting. These facts imply the existence of recognition mechanism specific to the subpopulation of white blood cells, further, recognition mechanism specific to the state of cells, and, in particular, cell adhesion molecules (cell adhesion proteins).

Cell adhesion molecules, or cell adhesion proteins are, in general, the molecules that adhere cells to each other in the development and differentiation of individuals or in migration of cells to local site, and are known to be essential molecules for organic and functional contacts in a living body.

Cell adhesion molecules are roughly classified from their structural characteristics into five (5) families, immunoglobulin superfamily, integrin family, selectin family, cadherin family, and CD44 family. Adhesion molecules belonging to immunoglobulin superfamily are characterised by the existence of repeated loop-like domains formed with disulfide bonds. Examples thereof are intercellular adhesion molecule-1 "ICAM-1" and vascular cell adhesion molecule-1 "VCAM-1." In addition, adhesion molecules belonging to integrin family are characterised by α/β heterodimer structure. Examples thereof are very late antigen-1 to 6 "VLA-1 to 6," lymphocyte function-associated antigen-1 "LFA-1", "Mac-1," and "p150/90." Molecules belonging to selectin family have lectin-like domain, EGF-like domain, and complement domain in this order from N terminus. Examples thereof are "E-selectin" and "P-selectin." Examples of cadherin family are "E-cadherin," "N-cadherin," and "P-cadherin," and an example of CD44 family is "CD44".

The specific function of these adhesion molecules is known to be adhesion of white blood cells to vascular endothelial cells or of lymphocytes to antigen-presenting cells. From recent various studies, it has been gradually revealed that adhesion molecules are involved not only in these functions but also in various diseases.

In particular, there are many reports on diseases and expression abnormality of adhesion molecules. For example, as for rheumatoid arthritis (RA), the expression of both "Mac-1" and "p150/95" was reportedly strengthened in RA synoviocytes (Allen, C. *et al.*, Arthritis Rheum., Vol.32,

p.947 (1989)). It has also been reported that various cells expressed "ICAM-1" strongly and ectopically on RA synovial membrane (Hale, L. *et al.*, Arthritis Rheum., Vol.32, p.22 (1989)). Another report implied that "ELAM-1" was also involved in the adhesion of neutrophils to vascular endothelial cells and that the overexpression of these molecules was involved in infiltration of neutrophils (especially, into synovial fluid), which is observed in RA synovial fluid (Laffon, A. *et al.*, Arthritis Rheum., Vol.32, p.386 (1989)). Strong expression of "CD44" in vascular endothelial cells and A-type synoviocytes on RA synovial membrane was reported (Heynes, B. *et al.*, Arthritis Rheum., Vol.34, p.1434 (1991)).

There are reports on the relationship between systemic lupus erythematosus (SLE) and the expression abnormality of adhesion molecules. For example, adhesion ability of T lymphocytes to cultured vascular endothelial cells was reportedly lowered in SLE patients, compared to healthy volunteers. In peripheral lymphocytes of SLE patients, adhesion molecules "ICAM-1", "VLA-4", and "IFA-1" were strongly expressed (Haskard, D. O. *et al.*, Rheumatol. Int., Vol.9, p.33 (1989)).

In autoimmune thyroid diseases, it was reported that "ICAM-1" was expressed when a thyroid follicular cells were stimulated with interferon-γ, interleukin-1, and tumour necrosis factor, and that the formation of cluster of follicular cells and mononuclear cells was inhibited by anti-"ICAM-1" antibody (Weetman, A. P. *et al.*, Eur. J. Immunol., Vol.20, p.271 (1990)).

In hepatitis, it is thought that the chances of adhesion between hepatocytes and inflammatory cells increases since there are two pathways of adhesion, "ICAM-1" and "LFA-3", and "LFA-1" and "CD2", to thereby promote presentation of antigens and activation of inflammatory cells. In particular, in hepatitis B, "LFA-3" molecules are strongly expressed in hepatocytes, in which viruses are actively proliferating, and "ICAM-1" well correlates with the degree of hepatitis. It is thus implied that "LFA-3" is involved in the exclusion of viruses and "ICAM-1" promotes T cells to present antigen and regulates inflammation reaction. In "ICAM-1"-negative and HBc antigen-positive hepatocytes, chronic virus infection, a kind of immunounresponsiveness, may occur due to no interaction between lymphocytes and hepatocytes. It has also been reported that serum "ICAM-1" in chronic liver disease may correlate with the degree of hepatocyte damage because the serum "ICAM-1" concentrations in acute hepatitis patients, chronic active hepatitis patients, and liver cirrhosis patients were higher than that in healthy volunteers and chronic persisting hepatitis patients, and the concentration was high in the case of histologically progressing active hepatitis (Mod. Phys., Vol.15, No.1, pp.73-76 (1995)).

In a model animal of arteriosclerosis, adhesion and invasion of monocytes and lymphocytes to vascular endothelium were observed at very early stages of the onset of the disease. It is thus thought that the interaction of these haemocytes with endothelium is the first step of the onset of arteriosclerosis. Various reports show the expression of adhesion molecules in actual arteriosclerosis nidus including the expression of "ICAM-1" in human arteriosclerosis nidus (Poston, R. N. *et al.*, Am. J. Pathol., Vol.140, p.665 (1992)) and the expression of "VCAM-1" in arteriosclerosis nidus of a hypercholesterolemia rabbit (Cybulsky, M. I. & Gimbrone, M. A. Jr., Science, Vol.251, p.788 (1991)). A recent report revealed that the expression of "VCAM-1" was observed in human arteriosclerosis nidus, and, in particular, strong expression in smooth muscle cells migrating to intima and in monocytes/macrophages. In addition, the expression of "MCP-1" was enhanced in rabbit and human

arteriosclerosis nidus, suggesting that "MCP-1" promotes the formation of arteriosclerosis nidus through the migration of monocytes/macrophages (Current Therapy, Vol.12, No.8 pp.1485-1488 (1994)).

The relationship between tumour metastasis and adhesion molecule abnormality has also been reported. For example, E-cadherin-decreased cancer cells showed strong invasiveness, but the invasiveness was inhibited by introducing the cDNA of E-cadherin into the cancer cells, and invasiveness was recovered when E-cadherin antiserum was added to the cells. This suggests the tight relationship between the decrease in the expression of E-cadherin and invasiveness of tumour cells (Frixen, U. H. *et al.*, Vol.113, p.173 (1991)). In actual clinical cases, the relationship between the decrease of the expression of E-cadherin and metastasis is pointed out in various kinds of cancer such as hepatoma, oesophageal cancer, gastric cancer, and breast cancer. It has also been reported that "VLA-4" molecules, a ligand for "VCAM-1", were highly expressed in metastatic melanoma, gastric cancer, and breast cancer, suggesting that this molecule could be utilised for the implantation to vascular endothelial cells in metastasis. In addition, based on experiments using various tumour cell lines, it has been reported that epithelial cancer, such as gastric cancer, large intestine cancer, lung cancer, hepatoma, or pancreatic cancer, adhered to vascular endothelial cells through E-selectin (Takada, A. *et al.*, Cancer Res., Vol.53, p.354 (1993)).

On the other hand, therapeutic approach to treat diseases by targeting these adhesion molecules have been made. For example, it was reported that anti-rat "ICAM-1" antibody strongly inhibited inflammatory reaction in rat autoimmune arthritis model. It has also been reported that the administration of anti-"ICAM-1" antibody inhibited the onset of arthritis in adjuvant synovitis in one of animal models of RA (Nihon Rinsho Meneki Gakkai Kaishi, Vol.14, No.6, pp.571-577 (1991)). It was further reported that the metastasis formation of inoculated tumour was remarkably inhibited if a large amount of peptides having REG sequence, which is an amino acid sequence in an extracellular matrix protein recognised and bound by some integrins, were administered to a gallbladder cancer mouse, and that in *in vitro* system RGD peptides and anti-β1 subunit antibody inhibited the motion and infiltration of tumour cells (Yamada, K. M. *et al.*, Cancer Res., Vol.50, p.4485, (1990)).

In the following, the present invention is described in detail by clarifying the meanings of terms used herein and the general production methods of polypeptides, fusion polypeptides, genes, antibodies, transgenic mice, and knockout mice of the present invention. However, it is needless to say that the meanings of the terms are not to be interpreted limitedly by the definition given herein.

"Mitogen" used herein is also called mitogenic factor and means a substance which induces cell division. Immunologically, it means a substance inducing blastogenesis of lymphocytes polyclonally and inducing cell division. Examples thereof are lectins such as PHA and PWM, Concanavalin A (ConA), lipopolysaccharides, streptolysin S, and anti-lymphocyte antibody. It is known that Concanavalin A and PHA act only on T lymphocytes, that lipopolysaccharides act only on B lymphocytes, and that PWM acts on both lymphocytes.

The term "lymphoblast cell" used herein is also called a large lymphocyte, lymphoblast, or immunoblast, and means a lymphocyte belonging to a large lymphocyte among lymphocytes existing in lymphoid tissues (lymph node, spleen, thymus, bone marrow, lymph duct, tonsil, etc.) and blood.

The term "activated lymphocyte" used herein means, for example, a lymphocyte mentioned below, but is not limited thereto. For example, the term means a lymphocyte activated by some stimulation. As mentioned above, lymphocytes are classified into T cells, B cells, and natural killer cells. T cells are classified into CD4-positive cells and CD8-positive cells. Therefore, the "activated lymphocytes" of the present invention include mainly activated T cells, activated B cells, and activated natural killer cells, and activated T cells include activated CD4-positive cells and activated CD8-positive cells.

Upon reacting with antigens presented by antigen-presenting cells, CD4-positive T cells secrete various cytokines, newly express receptors for these cytokines, enlarge their own size, start cell dividing, proliferate, and are activated. Activated CD4-positive T cells include those in such a state.

CD8-positive T cells express IL-2R when they react with antigens. When IL-2 acts on IL-2R, the cells are differentiated into CTL, which has cellular cytotoxicity. CTL destroy its target cells to kill them when they meet the same antigen peptide/MHC class I complex. When CD8-positive T cells are differentiated into CTL, granules increase in the cytoplasm. These granules comprise various high molecular weight proteins, represented by perforin. Perforin resembles MAC composed of the fifth to ninth components of complement, and makes holes in the cell membrane of target cells. The granules also comprise serine proteases, LT, and proteoglycan. If CD8-positive cells receive antigen stimulation and are differentiated into CTL, they also secrete lymphokines such as IFNγ, LT, TNF, or IL-2. Activated CD8-positive T cells include those in such a state.

T cells show blast formation phenomenon when they react with hemagglutinin (phytohemagglutinin, PHA) or Concanavalin A (ConA). Activated T cells include those in such a state.

B cells express B7 molecules, activate helper T cells by stimulating CD28 on their surface with TCR, allow the helper T cells to express CD40L or produce lymphokines. When the cells receive stimulation, they change to expand their cell size or proliferate. Activated B cells include those in such a state. In the present invention, activated B cells include those secreting antibodies (antibody-secreting cells and plasma cells).

Activated natural killer cells mean those showing cytotoxic action on tumour cells or virus-infected cells as mentioned above. In the present invention, activated lymphocytes include thymus cells stimulated by Concanavalin A (ConA).

The "activated lymphoblast cell" used herein includes an activated "lymphoblast" that is generated when the lymphoblast mentioned above is stimulated with "mitogen" mentioned above such as Concanavalin A.

The term "resting lymphocyte" used herein means, in some case, an non-activated lymphocyte, which has not received the stimulation to activate cells, in contrast to an activated lymphocyte mentioned above.

The "gene" of the present invention includes a genomic DNA and a cDNA.

The "human-derived" substance of the present invention includes natural substance isolated from a human body component (organ, tissue, cell, body fluid, etc.), and recombinant substance produced by recombinant DNA technology. When the substance is protein or polypeptide, the

substance includes an artificial protein and polypeptide having an amino acid sequence where one or more amino acids are substituted, deleted, or added.

The "cell surface molecule" of the present invention is that derived from a mammal such as human, rat, mouse, guinea pig, and rabbit, preferably that derived from human, rat, or mouse, and more preferably that derived from human.

Specifically, the "cell surface molecule" of the present invention is that characterised by having, at least, properties described below.

(a) the cell surface molecule is expressed in, at least, thymocytes and mitogen-stimulated lymphoblast cells;

(b) an antibody reactive to the cell surface molecule induces adhesion between mitogen-stimulated lymphoblast cells;

(c) an antibody reactive to the cell surface molecule induces proliferation of peripheral blood lymphocytes in the presence of an antibody against CD3;

(d) the cell surface molecule has a partial amino acid sequence represented by Phe-Asp-Pro-Pro-Pro-Phe in its extracellular region; and

(e) the cell surface molecule has a partial amino acid sequence represented by Tyr-Met-Phe-Met in its cytoplasmic region.

Preferably, the "cell surface molecule" comprises the following "polypeptide" of the present invention.

The "polypeptide" of the present invention is that which constitutes the above-mentioned "cell surface molecule" of the present invention. Examples thereof are as follows.

(1) A polypeptide encoded by a DNA hybridising with a DNA comprising a nucleotide sequence of SEQ ID NO: 1 under stringent conditions;

(2) A polypeptide having an amino acid sequence having 60% or more homology with an amino acid sequence of SEQ ID NO: 2;

(3) A polypeptide having an amino acid sequence of SEQ ID NO: 2 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting "human JTT-1 antigen" and its derivative);

(4) A polypeptide having an amino acid sequence encoded by a nucleotide sequence corresponding to nucleotide residues 26 to 625 of SEQ ID NO: 3 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting "human JTT-1 antigen" and its derivative);

(5) A polypeptide having an amino acid sequence encoded by a nucleotide sequence corresponding to nucleotide residues 35 to 637 of SEQ ID NO: 4 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting "rat JTT-1 antigen" and its derivative);

(6) A polypeptide having an amino acid sequence encoded by a nucleotide sequence corresponding to nucleotide residues 1 to 603 of SEQ ID NO: 5 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting "mouse JTT-1 antigen" and its derivative);

(7) A polypeptide having an amino acid sequence encoded by a nucleotide sequence corresponding to nucleotide residues 35 to 685 of SEQ ID NO: 6 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting a "mutant of rat JTT-1 antigen" and its derivative); and

(8) A polypeptide having an amino acid sequence encoded by a DNA encoding a polypeptide constituting the cell surface molecule of the present invention, wherein the DNA is introduced into the transformant identified by an international deposit accession No. FERM BP-5725 or, having amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting a "human JTT-1 antigen" and its derivative).

Examples of "stringent conditions" are as follows. When a probe with 50 or more nucleotides is used and hybridisation is performed in 0.9% NaCl, the standard of temperature where 50% dissociation occurs (Tm) is calculated using the following formula and the temperature for hybridisation can be determined according to the following formula.

$Tm = 82.3^{o}C + 0.41 \times (G+C)\ \% - 500/n - 0.61 \times (\text{formamide})\ \%$

(n means the number of the nucleotide of probe).

$\text{Temperature} = Tm - 25^{o}C$.

In addition, when a probe with 100 or more nucleotides (G+C = 40 to 50%) is used, it should be considered that Tm varies as (1) and (2) mentioned below.

(1) Tm descends by about 1°C per 1% mismatch.

(2) Tm descends by 0.6 to 0.7°C per 1% formamide.

Accordingly, the temperature conditions for the combination of completely complementary strands can be set as follows.

(A) 65 to 75°C (formamide not added)

(B) 35 to 45°C (in the presence of 50% formamide)

The temperature conditions for the combination of incompletely complementary strands can be set as follows.

(A) 45 to 55°C (formamide not added)

(B) 35 to 42°C (in the presence of 30% formamide)

The temperature conditions when a probe with 23 or less nucleotides is used can be 37°C or can be calculated using the following formula.

$\text{Temperature} = 2^{o}C \times (\text{the number of A+T}) + 4^{o}C \times (\text{the number of C+G}) - 5^{o}C$.

Here, "having substantially the same amino acid sequence" means to include a polypeptide having an amino acid sequence where multiple amino acids, preferably 1 to 10 amino acids, particularly preferably 1 to 5 amino acids, in the amino acid sequence shown in Sequence Listing are substituted, deleted, and/or modified, and a polypeptide having an amino acid sequence where multiple amino acids, preferably 1 to 10 amino acids, particularly preferably 1 to 5 amino acids, are added to the amino acid sequence shown in Sequence Listing, as long as the polypeptide has substantially the same biological properties as the polypeptide having the amino acid sequence shown in Sequence Listing.

Such substitution, deletion, or insertion of amino acids can be performed by the usual method (Experimental Medicine: SUPPLEMENT, "Handbook of Genetic Engineering" (1992); and so on).

Examples thereof are synthetic oligonucleotide-directed mutagenesis (gapped duplex method), point metagenesis by which a point mutation is introduced at random by treatment with nitrite or sulfite, the method by which a deletion mutant is prepared with Bal31 enzyme and the like, cassette mutagenesis, linker scanning method, miss incorporation method, mismatch primer method, DNA segment synthesis method, etc.

Synthetic oligonucleotide-directed mutagenesis (gapped duplex method) can be, for example, performed as follows. The region desired to be mutagenised is cloned into M13 phage vector having amber mutation to prepare the single-stranded phage DNA. After RF I DNA of M13 vector without amber mutation is linearised by restriction enzyme treatment, DNA is mixed with the single-stranded phage DNA mentioned above, denatured, and annealed thereby forming "gapped duplex DNA." A synthetic oligonucleotide into which mutations are introduced is hybridised with the gapped duplex DNA and the closed-circular double-stranded DNAs are prepared by the reactions with DNA polymerase and DNA ligase. *E. coli* mutS cells, deficient in mismatch repair activity, are transfected with this DNA. *E. coli* cells without suppressor activity are infected with the grown phages, and only phages without amber mutation are screened.

The method by which a point mutation is introduced with nitrite utilises, for example the principle as mentioned below. If DNA is treated with nitrite, bases are deaminated to change adenine into hypoxanthine, cytosine into uracil, and guanine into xanthine. If deaminated DNA is introduced into cells, "A:T" and "G:C" are replaced with "G:C" and "A:T", respectively, because hypoxanthine, uracil, and xanthine form a base pair with cytosine, adenine, and thymine, respectively, in the DNA replication. Actually, single-stranded DNA fragments treated with nitrite are hybridised with "gapped duplex DNA", and thereafter mutant strains are separated by manipulating in the same way as synthetic oligonucleotide-directed mutagenesis (gapped duplex method).

Alphabetical triplet or single letter codes used to represent amino acids in the present specification or figures mean amino acids as follows. (Gly/G) glycine, (Ala/A) alanine, (Val/V) valine, (Leu/L) leucine, (Ile/I) isoleucine, (Ser/S) serine, (Thr/T) threonine, (Asp/D) aspartic acid, (Glu/E) glutamic acid, (Asn/N) asparagine, (Gln/Q) glutamine, (Lys/K) lysine, (Arg/R) arginine, (Cys/C) cysteine, (Met/M) methionine, (Phe/F) phenylalanine, (Tyr/Y) tyrosine, (Trp/W) tryptophane, (His/H) histidine, (Pro/P) proline.

The "polypeptide" constituting the above-mentioned "cell surface molecule" of the present invention is a transmembrane protein, which penetrates cell membrane, and the "cell surface molecule" is composed of one or two of these transmembrane polypeptides.

Here, a "transmembrane protein" means a protein that connects with membrane through the hydrophobic peptide region penetrating the lipid bilayer of the membrane once or several times and whose structure is, as a whole, composed of three main regions, that is, extracellular region, transmembrane region, and cytoplasmic region, as seen in many receptors or cell surface molecules. Such a transmembrane protein constitutes each receptor or cell surface molecule in the form of a

monomer, homodimer, heterodimer or oligomer with another chain(s) having the same or different amino acid sequence.

The "polypeptide fragment" of the present invention is a fragment from the above-defined "polypeptide" of the present invention, and preferably the extracellular region of the polypeptide. One to five amino acids, if desired, can be added to the N terminus and/or C terminus of this region.

Here, an "extracellular region" means the whole or a portion from the partial structure (partial region) from the entire structure of the above-mentioned transmembrane protein where the partial structure exists outside of the membrane. In other words, it means the whole or a portion of the region of the transmembrane protein except the region incorporated into the membrane (transmembrane region) and the region existing in the cytoplasm following the transmembrane region (cytoplasmic region).

"The constant region or a portion of the constant region of human immunoglobulin (Ig) heavy chain" used herein means the constant region or the Fc region of human-derived immunoglobulin heavy chain (H chain) as described, or a portion of them. The immunoglobulin can be any immunoglobulin belonging to any class and any subclass. Specifically, examples of the immunoglobulin are IgG (IgG1, IgG2, IgG3, and IgG4), IgM, IgA (IgA1 and IgA2), IgD, and IgE. Preferably, the immunoglobulin is IgG (IgG1, IgG2, IgG3, or IgG4), or IgM. Examples of particularly preferable immunoglobulin of the present invention are those belonging to human-derived IgG (IgG1, IgG2, IgG3, or IgG4).

Immunoglobulin has a Y-shaped structural unit in which four chains composed of two homologous light chains (L chains) and two homologous heavy chains (H chains) are connected through disulfide bonds (S-S bonds). The light chain is composed of the light chain variable region (VL) and the light chain constant region (CL). The heavy chain is composed of the heavy chain variable region (VH) and the heavy chain constant region (CH).

The heavy chain constant region is composed of some domains having the amino acid sequences inherent in each class (IgG, IgM, IgA, IgD, and IgE) and each subclass (IgG1, IgG2, IgG3, and IgG4, IgA1, and IgA2).

The heavy chain of IgG (IgG1, IgG2, IgG3, and IgG4) is composed of VH, CH1 domain, hinge region, CH2 domain, and CH3 domain in this order from N terminus.

Similarly, the heavy chain of IgG1 is composed of VH, $C\gamma_1 1$ domain, hinge region, $C\gamma_1 2$ domain, and $C\gamma_1 3$ domain in this order from N terminus. The heavy chain of IgG2 is composed of VH, $C\gamma_2 1$ domain, hinge region, $C\gamma_2 2$ domain, and $C\gamma_2 3$ domain in this order from N terminus. The heavy chain of IgG3 is composed of VH, $C\gamma_3 1$ domain, hinge region, $C\gamma_3 2$ domain, and $C\gamma_3 3$ domain in this order from N terminus. The heavy chain of IgG4 is composed of VH, $C\gamma_4 1$ domain, hinge region, $C\gamma_4 2$ domain, and $C\gamma_4 3$ domain in this order from N terminus.

The heavy chain of IgA is composed of VH, Cα1 domain, hinge region, Cα2 domain, and Cα3 domain in this order from N terminus.

Similarly, the heavy chain of IgA1 is composed of VH, $C\alpha_1 1$ domain, hinge region, $C\alpha_1 2$ domain, and $C\alpha_1 3$ domain in this order from N terminus. The heavy chain of IgA2 is composed of VH, $C\alpha_2 1$ domain, hinge region, $C\alpha_2 2$ domain, and $C\alpha_2 3$ domain in this order from N terminus.

The heavy chain of IgD is composed of VH, Cδ1 domain, hinge region, Cδ2 domain, and Cδ3 domain in this order from N terminus.

The heavy chain of IgM is composed of VH, Cμ1 domain, Cμ2 domain, Cμ3 domain, and Cμ4 domain in this order from N terminus and have no hinge region as seen in IgG, IgA, and IgD.

The heavy chain of IgE is composed of VH, Cε1 domain, Cε2 domain, Cε3 domain, and Cε4 domain in this order from N terminus and have no hinge region as seen in IgG, IgA, and IgD.

If, for example, IgG is treated with papain, it is cleaved at the slightly N terminal side beyond the disulfide bonds existing in the hinge region where the disulfide bonds connect the two heavy chains to generate two homologous Fab, in which a heavy chain fragment composed of VH and CH1 is connected with one light chain through a disulfide bond, and one Fc, in which two homologous heavy chain fragments composed of the hinge region, CH2 domain, and CH3 domain are connected through disulfide bonds (See "Immunology Illustrated", original 2nd ed., Nankodo, pp.65-75 (1992); and "Focus of Newest Medical Science 'Recognition Mechanism of Immune System'", Nankodo, pp.4-7 (1991); and so on).

Namely, "a portion of a constant region of immunoglobulin heavy chain" of the present invention means a portion of a constant region of an immunoglobulin heavy chain having the structural characteristics as mentioned above, and preferably, is the constant region without C1 domain, or the Fc region. Specifically, examples thereof are the region composed of hinge region, C2 domain, and C3 domain from each of IgG, IgA, and IgD, and are the region composed of C2 domain, C3 domain, and C4 domain from each of IgM and IgE. A particularly preferable example thereof is the Fc region of human-derived IgG1.

The "fusion polypeptide" of the present invention is that composed of the extracellular region of the "polypeptide" constituting the above-described "cell surface molecule" of the present invention and "a constant region or a portion of a constant region of human immunoglobulin (Ig) heavy chain." Preferably, it is a fusion polypeptide composed of an extracellular region of a polypeptide of the present invention and a portion of a constant region of human IgG heavy chain, and particularly preferably, it is a fusion polypeptide composed of an extracellular region of a polypeptide of the present invention and the region (Fc) composed of a hinge region, CH2 domain, and CH3 domain of human IgG heavy chain. Moreover, IgG1 is preferable among IgG. In addition, a polypeptide derived from human, mouse, or rat (preferably, human) is preferable as the polypeptide of the present invention.

The fusion polypeptide of the present invention has the advantage that the fusion polypeptide can be purified extremely easily by using affinity column chromatography using the property of protein A, which binds specifically to the immunoglobulin fragment because the fusion polypeptide of the present invention has a portion of a constant region (for example Fc) of an immunoglobulin such as IgG as mentioned above as a fusion partner. Moreover, since various antibodies against the Fc of various immunoglobulin are available, an immunoassay for the fusion polypeptides can be easily performed with antibodies against the Fc.

The polypeptide, polypeptide fragment, and fusion polypeptide of the present invention can be produced not only by recombinant DNA technology as mentioned below but also by a method well

known in the art such as a chemical synthetic method and a cell culture method, or a modified method thereof.

The "gene" of the present invention comprises a DNA encoding the above-mentioned polypeptide or polypeptide fragment of the present invention, and includes any gene having a nucleotide sequence encoding the polypeptide or polypeptide fragment of the present invention.

Examples of the gene are those encoding the polypeptide or polypeptide fragment mentioned below.

(1) A polypeptide encoded by a DNA hybridising with a DNA comprising a nucleotide sequence of SEQ ID NO: 1 under stringent conditions;

(2) A polypeptide having an amino acid sequence having 60% or more homology with an amino acid sequence of SEQ ID NO: 2;

(3) A polypeptide having an amino acid sequence of SEQ ID NO: 2 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting "human JTT-1 antigen" and its derivative);

(4) A polypeptide having an amino acid sequence encoded by a nucleotide sequence corresponding to nucleotide residues 26-625 of SEQ ID NO: 3 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting "human JTT-1 antigen" and its derivative);

(5) A polypeptide having an amino acid sequence encoded by a nucleotide sequence corresponding to nucleotide residues 35-637 of SEQ ID NO: 4 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting "rat JTT-1 antigen" and its derivative);

(6) A polypeptide having an amino acid sequence encoded by a nucleotide sequence corresponding to nucleotide residues 1-603 of SEQ ID NO: 5 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting "mouse JTT-1 antigen" and its derivative);

(7) A polypeptide having an amino acid sequence encoded by a nucleotide sequence corresponding to nucleotide residues 35-685 of SEQ ID NO: 6 or an amino acid sequence substantially the same as the amino acid sequence (namely, a polypeptide constituting a "mutant of rat JTT-1 antigen" and its derivative); and

(8) A polypeptide having an amino acid sequence encoded by a DNA encoding a polypeptide constituting the cell surface molecule of the present invention, wherein the DNA is introduced into the transformant identified by an international deposit accession No. FERM BP-5725 or, having an amino acid sequence substantially the same as said amino acid sequence (namely, a polypeptide constituting a "human JTT-1 antigen" and its derivative).

Here, "substantially the same amino acid sequence" means as defined above.

Specific examples of the gene of the present invention are DNAs or their fragments mentioned below.

(1) A DNA comprising a nucleotide sequence of SEQ ID NO: 1, and a DNA hybridising with the DNA under stringent conditions;

(4) A DNA comprising a nucleotide sequence corresponding to nucleotide residues 26-625 of SEQ ID NO: 3;

(5) A DNA comprising a nucleotide sequence corresponding to nucleotide residues 35-637 of SEQ ID NO: 4;

(6) A DNA comprising a nucleotide sequence corresponding to nucleotide residues 1-603 of SEQ ID NO: 5;

(7) A DNA comprising a nucleotide sequence corresponding to nucleotide residues 35-685 of SEQ ID NO: 6;

(8) A DNA encoding a polypeptide constituting a cell surface molecule of the present invention, wherein the DNA is introduced into a transformant identified by an international deposit accession No. FERM BP-5725.

The DNA encoding a portion of a constant region of immunoglobulin heavy chain, which is a part of a fusion polypeptide of the present invention, can be cDNA, or genomic DNA comprised of introns between every exon (the DNA encoding, for example, CH1 domain, hinge region, CH2 domain, CH3 domain, CH4 domain and so on).

The DNA of the present invention includes any DNA comprised of any codons as long as the codons encode the same amino acids.

The DNA of the present invention can be a DNA obtained by any method. For example, the DNA includes complementary DNA (cDNA) prepared from mRNA, DNA prepared from genomic DNA, DNA prepared by chemical synthesis, DNA obtained by PCR amplification with RNA or DNA as a template, and DNA constructed by appropriately combining these methods.

The DNA encoding the polypeptide of the present invention can be obtained by the usual method such as a method to clone cDNA from mRNA encoding the polypeptide of the present invention, a method to isolate genomic DNA and then splice them, chemical synthesis and so on.

(1) cDNA can be cloned from the mRNA encoding the polypeptide of the present invention by, for example, the method described below.

First, the mRNA encoding a cell surface molecule (polypeptide) of the present invention is prepared from tissues or cells (for example, thymus cells or spleen-derived lymphoblast cells stimulated with ConA) expressing and producing a cell surface molecule (polypeptide) of the present invention. mRNA can be prepared isolating total RNA by a known method such as quanidine-thiocyanate method (Chirgwin, J.M. *et al.*, Biochemistry, Vol.18, p5294, 1979), hot phenol method, or AGPC method, and subjecting it to affinity chromatography using oligo-dT cellulose or poly-U Sepharose.

Then, with the mRNA obtained as a template, cDNA is synthesised, for example, by a well-known method using reverse transcriptase such as the method of Okayama *et al.* (Mol. Cell. Biol. Vol.2, p.161 (1982); ibid. Vol.3, p.280 (1983)) or the method of Hoffman *et al.* (Gene Vol.25, p.263 (1983)), and converted into double-stranded cDNA. A cDNA library is prepared by transforming *E. coli* with plasmid vectors, phage vectors, or cosmid vectors having this cDNA or by transfecting *E. coli* after *in vitro* packaging.

The plasmid vectors used in this invention are not limited as long as they are replicated and maintained in hosts. Any phage vectors that can be replicated in hosts can also be used. Examples of usually used cloning vectors are pME18S, λZAPII(IZAPII), pUC19, λgt10,λgt11, and so on. When the vector is applied to immunological screening as mentioned below, the vector having a promoter that can express a gene encoding the polypeptide of the present invention in a host is preferably used.

cDNA can be inserted into a plasmid by, for example, the method of Maniatis *et al.* (Molecular Cloning, A Laboratory Manual, second edition, Cold Spring Harbor Laboratory, p.1.53, 1989). cDNA can be inserted into a phage vector by, for example, the method of Hyunh *et al.* (DNA cloning, a practical approach, Vol.1, p.49 (1985)). These methods can be simply performed by using a commercially available cloning kit (for example, a product from Takara Shuzo). The recombinant plasmid or phage vector thus obtained is introduced into appropriate host cells such as a prokaryote (for example, *E. coli*: XL1Blue MRF', DH5α, HB101, MC1061/P3, etc.).

Examples of a method for introducing a plasmid into a host are calcium chloride method, calcium chloride/rubidium chloride method described in Molecular Cloning, A Laboratory Manual (second edition, Cold Spring Harbor Laboratory, p.1.74 (1989)), and electroporation method. Phage vectors can be introduced into host cells by, for example, a method in which the phage DNAs are introduced into grown hosts after *in vitro* packaging. *In vitro* packaging can be easily performed with a commercially available *in vitro* packaging kit (for example, a product from Stratagene or Amersham).

The cDNA encoding the polypeptide of the present invention can be isolated from the cDNA library so prepared according to the method mentioned above by combining general cDNA screening methods.

For example, a clone comprising the desired cDNA can be screened by a known colony hybridisation method (Crunstein *et al.* Proc. Natl. Acad. Sci. USA, Vol.72, p.3961 (1975)) or plaque hybridisation method (Molecular Cloning, A Laboratory Manual, second edition, Cold Spring Harbor Laboratory, p.2.108 (1989)) using ^{32}P-labelled chemically synthesised oligonucleotides as probes, which are corresponding to the amino acid sequence of the polypeptide of the present invention. Alternatively, a clone having a DNA fragment encoding a specific region within the polypeptide of the present invention can be screened by amplifying the region by PCR with synthetic PCR primers.

When a cDNA library prepared using a cDNA expression vector (for example, λZAPII phage vector) is used, the desired clone can be screened by the antigen-antibody reaction using an antibody against the polypeptide of the present invention. A screening method using PCR method is preferably used when many clones are subjected to screening.

The nucleotide sequence of the DNA thus obtained can be determined by Maxam-Gilbert method (Maxam *et al.* Proc. Natl. Acad. Sci. USA, Vol.74, p.560 (1977)) or the dideoxynucleotide synthetic chain termination method using phage M13 (Sanger *et al.* Proc. Natl. Acad. Sci. USA, Vol.74, pp.5463-5467 (1977)). The whole or a portion of the gene encoding the polypeptide of the present invention can be obtained by excising the clone obtained as mentioned above with restriction enzymes and so on.

(2) The DNA encoding the polypeptide of the present invention can be isolated from the genomic DNA derived from the cells expressing the polypeptide of the present invention as mentioned above by the following methods.

Such cells are solubilised preferably by SDS or proteinase K, and the DNAs are deproteinised by repeating phenol extraction. RNAs are digested preferably with ribonuclease. The DNAs obtained are partially digested with appropriate restriction enzymes, and the DNA fragments obtained are amplified with appropriate phage or cosmid to generate a library. Then, clones having the desired sequence are detected, for example, by using radioactively labelled DNA probes, and the whole or a portion of the gene encoding the polypeptide of the present invention is obtained from the clones by excision with restriction enzyme and so on.

cDNA encoding a human-derived polypeptide can be obtained as follows. After a cosmid library into which human genomic DNA (chromosomal DNA) is introduced is prepared ("Laboratory Manual: Human Genome Mapping", Maruzen press), positive clones comprising the DNA of the coding region of the desired protein are obtained by screening the cosmid library. Then, the cDNA library mentioned above is screened with the coding DNA excised from the positive clone as a probe to prepare the human cDNA.

(3) The DNA of the present invention can also be chemically synthesised by the usual method, based on the nucleotide sequence of SEQ ID NO: 1, 3, 4, 5, or 6.

The present invention also relates to a recombinant vector comprising the DNA encoding an above-mentioned cell surface molecule (polypeptide) of the present invention. The recombinant vector of the present invention is not limited as long as it can be replicated and maintained or can autonomously replicate in various prokaryotic and/or eukaryotic hosts. The vector of the present invention includes plasmid vectors and phage vectors.

The recombinant vector can easily be prepared by ligating the DNA encoding the polypeptide of the present invention with a vector for recombination available in the art (plasmid DNA and bacteriophage DNA) by the usual method. Specific examples of the vectors for recombination used are *E. coli*-derived plasmids such as pBR322, pBR325, pUC12, pUC13, and pUC19, yeast-derived plasmids such as pSH19 and pSH15, and *Bacillus subtilis*-derived plasmids such as pUB110, pTP5, and pC194. Examples of phages are a bacteriophage such as λ phage, and an animal or insect virus (pVL1393, Invitrogen) such as a retrovirus, vaccinia virus, and nuclear polyhedrosis virus.

An expression vector is useful for expressing the DNA encoding the polypeptide of the present invention and for producing the polypeptide of the present invention. The expression vector is not limited as long as it expresses the gene encoding the polypeptide of the present invention in various prokaryotic and/or eukaryotic host cells and produces this protein. Examples thereof are pEFneo (Proc. Natl. Acad. Sci. USA, Vol.91, pp.158-162 (1994)), pEF-BOS (Nucleic Acids Res. Vol.18, p.5322 (1990)), pME18S (Experimental Medicine: SUPPLEMENT, "Handbook of Genetic Engineering" (1992)), pMAL C2, and so on.

When bacteria, particularly *E. coli* are used as host cells, an expression vector is generally comprised of, at least, a promoter/operator region, an initiation codon, the DNA encoding the polypeptide of the present invention, termination codon, terminator region, and replicon.

When yeast, animal cells, or insect cells are used as hosts, an expression vector is preferably comprised of, at least, a promoter, an initiation codon, the DNA encoding the polypeptide of the present invention, and a termination codon. It may also comprise the DNA encoding a signal peptide, enhancer sequence, 5'- and 3'-untranslated region of the gene encoding the polypeptide of the present invention, splicing junctions, polyadenylation site, selectable marker region, and replicon. The expression vector may also contain, if required, a gene for gene amplification (marker) that is usually used.

A promoter/operator region to express the polypeptide of the present invention in bacteria comprises a promoter, an operator, and a Shine-Dalgarno (SD) sequence (for example, AAGG). For example, when the host is *Escherichia*, it preferably comprises Trp promoter, lac promoter, recA promoter, λPL promoter, lpp promoter, tac promoter, or the like. Examples of a promoter to express the polypeptide of the present invention in yeast are PH05 promoter, PGK promoter, GAP promoter, ADH promoter, and so on. When the host is *Bacillus*, examples thereof are SL01 promoter, SP02 promoter, penP promoter and so on. When the host is a eukaryotic cell such as a mammalian cell, examples thereof are SV40-derived promoter, retrovirus promoter, heat shock promoter, EF promoter, and so on, and preferably SV-40, SRα, and retrovirus-derived one. As a matter of course, the promoter is not limited to the above examples. In addition, to use an enhancer is effective for expression.

A preferable initiation codon is, for example, a methionine codon (ATG).

The commonly used termination codon (for example, TAG, TGA, TAA, and so on) is illustrated as a termination codon.

Usually used natural or synthetic terminators are used as a terminator region.

A replicon means a DNA capable of replicating the whole DNA sequence in host cells, and includes a natural plasmid, an artificially modified plasmid (DNA fragment prepared from a natural plasmid), a synthetic plasmid, and so on. Examples of a preferable plasmids are pBR322 or its artificial derivatives (DNA fragment obtained by treating pBR322 with appropriate restriction enzymes) for *E. coli*, yeast 2 μplasmid or yeast chromosomal DNA for yeast, and pEFneo, pME18S, pRSVneo ATCC 37198, pSV2dhfr ATCC 37145, pdBPV-MMTneo ATCC 37224, pSV2neo ATCC 37149, etc. for mammalian cells.

An enhancer sequence, polyadenylation site, and splicing junction that are usually used in the art, such as those derived from SV40 can be also used.

A selectable marker usually used can be used according to the usual method. Examples thereof are resistance genes for antibiotics, such as tetracycline, neomycin, ampicillin, or kanamycin, and thymidine kinase gene.

Examples of a gene for gene amplification are dihydrofolate reductase (DHFR) gene, thymidine kinase gene, neomycin resistance gene, glutamate synthase gene, adenosine deaminase gene, ornithine decarboxylase gene, hygromycin-B-phophotransferase gene, aspartate transcarbamylase gene, etc.

The expression vector of the present invention can be prepared by continuously and circularly linking at least the above-mentioned promoter, initiation codon, DNA (gene) encoding the polypeptide

of the present invention, termination codon, and terminator region, to an appropriate replicon. If desired, appropriate DNA fragments (for example, linkers, restriction sites generated with other restriction enzyme), can be used by the usual method such as digestion with a restriction enzyme or ligation using T4 DNA ligase.

Transformants of the present invention can be prepared by introducing the expression vector mentioned above into host cells.

Host cells used in the present invention are not limited as long as they are compatible with an expression vector mentioned above and can be transformed. Examples thereof are various cells such as natural cells or artificially established recombinant cells usually used in technical field of the present invention (for example, bacteria (*Escherichia* and *Bacillus*), yeast (*Saccharomyces*, *Pichia*, etc.), animal cells, or insect cells.

E. coli or animal cells are preferably used. Specific examples are *E. coli* (DH5α, XL1Blue MRF', TB1, HB101, etc.), mouse-derived cells (COP, L, C127, Sp2/0, NS-1, NIH 3T3, etc.), rat-derived cells, hamster-derived cells (BHK, CHO-K1, CHO, etc.), monkey-derived cells (COS1, COS3, COS7, CV1, Velo, etc.), and human-derived cells (HEK293, Hela, diploid fibroblast-derived cells, myeloma, Namalwa, etc.).

An expression vector can be introduced (transformed (transduced)) into host cells by known method.

Transformation can be performed, for example, according to the method of Cohen *et al.* (Proc. Natl. Acad. Sci. USA, Vol.69, p.2110 (1972)), protoplast method (Mol. Gen. Genet., Vol.168, p.111 (1979)), or competent method (J. Mol. Biol., Vol.56, p.209 (1971)) when the hosts are bacteria (*E. coli*, *Bacillus subtilis*, etc.), the method of Hinnen *et al.* (Proc. Natl. Acad. Sci. USA, Vol.75, p.1927 (1978)), or lithium method (J. Bacteriol., Vol.153, p.163 (1983)) when the host is *Saccharomyces cerevisiae*, the method of Graham (Virology, Vol.52, p.456 (1973)) when the hosts are animal cells, and the method of Summers *et al.* (Mol. Cell. Biol., Vol.3, pp.2156-2165 (1983)) when the hosts are insect cells.

The polypeptide of the present invention can be produced by cultivating transformants (in the following this term includes transductants) comprising an expression vector prepared as mentioned above in nutrient media.

The nutrient media preferably comprise carbon source, inorganic nitrogen source, or organic nitrogen source necessary for the growth of host cells (transformants). Examples of the carbon source are glucose, dextran, soluble starch, and sucrose, and examples of the inorganic or organic nitrogen source are ammonium salts, nitrates, amino acids, corn steep liquor, peptone, casein, meet extract, soy bean cake, and potato extract. If desired, they may comprise other nutrients (for example, an inorganic salt (for example, calcium chloride, sodium dihydrogenphosphate, and magnesium chloride), vitamins, antibiotics (for example, tetracycline, neomycin, ampicillin, kanamycin, etc.).

Cultivation is performed by a method known in the art. Cultivation conditions such as temperature, pH of the media, and cultivation time are selected appropriately so that the polypeptide of the present invention is overproduced.

Specific media and cultivation conditions used depending on host cells are illustrated below, but are not limited thereto.

When the hosts are bacteria, actinomycetes, yeasts, filamentous fungi, liquid media comprising the nutrient source mentioned above are appropriate. The media with pH 5 to 8 are preferably used.

When the host is *E. coli*, examples of preferable media are LB media, and M9 media (Miller *et al.* Exp. Mol. Genet., Cold Spring Harbor Laboratory, p.431 (1972)). Using these media, cultivation can be performed usually at 14 to 43 °C for about 3 to 24 hours with aeration and stirring, if necessary.

When the host is *Bacillus*, cultivation can be performed usually at 30 to 40 °C for about 16 to 96 hours with aeration and stirring, if necessary.

When the host is yeast, examples of media are Burkholder minimal media (Bostian, Proc. Natl. Acad. Sci. USA, Vol.77, p.4505 (1980)). The pH of the media is preferably 5 to 8. Cultivation can be performed usually at 20 to 35°C for about 14 to 144 hours with aeration and stirring, if necessary.

When the host is an animal cell, examples of media are MEM media containing about 5 to 20% foetal bovine serum (Science, Vol.122, p.501 (1952)), DMEM media (Virology, Vol.8, p.396 (1959)), RPMI1640 media (J. Am. Med. Assoc., Vol.199, p.519 (1967)), and 199 media (Proc. Soc. Exp. Biol. Med., Vol.73, p.1 (1950)). The pH of the media is preferably about 6 to 8. Cultivation can be performed usually at about 30 to 40°C for about 15 to 72 hours with aeration and stirring, if necessary.

When the host is an insect cell, an example of media is Grace's media containing foetal bovine serum (Proc. Natl. Acad. Sci. USA, Vol.82, p.8404 (1985)). The pH thereof is preferably about 5 to 8. Cultivation can be performed usually at about 20 to 40°C for 15 to 100 hours with aeration and stirring, if necessary.

Cultivation of transformants as mentioned above, in particular animal cells can overexpress the polypeptide of the present invention on the surface of the cells.

The polypeptide of the present invention can be produced as a soluble polypeptide fragment such as an extracellular region fragment by preparing the transformants as mentioned above using the DNA encoding the extracellular region or each domain and by cultivating the transformants to allow them to secrete the soluble polypeptide into the culture supernatant. In addition, a fusion polypeptide of the present invention can be prepared similarly.

Namely, a culture filtrate (supernatant) is obtained by the method such as filtration or centrifugation of the obtained culture, and the polypeptide or polypeptide fragment of the present invention is purified and isolated from the culture filtrate by the usual method commonly used in order to purify and isolate a natural or synthetic protein.

Examples of the isolation and purification method are a method utilising solubility, such as salting out and solvent precipitation method, a method utilising the difference in molecular weight, such as dialysis, ultrafiltration, gel filtration, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, a method utilising charges, such as ion exchange chromatography and hydroxylapatite chromatography, a method utilising specific affinity, such as affinity chromatography, a method utilising the difference in hydrophobicity, such as reverse phase high performance liquid chromatography, and a method utilising the difference in isoelectric point, such as isoelectric focusing.

When the polypeptide or a polypeptide fragment of the present invention exists in the periplasm or cytoplasm of cultured transformants, first, the fungus bodies or cells are harvested by the usual method such as filtration or centrifugation and suspended in appropriate buffer. After the cell wall and/or cell membrane of the cells and so on are disrupted by the method such as lysis with sonication, lysozyme, and freeze-thawing, the membrane fraction comprising the polypeptide of the present invention is obtained by the method such as centrifugation or filtration. The membrane fraction is solubilised with a detergent such as Triton-X100 to obtain the crude extract. Finally, the polypeptide or the polypeptide fragment is isolated and purified from the crude extract by the usual method as illustrated above.

The "transgenic mouse" of the present invention is a transgenic mouse wherein the DNA (cDNA or genomic DNA) prepared as mentioned above encoding the polypeptide of the present invention derived from animals except mice (non-self polypeptide) have been integrated into its endogenous locus of the mouse. The transgenic mouse expresses the non-self polypeptide and secretes the polypeptide into its body.

The transgenic mouse can be prepared according to the method as usually used for producing a transgenic animal (for example, see "Newest Manual of Animal Cell Experiment", LIC press, Chapter 7, pp.361-408, (1990)).

Specifically, for example, embryonic stem cells (ES cells) obtained from normal mouse blastocysts are transformed with an expression vector in which the gene encoding human-derived polypeptide of the present invention (ie. "human JTT-1 antigen") has been operably inserted. ES cells in which the gene encoding the human-derived polypeptide of the present invention has been integrated into the endogenous gene are screened by the usual method. Then, the ES cells screened are microinjected into a fertilised egg obtained from another normal mouse (blastocyst) (Proc. Natl. Acad. Sci. USA, Vol.77, No.12, pp.7380-7384 (1980); U.S. Pat. No. 4,873,191). The blastocyst is transplanted into the uterus of another normal mouse as the foster mother. Then, founder mice (progeny mice) are born from the foster mother mouse. By mating the founder mice with normal mice, heterogeneic transgenic mice are obtained. By mating the heterogeneic transgenic mice with each other, homogeneic transgenic mice are obtained according to Mendel's laws.

"Knockout mouse" of the present invention is a mouse wherein the endogenous gene encoding the mouse-derived polypeptide of the present invention (ie. "mouse JTT-1 antigen") has been knocked out (inactivated). It can be prepared, for example, by positive-negative selection method in which homologous recombination is applied (U.S. Pat. No. 5,464,764; No. 5,487,992; No. 5,627,059; Proc. Natl. Acad. Sci. USA, Vol.86, pp.8932-8935 (1989); Nature, Vol.342, pp.435-438 (1989); etc.).

The "antibody" of the present invention can be a polyclonal antibody (antiserum) or a monoclonal antibody, and preferably a monoclonal antibody.

Specifically, it is an antibody reactive to (against, which binds to) the above-mentioned polypeptide or polypeptide fragment of the present invention.

The antibody of the present invention can be natural antibodies obtained by immunising mammals such as mice, rats, hamsters, guinea pigs, and rabbits with the antigen, such as cells (natural cells, cell lines, tumour cells, etc.) expressing "cell surface molecules" of the present

invention, transformants overexpressing the polypeptide or cell surface molecules of the present invention on the surface thereof prepared using recombinant DNA technology on the cell surface, or "polypeptide fragments" or "fusion polypeptides" of the present invention. The antibody of the present invention also includes chimeric antibodies and humanised antibodies (CDR-grafted antibodies) that can be produced by recombinant DNA technology, and human antibodies that can be produced using human antibody-producing transgenic animals.

The monoclonal antibody includes those having any one isotype of IgG, IgM, IgA, IgD, or IgE. IgG or IgM is preferable.

The polyclonal antibody (antisera) or monoclonal antibody of the present invention can be produced by the known methods. Namely, a mammal, preferably, a mouse, rat, hamster, guinea pig, rabbit, cat, dog, pig, goat, horse, or cattle, or more preferably, a mouse, rat, hamster, guinea pig, or rabbit is immunised, for example, with an antigen mentioned above with Freund's adjuvant, if necessary.

The polyclonal antibody can be obtained from the antiserum obtained from the animal so immunised. In addition, the monoclonal antibodies are produced as follows. Hybridomas are prepared from the antibody-producing cells obtained from the animal so immunised and myeloma cells that are not capable of producing autoantibodies. The hybridomas are cloned, and clones producing the monoclonal antibodies showing the specific affinity to the antigen used for immunising the mammal are screened.

Specifically, the monoclonal antibody can be produced as follows. Immunisations are performed by injecting or implanting once or several times the antigen as mentioned above as an immunogen, if necessary, with Freund's adjuvant, subcutaneously, intramuscularly, intravenously, through the footpad, or intraperitoneally into a non-human mammal, specifically a mouse, rat, hamster, guinea pig, or rabbit, preferably a mouse, rat, or hamster (including a transgenic animal generated so as to produce antibodies derived from another animal such as the transgenic mouse producing human antibody mentioned below). Usually, immunisations are performed once to four times every one to fourteen days after the first immunisation. Antibody-producing cells are obtained from the mammal so immunised in about one to five days after the last immunisation. The frequency and interval of immunisations can be appropriately arranged depending on property of the immunogen used. Hybridomas that secrete a monoclonal antibody can be prepared by the method of Köhler and Milstein (Nature, Vol.256, pp.495-497 (1975)) and by its modified method. Namely, hybridomas are prepared by fusing antibody-producing cells contained in a spleen, lymph node, bone marrow, or tonsil obtained from the non-human mammal immunised as mentioned above, preferably a spleen, with myelomas without autoantibody-producing ability, which are derived from, preferably, a mammal such as a mouse, rat, guinea pig, hamster, rabbit, or human, or more preferably, a mouse, rat, or human.

For example, mouse-derived myeloma P3/X63-AG8.653 (653), P3/NSI/1-Ag4-1 (NS-1), P3/X63-Ag8.U1 (P3U1), SP2/0-Ag14 (Sp2/0, Sp2), PAI, F0, or BW5147, rat-derived myeloma 210RCY3-Ag.2.3., or human-derived myeloma U-266AR1, GM1500-6TG-A1-2, UC729-6, CEM-AGR, D1R11, or CEM-T15 can be used as a myeloma used for the cell fusion.

Hybridoma clones producing monoclonal antibodies can be screened by cultivating hybridomas, for example, in microtiter plates and by measuring the reactivity of the culture supernatant in the well in which hybridoma growth is observed, to the immunogen used for the immunisation mentioned above, for example, by enzyme immunoassay such as RIA and ELISA.

The monoclonal antibodies can be produced from hybridomas by cultivating the hybridomas *in vitro* or *in vivo* such as in the ascites fluid of a mouse, rat, guinea pig, hamster, or rabbit, preferably a mouse or rat, more preferably mouse and isolating the antibodies from the resulting the culture supernatant or ascites fluid of a mammal.

Cultivating hybridomas *in vitro* can be performed depending on the property of cells to be cultured, on the object of a test study, and on the various conditions of a cultivating method, by using known nutrient media or any nutrient media derived from known basal media for growing, maintaining, and storing the hybridomas to produce monoclonal antibodies in culture supernatant.

Examples of basal media are low calcium concentration media such as Ham'F12 medium, MCDB153 medium, or low calcium concentration MEM medium, and high calcium concentration media such as MCDB104 medium, MEM medium, D-MEM medium, RPMI1640 medium, ASF104 medium, or RD medium. The basal media can contain, for example, sera, hormones, cytokines, and/or various inorganic or organic substances depending on the objective.

Monoclonal antibodies can be isolated and purified from the culture supernatant or ascites fluid mentioned above by saturated ammonium sulfate precipitation, euglobulin precipitation method, caproic acid method, caprylic acid method, ion exchange chromatography (DEAE or DE52), affinity chromatography using anti-immunoglobulin column or protein A column.

Preferable examples of monoclonal antibodies of the present invention are as follows.

(1) A monoclonal antibody reactive to a polypeptide having an amino acid sequence of SEQ ID NO: 2, a polypeptide fragment derived from the polypeptide, or a human-derived cell surface molecule composed of the polypeptide;

(2) A monoclonal antibody reactive to a polypeptide of the present invention, a polypeptide fragment derived from the polypeptide, or a cell surface molecule composed of the polypeptide, wherein the effect of the monoclonal antibody on mitogen-stimulated lymphoblast cells is substantially the same as the effect of a monoclonal antibody produced by a hybridoma identified by an international deposit accession No. FERM BP-5707 on mitogen-stimulated rat lymphoblast cells; and

(3) A monoclonal antibody reactive to a polypeptide of the present invention, a polypeptide fragment derived from the polypeptide, or a cell surface molecule composed of the polypeptide, wherein the effect of the monoclonal antibody on mitogen-stimulated lymphoblast cells is substantially the same as the effect of a monoclonal antibody produced by a hybridoma identified by an international deposit accession No. FERM BP-5708 on mitogen-stimulated rat lymphoblast cells.

In addition, the monoclonal antibody of the present invention includes that produced by the hybridoma identified by an international deposit accession No. FERM BP-5707 or No. FERM BP-5708.

The "chimeric monoclonal antibody" of the present invention is a monoclonal antibody prepared by genetic engineering, and specifically means a chimeric antibody such as mouse/human chimeric

monoclonal antibody whose variable regions are derived from immunoglobulin of an non-human mammal (mouse, rat, hamster, etc.) and whose constant regions are derived from human immunoglobulin.

The constant region derived from human immunoglobulin has the amino acid sequence inherent in each isotype such as IgG (IgG1, IgG2, IgG3, IgG4), IgM, IgA, IgD, and IgE. The constant region of the recombinant chimeric monoclonal antibody of the present invention can be that of human immunoglobulin belonging to any isotype. Preferably, it is the constant region of human IgG.

The chimeric monoclonal antibody of the present invention can be produced, for example, as follows. Needless to say, the production method is not limited thereto.

A mouse/human chimeric monoclonal antibody can be prepared, referring to Experimental Medicine: SUPPLEMENT, Vol.1.6, No.10 (1988); and examined published Japanese patent application (JP-B) No. Hei 3-73280. Namely, it can be prepared by operably inserting CH gene (C gene encoding the constant region of H chain) obtained from the DNA encoding human immunoglobulin downstream of active VH genes (rearranged VDJ gene encoding the variable region of H chain) obtained from the DNA encoding a mouse monoclonal antibody isolated from the hybridoma producing the mouse monoclonal antibody, and CL gene (C gene encoding the constant region of L chain) obtained from the DNA encoding human immunoglobulin downstream of active VL genes (rearranged VJ gene encoding the variable region of L chain) obtained from the DNA encoding the mouse monoclonal antibody isolated from the hybridoma, into the same or different vectors so as for them to be expressed, following by transforming host cells with the expression vector, and then by cultivating the transformants.

Specifically, DNAs are first extracted from mouse monoclonal antibody-producing hybridomas by the usual method, digested with appropriate restriction enzymes (for example, EcoRI and HindIII), electrophoresed (using, for example, 0.7% agarose gel), and analysed by Southern blotting. After an electrophoresed gel is stained, for example, with ethidium bromide and photographed, the gel is given with marker positions, washed twice with water, and soaked in 0.25 M HCl for 15 minutes. Then, the gel is soaked in 0.4 N NaOH solution for 10 minutes with gently stirring. The DNAs are transferred to a filter for 4 hours by the usual method. The filter is recovered and washed twice with 2xSSC. After the filter is sufficiently dried, it is baked at 75°C for 3 hours. After baking, the filter is treated with 0.1 x SSC/0.1% SDS at 65°C for 30 minutes. Then, it is soaked in 3 x SSC/0.1% SDS. The filter obtained is treated with prehybridisation solution in a plastic bag at 65°C for 3 to 4 hours.

Next, ^{32}P-labelled probe DNA and hybridisation solution are added to the bag and reacted at 65°C about 12 hours. After hybridisation, the filter is washed under appropriate salt concentration, reaction temperature, and time (for example, 2 x SSC-0.1% SDS, room temperature, 10 minutes). The filter is put into a plastic bag with a little 2 x SSC, and subjected to autoradiography after the bag is sealed.

Rearranged VDJ gene and VJ gene encoding H chain and L chain of a mouse monoclonal antibody are identified by Southern blotting mentioned above. The region comprising the identified DNA fragment is fractioned by sucrose density gradient centrifugation and inserted into a phage vector (for example, Charon 4A, Charon 28, λEMBL3, λEMBL4, etc.). *E. coli* (for example, LE392,

NM539, etc.) is transformed with the phage vector to generate a genomic library. The genomic library is screened by plaque hybridisation such as Benton-Davis method (Science, Vol.196, pp.180-182 (1977)) using appropriate probes (H chain J gene, L chain (κ) J gene, etc.) to obtain positive clones comprising rearranged VDJ gene or VJ gene. By making the restriction map and determining the nucleotide sequence of the clones obtained, it is confirmed that genes comprising the desired, rearranged VH (VDJ) gene or VL (VJ) gene are obtained.

Separately, human CH gene and human CL gene used for chimerisation are isolated. For example, when a chimeric antibody with human IgG1 is produced, Cγ1 gene as a CH gene, and Cκ gene as a CL gene, are isolated. These genes can be isolated from human genomic library with mouse Cγ1 gene and mouse Cκ gene, corresponding to human Cγ1 gene and human Cκ gene, respectively, as probes, taking advantage of high homology between the nucleotide sequences of mouse immunoglobulin gene and that of human immunoglobulin gene.

Specifically, DNA fragments comprising human Cκ gene and an enhancer region are isolated from human λ Charon 4A HaeIII-AluI genomic library (Cell, Vol.15, pp.1157-1174 (1978)), for example, with a 3 kb HindIII-BamHI fragment of clone Ig146 (Proc. Natl. Acad. Sci. USA, Vol.75, pp.4709-4713 (1978)) and a 6.8 kb EcoRI fragment of clone MEP10 (Proc. Natl. Acad. Sci. USA, Vol.78, pp.474-478 (1981)) as probes. In addition, for example, after human foetal hepatocyte DNA is digested with HindIII and fractioned by agarose gel electrophoresis, a 5.9 kb fragment is inserted into λ788 and then human Cγ1 gene is isolated with the probes mentioned above.

Using mouse VH gene, mouse VL gene, human CH gene, and human CL gene so obtained, and taking promoter region and enhancer region into consideration, human CH gene is inserted downstream mouse VH gene and human CL gene is inserted downstream mouse VL gene into an expression vector such as pSV2gpt or pSV2neo with appropriate restriction enzymes and DNA ligase by the usual method. In this case, chimeric genes of mouse VH gene/human CH gene and mouse VL gene/human CL gene can be respectively inserted in the same expression vector or in different expression vectors.

Chimeric gene-inserted expression vector(s) thus prepared are introduced into myelomas that do not produce antibodies, for example, P3X63.Ag8.653 cells or SP210 cells by protoplast fusion method, DEAE-dextran method, calcium phosphate method, or electroporation method. The transformants are screened by cultivating in media containing a drug corresponding to the drug resistance gene inserted into the expression vector and, then, cells producing desired chimeric monoclonal antibodies are obtained.

Desired chimeric monoclonal antibodies are obtained from the culture supernatant of antibody-producing cells thus screened.

The "humanised monoclonal antibody (CDR-grafted antibody)" of the present invention is a monoclonal antibody prepared by genetic engineering and specifically means a humanised monoclonal antibody wherein a portion or the whole of the complementarity determining regions of the hypervariable region are derived from the complementarity determining regions of the hypervariable region from a monoclonal antibody of an non-human mammal (mouse, rat, hamster, etc.), the framework regions of the variable region are derived from the framework regions of the variable region

from human immunoglobulin, and the constant region is derived from human a constant region from immunoglobulin.

The complementarity determining regions of the hypervariable region exists in the hypervariable region in the variable region of an antibody and means three regions which directly and complementary binds to an antigen (complementarity-determining residues, CDR1, CDR2, and CDR3). The framework regions of the variable region means four comparatively conserved regions lying upstream, downstream or between the three complementarity determining regions (framework region, FR1, FR2, FR3, and FR4).

In other words, a humanised monoclonal antibody means that in which the whole region except a portion or the whole of the complementarity determining regions of the hypervariable region of a nonhuman mammal-derived monoclonal antibody have been replaced with their corresponding regions derived from human immunoglobulin.

The constant region derived from human immunoglobulin has the amino acid sequence inherent in each isotype such as IgG (IgG1, IgG2, IgG3, IgG4), IgM, IgA, IgD, and IgE. The constant region of a humanised monoclonal antibody in the present invention can be that from human immunoglobulin belonging to any isotype. Preferably, it is the constant region of human IgG. The framework regions of the constant region derived from human immunoglobulin are not particularly limited.

The humanised monoclonal antibody of the present invention can be produced, for example, as follows. Needless to say, the production method is not limited thereto.

For example, a recombinant humanised monoclonal antibody derived from mouse monoclonal antibody can be prepared by genetic engineering, referring to unexamined Japanese patent publication (JP-WA) No. Hei 4-506458 and unexamined Japanese patent publication (JP-A) No. Sho 62-296890. Namely, at least one mouse H chain CDR gene and at least one mouse L chain CDR gene corresponding to the mouse H chain CDR gene are isolated from hybridomas producing mouse monoclonal antibody, and human H chain gene encoding the whole regions except human H chain CDR corresponding to mouse H chain CDR mentioned above and human L chain gene encoding the whole region except human L chain CDR correspond to mouse L chain CDR mentioned above are isolated from human immunoglobulin genes.

The mouse H chain CDR gene(s) and the human H chain gene(s) so isolated are operably inserted into an appropriate vector so that they can be expressed. Similarly, the mouse L chain CDR gene(s) and the human L chain gene(s) are operably inserted into another appropriate vector so that they can be expressed. Alternatively, the mouse H chain CDR gene(s)/human H chain gene(s) and mouse L chain CDR gene(s)/human L chain gene(s) can be operably inserted into the same expression vector so that they can be expressed. Host cells are transformed with the expression vector thus prepared to obtain transformants producing humanised monoclonal antibody. By cultivating the transformants, desired humanised monoclonal antibody is obtained from culture supernatant.

The "human monoclonal antibody" of the present invention is immunoglobulin in which the entire regions comprising the variable and constant region of H chain, and the variable and constant

region of L chain constituting immunoglobulin are derived from the gene encoding human immunoglobulin.

The human antibody can be produced in the same way as the production method of polyclonal or monoclonal antibodies mentioned above by immunising, with an antigen, a transgenic animal which for example, at least human immunoglobulin gene(s) have been integrated into the locus of a non-human mammal such as a mouse by the usual method.

For example, a transgenic mouse producing human antibodies is prepared by the methods described in Nature Genetics, Vol.7, pp.13-21 (1994); Nature Genetics, Vol.15, pp.146-156 (1997); JP-WA Nos. Hei 4-504365 and Hei 7-509137; Nikkei Science, No.6, pp.40-50 (1995); International patent publication No. WO94/25585; Nature, Vol.368, pp.856-859 (1994); and JP-WA No. Hei 6-500233.

In addition, recently developed technique for producing a human-derived protein from the milk of a transgenic cow or pig can also be applied (Nikkei Science, pp.78-84 (April, 1997)).

The "portion of an antibody" used in the present invention means a partial region of the monoclonal antibody as mentioned above, and specifically, means $F(ab')_2$, Fab', Fab, Fv (variable fragment of antibody), sFv, dsFv (disulfide stabilised Fv), or dAb (single domain antibody) (Exp. Opin. Ther. Patents, Vol.6, No.5, pp.441-456 (1996)).

"$F(ab')_2$" and "Fab'" can be produced by treating immunoglobulin (monoclonal antibody) with a protease such as pepsin and papain, and means an antibody fragment generated by digesting immunoglobulin near the disulfide bonds existing between the hinge regions in each of the two H chains. For example, papain cleaves IgG upstream of the disulfide bonds existing between the hinge regions in each of the two H chains to generate two homologous antibody fragments in which an L chain composed of VL (L chain variable region) and CL (L chain constant region), and an H chain fragment composed of VH (H chain variable region) and CHγ1 (γ1 region in the constant region of H chain) are connected at their C terminal regions through a disulfide bond. Each of such two homologous antibody fragments is called Fab'. Pepsin also cleaves IgG downstream of the disulfide bonds existing between the hinge regions in each of the two H chains to generate an antibody fragment slightly larger than the fragment in which the two above-mentioned Fab' are connected at the hinge region. This antibody fragment is called $F(ab')_2$.

The "pharmaceutical composition" of the present invention comprises any one of the "polypeptides" of the present invention as defined above; "homodimer molecule", "polypeptide fragment", "fusion polypeptide" comprising the polypeptide; "homodimer molecule" comprising the fusion polypeptides, "antibody", or "portion of an antibody"; and a pharmaceutically acceptable carrier.

The "pharmaceutically acceptable carrier" includes a excipient, a diluent, an expander, a decomposition agent, a stabiliser, a preservative, a buffer, an emulsifier, an aromatic, a colorant, a sweetener, a viscosity increasing agent, a flavour, a solubility increasing agent, or other additives. Using one or more of such carriers, a pharmaceutical composition can be formulated into tablets, pills, powders, granules, injections, solutions, capsules, troches, elixirs, suspensions, emulsions, or syrups. The pharmaceutical composition can be administered orally or parenterally. Other forms for parenteral administration include a solution for external application, suppository for rectal

administration, and pessary, prescribed by the usual method, which comprises one or more active ingredient.

The dosage can vary depending on the age, sex, weight, and symptom of a patient, effect of treatment, administration route, period of treatment, or the kind of active ingredient (polypeptide or antibody mentioned above) contained in the pharmaceutical composition. Usually, the pharmaceutical composition can be administered to an adult in a dose of 10μg to 1000mg (or 10μg to 500mg) per one administration. Depending on various conditions, the dosage less than that mentioned above may be sufficient in some cases, and the dosage more than that mentioned above may be necessary in other cases.

In particular, the injection can be produced by dissolving or suspending the antibody in a non-toxic, pharmaceutically acceptable carrier such as physiological saline or commercially available distilled water for injection with adjusting a concentration to 0.1μg antibody/mL carrier to 10mg antibody/mL carrier. The injection thus produced can be administered to a human patient in need of treatment in a dose of 1μg to 100mg/kg body weight, preferably 50g to 50mg/kg body weight once or more times a day. Examples of administration route are medically appropriate administration routes such as intravenous injection, subcutaneous injection, intradermal injection, intramuscular injection, or intraperitoneal injection, preferably intravenous injection.

The injection can also be prepared into a non-aqueous diluent (for example, propylene glycol, polyethylene glycol, vegetable oil such as olive oil, and alcohol such as ethanol), suspension, or emulsion.

The injection can be sterilised by filtration with a bacteria-non-penetrated filter, by mixing bactericide, or by irradiation. The injection can be produced in the form that is prepared upon use. Namely, it is freeze-dried to be a sterile solid composition, and can be dissolved in sterile distilled water for injection or another solvent before use.

The pharmaceutical composition of the present invention can be applied to treating or preventing various autoimmune diseases, allergic diseases, or inflammatory diseases caused by the activation of lymphocytes such as T cells and the regulation of activated lymphocyte functions. Examples of the diseases are rheumatoid arthritis, multiple sclerosis, autoimmune thyroiditis, allergic contact dermatitis, chronic inflammatory dermatosis such as lichen planus, systemic lupus erythematosus, insulin dependent diabetes mellitus, and psoriasis.

The therapeutic effect of the pharmaceutical composition of the present invention for symptom of various diseases can be tested by the usual method by administering it to an known disease model animal.

Examples of the model include (1) a (NZB/NZW)F1 mouse, a model for human systemic lupus erythematosus (SLE) (Science, Vol.125, p.1225-1227 (1994)), (2) experimental allergic encephalomyelitis (EAE), a model for multiple sclerosis (MS) (J. Clin. Invest., Vol.95, pp.2783-2789 (1995)), (3) an NOD (non-obese diabetes) mouse, a model for insulin dependent diabetes mellitus (IDDM) (J. Exp. Med., Vol.181, pp.1145-1155 (1995)), (4) rat nephritis model by renal glomerulus basement membrane immunity, Goodpasture's nephritis model (Eur. J. Immunol., Vol.24, No.6,

pp.1249-1254 (1994)), and (5) a DBA/1 mouse, a model for human rheumatoid arthritis (Eur. J. Immunol., Vol.26, pp.2320-2328 (1996)).

Brief Description of the Drawings

Figure 1 are micrographs showing the state of aggregation of FTL435 cells induced by "JTT-1 antibody" and the state of inhibition of the cell aggregation by "JTT.2 antibody."

Subfigure (a) shows the state of the cells in the absence of any hybridoma supernatant, subfigure (b) shows the state of cell aggregation induced by "JTT-1 antibody," subfigure (c) shows the state of the cell aggregation in the presence of "anti-ICAM-1 antibody" together with "JTT-1 antibody," and subfigure (d) shows the state of the cell aggregation in the presence of "JTT-2 antibody" together with "JTT-1 antibody."

Figure 2 are micrographs showing the state of aggregation of FTL435 cells and rat activated lymphoblasts induced by "JTT-1 antibody" and the state of inhibition of the cell aggregation by "JTT.2 antibody."

Subfigure (a) shows the state of FTL435 cells in the absence of any antibody, subfigure (b) shows the state of FTL435 cells in the presence of PMA, subfigure (c) shows the state of FTL435 cells in the presence of "JTT-1 antibody," subfigure (d) shows the state of FTL435 cells in the presence of anti-LFA-1 antibody together with "JTT-1 antibody," subfigure (e) shows the state of FTL435 cells in the presence of anti-CD18 antibody together with "JTT-1 antibody," subfigure (f) shows the state of FTL435 cells in the presence of anti-ICAM-1 antibody together with "JTT-1 antibody," subfigure (g) shows the state of activated lymphoblasts in the absence of any antibody, subfigure (h) shows the state of activated lymphoblasts in the presence of PMA, subfigure (i) shows the state of activated lymphoblasts in the presence of "JTT-1 antibody," subfigure (j) shows the state of activated lymphoblasts in the presence of anti-LFA-1 antibody together with "JTT-1 antibody," subfigure (k) shows the state of activated lymphoblasts in the presence of anti-CD18 antibody together with "JTT-1 antibody," and subfigure (l) shows the state of activated lymphoblasts in the presence of anti-ICAM-1 antibody together with "JTT-1 antibody."

Figure 3 shows the expression state of "JTT-1 antigen" and "JTT-2 antigen" in various cells measured with a flow-cytometer.

Figure 4 shows the expression state of "JTT-1 antigen" in various lymphocytic cells measured with a flow-cytometer.

Figure 5 is a photograph showing electrophoretogram of "JTT-1 antigen" analysed by SDS-PAGE.

Figure 6 are micrographs showing the state of adhesion of rat thymocytes to the microtiter plate coated with purified "JTT-1 antigen," where the adhesion is induced in the presence of "JTT-1 antibody," and the state of inhibition of the cell adhesion by "JTT-2 antibody."

Subfigure (a) shows the state of adhesion of the cells to the plate which has not been coated with "JTT-1 antigen," subfigure (b) shows the state of adhesion of the cells to the plate coated with "JTT-1 antigen" in the absence of any antibody, subfigure (c) shows the state of adhesion of the cells to the plate coated with "JTT-1 antigen" in the presence of the Fab fragments of "JTT-1 antibody," and

subfigure (d) shows the state of adhesion of the cells to the plate coated with "JTT-1 antigen" in the presence of "JTT-2 antibody" together with the Fab fragments of "JTT-1 antibody."

Figure 7 shows the relative cell number of thymocytes adhering to the plate coated with purified "JTT-1 antigen" measured in terms of fluorescence intensity.

"Ag(-)" shows the relative cell number in the plate which has not been coated with "JTT-1 antigen," "Ag(+)" shows the relative cell number in the plate coated with "JTT-1 antigen" in the absence of any antibody, "Ag(+) + JTT-1 Fab" shows the relative cell number in the plate coated with "JTT-1 antigen" in the presence of the Fab fragments of "JTT-1 antibody", and "Ag(+) + JTT-1 Fab + JTT-2" shows the relative cell number in the plate coated with "JTT-1 antigen" in the presence of "JTT-2 antibody" together with the Fab fragments of "JTT-1 antibody."

Figure 8 shows the expression state of "rat JTT-1 antigen" and "rat JTT-2 antigen" in COS cells transformed with cDNA encoding "rat JTT-1 antigen" with a flow-cytometer.

Figure 9 shows the structural characteristics of amino acid sequence of "JTT-1 antigen" revealed by hydropathy plot analysis.

Figure 10 shows the homology among amino acid sequences of human, rat, and mouse "JTT-1 antigen" and "rat JTT-1 antigen" mutant.

Figure 11 shows the homology among amino acid sequences and conservation state of motifs in "human JTT-1 antigen," "human CD28 molecule", and "human CTLA-4 molecule."

Figure 12 schematically shows the protein secondary structure of, and their similarity among "human JTT-1 antigen," "human CD28 molecule," and "human CTLA-4 molecule."

Figure 13 schematically shows the structure of the genomic DNA encoding "mouse JTT-1 antigen."

Figure 14 shows the difference in amino acid sequences between "rat JTT-1 antigen" and its alternative splicing mutant.

Figure 15 shows the degree of the growth of human peripheral blood lymphocytes induced by the monoclonal antibody against "human JTT-1 antigen," where the degree of the growth was measured by [^{3}H] thymidine uptake.

The ordinate shows the amount of uptake (dpm) of [^{3}H] thymidine into the cells.

Figure 16 shows the therapeutic effect of the monoclonal antibody against "JTT-1 antigen" on experimental allergic encephalomyelitis (EAE) in a disease model rat.

The ordinate shows the scored degree of disease symptom, and the abscissa shows the days after immunisation for induction of EAE.

Figure 17 shows the therapeutic effect of the monoclonal antibody against "JTT-1 antigen" on glomerulonephritis in a disease model rat.

The ordinate shows the amount of urinary excretion of proteins, and the abscissa shows the time course (week) after immunisation for induction of glomerulonephritis

Figure 18 shows a column histogram in purification of fusion polypeptide between "rat JTT-1 antigen" extracellular region and human IgFc (rJTT-1-IgFc) with protein A Sepharose column.

Figure 19 is a photograph showing electrophoretogram of rJTT-1-IgFc analysed by SDS-PAGE.

Figure 20 shows a column histogram in purification of fusion polypeptide between "human JTT-1 antigen" extracellular region and human IgFc (hJTT-1-IgFc) with protein A Sepharose column.

Figure 21 is a photograph showing electrophoretogram of hJTT-1-IgFc analysed by SDS-PAGE.

Figure 22 schematically shows the structure of the gene transfer (targeting) vector used for preparation of a transgenic mouse into which the cDNA encoding "rat JTT-1 antigen" has been introduced.

Best Mode for Implementing the Invention

The present inventions are described in more detail with reference to Examples below, but are not to be construed to be limited thereto.

Example 1 Preparation of monoclonal antibodies

Antibody-producing hybridomas were prepared according to the method of Kohler *et al.* (Omori *et al.*, Blood, Vol.81, pp.101-111 (1993)), and monoclonal antibodies were prepared according to the method of Kannagi *et al.* (Handbook of Experimental Immunology, Vol.4, 117.21-117.21 (1986)).

First, rat thymoma cell line FTL435 cells were administered as an immunising antigen to BALB/c mice into their footpad in an amount of 10^7 cells/mouse at intervals of 0, 7, 14, and 28 days. The mixture of the antigen with Freund's complete adjuvant was administered only in the first immunisation. Two days after the last immunisation, the lymph nodes of the mice were taken out and fused with mouse myeloma cells PAI (JCR No. B0113; Stocker, J. W. *et al.*, Res. Disclosure, Vol.217, p.155 (1982)) by the usual method to obtain many hybridomas producing monoclonal antibodies.

Example 2 Screening of hybridomas and characterisation of monoclonal antibodies

The hybridomas prepared in Example 1 were screened by analysing the effect of the antibodies produced in the culture supernatant of the hybridomas on FTL435 cells, which were used as the immunogen. FTL435 cells (5 x 10^6 cells/mL, 0.1mL) were seeded into each well of a 96-well microtiter plate and cultivated at 37°C for an hour in the presence of culture supernatant of each hybridoma (10 µg/mL each). The results obtained for hybridoma clones "JTT-1" and "JTT-2" are shown in Figure 1 and Figure 2.

It was revealed that a monoclonal antibody produced by hybridoma clone "JTT-1" ("JTT-1 antibody") strongly agglutinated FTL435 cells (Figure 1 (b) and Figure 2 (c)) and that addition of "JTT-2 antibody" strongly inhibited the aggregation of FTL435 cells induced by "JTT-1 antibody" stimulation (Figure 1 (d)). The assays, in which no hybridoma supernatant was added, were used as controls (Figure 1 (a) and Figure 2 (a)).

In order to determine whether the aggregation of FTL435 cells induced by "JTT-1 antibody" stimulation was caused by the cell adhesion between intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1), which is a representative known pathway of cell adhesion, FTL435 cells were cultivated at 37°C for an hour in the presence of anti-rat ICAM-1 antibody 1A29 (10 µg/mL; IgG1) or anti-rat LFA-1 antibody (10 µg/mL; IgG2a) together with "JTT-1 antibody."

The aggregation of FTL435 cells by "JTT-1 antibody" stimulation was inhibited by neither anti-ICAM-1 antibody nor anti-LFA-1 antibody (anti-ICAM-1 antibody, Figure 1 (c) and Figure 2 (f); anti-LFA-1 antibody, Figure 2 (d)).

In order to further analyse the cell agglutination ability of "JTT-1 antibody," the ability to agglutinate rat lymphoblast cells activated with concanavalin A stimulation was analysed in the same manner as mentioned above. The results are shown in Figure 2.

Similar to the effect on FTL435 cells, the aggregation of activated lymphoblast cells was induced by "JTT-1 antibody" stimulation (Figure 2 (i)). The aggregation of activated lymphoblast cells by "JTT-1 antibody" stimulation was mostly inhibited by anti-LFA-1 antibody (Figure 2 (j)) and anti-ICAM-1 antibody (Figure 2 (l)). (However, partial aggregation occurred.)

As understood from the control assay (Figure 2 (g)), in which no antibody was added, activated lymphocytes such as activated lymphoblasts showed no aggregation through cell adhesion unless they receive the stimulation with phorbol myristate acetate (PMA, which activates LFA-1) (Figure 2 (h)) or "JTT-1 antibody" (Figure 2 (l)). Therefore, the fact that anti-LFA-1 antibody partially inhibited cell aggregation by "JTT-1 antibody" stimulation indicates that LFA-1 in activated lymphoblast cells was activated by "JTT-1 antibody" stimulation. This also indicates that molecules recognised by "JTT-1 antibody" are involved in some signal transmission.

Hybridoma clones "JTT-1" and "JTT-2" have been deposited under the Budapest Treaty with international depository authority, National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan (1-1-3, Higashi, Tsukuba-shi, Ibaraki, Japan) since October 11, 1996 with an international accession Nos. FERM BP-5707 and FERM BP-5708, respectively.

Analysis using mouse monoclonal antibody isotype identification kit (Amersham) determined that the isotype of monoclonal antibodies produced from each hybridoma (JTT-1 antibody and JTT-2 antibody) were both IgG1.

Example 3 Reactivity of "JTT-1 antibody" and "JTT-2 antibody" to various cells

In order to analyse the expression pattern of molecules recognised by "JTT-1 antibody" and "JTT-2 antibody" in various cells, the reactivities of the antibodies to various cells were examined. Molecules recognised by "JTT-1 antibody" or "JTT-2 antibody" are designated "JTT-1 antigen" or "JTT-2 antigen", respectively.

A five- to ten-week-old Wistar rat (150 to 250 g) was killed by anaesthesia with diethyl ether. The thymus and spleen were taken out of its chest and abdomen, respectively, by celiotomy, and homogenised to prepare cell suspension. The resulting spleen cells were cultivated in RPMI1640 medium containing 2μg/mL concanavalin A and 10% FCS at 37°C for 3 days to prepare activated lymphoblasts.

FTL435 cells, thymocytes, spleen cells, and activated lymphoblasts (5 x 10^5 cells each) were reacted with "JTT-1 antibody" or "JTT-2 antibody" and then with FITC-labelled anti-mouse IgG (Cappel). The fluorescence intensity of the stained cells was measured with EPICS-Elite flow cytometer.

The results are shown in Figure 3. In FTL435 cells, the strong expression of each "JTT-1 antigen" and "JTT-2 antigen" was observed. While the antigens were expressed in thymocytes, they were expressed only a little in spleen cells. However, in activated lymphoblasts obtained by simulating spleen cells with concanavalin A, "JTT-1 antigen" and "JTT-2 antigen" were strongly expressed. In addition, in each kind of cells, the expression pattern of "JTT-1 antigen" and "JTT-2 antigen" coincided with each other. These results indicate that "JTT-1 antigen" and "JTT-2 antigen" are the same molecules.

Example 4 Reactivity of "JTT-1 antibody" to various lymphocytic cells

In order to analyse the expression pattern of molecules ("JTT-1 antigen") recognised by "JTT-1 antibody" in various lymphocytic cells, the reactivity of "JTT-1 antibody" to lymph nodes, T lymphoblasts derived from spleen, and B lymphoblasts derived from spleen of two kinds of rats (Wistar rat and F344 rat) was analysed.

A five- to ten-week-old Wistar rat and F344 rat (150 to 250g) were killed by anaesthesia with diethyl ether. The lymph nodes and spleen were taken out of each rat by celiotomy, and homogenised to prepare cell suspension. The resulting cell suspension from spleen was cultivated in RPMI1640 medium containing 2μg/mL concanavalin A (ConA) and 10% FCS at 37°C for 3 days. Activated T lymphoblasts and activated B lymphoblasts were obtained from each rat after 1-day and 3-day cultivation. In addition, spleen-derived T lymphoblasts and B lymphoblasts obtained before lymph node cells and ConA were added were used as controls.

Each cells (5 x 10^5 cells each) were reacted with biotin-labelled anti-rat T cell antibody or biotin-labelled anti-rat B cell antibody (10μg/mL, Seikagaku Corporation), and subsequently with phycoerythrin-labelled streptavidin. The cells were then reacted with 10μg/mL FITC-labelled "JTT-1 antibody." The fluorescence intensity of the stained cells was measured with EPICS-Elite flow cytometer.

The results are shown in Figure 4. In both activated T lymphoblasts and activated B lymphoblasts from Wistar rat and F344 rat, the strong expression of "JTT-1 antigen" was observed from day 1 of activation with ConA stimulation. In addition, the expression pattern of "JTT-1 antigen" in each kind of cells almost perfectly coincided with each other.

Example 5 Characterisation of "JTT-1 antigen" and "JTT-2 antigen" by immunoprecipitation

"JTT1 antigen" and "JTT-2 antigen" were characterised by immunoprecipitation using FTL435 cells.

(1) Preparation of biotinylated soluble cell surface molecules

FTL435 cells were washed with PBS, suspended in physiological saline containing 100μg/mL NHS-biotin and 0.1M HEPES (pH 8.0) to adjust 1 x 10^7 cells/mL, and incubated at room temperature for 40 minutes. The cells were washed three times with PBS, lysis buffer (1% NP-40, 10mM Tris-HCl (pH7.4), 0.15M NaCl) was added thereto to adjust 5 x 10^7 cells/mL, and the mixture was allowed to react at 4°C for 30 minutes to lyse the cells. The cell lysate obtained was centrifuged, and the supernatant comprising biotinylated soluble cell surface molecules was stored at –80°C.

(2) Immunoprecipitation and SDS-PAGE analysis

The purified sample of "JTT-1 antibody" purified by the usual method from the culture supernatant of the hybridoma clone "JTT-1" prepared in Example 1 was mixed with protein G-Sepharose beads to adjust 2mg/mL, and allowed to react at 4 °C for an hour to bind the antibody with the beads. After the beads were washed, 500μL of the biotinylated FTL435 cell lysate was added to 10μL of the beads, and the mixture was allowed to react at 4 °C for 2 hours. After the beads were washed with lysis buffer three times, 50μL of glycanase buffer (sodium phosphate buffer (pH7.0) containing 0.15% SDS) was added to the beads, and the mixture was boiled to elute the bound molecules trapped by the antibody-bound beads. 1.25% NP-40 and 20U/mL N-glycanase were added to a fraction of the sample so eluted, and the mixture was allowed to react overnight to digest N-linked sugar chains.

An equal volume of sample buffer (Enprotech) for SDS-PAGE was added to 5μL of the eluted sample in the presence or absence of 2-mercaptoethanol, and the mixture was boiled. After electrophoresis, the gel was transferred to a PVDF membrane. The membrane was blocked with 3% BSA-PBS and reacted with peroxidase-labelled streptavidin to detect biotinylated soluble cell surface molecules trapped by "JTT-1 antibody" with ECL system (Amersham) as described in the manual.

The results are shown in Figure 5. The "JTT-1 antibody"-recognised molecule ("JTT-1 antigen") on FTL435 cells showed the molecular weight of about 47kD under the non-reduced conditions ("(-)" in Figure 5) and about 24kD and 28kD under the reduced conditions ("(+)" in Figure 5). As the result of digestion of N-linked sugar chains ("+N-gly" in Figure 5), "JTT-1 antigen" was converged on a single band of about 36kD under the non-reduced conditions and about 20kD under the reduced conditions. These results suggest that "JTT-1 antigen" forms a dimer in which the same core proteins have different sugar chains. Completely the same results were obtained in the experiment performed as mentioned above using "JTT-2 antibody." Considering these results together with the results of Example 3 and Example 7 below, "JTT-1 antigen" (molecule recognised by "JTT-1 antibody") and "JTT-2 antigen" (molecule recognised by "JTT-2 antibody") have been thought to be identical to each other.

Example 6 Adhesion experiment of rat thymocytes to purified "JTT-1 antigen" and N terminal amino acid analysis

The following experiments were performed to analyse whether the molecule that "JTT-1 antibody" recognises ("JTT-1 antigen") functions as an adhesion molecule. N-terminal amino acid analysis was also performed.

(1) Preparation of "JTT-1 antibody"-affinity column

The purified sample (2mg in 2mL) of "JTT-1 antibody" purified by the usual method from the culture supernatant of the hybridoma clone "JTT-1" prepared in Example 1 was mixed with 1mL of protein G- Sepharose resin, and the mixture was allowed to react at 4°C for an hour. The resin was washed three times with 200mM triethanolamine (pH 8.2). The resin was then incubated in triethanolamine (pH8.2) containing 10mM dimethyl pimelimidate (DMP) at room temperature for an hour to covalently bind "JTT-1 antibody" to the resin.

(2) Purification of "JTT-1 antigen"

FTL435 cells were cultivated in RPMI1640 medium containing 10% FCS. The cells were harvested by centrifugation to obtain a pellet and washed with PBS three times. Lysis buffer (1% NP-40, 10mM Tris-HCl (pH7.4), 0.15M NaCl) was added to the washed pellet to adjust 5 x 10^7 cells/mL, and the mixture was allowed to react at 4°C for 30 minutes to lyse the cells. The cell lysate obtained was centrifuged, and the supernatant containing soluble cell surface molecules was stored at –80°C.

The lysate (400mL) was loaded onto "JTT-1 antibody"-affinity column. After the column was washed with 50mL of the lysis buffer and 20mL of PBS, "JTT-1 antigen" was eluted with 0.2M glycine buffer (pH2.8). 1 M Tris buffer was added to the "JTT-1 antigen" so eluted for neutralisation. "JTT-1 antigen" obtained was stored at –80°C.

(3) Determination of N terminal amino acid sequence

After the purified "JTT-1 antigen" was subjected to SDS-PAGE, the N-terminal amino acid sequence was determined by the usual method. The result revealed that "JTT-1 antigen" contained an amino acid sequence Glu-Leu-Asn-Asp-Leu-Ala-Asn-His-Arg.

(4) Adhesion experiment

A five- to ten-week-old Wistar rat (150 to 250g) was killed by anaesthesia with diethyl ether. The thymus was taken out of its chest by celiotomy and homogenised to prepare thymocyte suspension. 10μM 2',7'-bis(carboxyethyl)carboxyfluorescein tetraacetoxy-methyl ester (BCECF-AM; Molecular Probes) was added to the suspension, and the mixture was incubated at 37°C for 30 minutes to fluorescently label the thymocytes. The cells were washed with PBS and suspended in RPMI1640 medium containing 10% FCS to adjust 2 x 10^7 cells/mL.

The purified "JTT-1 antigen" obtained in (2) was coated on a 96-well ELISA plate at the concentration of 10μL/well overnight. After the plate was washed with PBS, 200μL/well of PBS containing 3% BSA was added to the plate, and blocking was performed for 2 hours. After the plate was washed with PBS, (1) only fluorescence-labelled thymocytes (2 x 10^7 cells/mL, 0.1mL), (2) fluorescence-labelled thymocytes (same concentration) and "JTT-1 antibody" Fab fragments prepared by the usual method (5μg/mL), or (3) fluorescence-labelled thymocytes (same concentration), the "JTT-1 antibody" Fab fragments (same concentration), and "JTT-2 antibody" (10μg/mL), were added to each well, and cultivated at 37°C for an hour. In order to remove unbound cells, each well was washed once with RPMI1640 medium containing 10% FCS. Each well was observed with light microscope. Then, 100μL of 0.1% NP-40 was added to each well, and the cells adhered to the plate were lysed. The relative cell number of fluorescence-labelled thymocytes adhered to each well was counted by measuring the fluorescence intensity at 538nm (excited at 485nm) with Fluoroscan II Microplate Fluorometer (Flow Laboratories). The assay in which a plate was not coated with purified "JTT-1 antigen" was used as a control.

The results of light microscopy observation are shown in Figure 6.

Thymocytes significantly adhered to purified "JTT-1 antigen" only in the presence of "JTT-1 antibody" Fab fragments (Figure 6 (c)). The adhesion was significantly inhibited by "JTT-2 antibody" (Figure 6 (d)).

Figure 7 shows the relative cell number of thymocytes adhered to "JTT-1 antigen" coated on each well in terms of fluorescent intensity.

From these results, it was revealed that "JTT-1 antigen" functions as an adhesion molecule.

Example 7 Cloning of cDNA encoding rat "JTT-1 antigen"

1. Preparation of cDNA library

1-(1) Extraction of poly(A)$^+$ RNA from ConA-stimulated rat lymphoblasts

ConA-stimulated lymphoblasts (ConA blast) derived from rat spleen (about 1 x 10^6 cells/mL) were centrifuged (2,000 x g) at 4°C for 5 minutes. The precipitated cells were suspended with ISOGEN (Nippon Gene) and extracted with chloroform with shaking to collect the supernatant. After isopropanol was added to the obtained supernatant, the mixture was allowed to stand at room temperature for 10 minutes and centrifuged at 12,000 x g at 4°C for 10 minutes to precipitate RNA. The precipitated RNA was washed with ethanol and dissolved in TE buffer. Poly(A)$^+$ RNA was purified from the total RNA so obtained with "mRNA Purification Kit" (Pharmacia).

1-(2) Preparation of cDNA

With 5 μg of the poly(A)$^+$ RNA prepared above as a template, cDNA was synthesised with "Time Saver cDNA Synthesis Kit" (Pharmacia). "Oligo dT primer" (Pharmacia) having NotI site was used to increase the efficiency of screening. EcoRI adapter was added, and digestion with NotI was performed to obtain cDNA with unidirectionality. Size fractionation was then performed with Spun Column (Pharmacia).

1-(3) Insertion into a vector

The obtained cDNA having EcoRI- and NotI-ends was ligated with pME18S (Hara, T. & Miyajima, A., EMBO J., Vol.11, pp.1875-1884 (1992)) digested with EcoRI and NotI. "DNA Ligation Kit" (Takara Shuzo) was used for the ligation. *E. coli* DH5 cells (Toyobo) were transformed with the reaction product so obtained. Transformants were cultivated until O.D. value (at 600nm) reached 0.6 and harvested to recover plasmid DNAs with a library. QUIAGEN-Tip (QUIAGEN) was used for purification of plasmid DNAs.

2. Screening of cDNA library

Screening was performed according to panning method (Seed, B. *et al.*, Proc. Natl. Acad. Sci. USA, Vol.84, pp.3365-3369 (1987)).

2-(1) Gene transfer into COS cells

The library so obtained was introduced into COS7 cells by electroporation (Potter, H. *et al.*, Proc. Natl. Acad. Sci. USA, Vol.85, pp.2288-2292). The transformants were cultivated for 60 hours after introduction, the supernatant was removed, and the pellet was washed with PBS three times. After the pellet was treated with PBS (containing 0.5mM EDTA) at 37°C for 30 minutes, the cells were removed by pipetting. Only living cells were then collected with "Lymphprep" (NYCOMED).

2-(2) Concentration of gene-expressing cells by panning

The living cells obtained above were suspended in PBS (containing 5% FCS and 0.5mM EDTA). The cell suspension was transferred to a culture dish coated with "JTT-1 antibody" and incubated at room temperature for 3 hours. After cells not binding to the culture dish were removed and the culture dish was washed with PBS three times, plasmid DNAs were collected from the cells

binding to the culture dish by Hirt method (Hirt, B., J. Mol. Biol., Vol.26, pp.365-369). *E. coli* DH10B (GIBCO BRL) were transformed with the plasmid DNA so obtained. The plasmid DNAs were amplified and purified with the transformants as in 1-(3) mentioned above. The procedures described in (1) and (2) were then repeated twice.

2-(3) Isolation of the positive clone

After the third panning, transformed *E. coli* DH10B cells were cultivated overnight on LB plates containing ampicillin to obtain colonies. Twenty drug-resistant colonies were cultivated, plasmid DNAs were collected by alkaline miniprep method (Maniatis, T. *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), and the insert DNA was analysed. Agarose gel electrophoresis revealed that the clone having about 0.9kb cDNA (designated "T132A7") was concentrated.

"T132A7" was transiently expressed in COS7 cells again with the method described in (1). After "T132A7"-introduced cells were reacted with "JTT-1 antibody" or "JTT-2 antibody", and then with FITC-labelled anti-mouse IgG (Cappel), the fluorescence intensity of the stained cells was measured with EPICS-Elite flow cytometer (Coulter). "JTT-1 antibody" and "JTT-2 antibody" strongly recognised the "T132A7" gene product. The results are shown in Figure 8.

3. Determination of the nucleotide sequence and the amino acid sequence

The nucleotide sequence of clone "T132A7" was determined by dideoxy method with "Auto Read Sequencing Kit" (Pharmacia) and "A.L.F. DNA Sequencer" (Pharmacia). In addition, the deduced amino acid sequence of "rat JTT-1 antigen" encoded by the nucleotide sequence was analysed with gene analysis software "GENEWORKS" (IntelliGenetics). The nucleotide sequence and the deduced amino acid sequence were shown in SEQ ID NO: 4.

The amino acid sequence (composed of 200 amino acid residues) deduced from the cloned gene comprises the same amino acid sequence as the N terminal amino acid sequence determined in Example 6-(3). Considering that clone "T132A7"-introduced cells strongly react with "JTT-1 antibody," it can be concluded that clone "T132A7" comprises the cDNA encoding "rat JTT-1 antigen."

4. Computer analysis

Hydropathy analysis of the primary structure of the deduced amino acid sequence of "JTT-1 antigen" was performed according to the method of Kite and Doolittle (Kite, J. & Doolittle, R. F., J. Mol. Biol., Vol.157, pp.105-132 (1982)) (Figure 9). The results revealed that "JTT-1 antigen" is a transmembrane protein having a signal sequence at the N-terminus. In addition, the results of motif analysis revealed that "JTT-1 antigen" has two Asn-linked sugar chain binding sites in the extracellular domain, and two casein kinase phosphorylation sites and one protein kinase C phosphorylation site in the cytoplasmic domain. In Figure 9 "CHO" means N-linked sugar chain binding site; "P", phosphorylation site; "CKII", casein kinase II; and "PKC", protein kinase C.

Example 8 Cloning of cDNA encoding "human JTT-1 antigen"

1. Preparation of a probe

The cDNA (about 0.9kb) encoding "rat JTT-1 antigen" was generated by digesting the clone "T132A7" obtained in Example 7 with restriction enzymes EcoRI and NotI, and separated by agarose gel electrophoresis. The separated DNA fragments were purified with "QUIAEX gel extraction kit"

(QUIAGEN), and the obtained DNA fragments were labelled with ^{32}P using "Ready-To-Go DNA labelling kit" (Pharmacia). These labelled DNA fragments were used as probes for plaque hybridisation.

2. Preparation of cDNA library

2-(1) Extraction of poly(A)$^+$ RNA

Poly(A)$^+$ RNA was extracted from ConA-stimulated lymphoblasts (ConA blast) derived from human peripheral blood in the same manner as in Example 7-1-(1).

2-(2) Preparation of cDNA

With 5 µg of the poly(A)$^+$ RNA so prepared as a template, cDNAs were synthesised with "oligo dT primer" (Pharmacia) and "Time Saver cDNA Synthesis Kit" (Pharmacia). EcoRI adapter was then added, and size fractionation was performed with Spun Column (Pharmacia).

2-(3) Insertion into a vector and packaging

The cDNAs so obtained having EcoRI-ends were ligated with the vector "λZAPII" (Stratagene) digested with EcoRI. "DNA Ligation Kit" (Takara Shuzo) was used for the ligation. After *in vitro* packaging of the ligated DNA was performed with "GIGA PACK II GOLD" (Stratagene), *E. coli* XL1Blue MRF' cells (Stratagene) were transfected with the obtained phage particle to generate a cDNA library composed of plaque comprising recombinant phage.

3. Screening of cDNA library

cDNA library was screened by plaque hybridisation method (Maniatis, T. *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) with "Rapid hybridisation buffer" (Amersham). First, the cDNA library so obtained (1 x 10^4) was plated onto agar plates and the replica was produced with "Hybond-N nylon membrane" (Amersham). Plaque hybridisation was performed in "Rapid hybridisation buffer" (Amersham) using the replica and the ^{32}P-labelled probe prepared in Example 8-1. First and second screenings were performed to obtain eight positive clones. Single plaques of each clone were isolated and subjected to *in vivo* excision in accordance with the manual (Stratagene) and seven positive clones were collected as plasmid DNA.

4. Determination of the nucleotide sequence

The nucleotide sequences of the seven clones were determined by dideoxy method with "Auto Read Sequencing Kit" (Pharmacia) and "A.L.F. DNA Sequencer" (Pharmacia). The seven clones comprise the same nucleotide sequence. It was found that clone "pBSh41" encodes the full length "human JTT-1 antigen." The cDNA sequence corresponding to the open reading frame (ORF) of "human JTT-1 antigen" is shown in SEQ ID NO: 1, the full length of the deduced amino acid sequence of "human JTT-1 antigen" is shown in SEQ ID NO: 2, and the nucleotide sequence comprising 5' and 3' sequences is shown in SEQ ID NO: 3 (ORF corresponds to the nucleotide residues 26 to 625). It is understood that the nucleotide sequence contained in the clone encodes the full length of "human JTT-1 antigen" because the amino acid sequence (composed of 199 amino acid residues) deduced from the nucleotide sequence shows significant homology with the amino acid sequence of "rat JTT-1 antigen" (Figure 10). As shown in Figure 10, the homology between the amino acid sequences of human and rat "JTT-1 antigen" is 60% or more.

E. coli DH10B (GIBCO BRL) transformed with the clone "pBSh41" has been deposited under the Budapest Treaty with international depository authority, National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan (1-1-3, Higashi, Tsukuba-shi, Ibaraki, Japan) since October 25, 1996 (an international deposit accession No. FERM BP-5725).

5. Structural characteristics and biological function of "JTT-1 antigen"

The results of motif search for the deduced amino acid sequence of "human JTT-1 antigen" in known human proteins revealed that "human JTT-1 antigen" has structural similarity to "CD28" and "CTLA-4," human-derived cell membrane proteins belonging to the immunoglobulin superfamily, mentioned in detail above (Figures 11 and 12). As mentioned above, "CD28" and "CTLA-4" are extremely important molecules regulating the activation and inhibition of T cells in immune system.

The structural similarity is as follows

1. 20 or more amino acid residues including cysteine residues are highly conserved.

2. Proline repeating sequence, "Pro-Pro-Pro (PPP)", which is essential as the ligand binding region in CD28 and CTLA-4, is conserved.

3. "Tyr-Xaa-Xaa-Met (YxxM)" (Xaa and x represents any amino acid) sequence essential as the signal transmitting region in CD28 and CTLA-4 is conserved in the cytoplasmic region.

From the fact that the same structure with the specific structure of "CD28" and "CTLA-4", which play an important role in regulation of activation of T cells that are main actor in immune mechanism, "JTT-1 antigen" of the present invention is inferred to play an important role like those molecules in regulation of activation of lymphocytes such as T cells which are main actor in immune response.

Example 9 Cloning of cDNA encoding "mouse JTT-1 antigen"

1. Preparation of a probe

The cDNA (about 0.9kb) encoding "rat JTT-1 antigen" was obtained by digesting the clone "T132A7," cloned in Example 7, with restriction enzymes EcoRI and NotI, and separated by agarose gel electrophoresis. The DNA fragments so separated were purified with "QUIAEX gel extraction kit" (QUIAGEN), and the DNA fragments were labelled with ^{32}P using "Ready-To-Go DNA labelling kit" (Pharmacia). These labelled DNA fragments were used as a probe for plaque hybridisation.

2. Preparation of cDNA library

2-(1) Extraction of poly(A)$^+$ RNA

As Example 7-1-(1), poly(A)$^+$ RNAs were extracted from ConA-stimulated lymphoblasts derived from mouse spleen (about 1 x 10^6 cells/mL).

2-(2) Preparation of cDNA library

With 5mg of poly(A)$^+$ RNAs prepared in the above as a template, cDNAs were synthesised with oligo dT primer (Pharmacia) and "Time Saver cDNA Synthesis Kit" (Pharmacia). After EcoRI adapter was added to the cDNA, size fractionation was performed with Spun Column (Pharmacia).

2-(3) Insertion of cDNA into a vector and packaging

The cDNA so obtained having EcoRI-ends was ligated with the vector lZAPII (Stratagene) digested with EcoRI. "DNA Ligation Kit" (Takara Shuzo) was used for the ligation. After *in vitro* packaging of the ligated DNA was performed with GIGA PACK II GOLD (Stratagene), *E. coli* XL1Blue

MRF' cells (Stratagene) were transfected with the phage particle so obtained to generate a cDNA library composed of plaque comprising recombinant phage.

3. Screening of cDNA library

Screening was performed by plaque hybridisation method (Maniatis, T. *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) using Rapid hybridisation buffer (Amersham).

The above-obtained cDNA library (1 x 10^4) was plated onto agar plates and the replica was produced using Hybond-N nylon membrane (Amersham). Plaque hybridisation was performed in Rapid hybridisation buffer (Amersham) using the replica and the ^{32}P-labelled probe prepared in Example 9-1. First and second screenings were performed to obtain five positive clones. After single plaque of each clone was isolated, *in vivo* excision was performed in accordance with Instruction Manual (Stratagene) and five positive clones were collected as plasmid DNA.

4. Determination of the nucleotide sequence

The nucleotide sequences of each of the five clones were determined by dideoxy method with "Auto Read Sequencing Kit" (Pharmacia) and "A.L.F. DNA Sequencer" (Pharmacia). The four of the five clones comprise the same nucleotide sequence. The nucleotide sequence of cDNA encoding the full length of "mouse JTT-1 antigen" and the deduced amino acid sequence are shown in SEQ ID NO: 5.

As understood from Figure 10, "mouse JTT-1 antigen" is composed of 200 amino acid residues like "rat JTT-1 antigen." The homology among the amino acid sequences of mouse, rat, and human "JTT-1 antigens" is significant (60% or more).

5. Analysis of the locus of "mouse JTT-1 antigen" gene

The locus of the gene encoding "mouse JTT-1 antigen" was analysed by fluorescence *in situ* hybridisation method.

The cDNA so obtained encoding "mouse JTT-1 antigen" was labelled with ^{32}P to prepare hybridisation probes by the usual method. Using these probes, the 129 SVJ mouse genomic DNA library (Stratagene) was screened to obtain mouse genomic DNA clones comprising the exons encoding "mouse JTT-1 antigen." The structure of the genomic DNA is schematically shown in Figure 13.

The above-obtained genomic DNA clones were labelled with digoxigenin dUTP by nick translation to prepare probes. The labelled probes were bound to cleaved mouse DNA and hybridised with normal metaphase chromosomes derived from mouse embryonic fibroblasts in the solution containing 50% formaldehyde, 10% dextran sulfate, and 2 x SSC. After a slide glass for hybridisation was incubated in fluorescence-labelled anti-digoxigenin antibody, specific hybridisation signal was detected by staining with DAPI. In the first test, the part near the largest chromosome that was thought to be the chromosome 1, judging from the DNA size and emerged band, was specifically labelled. Based on this information, the above-described genomic DNA clone was co-hybridised with probes specific to the centromere region of the chromosome 1. As a result, the centromere region of the chromosome 1 and the regions near them were specifically labelled. Ten samples of the chromosome 1 showing the specific hybridisation were analysed, and it was revealed that the above-

mentioned genomic DNA clone was located at the position of 33% of the distance from the border between heterochromatin and euchromatin to the telomere of the chromosome 1, namely, on the same band "1C3" as the loci of mouse "CD28" and "CTLA-4" genes. As the result that 80 metaphase cells were analysed, specific labelling was identified at said position for 79 cells.

These results and the results obtained in Example 8 indicating the structural similarity of "JTT-1 antigen" to "CD28" and "CTLA-4" suggest that "JTT-1 antigen," like "CD28" and "CTLA-4," is an important molecule involved in the regulation of the transmission of costimulatory signal and/or activation of lymphocytes.

Example 10 Cloning of cDNA encoding a mutant of "rat JTT-1 antigen"

Another cDNA that is thought to encode alternative splicing variant of "rat JTT-1 antigen" cloned in Example 7 was cloned as follows.

1. Preparation of a probe

The cDNA (about 0.9kb) encoding "rat JTT-1 antigen" was generated by digesting the clone "T132A7," obtained in Example 7, with restriction enzymes EcoRI and NotI, and separated by agarose gel electrophoresis. The separated DNA fragments were purified with "QUIAEX gel extraction kit" (QUIAGEN), and the obtained DNA fragments were labelled with ^{32}P using "Ready-To-Go DNA labelling kit" (Pharmacia). These labelled DNA fragments were used as probes for plaque hybridisation.

2. Preparation of cDNA library

2-(1) Extraction of poly(A)$^+$ RNA

As in Example 7-1-(1), poly(A)$^+$ RNA was extracted from rat thymoma cell line FTL435 (about 1 x 10^6 cells/mL).

2-(2) Preparation of cDNA library

With 5mg of the poly(A)$^+$ RNA prepared as mentioned above as a template, cDNAs were synthesised using oligo dT primer (Pharmacia) and "Time Saver cDNA Synthesis Kit" (Pharmacia). After EcoRI adapter was added to the cDNA, size fractionation was performed with Spun Column (Pharmacia).

2-(3) Insertion of cDNA into a vector and packaging

The cDNA having EcoRI-end obtained above was ligated with the vector lZAPII (Stratagene) digested with EcoRI. "DNA Ligation Kit" (Takara Shuzo) was used for ligation. After *in vitro* packaging of the ligated DNA was performed with GIGA PACK II GOLD (Stratagene), *E. coli* XL1Blue MRF' (Stratagene) was transfected with the obtained phage particle to generate a cDNA library composed of plaque comprising recombinant phage.

3. Screening of cDNA library

Screening was performed by plaque hybridisation method (Maniatis, T. *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) with Rapid hybridisation buffer (Amersham).

The above-prepared cDNA library (1 x 10^4) was plated onto agar plates and the replica was produced with Hybond-N nylon membrane (Amersham). Plaque hybridisation was performed in Rapid hybridisation buffer (Amersham) using the replica and the ^{32}P-labelled probe prepared in Example 10-

1. First and second screenings were performed to obtain two positive clones. After single plaque of each clone was isolated, *in vivo* excision was performed in accordance with Instruction Manual (Stratagene), and two positive clones were collected as plasmid DNA.

4. Determination of the nucleotide sequence

The nucleotide sequences of the two clones were determined by dideoxy method with "Auto Read Sequencing Kit" (Pharmacia) and A.L.F. DNA Sequencer (Pharmacia). The two clones comprise the same nucleotide sequence. The nucleotide sequence of cDNA encoding the full length of the obtained "rat JTT-1 antigen" and the deduced amino acid sequence are shown in SEQ ID NO: 6. The amino acid sequence (SEQ ID NO: 6) deduced from the obtained cDNA sequence was compared with the amino acid sequence (SEQ ID NO: 4) deduced from the obtained cDNA sequence encoding "rat JTT-1 antigen" cloned in Example 7 (Figure 14). As shown in Figure 14, the amino acid sequence encoded by the cDNA cloned in this test was completely the same as that encoded by the cDNA encoding "rat JTT-1 antigen" obtained in Example 7, except that (1) C-terminal three continuous amino acid residues (Met-Thr-Ser) changes into Thr-Ala-Pro, and that (2) subsequent to the Thr-Ala-Pro, 16 continuous amino acid residues (Leu-Arg-Ala-Leu-Gly-Arg-Gly-Glu-His-Ser-Ser-Cys-Gln-Asp-Arg-Asn) are added. This indicates that the cDNA cloned in this test encodes the alternative splicing variant of "rat JTT-1 antigen" obtained in Example 7.

Example 11 Preparation of recombinant "human JTT-1 antigen"-expressing cells

The plasmid clone pBSh41 obtained in Example 8 was digested with a restriction enzyme EcoRI, and a DNA fragment comprising the cDNA encoding the full length of "human JTT-1 antigen" was excised. This DNA fragment was inserted with DNA Ligation Kit (Takara Shuzo) into a plasmid pEFneo (Proc. Natl. Acad. Sci. USA, Vol.91, pp.158-162 (1994)) treated with the same restriction enzyme EcoRI to prepare the expression vector. CHO-K1 cells (ATCC: CCL-61) were transformed with the vector by electroporation. By cultivating the cells in RPMI1640 medium containing 0.8mg/mL Geneticin (GIBCO BRL) and 10% foetal calf serum for about two weeks, Geneticin-resistant transformants were selected. The expression of recombinant "human JTT-1 antigen" was confirmed by Northern blotting by the usual method.

Example 12 Preparation of monoclonal antibodies against "human JTT-1 antigen"

The recombinant "human JTT-1 antigen"-expressing transformants prepared in Example 11 were homogenised and ultracentrifuged (100,000 x g). The pellet containing the cell membrane fraction was collected and suspended in PBS. The resulting suspension comprising the cell membrane fraction was injected into the footpad of a BALB/c mouse with complete Freund's adjuvant for the first immunisation (day 0). The cell membrane fraction antigen was further administered into its footpad at intervals of 7, 14, and 28 days. Two days after the last immunisation, the lymph node cells was taken out. The lymph node cells and mouse myeloma cells PAI (JCR No. B0113; Res. Disclosure, Vol.217, p.155 (1982)) were mixed at a ratio of 5:1, and fused using polyethyleneglycol 4000 (GIBCO) as a fusing agent to prepare monoclonal antibody-producing hybridomas. The hybridomas were screened by cultivating them in HAT-containing ASF104 medium (Ajinomoto) supplemented with 10% foetal calf serum and aminopterin. The culture supernatant of each hybridoma was reacted with the recombinant "human JTT-1 antigen"-expressing transformants

prepared in Example 11, and the fluorescence intensity of cells stained by reacting them with FITC-labelled anti-mouse IgG (Cappel) was measured with EPICS-ELITE flow cytometer to confirm the reactivity of the monoclonal antibody generated in each culture supernatant to "human JTT-1 antigen." It has been confirmed that 10 or more kinds of hybridomas producing monoclonal antibodies reactive to "human JTT-1 antigen" were obtained.

Each of two kinds (designated clone SA12 and SG430) among these hybridomas (10^6 to 10^7 cells/0.5mL/mouse) was injected into a ICR nu/nu mouse (female, 7-8 weeks old) intraperitoneally. After 10 to 20 days, celiotomy of the mice was performed under anaesthesia, and the two kinds of monoclonal antibodies (SA12 and SG430) reactive to "human JTT-1 antigen" were prepared in a large amount from the ascites fluid extracted by the usual method.

Example 13 Effect of the monoclonal antibodies against "human JTT-1 antigen" on human peripheral blood lymphocytes

As mentioned in Example 8, it is thought that "JTT-1 antigen" can be involved in the regulation of the activation of lymphocytes in immune reaction like "CD28" and "CTLA-4." In order to prove this, the effect of the monoclonal antibodies against "human JTT-1 antigen" on human lymphocytes was analysed in light of cell growth as an indication.

To each well of 96-well microtiter plate were added (1) either SA12 or SG430 (1 μg/mL), the monoclonal antibody against "human JTT-1 antigen" prepared in Example 12, or (2) a mixture of either monoclonal antibody SA12 or SG430 (1 μg/mL) with anti-CD3 monoclonal antibody OKT-3 (1 μg/mL, Orthodiagnostic Systems), which is used for adding the primary signal in the activation of lymphocytes. The plate was incubated at 37°C for 1 hour to coat each well with the antibody. After the plate was washed with RPMI1640 medium, normal human peripheral blood lymphocytes (1×10^5 cells/well) were added to each well and incubated in RPMI1640 medium containing 10% foetal calf serum for 3 days. If necessary, 1ng/mL phorbol myristate acetate (PMA) was added. Then, [^{3}H] thymidine (3.7 μkBq/well) was added to each well, and the plate was incubated at 37°C for 6 hours. The cells were harvested, and the amount of [^{3}H] thymidine incorporated into DNA was measured with a liquid scintillation counter (Beckman). The assay without any antibody was used as a control. The results are shown in Figure 15.

In the assay using the plates coated with either monoclonal antibody SA12 or SG430, the number of lymphocytes increased about 10 times compared to the control. In the co-presence of OKT3, the number of lymphocytes increased about 100 times when either monoclonal antibody SA12 or SG430 was used.

These results indicate that "JTT-1 antigen" functions in the regulation of the lymphocyte activation. The fact that the cell growth rate was increased by using together with OKT3 indicates that "JTT-1 antigen" is involved in the transmission of costimulatory signal like "CD28" and "CTLA-4."

Example 14 Effect of "JTT-2 antibody" on experimental allergic encephalomyelitis (EAE)

As above mentioned in detail, recently, many attempts to treat various autoimmune diseases (rheumatoid arthritis, multiple sclerosis, autoimmune thyroiditis, allergic contact dermatitis, chronic inflammatory dermatosis such as lichen planus, systemic lupus erythematosus, insulin dependent diabetes mellitus, psoriasis, etc.) have been made by regulating the transmission of between

CD28/CTLA-4 and CD80/CD86. The effect has been already confirmed in various model animals of autoimmune diseases ((1) a model for human systemic lupus erythematosus (SLE), (2) experimental allergic encephalomyelitis (EAE), a model for multiple sclerosis (MS), (3) a model for insulin dependent diabetes mellitus (IDDM), (4) Goodpasture's nephritis model, and (5) human rheumatoid arthritis).

In order to determine whether "JTT-1 antigen" of the present invention is a molecule involved in the activation or inhibition of lymphocytes such as "CD28" and "CTLA-4," model rats for experimental allergic encephalomyelitis (EAE), a model for multiple sclerosis (MS), were produced, and the effect of the titled monoclonal antibody on "JTT-1 antigen" in the model was analysed.

An emulsion to be used as immunogen was prepared by mixing Hartley guinea pig cerebrospinal homogenate (800mg/mL physiological saline) with the same amount of Freund's complete adjuvant. Immunisation was performed by intradermally injecting the emulsion into left and right foot pads of 15 Lewis rats (female, 6-week-old) in an amount of 0.25mL per footpad. The administration (immunisation) was adjusted so as for the dosages of the homogenate prepared to be 200mg per rat. This immunisation so induces experimental allergic encephalomyelitis (EAE).

The rats so immunised were divided into three groups of five rats each, and any one of (1) to (3) below was intravenously injected into mice of each group immediately after immunisation (day 0), and 3, 6, 9, and 12 days after the immunisation.

(1) Monoclonal antibody "JTT-2 antibody" against "rat JTT-1 antigen" prepared in Example 2 (dosage: 2mg/mL PBS, 5mg/kg)

(2) Prednisolone, steroid agent (dosage: 4mg/mL PBS, 10mg/kg)

(3) Control antibody non-reactive to "rat JTT-1 antigen" (dosage: 2mg/mL PBS, 5mg/kg)

Symptom was observed in the course of time after the immunisation. After the onset of EAE had been found, the degree of the symptom was estimated by scoring the symptom based on the following criteria.

(Score 1) Disappearance of tension of a tail

(Score 2) Dragging of hind legs, and slight paralysis

(Score 3) Dragging of hind legs, and serious paralysis

(Score 4) Paralysis of the whole body, or death

The results are shown in Figure 16. In the group to which the control antibody was administered, the symptom of EAE reached the peak (maximum score) at day 11 to 15 after the immunisation, and then gradually recovered. In contrast, in the "JTT-2 antibody"-administered group, the symptom of EAE at day 11 after the immunisation was significantly inhibited. This inhibitory effect was significantly higher than that in the prednisolone-administered group.

These results indicate that "JTT-1 antigen" is a molecule that functions in the induction of immune response such as the lymphocyte activation induced by immunisation by foreign antigens, and that the regulation of the function of "JTT-1 antigen" or its ligands can inhibit the symptom of various autoimmune diseases.

Example 15 Effect of "JTT-2 antibody" on glomerulonephritis

For the same purpose as Example 14, glomerulus basement membrane (GBM) nephritis model rats were produced, and the effect of the titled monoclonal antibody on "JTT-1 antigen" in the model was analysed.

After bovine glomerulus basement membrane (Shigei Medical Institute) digested with collagenase was diluted with physiological saline to 200μg/mL, the dilution was mixed with Freund's complete adjuvant to prepare an emulsion to be used as immunogen. Immunisation was performed by intradermally injecting the emulsion into both hind soles of 48 Wistar kyoto rats (about 200g) under anaesthesia in an amount of about 0.2mL per footpad (dosage: about 15μg). This immunisation so induces glomerulus basement membrane (GBM) nephritis.

The immunised rats were divided into eight groups of six rats each, and any one of (1) to (3) below was injected into rats of each group immediately after immunisation (day 0), and three times a week for 5 consecutive weeks.

(1) Monoclonal antibody "JTT-2 antibody" against "rat JTT-1 antigen" prepared in Example 2 (dosage: 3mg/kg (2mL PBS/kg), intravenous injection)

(2) Prednisolone, steroid agent, as a positive control (suspended in 0.5% carboxymethylcellulose (CMC)) (dosage: 3mg/kg (5mL/kg), oral administration)

(3) 0.5% CMC as a negative control (dosage: 5mL/kg, oral administration)

After the administration of a test substrate, sterilised water (25mL/kg) was orally administered into each rat forcedly, and urine was collected for 5 hours from each rat which had been kept in a metabolism cage without eating and drinking. After the volume of the collected urine was measured, the urinary protein concentration was measured using Tonein TP-II (Otuka), and the urinary excretion of protein per five hours was calculated (unit: mg protein/5 hours). The above-mentioned urinary collection and urinary protein measurement were performed in the same manner at 1, 2, 3, and 4 weeks after the immunisation (day 0).

The results are shown in Figure 17. Compared to the control group, the urinary excretion of protein at 3 weeks after the immunisation was significantly reduced in the "JTT-2 antibody"-administered group.

These results indicate that "JTT-1 antigen" is a molecule that induces immune response such as the lymphocyte activation induced by immunisation by foreign antigens, and that the regulation of the function of "JTT-1 antigen" or its ligands can inhibit the symptom of various autoimmune diseases.

Example 16 Preparation of the fusion protein between "JTT-1 antigen" and IgFc

As mentioned in Examples 8, and 13 to 15, "JTT-1 antigen" of the present invention is thought to be a molecule such as "CD28" and "CTLA-4" involved in the transmission of costimulatory signal involved in the regulation of the activation of lymphocytes. In addition, as mentioned in Example 14, a fusion protein (CTLA-4-IgFc) composed of the extracellular domain of "CTLA-4" and the Fc region of human immunoglobulin IgG1 reportedly has therapeutic effects on various autoimmune diseases. In this Example, a fusion protein composed of the extracellular region of "JTT-1 antigen" and human IgGFc was prepared as follows in order to examine whether soluble JTT-1 antigen, like CTLA-4-IgFc, could be applied to therapy of various autoimmune diseases.

(1) Preparation of the fusion protein between "rat JTT-1 antigen" and human IgG1-Fc (rJTT-1-IgFc)

In order to amplify the cDNA encoding the extracellular region of "rat JTT-1 antigen" by PCR, 5' primer having XhoI restriction site (5'-CTGCTCGAGATGAAGCCCTACTTCTCG-3', SEQ ID NO: 7) and 3' primer having BamHI restriction site (5'-ACCCTACGGGTAACGGATCCTTCAGCTGGCAA-3', SEQ ID NO:8) at their terminus were designed and synthesised. Using cDNA clone "T132A7" obtained in Example 7 encoding the full length of "rat JTT-1 antigen" as a template, PCR was performed with the primers to prepare the cDNA comprising the cDNA encoding the extracellular region of "rat JTT-1 antigen" having XhoI and BamHI restriction sites at its both ends. The PCR products so obtained were digested with XhoI and BamHI and separated by agarose gel electrophoresis to isolate an about 450-bp band predicted to be the cDNA fragment encoding a desired extracellular region. The isolated cDNA fragment was subcloned into pBluescript II SK (+) (Stratagene) cleaved with XhoI and BamHI. Sequence analysis with an automated fluorescence DNA sequencer (Applied Biosystems) revealed that the cDNA fragment comprises the region encoding amino acid sequence corresponding to the amino acid residues 1 to 141 of "rat JTT-1 antigen" (SEQ ID NO: 4).

On the other hand, the DNA encoding the Fc of human IgG1 as the fusion partner was cut out as an about 1.3 kb BamHI-XbaI DNA fragment by digesting the plasmid (see Cell, Vol.61, pp.1303-1313 (1990)). Prepared by B. Seed *et al.* (Massachusetts General Hospital)) with BamHI and XbaI. This fragment comprises exons encoding human IgG1 hinge region, $C\gamma_1 2$, and $C\gamma_1 3$.

The XhoI-BamHI fragment encoding the extracellular region of "rat JTT-1 antigen," and BamHI-XbaI fragment comprising exons encoding the Fc of human IgG1 ("IgFc"), both prepared as mentioned above, were subcloned into pBluescript II SK (+) (Stratagene) cleaved with XhoI and XbaI.

Then, the plasmid was digested with XhoI and XbaI, and an about 1.8kb DNA fragment comprising the fusion DNA comprising the extracellular region of "rat JTT-1 antigen" and human IgFc was cut out. This fusion DNA fragment was inserted into the XhoI and XbaI sites of the expression vector pME18S (Medical Immunology, Vol.20, No.1, pp.27-32 (1990); Experimental Medicine: SUPPLEMENT, "Handbook of Genetic Engineering," Yodosha, pp.101-107 (1992)) with T4 DNA ligase to construct plasmid prJTT-1-IgFc.

HEK293 cells (ATCC CRL1573) subconfluently cultivated as monolayer in DMEM medium containing 10% foetal calf serum and ampicillin were transformed with prJTT-1-IgFc by electroporation to obtain transformants.

The transformants were cultured in serum-free ASF104 medium for 72 hours to express rJTT-1-IgFc.

Using a Protein G Sepharose affinity column (Pharmacia), rJTT-1-IgFc was purified as follows.

The supernatant obtained by centrifuging the culture medium mentioned above was loaded onto Protein G Sepharose affinity column previously equilibrated with binding buffer. After the column was washed with binding buffer, elution was performed with elution buffer. The eluate was collected and dialyzed against phosphate buffer with exchanging the external solution twice or more to obtain pure rJTT-1-IgFc.

The result of affinity chromatography is shown in Figure 18, and the result of SDS-PAGE of the pure rJTT-1-IgFc so obtained in Figure 19.

(2) Preparation of the fusion protein between "human JTT-1 antigen" and human IgG1-Fc (hJTT-1-IgFc)

hJTT-1-IgFc was prepared as mentioned above in (1), except for cDNA used as templates and primers for PCR. In this test, the clone "pBSh41" comprising the cDNA encoding the full length "human JTT-1 antigen" prepared in Example 8 was used as a template, and 5'-TAACTGTTTCTCGAGAACATGAAGTCAGGC-3' (SEQ ID NO: 9) and 5'-ATCCTATGGGTAACGGATCCTTCAGCTGGC-3' (SEQ ID NO: 10) were used as primers.

The result of affinity chromatography is shown in Figure 20, and the result of SDS-PAGE of the pure hJTT-1-IgFc so obtained in Figure 21.

Example 17 Preparation of a transgenic mouse in which cDNA encoding "rat JTT-1 antigen" has been integrated

The cDNA encoding the full length of "rat JTT-1 antigen" obtained in Example 7 was inserted into the expression vector pCAGGS (Gene, Vol.108, pp.193-200 (1991)) having chicken β actin promoter using DNA Blunting Kit (Takara) to obtain plasmid, prJTT-1. In order to prepare a transgenic mouse, prJTT-1 was linearised by restriction enzyme treatment.

A female ICR mouse having a vaginal plug, obtained by mating a white ICR mouse (Nihon LSC) with a male vasoligated white ICR mouse (Nihon SLC), was used as a foster mother mouse. A mouse for obtaining fertilised eggs for introducing "rat JTT-1 antigen" gene thereinto was prepared by mating a female BDF-1 mouse (Nihon SLC) that had been made to superovulate by administered PEAMEX (5 units, Sankyo Zoki) and Pregnil (5 units, Organon) with a male BDF-1 male (Nihon SLC). After mating, the oviduct was excised from the female BDF-1 mouse, and only fertilised eggs were obtained by hyaluronidase treatment and stored in a medium.

The "rat JTT-1 antigen" gene was introduced into the fertilised egg under microscopy using a manipulator according to the usual method. The fertilised egg was fixed with a retaining needle. A solution containing the above-mentioned linearised gene encoding "rat JTT-1 antigen," which was diluted with Tris-EDTA buffer, was microinjected into the male pronucleus of the fertilised eggs with a DNA introduction needle at 37°C.

After gene introduction, only fertilised eggs keeping normal state were selected, and then, the fertilised egg so selected in which the "rat JTT-1 antigen" genes have been introduced was inserted into the ovarian fimbria in the ovary of a foster mother mouse (white ICR mouse).

The tail of a progeny mouse (founder mouse) born from the foster mother mouse was cut off and the genomic gene was collected from it. It was confirmed by PCR that the "rat JTT-1 antigen" gene was integrated into the mouse genome. Then, heterozygous transgenic mice highly expressing "rat JTT-1 antigen" were prepared by mating this founder mouse with a normal mouse. Homozygous transgenic mice can be prepared by mating the heterozygous mice with each other.

The microinjected construct comprising the "rat JTT-1 antigen" gene is schematically shown in Figure 22.

Example 18 Preparation of a knockout mouse whose endogenous gene encoding "mouse JTT-1 antigen" has been inactivated

(1) Construction of a targeting vector

A targeting vector for inactivating (knocking out) the endogenous gene encoding "mouse JTT-1 antigen" through homologous recombination (Nikkei Science, pp.52-62 May 1994) was prepared as follows.

The PstI-HindIII fragment ("homologous DNA (1)") obtained by digesting the mouse genomic DNA clone comprising the region encoding "mouse JTT-1 antigen" cloned in Example 9-5 with PstI and HindIII was subcloned into pGEM-3 (Promega). Then, pGEM-3 was linerised with XhoI, and neomycin resistance gene ("neo") excised from pMC1-neo-polyA (Stratagene) by treating it with XhoI and SalI was inserted at the upstream of the "homologous DNA" (1) and then ligated them. The above-mentioned mouse genomic DNA clone was digested with XhoI and NotI to cut off an about 5.5kb gene ("homologous DNA (2)") located upstream of above-mentioned "homologous DNA (1)." Separately, the above-mentioned pGEM-3 into which "neo-homologous DNA (1)" has been inserted was digested with XhoI and HindIII to cut off "neo-homologous DNA (1)." "Homologous DNA (2)" and "neo-homologous DNA (1)" thus obtained were subcloned into pSEAP2-CONT (Clontech) linerised with NotI and HindIII.

After the obtained plasmid, in which "homologous (2)-neo-homologous (1)" has been inserted, was digested and linerised at the downstream of "homologous DNA (1) with NruI, thymidine kinase gene ("TK") obtained by digesting pMC1-TK (Stratagene) with PvuII was inserted at the downstream of "homologous DNA (1)" to obtain a targeting vector, in which "homologous DNA (2)-neo-homologous (1)" was inserted.

(2) Introduction of the targeting vector into ES cells

Mouse embryonic stem cells (Nature, Vol.362, pp.255-258 (1993); Nature, Vol.326, pp.292-295 (1987)) cultured in DMEM medium containing 15% foetal calf serum were treated with trypsin to be single cells, and the cells were washed three times, followed by adding phosphate buffer thereto to adjust 1 x 10^7 cells/mL. The targeting vector mentioned above (25μg per 1mL of the cell suspension) was added to the cell suspension, and electric pulse was delivered once under the condition of 350V/cm (25μF). Then, 1 x 10^7 of ES cells were plated on a 10cm dish and cultivated in maintenance medium for a day, and the medium was changed to selection medium (containing 250μg/mL G418 and 2μM ganciclovir). The cells were cultivated with the medium changed every two days. At the tenth day from the introduction of the targeting vector, 573 neomycin-resistant ES cell clones were obtained under microscopy with a micropipet. Each of the ES cell clones so obtained were cultivated independently on a 24-well plate coated by Feeder cells to obtain 768 neomycin-resistant ES cell replicas.

(3) Screening of knockout ES cells

It was confirmed by PCR whether the endogenous gene encoding "mouse JTT-1 antigen" was disrupted (knocked out) through homologous recombination in each of the neomycin-resistant ES cells.

For PCR, (1) primers designed and synthesised based on the sequence of above-mentioned neomycin-resistant gene ("neo") (5'-CGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGC-3', SEQ ID NO: 11) and (2) primers designed and synthesised based on the sequence of above-mentioned "homologous DNA (1)" (5'-CATTCAAGTTTCAGGGAACTAGTCCATGCGTTTC-3', SEQ ID NO: 12) were used.

Each genomic DNA was extracted from each of the neomycin-resistant ES cell, and PCRs were performed using the primers with the genomic DNA as a template. PCR was performed 1 cycle of reaction at 94°C for 3 minutes, 30 cycles of reaction at 94°C for 1 minute, at 60°C for 1 minute, and at 72°C for 3.5 minutes, and 1 cycle of reaction at 72°C for 10 minutes, and the resulting products were stored at 4°C. When a fragment less than about 4kb was amplified by this PCR, it could be judged that the endogenous gene encoding "mouse JTT-1 antigen" was disrupted (knocked out) through homologous recombination in the ES cell clone.

A desired PCR product was obtained from three of 768 ES cell clones tested. Genomic southern blottings were performed for these three clones for further screening and confirmation. After genomic DNA was extracted from the three clones and digested with restriction enzyme BamHI, the digested products were subjected to agarose gel electrophoresis. The resulting DNAs were transferred to nylon membrane, and hybridisation was performed with a probe prepared from the genomic DNA sequence comprising "mouse JTT-1." The probe was designed based on the sequence outside the site where homologous recombination occurred, which enables to distinguish mutant type genome from normal type genome in size.

As a result, two bands corresponding to mutant type and normal type were observed in one of the three clones. This ES cell clone was used for the preparation of a knockout mouse described below.

(4) Preparation of a knockout mouse

The above-obtained ES cells (15 ES cells per blastocyst) whose endogenous gene encoding "mouse JTT-1 antigen" has been inactivated (knocked out) through homologous recombination were microinjected into blastocysts to, which were obtained by mating a female C57BL6 mouse (Nihon Charles River) with male one. Immediately after the microinjection, the blastocysts (about 10 blastocysts per one side of the uterus) were transplanted in the uterus of a foster mother ICR mouse (CLEA Japan), which was 2.5 day-mouse from pseudopregnant treatment. As a result, 38 progeny mice in total were obtained, and 18 out of them were desired chimeric mice. Eleven (11) out of the chimeric mice were the chimeric-mouse in which the contribution to hair colour was 80% or more.

The chimeric mice so obtained were then mated with a normal C57BL6 mice to obtain agouti mice whose colour is derived from hair colour gene of the ES cells.

Example 19 Preparation of pharmaceutical composition comprising antibody

Each of the monoclonal antibody (50-150μg/mL), "JTT-1 antibody" and "JTT-2 antibody" against "rat JTT-1 antigen," prepared in Example 1, and monoclonal antibodies, "SA12" and "SG430" against "human JTT-1 antigen," prepared in Example 12, was added to injectable distilled water (10mL) to prepare injection.

Industrial Applicability

Novel cell surface molecules (called "JTT-1 antigen") of the present invention derived from mammals such as human, mouse, and rat are characterised as follows.

(1) "JTT-1 antigen" had the following similarity with "CD28," a cell surface molecule on lymphocytes such as T cells, which transmits costimulatory signal important for T cell activation through cell adhesion, and "CTLA-4," a cell surface molecule on lymphocytes such as T cells, which regulates the function of activated lymphocytes such as activated T cells, cooperating with the signal.

(i) 20 or more amino acid residues including cysteine residues are highly conserved;

(ii) Prorine repeating sequence, "Pro-Pro-Pro (PPP)," which is essential as the ligand binding region, is conserved in the extracellular region;

(iii) "Tyr-Xaa-Xaa-Met (YxxM)" (Xaa and x represents any amino acid) sequence essential as the signal transmitting region is conserved in the cytoplasmic region; and

(iv) The locus of the gene encoding "mouse JTT-1 antigen" on mouse chromosome is "1C3", like "CD28" and "CTLA-4."

(2) "JTT-1 antigen" can mediate cell adhesion of thymocytes, lymphoblasts stimulated with mitogen such as ConA, thymomas, like "CD28" and "CTLA-4" that mediate cell adhesion.

(3) "JTT-1 antigen" is strongly expressed, at least, in thymocytes, lymphoblast cells stimulated with mitogen such as ConA (activated T lymphoblast cells and activated B lymphoblast cells, etc.), peripheral blood lymphocytes, and thymomas.

(4) The antibody against "JTT-1 antigen" significantly proliferates human peripheral blood lymphocytes, and the proliferation is more enhanced in the presence of a monoclonal antibody against CD3 constituting TcR/CD3 complex on T cells that receive the primary signal essential for T cell activation from antigen-presenting cells.

(5) The administration of the antibody against "JTT-1 antigen" significantly inhibits the symptom of experimental allergic encephalomyelitis (EAE).

(6) The administration of the antibody against "JTT-1 antigen" to a model rat for glomerulus basement membrane (GBM) nephritis significantly inhibits the symptom of this disease.

"JTT-1 antigen" of the present invention is, like "CD28" and "CTLA-4," thought to be a molecule transmitting the secondary signal (costimulatory signal) essential for the activation of lymphocytes such as T cells, and regulating the function of activated lymphocytes such as activated T cells, cooperating with the signal.

Therefore, polypeptides constituting such cell surface molecules, its polypeptide fragment, and fusion polypeptides therefrom, and antibodies thereto of the present invention can provide extremely useful pharmaceuticals for therapy or prevention of various autoimmune diseases, allergic diseases, or inflammatory diseases, specifically, rheumatoid arthritis, multiple sclerosis, autoimmune thyroiditis, allergic contact dermatitis, chronic inflammatory dermatosis such as lichen planus, systemic lupus erythematosus, insulin dependent diabetes mellitus, and psoriasis, caused by the activation of lymphocytes such as T cells and the abnormality of regulation of activated lymphocyte functions.

Similarly, the genes encoding polypeptides or polypeptide fragments of the present invention can be used in not only gene therapy of various diseases as mentioned above but also preparation of antisense pharmaceuticals.

Among the antibodies of the present invention, human monoclonal antibodies and their pharmaceutical compositions have dramatically increased pharmaceutical value of antibody drugs because they have no antigenicity against human, which has been a serious problem (side effect) of antibody pharmaceuticals containing nonhuman mammal-derived antibodies such as mouse-derived antibodies.

The genes (DNA), polypeptides, polypeptide fragments and antibodies of the present invention are useful not only as pharmaceuticals but also as reagents for searching molecules (ligands) interacting with the cell surface molecules of the present invention, clarifying the function of the ligand, and developing drugs targeting the ligands.

Furthermore, the transgenic mouse of the present invention is extremely useful not only as a model animal for studying physiological function of "JTT-1 antigen" that is a cell surface molecule of the present invention but also as a tool for screening various drugs (low molecular weight compounds, antibodies, antisense substances, polypeptides, etc.) having activity regulating (inhibition, suppression, activation, stimulation, etc.) the function of "JTT-1 antigen." Specifically, such test substances can be administered to the transgenic mouse to measure and analyse various physiological, biological, or pharmacological parameters generated in the mouse, thereby assessing activity of the administered test substances.

In addition, the knockout mouse of the present invention can clarify the function of the cell surface molecules of the present invention by analysing the characteristics of the mouse from various viewpoints (physiological, biological, pharmacological, pathological, and genetic viewpoints).

Sequence listing

(1) Name of applicant: JAPAN TABACCO INC.

(2) Title of the invention: Cell Surface Molecule Mediating Cell Adhesion and Signal Transmission

(3) Reference number: J1-802PCT

(4) Application number:

(5) Filing date:

(6) Country where the priority application was filed and the application number of the application: Japan, No. Hei 9-062290 Japan, Patent Application filed on February 26, 1998 (reference number, J1-802DP1)

(7) Priority date: February 27, 1997

(8) Number of sequences: 12

SEQ ID NO: 1:

SEQUENCE LENGTH: 600

SEQUENCE TYPE: nucleic acid

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE:

ORGANISM: Homo sapiens

FEATURE:

NAME/KEY: CDS

LOCATION: 1 .. 600

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION: SEQ ID NO: 1

ATG	AAG	TCA	GGC	CTC	TGG	TAT	TTC	TTT	CTC	30
Met	Lys	Ser	Gly	Leu	Trp	Tyr	Phe	Phe	Leu	
1				5					10	
TTC	TGC	TTG	CGC	ATT	AAA	GTT	TTA	ACA	GGA	60
Phe	Cys	Leu	Arg	Ile	Lys	Val	Leu	Thr	Gly	
				15					20	
GAA	ATC	AAT	GGT	TCT	GCC	AAT	TAT	GAG	ATG	90
Glu	Ile	Asn	Gly	Ser	Ala	Asn	Tyr	Glu	Met	
				25					30	
TTT	ATA	TTT	CAC	AAC	GGA	GGT	GTA	CAA	ATT	120
Phe	Ile	Phe	His	Asn	Gly	Gly	Val	Gln	Ile	
				35					40	
TTA	TGC	AAA	TAT	CCT	GAC	ATT	GTC	CAG	CAA	150
Leu	Cys	Lys	Tyr	Pro	Asp	Ile	Val	Gln	Gln	
				45					50	
TTT	AAA	ATG	CAG	TTG	CTG	AAA	GGG	GGG	CAA	180
Phe	Lys	Met	Gln	Leu	Leu	Lys	Gly	Gly	Gln	
				55					60	
ATA	CTC	TGC	GAT	CTC	ACT	AAG	ACA	AAA	GGA	210
Ile	Leu	Cys	Asp	Leu	Thr	Lys	Thr	Lys	Gly	
				65					70	
AGT	GGA	AAC	ACA	GTG	TCC	ATT	AAG	AGT	CTG	240
Ser	Gly	Asn	Thr	Val	Ser	Ile	Lys	Ser	Leu	
				75					80	
AAA	TTC	TGC	CAT	TCT	CAG	TTA	TCC	AAC	AAC	270
Lys	Phe	Cys	His	Ser	Gln	Leu	Ser	Asn	Asn	
				85					90	
AGT	GTC	TCT	TTT	TTT	CTA	TAC	AAC	TTG	GAC	300
Ser	Val	Ser	Phe	Phe	Leu	Tyr	Asn	Leu	Asp	
				95					100	
CAT	TCT	CAT	GCC	AAC	TAT	TAC	TTC	TGC	AAC	330
His	Ser	His	Ala	Asn	Tyr	Tyr	Phe	Cys	Asn	
				105					110	
CTA	TCA	ATT	TTT	GAT	CCT	CCT	CCT	TTT	AAA	360
Leu	Ser	Ile	Phe	Asp	Pro	Pro	Pro	Phe	Lys	
				115					120	
GTA	ACT	CTT	ACA	GGA	GGA	TAT	TTG	CAT	ATT	390
Val	Thr	Leu	Thr	Gly	Gly	Tyr	Leu	His	Ile	
				125					130	
TAT	GAA	TCA	CAA	CTT	TGT	TGC	CAG	CTG	AAG	420
Tyr	Glu	Ser	Gln	Leu	Cys	Cys	Gln	Leu	Lys	
				135					140	
TTC	TGG	TTA	CCC	ATA	GGA	TGT	GCA	GCC	TTT	450
Phe	Trp	Leu	Pro	Ile	Gly	Cys	Ala	Ala	Phe	
				145					150	
GTT	GTA	GTC	TGC	ATT	TTG	GGA	TGC	ATA	CTT	480
Val	Val	Val	Cys	Ile	Leu	Gly	Cys	Ile	Leu	
				155					160	

					60					
ATT	TGT	TGG	CTT	ACA	AAA	AAG	AAG	TAT	TCA	510
Ile	Cys	Trp	Leu	Thr	Lys	Lys	Lys	Tyr	Ser	
				165					170	
TCC	AGT	GTG	CAC	GAC	CCT	AAC	GGT	GAA	TAC	540
Ser	Ser	Val	His	Asp	Pro	Asn	Gly	Glu	Tyr	
				175					180	
ATG	TTC	ATG	AGA	GCA	GTG	AAC	ACA	GCC	AAA	570
Met	Phe	Met	Arg	Ala	Val	Asn	Thr	Ala	Lys	
				185					190	
AAA	TCT	AGA	CTC	ACA	GAT	GTG	ACC	CTA	TAA	600
Lys	Ser	Arg	Leu	Thr	Asp	Val	Thr	Leu		
				195						

SEQUENCE ID NO: 2:

SEQUENCE LENGTH: 199

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

ORIGINAL SOURCE:

ORGANISM: Homo sapiens

SEQUENCE DESCRIPTION: SEQ ID NO: 2

Met	Lys	Ser	Gly	Leu	Trp	Tyr	Phe	Phe	Leu
1				5					10
Phe	Cys	Leu	Arg	Ile	Lys	Val	Leu	Thr	Gly
				15					20
Glu	Ile	Asn	Gly	Ser	Ala	Asn	Tyr	Glu	Met
				25					30
Phe	Ile	Phe	His	Asn	Gly	Gly	Val	Gln	Ile
				35					40
Leu	Cys	Lys	Tyr	Pro	Asp	Ile	Val	Gln	Gln
				45					50
Phe	Lys	Met	Gln	Leu	Leu	Lys	Gly	Gly	Gln
				55					60
Ile	Leu	Cys	Asp	Leu	Thr	Lys	Thr	Lys	Gly
				65					70
Ser	Gly	Asn	Thr	Val	Ser	Ile	Lys	Ser	Leu
				75					80
Lys	Phe	Cys	His	Ser	Gln	Leu	Ser	Asn	Asn
				85					90
Ser	Val	Ser	Phe	Phe	Leu	Tyr	Asn	Leu	Asp
				95					100
His	Ser	His	Ala	Asn	Tyr	Tyr	Phe	Cys	Asn
				105					110
Leu	Ser	Ile	Phe	Asp	Pro	Pro	Pro	Phe	Lys
				115					120
Val	Thr	Leu	Thr	Gly	Gly	Tyr	Leu	His	Ile
				125					130
Tyr	Glu	Ser	Gln	Leu	Cys	Cys	Gln	Leu	Lys
				135					140
Phe	Trp	Leu	Pro	Ile	Gly	Cys	Ala	Ala	Phe
				145					150
Val	Val	Val	Cys	Ile	Leu	Gly	Cys	Ile	Leu
				155					160
Ile	Cys	Trp	Leu	Thr	Lys	Lys	Lys	Tyr	Ser

				165					170
Ser	Ser	Val	His	Asp	Pro	Asn	Gly	Glu	Tyr
				175					180
Met	Phe	Met	Arg	Ala	Val	Asn	Thr	Ala	Lys
				185					190
Lys	Ser	Arg	Leu	Thr	Asp	Val	Thr	Leu	
				195					

SEQUENCE ID NO: 3:

SEQUENCE LENGTH: 2610

SEQUENCE TYPE: nucleic acid

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acids (cDNA containing 3'- and 5'-untranslated sequences)

ORIGINAL SOURCE:

ORGANISM: Homo sapiens

FEATURE:

NAME/KEY: CDS

LOCATION: 26 .. 625

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION: SEQ ID NO: 3

GGACTGTTAA		CTGTTTCTGG		CAAAC						25
ATG	AAG	TCA	GGC	CTC	TGG	TAT	TTC	TTT	CTC	55
Met	Lys	Ser	Gly	Leu	Trp	Tyr	Phe	Phe	Leu	
				5					10	
TTC	TGC	TTG	CGC	ATT	AAA	GTT	TTA	ACA	GGA	85
Phe	Cys	Leu	Arg	Ile	Lys	Val	Leu	Thr	Gly	
				15					20	
GAA	ATC	AAT	GGT	TCT	GCC	AAT	TAT	GAG	ATG	115
Glu	Ile	Asn	Gly	Ser	Ala	Asn	Tyr	Glu	Met	
				25					30	
TTT	ATA	TTT	CAC	AAC	GGA	GGT	GTA	CAA	ATT	145
Phe	Ile	Phe	His	Asn	Gly	Gly	Val	Gln	Ile	
				35					40	
TTA	TGC	AAA	TAT	CCT	GAC	ATT	GTC	CAG	CAA	175
Leu	Cys	Lys	Tyr	Pro	Asp	Ile	Val	Gln	Gln	
				45					50	
TTT	AAA	ATG	CAG	TTG	CTG	AAA	GGG	GGG	CAA	205
Phe	Lys	Met	Gln	Leu	Leu	Lys	Gly	Gly	Gln	
				55					60	
ATA	CTC	TGC	GAT	CTC	ACT	AAG	ACA	AAA	GGA	235
Ile	Leu	Cys	Asp	Leu	Thr	Lys	Thr	Lys	Gly	
				65					70	
AGT	GGA	AAC	ACA	GTG	TCC	ATT	AAG	AGT	CTG	265
Ser	Gly	Asn	Thr	Val	Ser	Ile	Lys	Ser	Leu	
				75					80	
AAA	TTC	TGC	CAT	TCT	CAG	TTA	TCC	AAC	AAC	295
Lys	Phe	Cys	His	Ser	Gln	Leu	Ser	Asn	Asn	
				85					90	
AGT	GTC	TCT	TTT	TTT	CTA	TAC	AAC	TTG	GAC	325
Ser	Val	Ser	Phe	Phe	Leu	Tyr	Asn	Leu	Asp	
				95					100	
CAT	TCT	CAT	GCC	AAC	TAT	TAC	TTC	TGC	AAC	355
His	Ser	His	Ala	Asn	Tyr	Tyr	Phe	Cys	Asn	

				105					110	
CTA	TCA	ATT	TTT	GAT	CCT	CCT	CCT	TTT	AAA	385
Leu	Ser	Ile	Phe	Asp	Pro	Pro	Pro	Phe	Lys	
				115					120	
GTA	ACT	CTT	ACA	GGA	GGA	TAT	TTG	CAT	ATT	415
Val	Thr	Leu	Thr	Gly	Gly	Tyr	Leu	His	Ile	
				125					130	
TAT	GAA	TCA	CAA	CTT	TGT	TGC	CAG	CTG	AAG	445
Tyr	Glu	Ser	Gln	Leu	Cys	Cys	Gln	Leu	Lys	
				135					140	
TTC	TGG	TTA	CCC	ATA	GGA	TGT	GCA	GCC	TTT	475
Phe	Trp	Leu	Pro	Ile	Gly	Cys	Ala	Ala	Phe	
				145					150	
GTT	GTA	GTC	TGC	ATT	TTG	GGA	TGC	ATA	CTT	505
Val	Val	Val	Cys	Ile	Leu	Gly	Cys	Ile	Leu	
				155					160	
ATT	TGT	TGG	CTT	ACA	AAA	AAG	AAG	TAT	TCA	535
Ile	Cys	Trp	Leu	Thr	Lys	Lys	Lys	Tyr	Ser	
				165					170	
TCC	AGT	GTG	CAC	GAC	CCT	AAC	GGT	GAA	TAC	565
Ser	Ser	Val	His	Asp	Pro	Asn	Gly	Glu	Tyr	
				175					180	
ATG	TTC	ATG	AGA	GCA	GTG	AAC	ACA	GCC	AAA	595
Met	Phe	Met	Arg	Ala	Val	Asn	Thr	Ala	Lys	
				185					190	
AAA	TCT	AGA	CTC	ACA	GAT	GTG	ACC	CTA	TAA	625
Lys	Ser	Arg	Leu	Thr	Asp	Val	Thr	Leu		
				195						

TATGGAACTC	TGGCACCCAG	GCATGAAGCA	CGTTGGCCAG	TTTTCCTCAA	675
CTTGAAGTGC	AAGATTCTCT	TATTTCCGGG	ACCACGGAGA	GTCTGACTTA	725
ACTACATACA	TCTTCTGCTG	GTGTTTTGTT	CAATCTGGAA	GAATGACTGT	775
ATCAGTCAAT	GGGGATTTTA	ACAGACTGCC	TTGGTACTGC	CGAGTCCTCT	825
CAAAACAAAC	ACCCTCTTGC	AACCAGCTTT	GGAGAAAGCC	CAGCTCCTGT	875
GTGCTCACTG	GGAGTGGAAT	CCCTGTCTCC	ACATCTGCTC	CTAGCAGTGC	925
ATCAGCCAGT	AAAACAAACA	CATTTACAAG	AAAAATGTTT	TAAAGATGCC	975
AGGGGTACTG	AATCTGCAAA	GCAAATGAGC	AGCCAAGGAC	CAGCATCTGT	1025
CCGCATTTCA	CTATCATACT	ACCTCTTCTT	TCTGTAGGGR	TGAGAATTCC	1075
TCTTTTAATC	AGTCAAGGGA	GATGCTTCAA	AGCTGGRGCT	ATTTTATTTC	1125
TGAGATGTTG	ATGTGAACTG	TACATTAGTA	CATACTCAGT	ACTCTCCTTC	1175
AATTGCTGAA	CCCCAGTTGA	CCATTTTACC	AAGACTTTAG	ATGCTTTCTT	1225
GTGCCCTCAA	TTTTCTTTTT	AAAAATACTT	CTACATGACT	GCTTGACAGC	1275
CCAACAGCCA	CTCTCAATAG	AGAGCTATGT	CTTACATTCT	TTCCTCTGCT	1325
GCTCAATAGT	TTTATATATC	TATGCATACA	TATATACACA	CATATGTATA	1375
TAAAATTCAT	AATGAATATA	TTTGCCTATA	TTCTCCCTAC	AAGAATATTT	1425
TTGCTCCAGA	AAGACATGTT	CTTTTCTCAA	ATTCAGTTAA	AATGGTTTAC	1475
TTTGTTCAAG	TTAGTGGTAG	GAAACATTGC	CCGGAATTGA	AAGCAAATTT	1525
AWWTTATTAT	CCTATTTTCT	ACCATTATCT	ATGTTTTCAT	GGTGCTATTA	1575
ATTACAAGTT	TAGTTCTTTT	TGTAGATCAT	ATTAAAATTG	CAAACAAAAT	1625
CATCTTTAAT	GGGCCAGCAT	TCTCATGGGG	TAGAGCAGAA	TATTCATTTA	1675
GCCTGAAAGC	TGCAGTTACT	ATAGGTTGCT	GTCAGACTAT	ACCCATGGTG	1725
CCTCTGGGCT	TGACAGGTCA	AAATGGTCCC	CATCAGCCTG	GAGCAGCCCT	1775
CCAGACCTGG	GTGGAATTCC	AGGGTTGAGA	GACTCCCCTG	AGCCAGAGGC	1825
CACTAGGTAT	TCTTGCTCCC	AGAGGCTGAA	GTCACCCTGG	GAATCACAGT	1875
GGTCTACCTG	CATTCATAAT	TCCAGGATCT	GTGAAGAGCA	CATATGTGTC	1925
AGGGCACAAT	TCCCTCTCAT	AAAAACCACA	CAGCCTGGAA	ATTGGCCCTG	1975
GCCCTTCAAG	ATAGCCTTCT	TTAGAATATG	ATTTGGCTAG	AAAGATTCTT	2025
AAATATGTGG	AATATGATTA	TTCTTAGCTG	GAATATTTTC	TCTACTTCCT	2075

GTCTGCATGC	CCAAGGCTTC	TGAAGCAGCC	AATGTCGATG	CAACAACATT	2125
TGTAACTTTA	GGTAAACTGG	GATTATGTTG	TAGTTTAACA	TTTTGTAACT	2175
GTGTGCTTAT	AGTTTACAAG	TGAGACCCGA	TATGTCATTA	TGCATACTTA	2225
TATTATCTTA	AGCATGTGTA	ATGCTGGATG	TGTACAGTAC	AGTACWTAAC	2275
TTGTAATTTG	AATCTAGTAT	GGTGTTCTGT	TTTCAGCTGA	CTTGGACAAC	2325
CTGACTGGCT	TTGCACAGGT	GTTCCCTGAG	TTGTTTGCAG	GTTTCTGTGT	2375
GTGGGGTGGG	GTATGGGGAG	GAGAACCTTC	ATGGTGGCCC	ACCTGGCCTG	2425
GTTGTCCAAG	CTGTGCCTCG	ACACATCCTC	ATCCCAAGCA	TGGGACACCT	2475
CAAGATGAAT	AATAATTCAC	AAAATTTCTG	TGAAATCAAA	TCCAGTTTTA	2525
AGAGGAGCCA	CTTATCAAAG	AGATTTTAAC	AGTAGTAAGA	AGGCAAAGAA	2575
TAAACATTTG	ATATTCAGCA	ACTGAAAAAA	AAAAA		2610

SEQ ID NO: 4:

SEQUENCE LENGTH: 2072

SEQUENCE TYPE: nucleic acid

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acids (cDNA containing 3'- and 5'-untranslated sequences)

ORIGINAL SOURCE:

ORGANISM: Rattus

FEATURE:

NAME/KEY: CDS

LOCATION: 35 .. 637

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION: SEQ ID NO: 4

CTGGAGGGGA	AGAGTGCAGC			TGTTCCTGGC		AGAC				34
ATG	AAG	CCC	TAC	TTC	TCG	TGC	GTC	TTT	GTC	64
Met	Lys	Pro	Tyr	Phe	Ser	Cys	Val	Phe	Val	
1				5					10	
TTC	TGC	TTC	CTA	ATC	AAA	CTT	TTA	ACA	GGA	94
Phe	Cys	Phe	Leu	Ile	Lys	Leu	Leu	Thr	Gly	
				15					20	
GAA	CTC	AAT	GAC	TTG	GCC	AAT	CAC	AGG	ATG	124
Glu	Leu	Asn	Asp	Leu	Ala	Asn	His	Arg	Met	
				25					30	
TTT	TCG	TTT	CAC	GAT	GGA	GGT	GTA	CAG	ATT	154
Phe	Ser	Phe	His	Asp	Gly	Gly	Val	Gln	Ile	
				35					40	
TCT	TGT	AAC	TAC	CCT	GAG	ACT	GTC	CAG	CAG	184
Ser	Cys	Asn	Tyr	Pro	Glu	Thr	Val	Gln	Gln	
				45					50	
TTA	AAA	ATG	CAG	TTG	TTC	AAA	GAC	AGA	GAA	214
Leu	Lys	Met	Gln	Leu	Phe	Lys	Asp	Arg	Glu	
				55					60	
GTC	CTC	TGC	GAC	CTC	ACC	AAG	ACC	AAG	GGA	244
Val	Leu	Cys	Asp	Leu	Thr	Lys	Thr	Lys	Gly	
				65					70	
AGC	GGA	AAC	ACC	GTG	TCC	ATC	AAG	AAT	CCG	274
Ser	Gly	Asn	Thr	Val	Ser	Ile	Lys	Asn	Pro	
				75					80	
ATG	TCC	TGT	CCA	TAT	CAG	CTG	TCC	AAC	AAC	304
Met	Ser	Cys	Pro	Tyr	Gln	Leu	Ser	Asn	Asn	
				85					90	
AGT	GTC	TCT	TTT	TTC	CTA	GAC	AAC	GCA	GAC	334

Ser	Val	Ser	Phe	Phe	Leu	Asp	Asn	Ala	Asp	
				95					100	
AGC	TCC	CAG	GGC	AGC	TAC	TTT	TTA	TGC	AGC	364
Ser	Ser	Gln	Gly	Ser	Tyr	Phe	Leu	Cys	Ser	
				105					110	
CTG	TCG	ATT	TTC	GAC	CCA	CCC	CCT	TTT	CAA	394
Leu	Ser	Ile	Phe	Asp	Pro	Pro	Pro	Phe	Gln	
				115					120	
GAA	AAG	AAC	CTT	AGT	GGA	GGA	TAT	TTG	CTT	424
Glu	Lys	Asn	Leu	Ser	Gly	Gly	Tyr	Leu	Leu	
				125					130	
ATT	TAT	GAA	TCC	CAG	CTT	TGT	TGC	CAG	CTG	454
Ile	Tyr	Glu	Ser	Gln	Leu	Cys	Cys	Gln	Leu	
				135					140	
AAG	CTT	TGG	TTA	CCC	GTA	GGG	TGT	GCA	GCT	484
Lys	Leu	Trp	Leu	Pro	Val	Gly	Cys	Ala	Ala	
				145					150	
TTT	GTG	GCA	GCG	CTC	CTT	TTT	GGA	TGC	ATA	514
Phe	Val	Ala	Ala	Leu	Leu	Phe	Gly	Cys	Ile	
				155					160	
TTT	ATC	GTC	TGG	TTT	GCA	AAA	AAG	AAG	TAC	544
Phe	Ile	Val	Trp	Phe	Ala	Lys	Lys	Lys	Tyr	
				165					170	
AGA	TCC	AGT	GTG	CAC	GAC	CCT	AAT	AGC	GAG	574
Arg	Ser	Ser	Val	His	Asp	Pro	Asn	Ser	Glu	
				175					180	
TAC	ATG	TTC	ATG	GCG	GCA	GTC	AAC	ACA	AAC	604
Tyr	Met	Phe	Met	Ala	Ala	Val	Asn	Thr	Asn	
				185				190		
AAA	AAG	TCC	AGA	CTT	GCA	GGT	ATG	ACC	TCA	634
Lys	Lys	Ser	Arg	Leu	Ala	Gly	Met	Thr	Ser	
				195					200	
TAA										637
TCTGGAACAC		GGGAACCCAT		GGAGGAACTA		CACTGTCTAG		TTCCCCTGAA		687
ACTTGAATGG		AGAAAGTCTT		CTATTTTCTG		GACCACAGGG		CATCTGACTT		737
GATTAACTAC		TGATACCTCC		TTTTGGKGTT		TTGTTTGTCT		GGATCAGTGA		787
CTATCAGTCA		CTCGGAATTT		CAGCAGACTG		CCCTGGGTTT		GCTGAGTCCT		837
TTTAAGGCAA		ACCCCTTCTT		ATAGAAGACC		CGGCTCATAT		GTATTCAACA		887
AACAGACCTC		ACTGGGATAC		AATCCCCTCT		TTCTGCGCCT		GCTTCTAGCT		937
ATGCACCGGC		CAGCAAGACA		AACATATCTC		CAGCATTTTT		ACAAAAATGC		987
CAGGGTATGA		ATCTGTAAAG		TACACAGGCA		GCCATTGACC		ACCGTCTGTC		1037
CTCGTTTTTT		CAGATTCTAT		TTTTTTCCAT		AGAGATCAGC		ATTCCTTCTA		1087
GAATCAGACA		GTAGAGGGAG		ATGCTTCACA		ACAGAAGCTC		TTATGTTTCT		1137
GAGATGTTGA		TGAATTCATG		CTTTAGTACC		ACCATGTTCT		CTAACAACTT		1187
CTATATTCCA		GCTGATCACT		GCTTCAGGGC		TTAGATGCCT		GCTTTTGCCT		1237
TCAAGTCTCC		CCTTAAAGAT		ACTCCCACAG		GTCTACTTGG		TGGCCTGCAG		1287
CCACTCTGAA		TAGGAAGTTT		GGTCTACAAT		TTCCCCCCTC		TGCTGCTCAA		1337
AAAAAAAAAT		TAGTAGATAT		GATTTTCCCA		TATTCTCCCT		GCCAAAGTAA		1387
TTTTTTCCAG		CAAAGACATC		TAAATTCAGT		TAATATGGTT		TACTGTGTTG		1437
ATATTAGTGG		CAGTAAACAT		TTCTCAGAAT		CAAAAGCAAA		TTAATTTTGC		1487
GGTGGTGTTT		TTCTACCATT		ATCTTGGGTT		TCCATGGTGC		TATTACTCAC		1537
AAGTTTAGCT		ATTTTTTTAT		GCATCATATT		AAAGTTGCAA		GCAAGCAGAG		1587
CAACCCTCGG		TTAATGGGCA		AACATTCTCC		TGGGGTAGAA		TGAATTGTCT		1637
ATTTAGCCCG		AAAACTGCAG		TTTCTGTGGG		TGGCTGCCAG		ACTACAGCCG		1687
TGCTTTGCTC		TGGCTTTGAC		AGGTTGAAAT		AGYCCCCATG		ASCSTGGAAC		1737
AGWACTCCAG		ACTGTGCTGG		AGTCCCAAAG		TTAGGAGGGC		CATGGAGCCT		1787
GGGACAGGCT		GCTGCTTTGG		TCTTTAGGAT		CTAGGAARAA		TTACAGAGGG		1837

65

GCCAAGACAG	AGTTCCCTCC	CCTAGAAACT	GTGCAGCCTG	GAAGTCAGCC	1887
CTGGCACTTT	AAGATAGCCT	TCTTTAGAAC	ATGAGTTAGT	TGGTAGTATT	1937
CTGACGTGTA	AACAGCCTAT	KGTTGCTCGG	AGCTGGACCA	TTTTCTCCAC	1987
TTCCCTGTCT	GCATGCCTAA	GACTTCTAGA	GCAGCCAACG	TATATGCAAC	2037
ATTAAAGAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAA		2072

SEQ ID NO: 5:

SEQUENCE LENGTH: 603

SEQUENCE TYPE: nucleic acid

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE:

ORGANISM: Mus

FEATURE:

NAME/KEY: CDS

LOCATION: 1 .. 603

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION: SEQ ID NO: 5

ATG	AAG	CCG	TAC	TTC	TGC	CAT	GTC	TTT	GTC	30
Met	Lys	Pro	Tyr	Phe	Cys	His	Val	Phe	Val	
1				5					10	
TTC	TGC	TTC	CTA	ATC	AGA	CTT	TTA	ACA	GGA	60
Phe	Cys	Phe	Leu	Ile	Arg	Leu	Leu	Thr	Gly	
				15					20	
GAA	ATC	AAT	GGC	TCG	GCC	GAT	CAT	AGG	ATG	90
Glu	Ile	Asn	Gly	Ser	Ala	Asp	His	Arg	Met	
				25					30	
TTT	TCA	TTT	CAC	AAT	GGA	GGT	GTA	CAG	ATT	120
Phe	Ser	Phe	His	Asn	Gly	Gly	Val	Gln	Ile	
				35					40	
TCT	TGT	AAA	TAC	CCT	GAG	ACT	GTC	CAG	CAG	150
Ser	Cys	Lys	Tyr	Pro	Glu	Thr	Val	Gln	Gln	
				45					50	
TTA	AAA	ATG	CGA	TTG	TTC	AGA	GAG	AGA	GAA	180
Leu	Lys	Met	Arg	Leu	Phe	Arg	Glu	Arg	Glu	
				55					60	
GTC	CTC	TGC	GAA	CTC	ACC	AAG	ACC	AAG	GGA	210
Val	Leu	Cys	Glu	Leu	Thr	Lys	Thr	Lys	Gly	
				65					70	
AGC	GGA	AAT	GCG	GTG	TCC	ATC	AAG	AAT	CCA	240
Ser	Gly	Asn	Ala	Val	Ser	Ile	Lys	Asn	Pro	
				75					80	
ATG	CTC	TGT	CTA	TAT	CAT	CTG	TCA	AAC	AAC	270
Met	Leu	Cys	Leu	Tyr	His	Leu	Ser	Asn	Asn	
				85					90	
AGC	GTC	TCT	TTT	TTC	CTA	AAC	AAC	CCA	GAC	300
Ser	Val	Ser	Phe	Phe	Leu	Asn	Asn	Pro	Asp	
				95					100	
AGC	TCC	CAG	GGA	AGC	TAT	TAC	TTC	TGC	AGC	330
Ser	Ser	Gln	Gly	Ser	Tyr	Tyr	Phe	Cys	Ser	
				105					110	
CTG	TCC	ATT	TTT	GAC	CCA	CCT	CCT	TTT	CAA	360
Leu	Ser	Ile	Phe	Asp	Pro	Pro	Pro	Phe	Gln	

				115					120	
GAA	AGG	AAC	CTT	AGT	GGA	GGA	TAT	TTG	CAT	390
Glu	Arg	Asn	Leu	Ser	Gly	Gly	Tyr	Leu	His	
				125					130	
ATT	TAT	GAA	TCC	CAG	CTC	TGC	TGC	CAG	CTG	420
Ile	Tyr	Glu	Ser	Gln	Leu	Cys	Cys	Gln	Leu	
				135					140	
AAG	CTC	TGG	CTA	CCC	GTA	GGG	TTG	CCA	GCT	450
Lys	Leu	Trp	Leu	Pro	Val	Gly	Leu	Pro	Ala	
				145					150	
TTC	GTT	GTG	GTA	CTC	CTT	TTT	GGA	TGC	ATA	480
Phe	Val	Val	Val	Leu	Leu	Phe	Gly	Cys	Ile	
				155					160	
CTT	ATC	ATC	TGG	TTT	TCA	AAA	AAG	AAA	TAC	510
Leu	Ile	Ile	Trp	Phe	Ser	Lys	Lys	Lys	Tyr	
				165					170	
GGA	TCC	AGT	GTG	CAT	GAC	CCT	AAT	AGT	GAA	540
Gly	Ser	Ser	Val	His	Asp	Pro	Asn	Ser	Glu	
				175					180	
TAC	ATG	TTC	ATG	GCG	GCA	GTC	AAC	ACA	AAC	570
Tyr	Met	Phe	Met	Ala	Ala	Val	Asn	Thr	Asn	
				185					190	
AAA	AAG	TCT	AGA	CTT	GCA	GGT	GTG	ACC	TCA	600
Lys	Lys	Ser	Arg	Leu	Ala	Gly	Val	Thr	Ser	
				195					200	
TAA										603

SEQ ID NO: 6:

SEQUENCE LENGTH: 836

SEQUENCE TYPE: nucleic acid

TOPOLOGY: linear

MOLECULE TYPE: other nucleic acids (cDNA containing 3'- and 5'-untranslated sequences)

ORIGINAL SOURCE:

ORGANISM: Rattus

FEATURE:

NAME/KEY: CDS

LOCATION: 35 .. 685

IDENTIFICATION METHOD: E

SEQUENCE DESCRIPTION: SEQ ID NO: 6

CTGGAGGGGA		AGAGTGCAGC		TGTTCCTGGC		AGAC				34
ATG	AAG	CCC	TAC	TTC	TCG	TGC	GTC	TTT	GTC	64
Met	Lys	Pro	Tyr	Phe	Ser	Cys	Val	Phe	Val	
1				5					10	
TTC	TGC	TTC	CTA	ATC	AAA	CTT	TTA	ACA	GGA	94
Phe	Cys	Phe	Leu	Ile	Lys	Leu	Leu	The	Gly	
				15					20	
GAA	CTC	AAT	GAC	TTG	GCC	AAT	CAC	AGG	ATG	124
Glu	Leu	Asn	Asp	Leu	Ala	Asn	His	Arg	Met	
				25					30	
TTT	TCG	TTT	CAC	GAT	GGA	GGT	GTA	CAG	ATT	154
Phe	Ser	Phe	His	Asp	Gly	Gly	Val	Gln	Ile	
				35					40	
TCT	TGT	AAC	TAC	CCT	GAG	ACT	GTC	CAG	CAG	184

Ser	Cys	Asn	Tyr	Pro	Glu	Thr	Val	Gln	Gln	
				45					50	
TTA	AAA	ATG	CAG	TTG	TTC	AAA	GAC	AGA	GAA	214
Leu	Lys	Met	Gln	Leu	Phe	Lys	Asp	Arg	Glu	
				55					60	
GTC	CTC	TGC	GAC	CTC	ACC	AAG	ACC	AAG	GGA	244
Val	Leu	Cys	Asp	Leu	Thr	Lys	Thr	Lys	Gly	
				65					70	
AGC	GGA	AAC	ACC	GTG	TCC	ATC	AAG	AAT	CCG	274
Ser	Gly	Asn	Thr	Val	Ser	Ile	Lys	Asn	Pro	
				75					80	
ATG	TCC	TGT	CCA	TAT	CAG	CTG	TCC	AAC	AAC	304
Met	Ser	Cys	Pro	Tyr	Gln	Leu	Ser	Asn	Asn	
				85					90	
AGT	GTC	TCT	TTT	TTC	CTA	GAC	AAC	GCA	GAC	334
Ser	Val	Ser	Phe	Phe	Leu	Asp	Asn	Ala	Asp	
				95					100	
AGC	TCC	CAG	GGC	AGC	TAC	TTT	TTA	TGC	AGC	364
Ser	Ser	Gln	Gly	Ser	Tyr	Phe	Leu	Cys	Ser	
				105					110	
CTG	TCG	ATT	TTC	GAC	CCA	CCC	CCT	TTT	CAA	394
Leu	Ser	Ile	Phe	Asp	Pro	Pro	Pro	Phe	Gln	
				115					120	
GAA	AAG	AAC	CTT	AGT	GGA	GGA	TAT	TTG	CTT	424
Glu	Lys	Asn	Leu	Ser	Gly	Gly	Tyr	Leu	Leu	
				125					130	
ATT	TAT	GAA	TCC	CAG	CTT	TGT	TGC	CAG	CTG	454
Ile	Tyr	Glu	Ser	Gln	Leu	Cys	Cys	Gln	Leu	
				135					140	
AAG	CTT	TGG	TTA	CCC	GTA	GGG	TGT	GCA	GCT	484
Lys	Leu	Trp	Leu	Pro	Val	Gly	Cys	Ala	Ala	
				145					150	
TTT	GTG	GCA	GCG	CTC	CTT	TTT	GGA	TGC	ATA	514
Phe	Val	Ala	Ala	Leu	Leu	Phe	Gly	Cys	Ile	
				155					160	
TTT	ATC	GTC	TGG	TTT	GCA	AAA	AAG	AAG	TAC	544
Phe	Ile	Val	Trp	Phe	Ala	Lys	Lys	Lys	Tyr	
				165					170	
AGA	TCC	AGT	GTG	CAC	GAC	CCT	AAT	AGC	GAG	574
Arg	Ser	Ser	Val	His	Asp	Pro	Asn	Ser	Glu	
				175					180	
TAC	ATG	TTC	ATG	GCG	GCA	GTC	AAC	ACA	AAC	604
Tyr	Met	Phe	Met	Ala	Ala	Val	Asn	Thr	Asn	
				185					190	
AAA	AAG	TCC	AGA	CTT	GCA	GGT	ACA	GCA	CCC	634
Lys	Lys	Ser	Arg	Leu	Ala	Gly	Thr	Ala	Pro	
				195					200	
CTT	AGG	GCT	TTG	GGG	AGA	GGA	GAA	CAC	TCT	664
Leu	Arg	Ala	Leu	Gly	Arg	Gly	Glu	His	Ser	
				205					210	
TCA	TGT	CAA	GAC	CGG	AAT	TAA	685			
Ser	Cys	Gln	Asp	Arg	Asn					
				215						
TTTGTTTATT		TCTATTTTAA		AAGAAAGACA		TTTTTTCCCC		TAAAGATAAT		735
TTTTGTATTT		TTATGTGAAA		GTCTGAATCT		TCATTTTAAC		TCGACTTATA		785
TACTCTGTGG		TATATTAAAA		ATAATGTTTG		TGAAAAAAAA		AAAAAAAAAA		835
A										836

SEQ ID NO: 7:
SEQUENCE LENGTH: 27
SEQUENCE TYPE: nucleic acid
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acids (synthetic DNA)
FEATURE:
NAME/KEY: primer bind
LOCATION: 1 .. 27
IDENTIFICATION METHOD: E
SEQUENCE DESCRIPTION: SEQ ID NO: 7
CTGCTCGAGA TGAAGCCCTA CTTCTCG 27
SEQ ID NO: 8:
SEQUENCE LENGTH: 32
SEQUENCE TYPE: nucleic acid
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acids (synthetic DNA)
FEATURE:
NAME/KEY: primer bind
LOCATION: 1 .. 32
IDENTIFICATION METHOD: E
SEQUENCE DESCRIPTION: SEQ ID NO: 8
ACCCTACGGG TAACGGATCC TTCAGCTGGC AA 32
SEQ ID NO: 9:
SEQUENCE LENGTH: 30
SEQUENCE TYPE: nucleic acid
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
FEATURE:
NAME/KEY: primer bind
LOCATION: 1 .. 30
IDENTIFICATION METHOD: E
SEQUENCE DESCRIPTION: SEQ ID NO: 9
TAACTGTTTC TCGAGAACAT GAAGTCAGGC 30
SEQ ID NO: 10:
SEQUENCE LENGTH: 30
SEQUENCE TYPE: nucleic acid
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
FEATURE:
NAME/KEY: primer bind

LOCATION: 1 .. 30
IDENTIFICATION METHOD: E
SEQUENCE DESCRIPTION: SEQ ID NO: 10
ATCCTATGGG TAACGGATCC TTCAGCTGGC 30

SEQ ID NO: 11:
SEQUENCE LENGTH: 35
SEQUENCE TYPE: nucleic acid
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
FEATURE:
NAME/KEY: primer bind
LOCATION: 1 .. 35
IDENTIFICATION METHOD: E
SEQUENCE DESCRIPTION: SEQ ID NO: 11
CGTGATATTG CTGAAGAGCT TGGCGGCGAA TGGGC 35

SEQ ID NO: 12:
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SEQUENCE TYPE: nucleic acid
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid (synthetic DNA)
FEATURE:
NAME/KEY: primer bind
LOCATION: 1 .. 34
IDENTIFICATION METHOD: E
SEQUENCE DESCRIPTION: SEQ ID NO: 12
CATTCAAGTT TCAGGGAACT AGTCCATGCG TTTC 34

The claims defining the invention are as follows:

1. An isolated polypeptide constituting a cell surface molecule having the following characteristics:

(a) said cell surface molecule is expressed in at least thymocytes and mitogen-stimulated lymphoblast cells;

(b) a specific antibody reactive to said cell surface molecule induces adhesion between mitogen-stimulated lymphoblast cells;

(c) a specific antibody reactive to said cell surface molecule induces proliferation of peripheral blood lymphocytes in the presence of an antibody against CD3;

(d) said cell surface molecule has a partial amino acid sequence represented by Phe-Asp-Pro-Pro-Pro-Phe in its extracellular region; and

(e) said cell surface molecule has a partial amino acid sequence represented by Tyr-Met-Phe-Met in its cytoplasmic region.

2. The polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO: 2 or the amino acid sequence of SEQ ID NO: 2 in which one or more amino acids are substituted, deleted, or added.

3. The polypeptide of claim 1, which is encoded by a DNA hybridising with a DNA having the nucleotide sequence of SEQ ID NO: 1 under stringent conditions.

4. The polypeptide of claim 1, comprising an amino acid sequence having 60% or more homology with an amino acid sequence of SEQ ID NO: 2.

5. The polypeptide of any one of claims 1 to 4, wherein said cell surface molecule is derived from human.

6. An isolated polypeptide constituting a cell surface molecule, said polypeptide being substantially as hereinbefore described with reference to any one of the examples.

7. An isolated nucleotide sequence encoding the polypeptide of any one of claims 1 to 6.

8. The nucleotide sequence of claim 7, wherein said nucleotide sequence is a cDNA.

9. The nucleotide sequence of claim 8, wherein said cDNA has a nucleotide sequence of SEQ ID NO: 1.

10. The nucleotide sequence of claim 8, wherein said cDNA comprises a nucleotide sequence corresponding to nucleotide residues 26 to 625 of SEQ ID NO: 3, nucleotide residues 35 to 637 of SEQ ID NO: 4, nucleotide residues 1 to 603 of SEQ ID NO: 5, or nucleotide residues 35 to 685 of SEQ ID NO: 6.

11. An isolated nucleotide sequence encoding a polypeptide constituting a cell surface molecule, substantially as hereinbefore described with reference to any one of the examples.

12. A vector comprising the nucleotide sequence of any one of claims 7 to 11.

13. A vector comprising a nucleotide sequence encoding a polypeptide constituting a cell surface molecule, substantially as hereinbefore described with reference to any one of the examples.

14. A method for transforming cells or organisms to express a polypeptide constituting a cell surface molecule, said method comprising transforming said cells or organisms with the nucleotide sequence of any one of claims 7 to 11, or the vector of claim 12 or claim 13.

15. A method for transforming cells or organisms with a nucleotide sequence encoding a polypeptide constituting a cell surface molecule, said method being substantially as hereinbefore described with reference to any one of the examples.

16. A transformant into which the vector of claim 12 or claim 13 has been introduced.

17. A transformant comprising the nucleotide sequence of any one of claims 7 to 11.

18. Transformed cells or organisms obtained by the method of claim 14 or claim 15.

19. A transformant identified by an international deposit accession No. FERM BP-5725.

20. Cells or organisms transformed with a nucleotide sequence encoding a polypeptide constituting a cell surface molecule, substantially as hereinbefore described with reference to any one of the examples.

21. A method for preparing a polypeptide constituting a cell surface molecule, said method comprising culturing the transformant of any one of claims 16 to 20 under conditions conducive to expression of said polypeptide.

22. The method of claim 21, further comprising isolating/purifying said polypeptide from said culture.

23. A method for preparing a polypeptide constituting a cell surface molecule, said method being substantially as hereinbefore described with reference to any one of the examples.

24. A polypeptide constituting a cell surface molecule prepared by the method of any one of claims 21 to 23.

25. A polypeptide fragment comprising an extracellular region of the polypeptide of any one of claims 1 to 6 or 24.

26. A polypeptide fragment comprising an extracellular region of a polypeptide constituting a cell surface molecule, substantially as hereinbefore described with reference to any one of the examples.

27. The polypeptide fragment of claim 25 or claim 26, wherein said polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

28. An isolated nucleotide sequence encoding the polypeptide fragment of any one of claims 25 to 27.

29. An isolated nucleotide sequence encoding a polypeptide fragment comprising an extracellular region of a cell surface molecule, substantially as hereinbefore described with reference to any one of the examples.

30. A vector comprising the nucleotide sequence of claim 28 or claim 29.

31. A method for transforming cells or organisms to express a polypeptide fragment comprising an extracellular region of a cell surface molecule, said method comprising transforming said cells or organisms with the nucleotide sequence of claim 28 or claim 29, or the vector of claim 30.

32. Transformed cells or organisms comprising the nucleotide sequence of claim 28 or claim 29, or the vector of claim 30.

33. Transformed cells or organisms obtained by the method of claim 31.

34. A method for preparing a polypeptide fragment comprising an extracellular region of a cell surface molecule, said method comprising culturing the transformed cells or organisms of claim 32 or claim 33 under conditions conducive to expression of said polypeptide fragment.

35. The method of claim 34, further comprising isolation and/or purification of said polypeptide fragment from said culture and/or culture supernatant.

36. A polypeptide fragment comprising an extracellular region of a cell surface molecule, prepared by the method of claim 34 or claim 35.

37. The polypeptide fragment of claim 36, wherein said polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

38. A homodimer molecule comprising two polypeptide fragments, wherein each of the fragments comprises an extracellular region of the polypeptide of any one of claims 1 to 6 or 24 and said two polypeptide fragments are bridged through disulfide bonds to each other.

39. The homodimer molecule of claim 37, wherein said polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

40. A fusion polypeptide comprising an extracellular region of the polypeptide of any one of claims 1 to 6 or 24 and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region.

41. The fusion polypeptide of claim 40, wherein the immunoglobulin is IgG.

42. The fusion polypeptide of claim 40, wherein the portion of the constant region comprises a hinge region, C2 domain, and C3 domain of IgG.

43. A fusion polypeptide comprising an extracellular region of a polypeptide constituting a cell surface molecule and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, substantially as hereinbefore described with reference to any one of the examples.

44. The fusion polypeptide of any one of claims 40 to 43, wherein said polypeptide of any one of claims 1 to 6 or 24 is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

45. A recombinant DNA molecule incorporating a nucleotide sequence according to claim 28 or claim 29, and further comprising a nucleotide sequence encoding a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region.

46. A recombinant DNA molecule incorporating a nucleotide sequence encoding a polypeptide fragment comprising an extracellular region of a cell surface molecule and a nucleotide sequence encoding a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, substantially as hereinbefore described with reference to any one of the examples.

47. A vector comprising the nucleotide sequence of claim 45 or claim 46.

48. A vector comprising a recombinant DNA molecule incorporating a nucleotide sequence encoding a polypeptide fragment comprising an extracellular region of a cell surface molecule and a nucleotide sequence encoding a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, substantially as hereinbefore described with reference to any one of the examples.

49. A method for transforming cells or organisms to express a fusion polypeptide incorporating a polypeptide fragment comprising an extracellular region of a cell surface molecule and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, said method comprising transforming said cells or organisms with the recombinant DNA molecule of claim 45 or claim 46, or the vector of claim 47 or claim 48.

50. A method for transforming cells or organisms to express a fusion polypeptide incorporating a polypeptide fragment comprising an extracellular region of a cell surface molecule and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, said method being substantially as hereinbefore described with reference to any one of the examples.

51. Transformed cells or organisms comprising the nucleotide sequence of claim 45 or claim 46, or the vector of claim 47 or claim 48.

52. Transformed cells or organisms obtained by the method of claim 49 or claim 50.

53. Transformed cells or organisms transformed to express a fusion polypeptide incorporating a polypeptide fragment comprising an extracellular region of a cell surface molecule and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, substantially as hereinbefore described with reference to any one of the examples.

54. A method for preparing a fusion polypeptide incorporating an extracellular region of a cell surface molecule and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, said method comprising culturing the transformed cells or organisms of any one of claims 51 to 53 under conditions conducive to expression of said fusion polypeptide.

55. The method of claim 54, further comprising isolation and/or purification of said fusion polypeptide from said culture and/or culture supernatant.

56. A method for preparing a fusion polypeptide comprising an extracellular region of a cell surface molecule and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, substantially as hereinbefore described with reference to any one of the examples.

57. A fusion polypeptide comprising an extracellular region of a cell surface molecule and a constant region of a human immunoglobulin (Ig) heavy chain or a portion of the constant region, obtained by the method of any one of claims 54 to 56.

58. The fusion polypeptide of claim 57, wherein said polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

59. A homodimer molecule comprising two fusion polypeptides of any one of claims 40 to 44, 57 or 58, wherein the two polypeptides are bridged through disulfide bonds to each other.

60. A homodimer molecule comprising two fusion polypeptides of claim 44 or claim 58, wherein the two polypeptides are bridged through disulfide bonds to each other.

61. A pharmaceutical composition comprising either the polypeptide fragment of claim 27 or claim 37, or the homodimer molecule of claim 39, or both said polypeptide fragment and said homodimer molecule, and a pharmaceutically acceptable carrier.

62. A pharmaceutical composition comprising either the fusion polypeptide of claim 44 or claim 58 or the homodimer molecule of claim 60, or both said polypeptide fragment and said homodimer molecule, and a pharmaceutically acceptable carrier.

63. The pharmaceutical composition of claim 61, wherein said pharmaceutical composition is utilised for treating autoimmune diseases or allergic diseases, or for preventing said disease symptom.

64. The pharmaceutical composition of claim 62, wherein said pharmaceutical composition is utilised for treating autoimmune diseases or allergic diseases, or for preventing said disease symptom.

65. An antibody or a portion thereof reactive to the polypeptide of any one of claims 1 to 6 or 24, the polypeptide fragment of any one of claims 25 to 27, 36 or 37, or the cell surface molecule comprising said polypeptide.

66. The antibody of claim 65 or a portion thereof, wherein said antibody is a monoclonal antibody.

67. A monoclonal antibody or a portion thereof reactive to the polypeptide having an amino acid sequence of SEQ ID NO: 2, the polypeptide fragment of claim 27 or claim 37, or the human-derived cell surface molecule comprising said polypeptide.

68. A monoclonal antibody or a portion thereof reactive to the polypeptide of any one of claims 1 to 6 or 24, or the cell surface molecule comprising said polypeptide, wherein the effect of said monoclonal antibody on mitogen-stimulated lymphoblast cells is substantially the same as the effect of a monoclonal antibody produced by a hybridoma identified by an international deposit accession No. FERM BP-5707 on mitogen-stimulated rat lymphoblast cells.

69. A monoclonal antibody or a portion thereof reactive to the polypeptide of any one of claims 1 to 6 or 24, or the cell surface molecule comprising said polypeptide, wherein the effect of said monoclonal antibody on mitogen-stimulated lymphoblast cells is substantially the same as the effect of a monoclonal antibody produced by a hybridoma identified by an international deposit accession No. FERM BP-5708 on mitogen-stimulated rat lymphoblast cells.

70. A process for preparing a monoclonal antibody or a portion thereof reactive to the polypeptide having an amino acid sequence of SEQ ID NO: 2, the polypeptide fragment as defined in claim 27 or claim 37, or the human-derived cell surface molecule comprising said polypeptide, substantially as hereinbefore described with reference to any one of the examples.

71. A monoclonal antibody or a portion thereof, prepared by the process of claim 70.

72. A pharmaceutical composition comprising the monoclonal antibody of claim 67 or claim 71, or a portion thereof and a pharmaceutically acceptable carrier.

73. The pharmaceutical composition of claim 72, wherein said pharmaceutical composition is utiiised for treating autoimmune diseases or allergic diseases, or for preventing said disease symptom.

74. A pharmaceutical composition comprising a monoclonal antibody or a portion thereof reactive to the polypeptide having an amino acid sequence of SEQ ID NO: 2, the polypeptide

fragment as defined in claim 27 or claim 37, or the human-derived cell surface molecule comprising said polypeptide, substantially as hereinbefore described with reference to any one of the examples.

75. A pharmaceutical composition comprising the nucleotide sequence of any one of claims 6 to 11, 28 or 29, or the vector of any one of claims 12, 13, 30 or 31, and a pharmaceutically acceptable carrier.

76. The pharmaceutical composition of claim 75, wherein the polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

77. The pharmaceutical composition of claim 75, wherein the polypeptide is encoded by the nucleotide sequence set forth in SEQ ID NO: 1.

78. The pharmaceutical composition of claim 75, wherein the nucleotide sequence is the sequence set forth in SEQ ID NO: 1, or a portion thereof encoding a polypeptide fragment comprising an extracellular region of a cell surface molecule.

79. A pharmaceutical composition comprising an antisense nucleotide sequence which is the antisense equivalent of the nucleotide sequence of any one of claims 6 to 11, 28 or 29, or a vector comprising said antisense nucleotide sequence, and a pharmaceutically acceptable carrier.

80. The pharmaceutical composition of claim 79, wherein the polypeptide is a human-derived polypeptide having an amino acid sequence of SEQ ID NO: 2.

81. The pharmaceutical composition of claim 79, wherein the polypeptide is encoded by the nucleotide sequence set forth in SEQ ID NO: 1.

82. The pharmaceutical composition of claim 79, wherein the antisense nucleotide sequence is antisense to the sequence set forth in SEQ ID NO: 1, or a portion thereof encoding a polypeptide fragment comprising an extracellular region of a cell surface molecule.

83. A method for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom, said method comprising administering to said patient a therapeutically or prophylactically effective amount of the polypeptide fragment of claim 27 or claim 37, the homodimer of claim 39, or the pharmaceutical composition of claim 61 or claim 63.

84. The polypeptide fragment of claim 27 or claim 37, the homodimer of claim 39, or the pharmaceutical composition of claim 61 or claim 63, when used for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

85. Use of the polypeptide fragment of claim 27 or claim 37, or the homodimer of claim 39 for the manufacture of a medicament for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

86. A method for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom, said method comprising administering to said patient a therapeutically or prophylactically effective amount of the fusion polypeptide of claim 44 or claim 58, the homodimer of claim 60, or the pharmaceutical composition of claim 62 or claim 64.

87. The fusion polypeptide of claim 44 or claim 58, the homodimer of claim 60, or the pharmaceutical composition of claim 62 or claim 64, when used for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

88. Use of the fusion polypeptide of claim 44 or claim 58, or the homodimer of claim 60 for the manufacture of a medicament for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

89. A method for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom, said method comprising administering to said patient a therapeutically or prophylactically effective amount of the antibody of any one of claims 65 to 69 or 71, or the pharmaceutical composition of any one of claims 72 to 74.

90. The antibody of any one of claims 65 to 69 or 71, or the pharmaceutical composition of any one of claims 72 to 74, when used for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

91. Use of the antibody of any one of claims 65 to 69 or 71 for the manufacture of a medicament for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

92. A method for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom, said method comprising administering to said patient a therapeutically or prophylactically effective amount of the nucleotide sequence of any one of claims 7 to 11, 28 or 29, the vector of any one of claims 12, 13, or 30, or the pharmaceutical composition of any one of claims 75 to 78.

93. The nucleotide sequence of any one of claims 7 to 11, 28 or 29, the vector of any one of claims 12, 13, or 30, or the pharmaceutical composition of any one of claims 75 to 78, when used for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

94. Use of the nucleotide sequence of any one of claims 7 to 11, 28 or 29, or the vector of any one of claims 12, 13, or 30 for the manufacture of a medicament for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

95. A method for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom, said method comprising administering to said patient a therapeutically or prophylactically effective amount of an antisense nucleotide sequence which is the antisense equivalent of the nucleotide sequence of any one of claims 6 to 11, 28 or 29, or a vector comprising said antisense nucleotide sequence, or the pharmaceutical composition of any one of claims 79 to 82.

96. An antisense equivalent of the nucleotide sequence of any one of claims 6 to 11, 28 or 29, or a vector comprising said antisense nucleotide sequence, or the pharmaceutical composition of any one of claims 79 to 82, when used for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

97. Use of an antisense equivalent of the nucleotide sequence of any one of claims 6 to 11, 28 or 29, or a vector comprising said antisense nucleotide sequence for the manufacture of a medicament for treating an autoimmune disease or allergic disease in a patient, or for preventing said disease symptom.

98. A hybridoma producing the monoclonal antibody of any one of claims 66 to 69 or 71.

99. A transgenic mouse in which a gene encoding the polypeptide of claim 1 is integrated into its endogenous gene, wherein said gene is a human-derived gene comprising a nucleotide sequence of SEQ ID NO: 1 or a rat-derived gene comprising a nucleotide sequence corresponding to nucleotide residues 35 to 637 of SEQ ID NO: 4.

100. A method for producing a transgenic mouse, substantially as hereinbefore described with reference to any one of the examples.

101. A transgenic mouse prepared by the method of claim 100.

102. A knockout mouse in which its endogenous gene encoding the mouse polypeptide of claim 1 comprising the amino acid sequence encoded by the gene of SEQ ID NO: 5 is inactivated so that said mouse polypeptide is not produced.

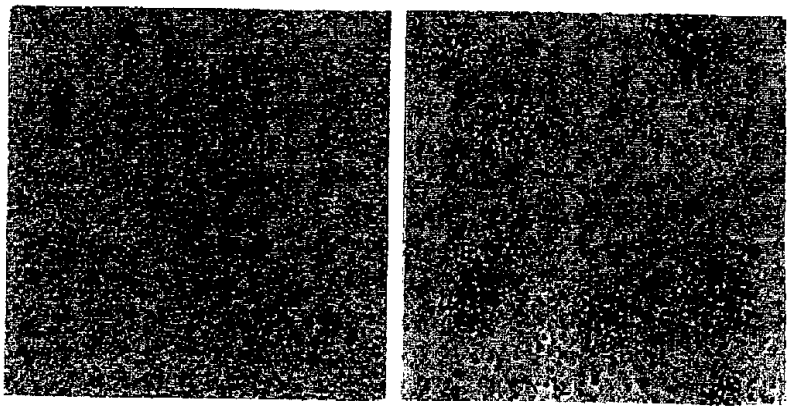
103. A method for producing a knockout mouse, substantially as hereinbefore described with reference to any one of the examples.

104. A knockout mouse prepared by the method of claim 103.

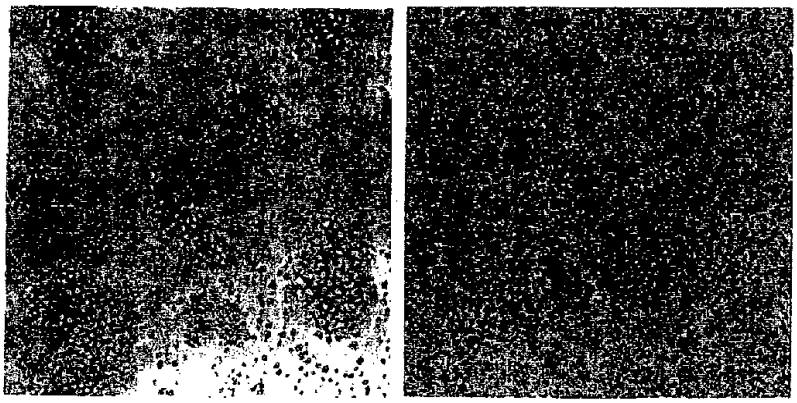
20 February 2001
JAPAN TOBACCO INC.

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

Figure 1



(a) (b)



(c) (d)

Figure 2

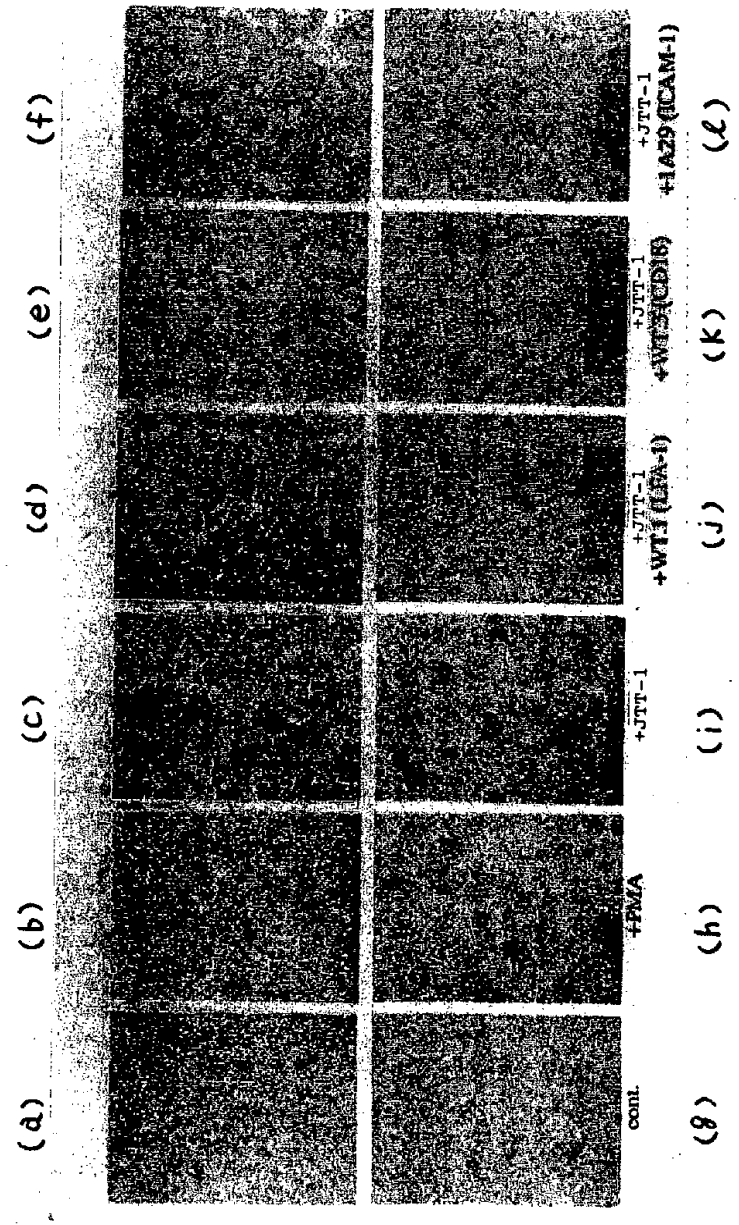

(f)
(e)
(d)
(c)
(b)
(a)
+JTT-1
+1A29 (ICAM-1)
+JTT-1
+WT3(CD18)
+JTT-1
+WT1 (LFA-1)
+JTT-1
+PMA
cont.
(ℓ)
(k)
(j)
(i)
(h)
(g)
FTL435
ConA-stimulated
lymphoblasts

Figure 3

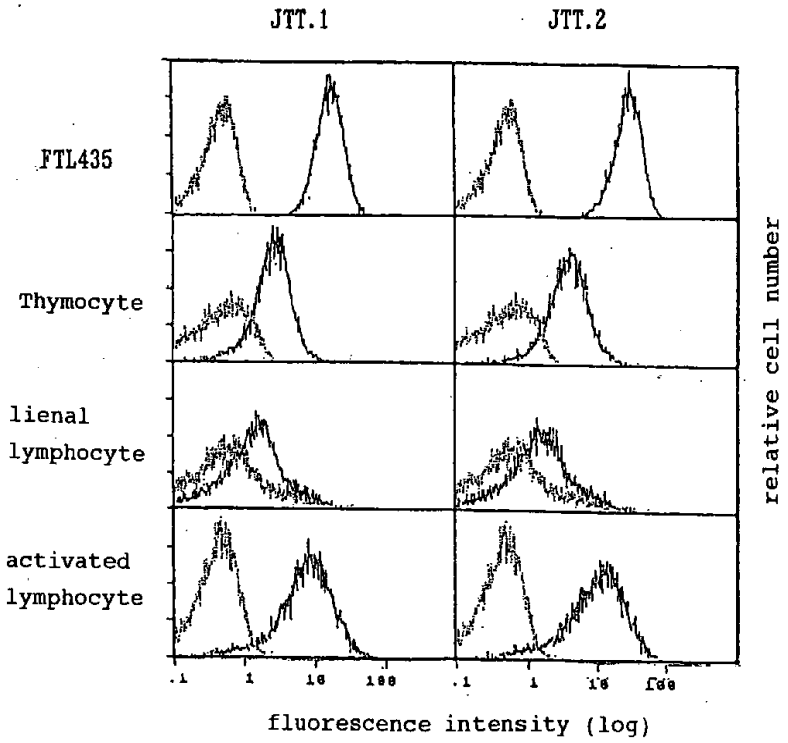

JTT.1
JTT.2
FTL435
Thymocyte
lienal lymphocyte
activated lymphocyte
relative cell number
.1
1
10
100
fluorescence intensity (log)

Figure 4

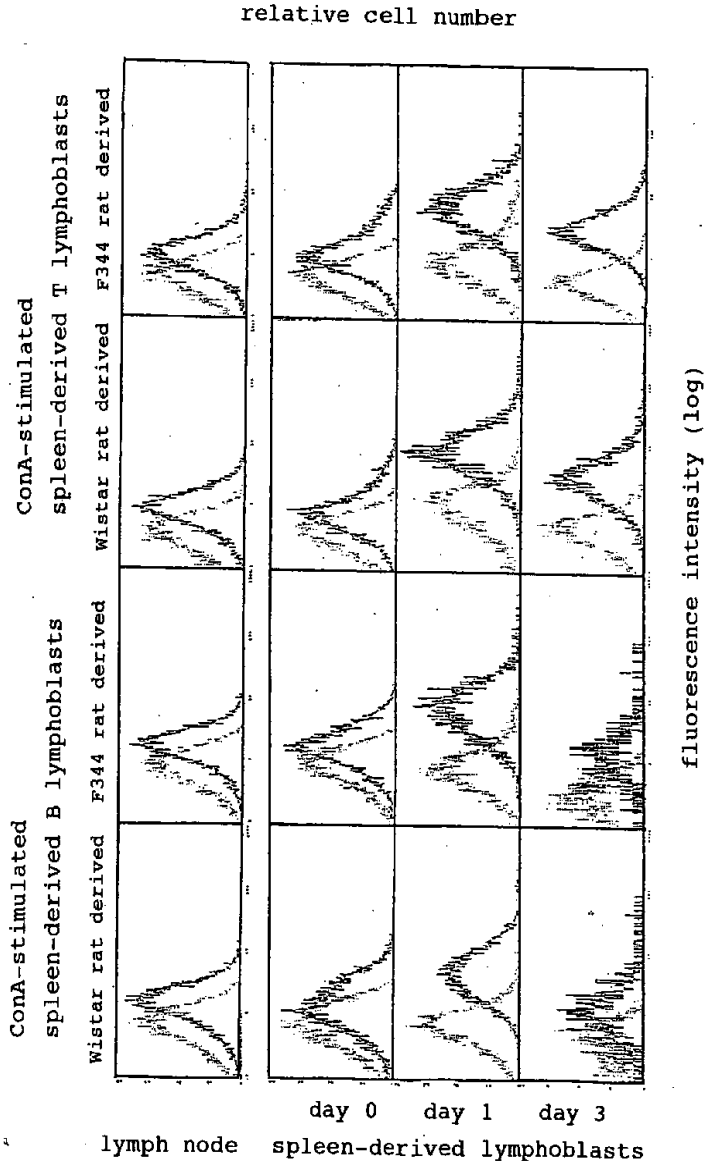

relative cell number
ConA-stimulated spleen-derived T lymphoblasts
ConA-stimulated spleen-derived B lymphoblasts
Wistar rat derived
F344 rat derived
Wistar rat derived
F344 rat derived
fluorescence intensity (log)
day 0
day 1
day 3
lymph node
spleen-derived lymphoblasts

Figure 5

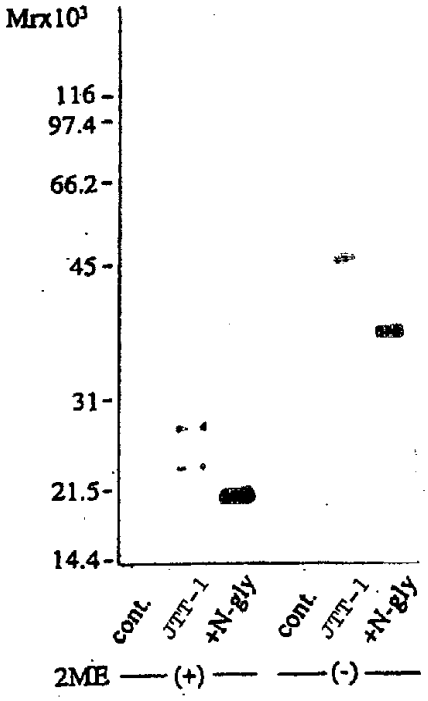

Mrx10³
116
97.4
66.2
45
31
21.5
14.4
cont.
JTT-1
+N-gly
cont.
JTT-1
+N-gly
2ME
(+)
(-)

Figure 6

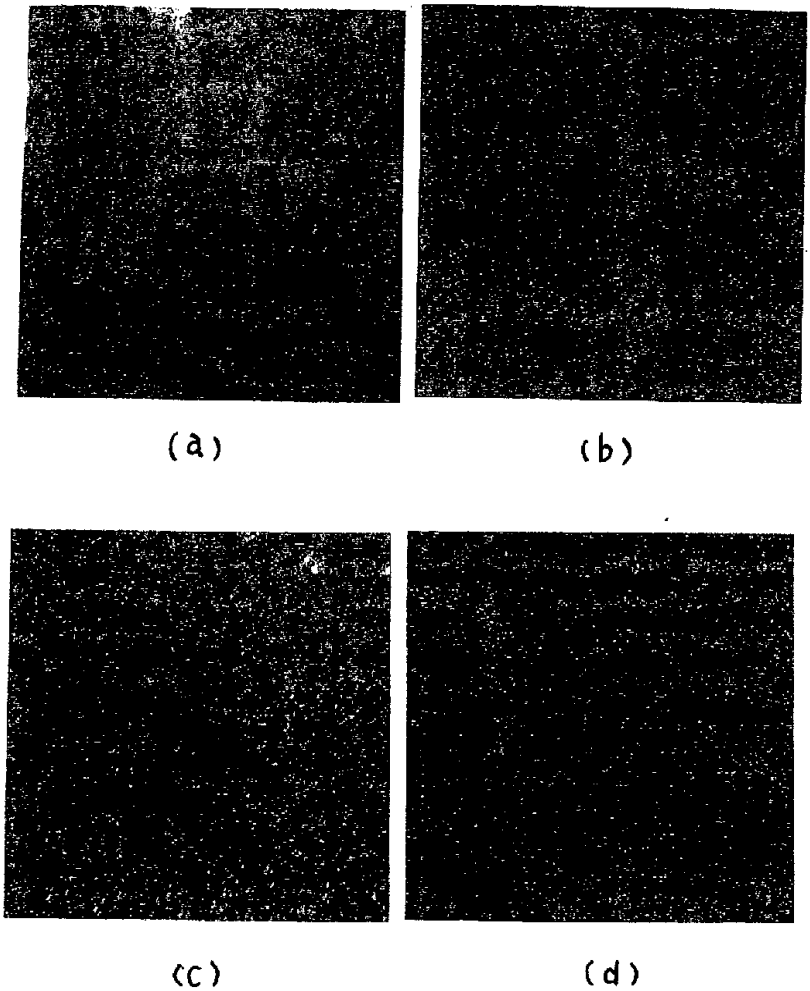


Figure 7

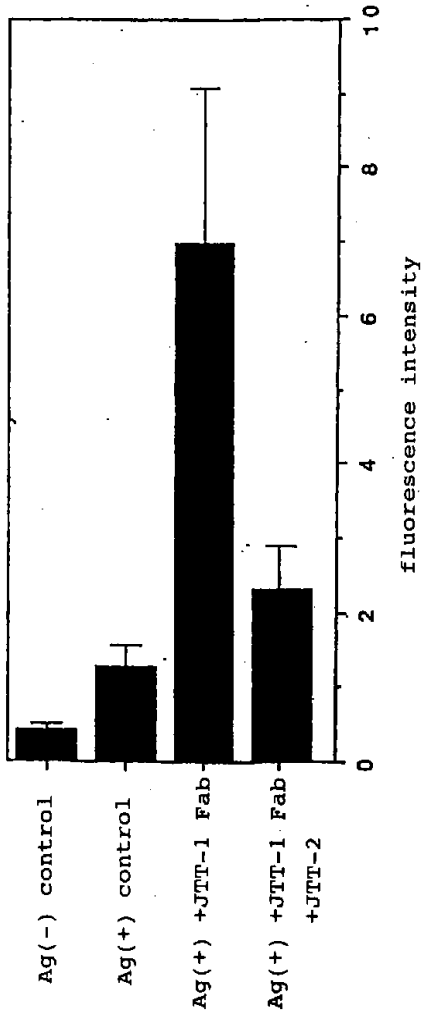

0
2
4
6
8
10
fluorescence intensity
Ag(-) control
Ag(+) control
Ag(+) +JTT-1 Fab
Ag(+) +JTT-1 Fab
+JTT-2

Figure 8

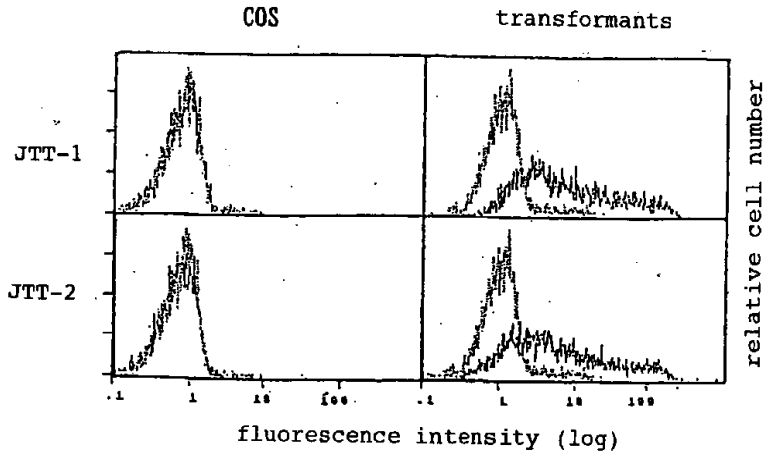

COS
transformants
JTT-1
JTT-2
relative cell number
fluorescence intensity (log)

Figure 9

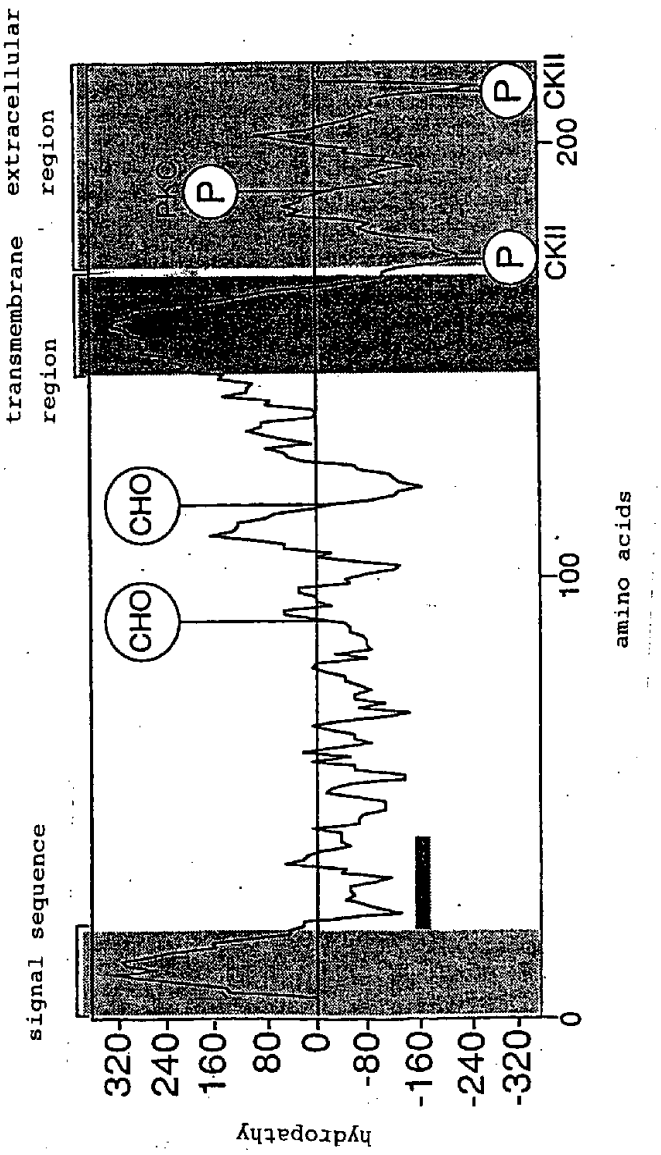

signal sequence
transmembrane region
extracellular region
CHO
CHO
P
P
P
CKII
CKII
hydropathy
320
240
160
80
0
-80
-160
-240
-320
0
100
200
amino acids

Figure 10

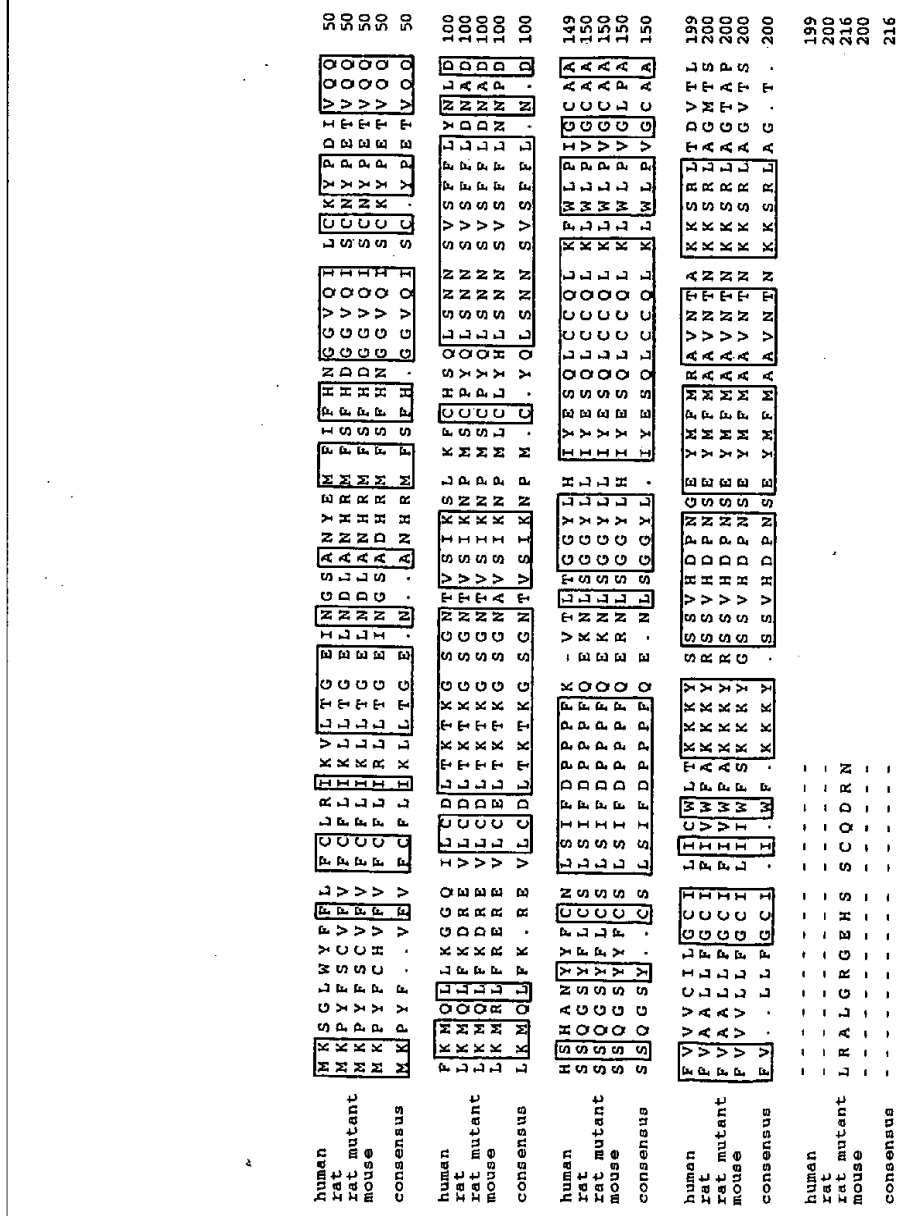

human
rat
rat mutant
mouse
consensus
50
100
149
150
199
200
216

Figure 11

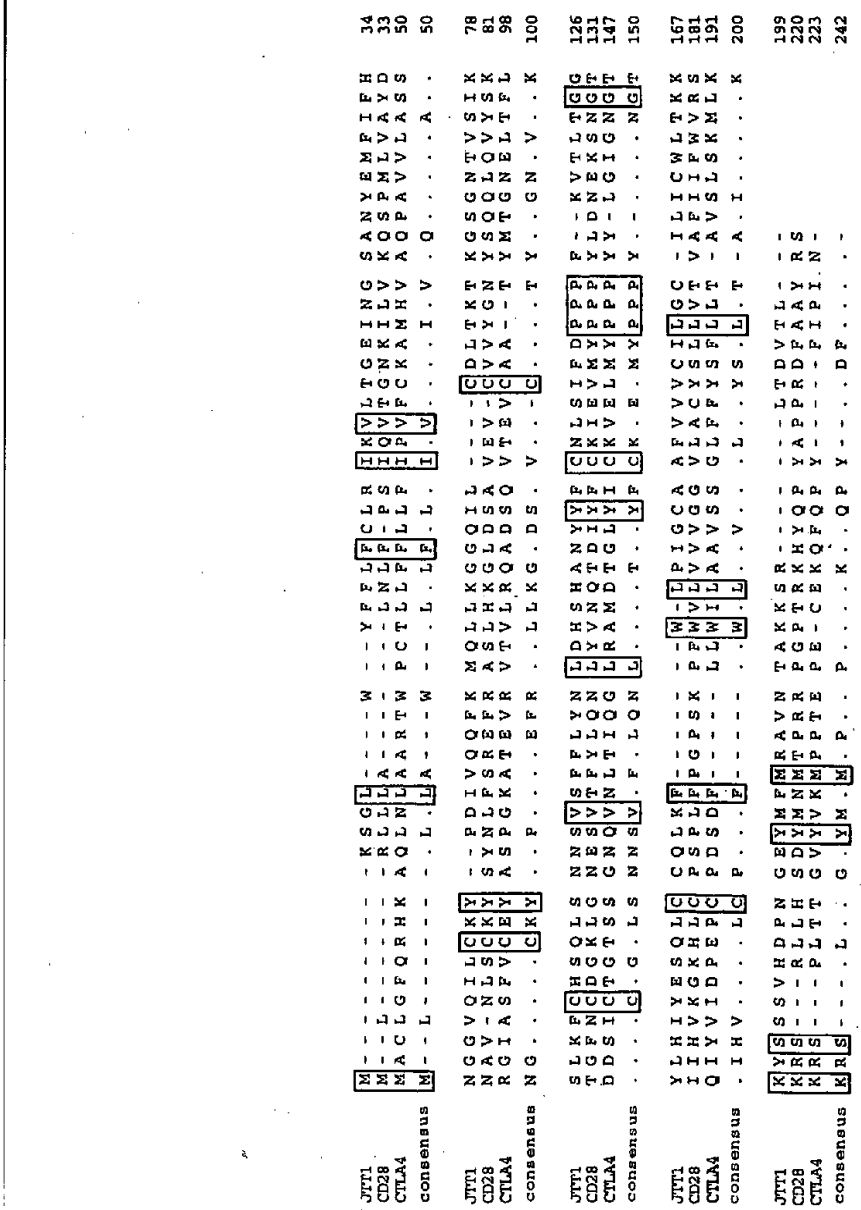


Figure 12

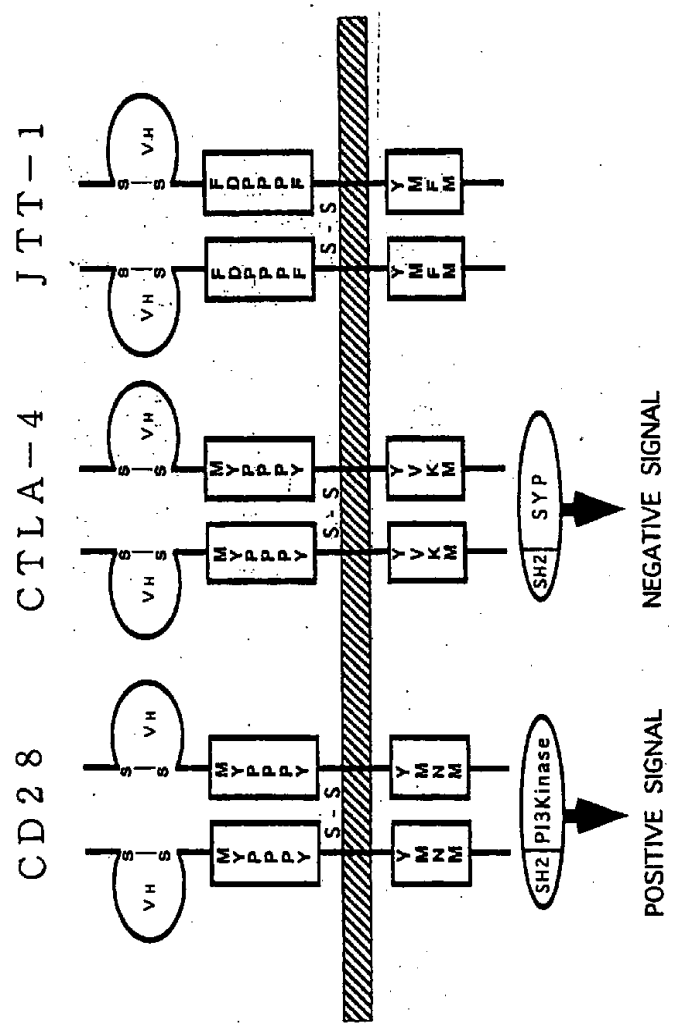

CD28
CTLA-4
JTT-1
NEGATIVE SIGNAL
POSITIVE SIGNAL
SH2 SYP
SH2 PI3Kinase

Figure 13

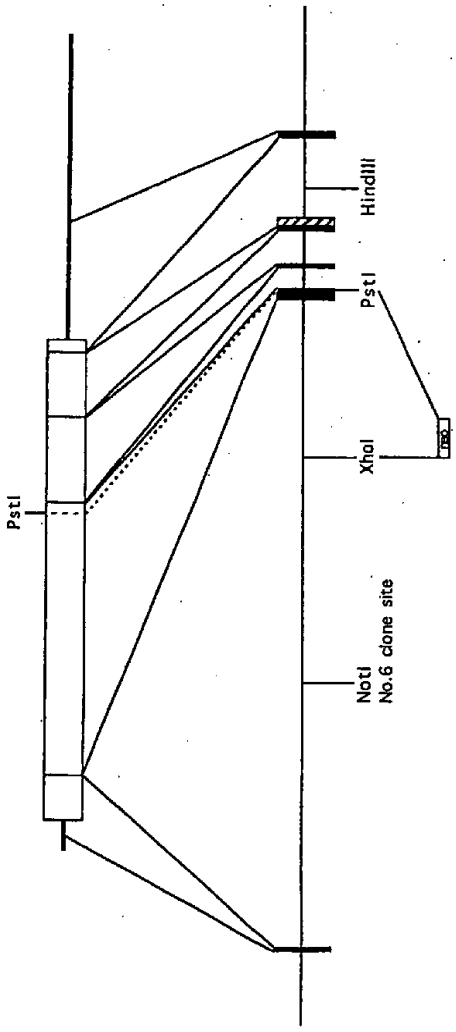


Figure 14

```
rat        MKPYFSCVFV FCFLIKLLTG ELNDLANHRM FSFHDGGVQI SCNYPETVQQ  50
rat mutant MKPYFSCVFV FCFLIKLLTG ELNDLANHRM FSFHDGGVQI SCNYPETVQQ  50
consensus  MKPYFSCVFV FCFLIKLLTG ELNDLANHRM FSFHDGGVQI SCNYPETVQQ  50

rat        LKMQLFKDRE VLCDLTKTKG SGNTVSIKNP MSCPYQLSNN SVSFFLDNAD 100
rat mutant LKMQLFKDRE VLCDLTKTKG SGNTVSIKNP MSCPYQLSNN SVSFFLDNAD 100
consensus  LKMQLFKDRE VLCDLTKTKG SGNTVSIKNP MSCPYQLSNN SVSFFLDNAD 100

rat        SSQGSYFLCS LSIFDPPPFQ EKNLSGGYLL IYESQLCCQL KLWLPVGCAA 150
rat mutant SSQGSYFLCS LSIFDPPPFQ EKNLSGGYLL IYESQLCCQL KLWLPVGCAA 150
consensus  SSQGSYFLCS LSIFDPPPFQ EKNLSGGYLL IYESQLCCQL KLWLPVGCAA 150

rat        FVAALLFGCI FIVWFAKKKY RSSVHDPNSE YMFMAAVNTN KKSRLAG MTS 200
rat mutant FVAALLFGCI FIVWFAKKKY RSSVHDPNSE YMFMAAVNTN KKSRLAG TAP 200
consensus  FVAALLFGCI FIVWFAKKKY RSSVHDPNSE YMFMAAVNTN KKSRLAG ... 200

rat        ---------- ------                                     216
rat mutant LRALGRGEHS SCQDRN                                     216
consensus  .......... ......                                     216
```

Figure 15

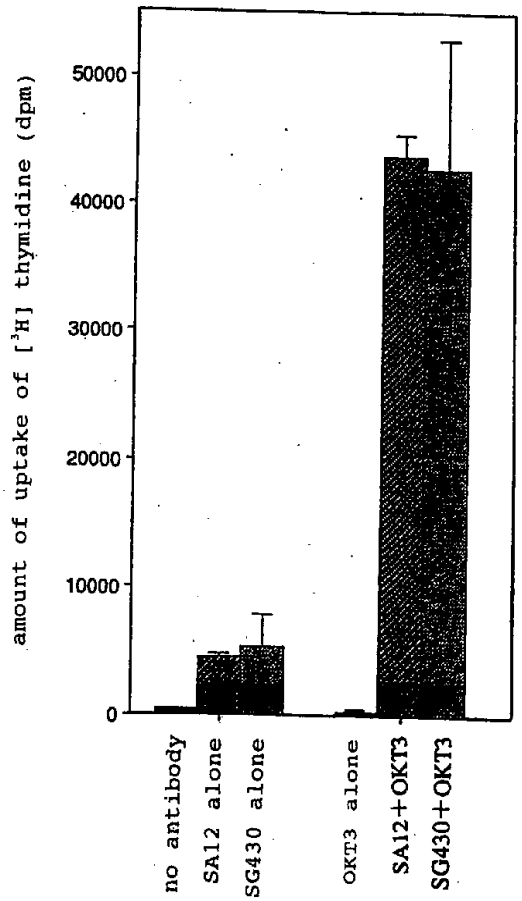

amount of uptake of [3H] thymidine (dpm)
50000
40000
30000
20000
10000
0
no antibody
SA12 alone
SG430 alone
OKT3 alone
SA12+OKT3
SG430+OKT3

Figure 16

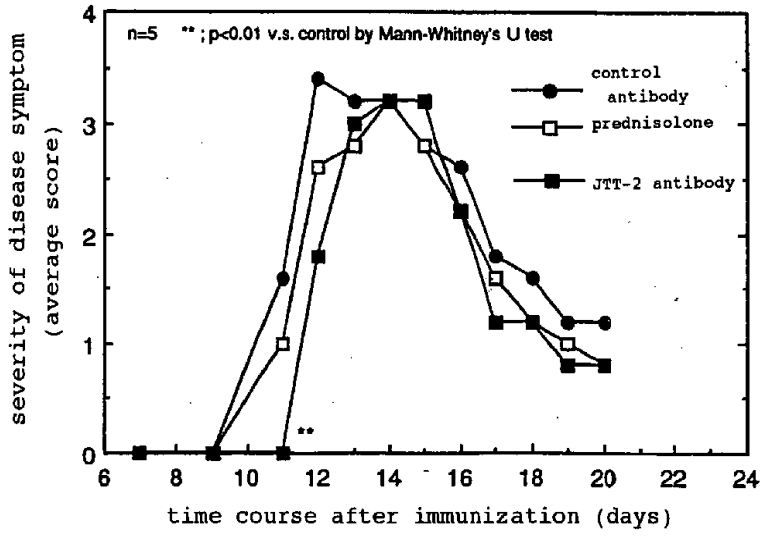

n=5 **; p<0.01 v.s. control by Mann-Whitney's U test
control antibody
prednisolone
JTT-2 antibody
severity of disease symptom (average score)
time course after immunization (days)
0
1
2
3
4
6
8
10
12
14
16
18
20
22
24
**

Figure 17

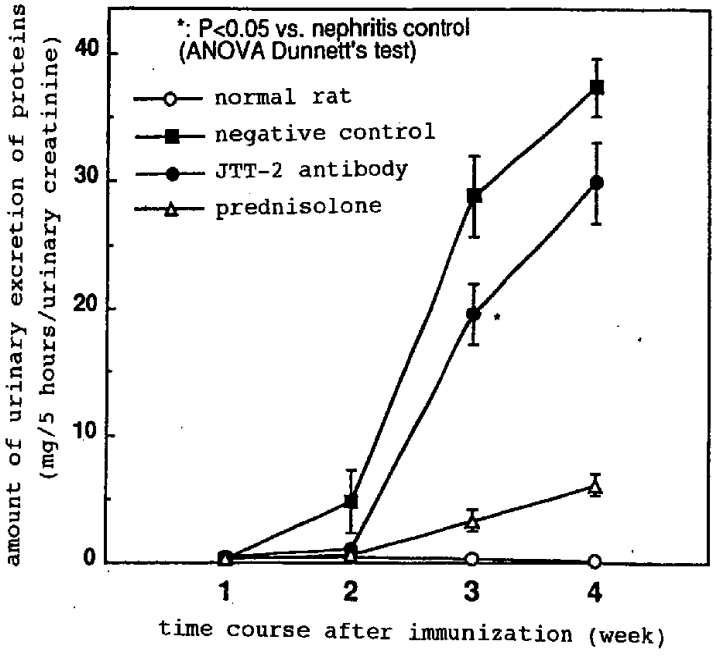

*: P<0.05 vs. nephritis control
(ANOVA Dunnett's test)
normal rat
negative control
JTT-2 antibody
prednisolone
amount of urinary excretion of proteins
(mg/5 hours/urinary creatinine)
0
10
20
30
40
1
2
3
4
time course after immunization (week)

Figure 18

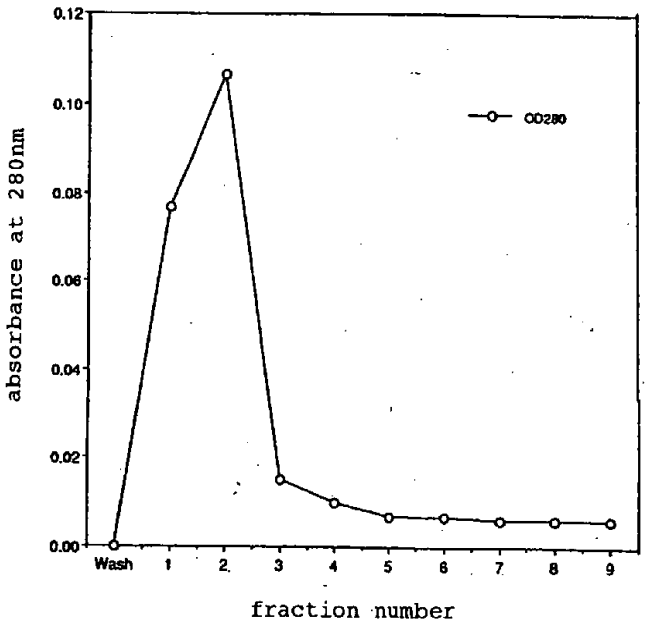

0.12
0.10
0.08
0.06
0.04
0.02
0.00
absorbance at 280nm
OD280
Wash
1
2
3
4
5
6
7
8
9
fraction number

Figure 19

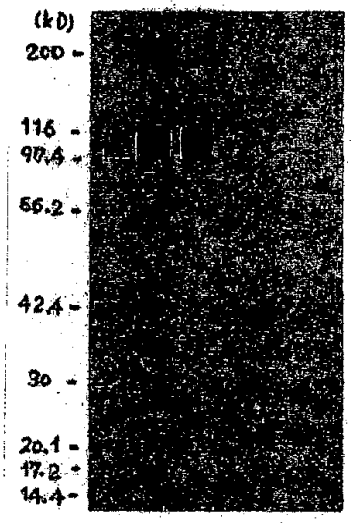


Figure 20

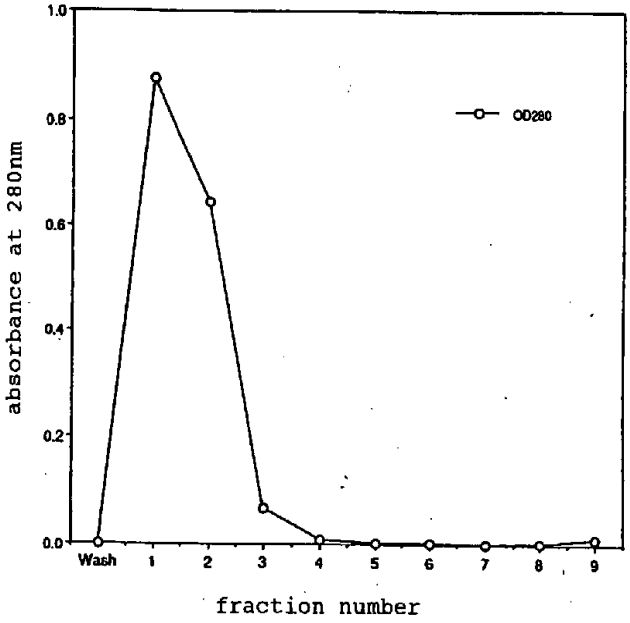

1.0
0.8
0.6
0.4
0.2
0.0
absorbance at 280nm
OD280
Wash
1
2
3
4
5
6
7
8
9
fraction number

Figure 21

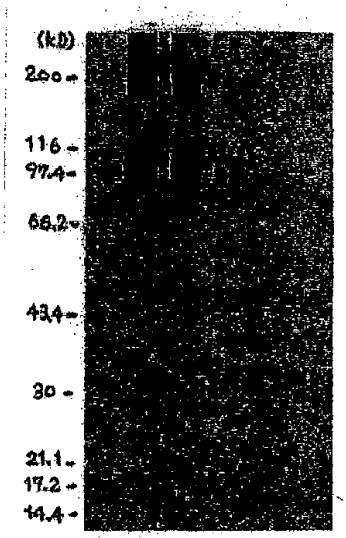
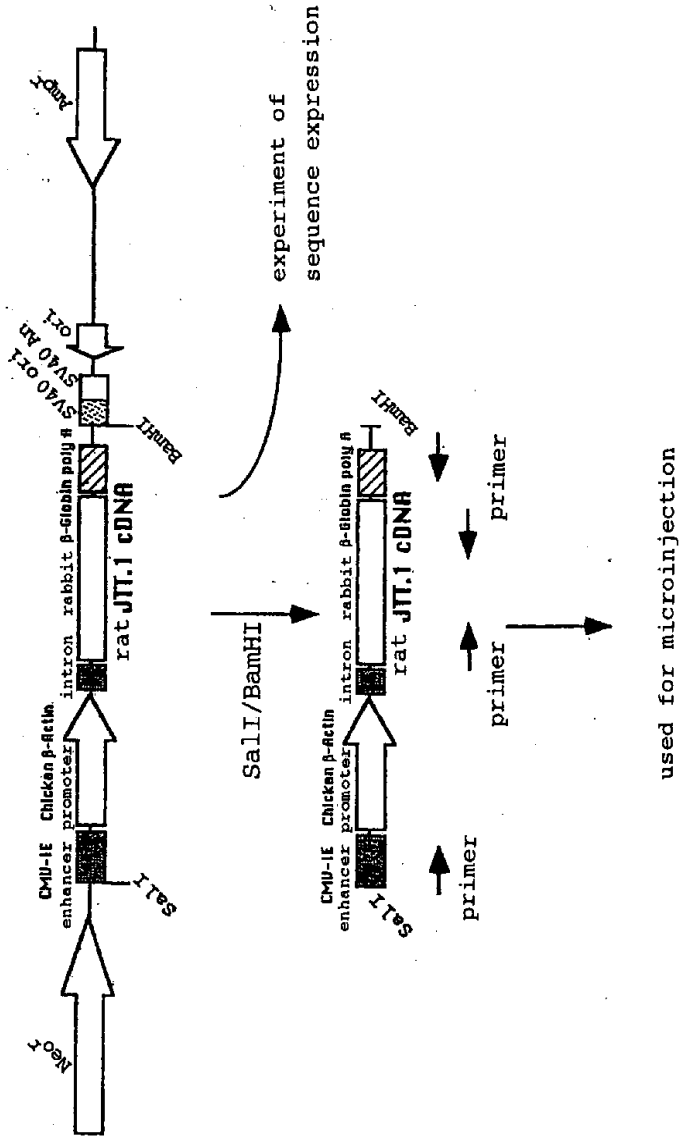


Figure 22


pUC
SV40 ori
SV40 Ap
BamHI
rabbit β-Globin poly A
intron
rat JTT.1 cDNA
Chicken β-Actin promoter
CMV-IE enhancer
SalI
Neo r
experiment of sequence expression
SalI/BamHI
primer
primer
primer
used for microinjection