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LFA-3-LIKE PROTEIN, DERIVATIVES THEREOF, GENES THEREOF AND PROCESSES FOR PREPARING THE SAME

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(57)

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 199 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

.../2

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Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr
 1               5                  10                  15
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys
            20                  25                  30
Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln
        35                  40                  45
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
    50                  55                  60
Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
65                  70                  75                  80
Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro
                85                  90                  95
Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile
            100                 105                 110
Thr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp
        115                 120                 125
Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Glu Cys
    130                 135                 140
His Arg Gly Ser Ile Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp
145                 150                 155                 160
Leu Ser Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr
                165                 170                 175
Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg
            180                 185                 190
His Arg Phe Ser Gly Thr Ser
        195
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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acids

(B) TYPE: amino acid

(vi) ORIGINAL SOURCE

(A) ORGANISM: Ovis

(G) CELL TYPE: leukocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

.../3

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

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 134 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Phe Ser Gln Glu Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His
1 5 10 15
Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys
20 25 30
Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser
35 40 45

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Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile
 50                  55                  60
Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro
 65                  70                  75                  80
Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His Ser
                 85                  90                  95
Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr Thr
            100                 105                 110
Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys Asp Arg Lys Pro
        115                 120                 125
Asp Arg Thr Asn Ser Asn
    130
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CLAIMS

1. A sheep LFA-3 like protein deficient in TM region which comprises the amino acid sequence of SEQ ID NO: 36.

5. A sheep LFA-3 like protein deficient in D2 region which comprises the amino acid sequence of SEQ ID NO: 1.

6. A human LFA-3 like protein deficient in D2 region which comprises the amino acid sequence of SEQ ID NO: 13.

660312

AUSTRALIA

PATENTS ACT 1990

COMPLETE SPECIFICATION

FOR A STANDARD PATENT

ORIGINAL

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Invention Title: "LFA-3-LIKE PROTEIN, DERIVATIVES THEREOF, GENES THEREOF AND PROCESSES FOR PREPARING THE SAME"--.

The following statement is a full description of this invention, including the best method of performing it known to us:-

"LFA-3-LIKE PROTEIN, DERIVATIVES THEREOF, GENES THEREOF AND PROCESSES FOR PREPARING THE SAME"

BACKGROUND OF THE INVENTION

The present invention relates to a novel cell adhesion protein, a gene coding for the same, a process for preparing the same and a carrier onto which the same is immobilized.

Forming rosette with sheep erythrocytes has been recognized to be one of specific responses of human T-cells. At present, it is understood that the forming rosette of sheep erythrocytes and human T-cells is a binding response due to high affinity of CD2 antigen receptor on a sheep erythrocyte for CD2 antigen on human T-cells (another name: T11 antigen). Any clear answer has not been obtained for the question why human T-cells form rosette with sheep erythrocytes easily. However, there is a possibility that in the structure of a sheep receptor for CD2 antigen itself there exists a function different from the function of human LFA-3, being a receptor for CD2 antigen in human. At present, it is known that the partly determined N-terminal amino acid sequence of a receptor on a sheep erythrocyte for CD2 antigen (SEQ ID NO:3) has about 50 % homology with the amino acid sequence of human LFA-3, a receptor for CD2 antigen (refer to Unexamined Japanese Patent Publication No. 150228/1988). However, it is not known what kind of whole structure the receptor on sheep erythrocyte for CD2 antigen has. As to human LFA-3 molecule, it is known that the molecule is classified as a cell adhesion protein belonging to immunoglobulin superfamily (refer to A. F. Williams and A. N. Barclay, Annu. Rev. Immunol. 6, 381, (1988)) and that the molecule is constructed by, from N-terminus, immunoglobulin-like domain 1 (D1 region), immunoglobulin-like domain 2 (D2 region), transmembrane region (TM region) and cytoplasm region (C region) (refer to B. P. Wallner et al., J. Exp. Med.

166, 923, (1987)). Further, there is also known human LFA-3 molecule which has D1 region and D2 region and binds to a membrane through glycosyl phosphatidylinositol (refer to B. Seed, Nature 329, 840, (1987)). Therefore, in the present specification, a CD2 antigen receptor having a structure of, from N-terminus, D1 region - D2 region - TM region - C region or a CD2 antigen receptor having D1 region and D2 region and binding to a membrane through glycosyl phosphatidylinositol is referred to as "LFA-3". A sheep receptor for CD2 antigen having unknown structure is kept to be referred to as a receptor for CD2 antigen.

A sheep receptor for CD2 antigen has various uses, such as a use as a reagent for detecting T-cells and a use as a ligand for separating T-cells from a mixture of various kinds of cells, because the receptor has high affinity for CD2 antigen of human T-cells. Further, it is known that CD2 antigen participates in various immune responses as functions of T-cells. Therefore, a sheep receptor for CD2 antigen which has affinity for CD2 antigen can be used as an immunoregulative agent and more, as a therapeutic agent which targets a tumor of T-cell family or a leukemia cell by utilizing affinity thereof for T-cells.

A sheep receptor for CD2 antigen can be obtained from sheep erythrocytes. As a process for preparing a sheep receptor for CD2 antigen and a derivative of the receptor, there are known a process comprising solubilizing the receptor from sheep erythrocytes by a surfactant and purifying by an affinity-chromatography using antibodies therefor (refer to Unexamined Japanese Patent Publication No. 150228/1988) and a process comprising solubilizing the receptor from sheep erythrocytes by trypsin (refer to T. Kitao et al., J, Immunol. 117, 310, (1976)). However, a amount of the sheep receptor for CD2 antigen is very small on sheep erythrocytes and it is hard work to prepare a large amount of the sheep receptor for CD2

antigen for the above-mentioned uses.

At present, a protein which naturally exists in very small amount can be prepared inexpensively and in large amount by genetic engineering techniques. For preparing the sheep receptor for CD2 antigen by genetic engineering techniques, a necessary gene coding for the sheep CD2 antigen receptor has to be isolated (cloned) first. However, at present, a whole amino acid sequence of the sheep receptor for CD2 antigen is not known. Further, it is not known at all whether the sheep receptor for CD2 antigen is LFA-3 which is a CD2 antigen receptor having the structure of, from N-terminus, D1 region - D2 region - TM region - C region or a CD2 antigen receptor having D1 region and D2 region and binding to a membrane through glycosyl phosphatidylinositol, or a receptor of other structures. Therefore, it has been impossible to detect a gene or mRNA coding for sheep LFA-3, to clone the sheep LFA-3 gene and to prepare sheep LFA-3 by genetic engineering techniques.

It can be considered that some processes can be used for cloning of sheep LFA-3 gene. For example, it may be possible, by using of DNA probe (mixed probe) which is deduced from the known N-terminal partial amino acid sequence of a sheep receptor for CD2 antigen (SEQ ID NO:3) consisting of 29 amino acid residues, to screen a cDNA of sheep LFA-3 from a cDNA library derived from cells in which the sheep LFA-3 gene is expressed. However, the cDNA screened by the mixed DNA probe can not be certified to be a true gene coding for sheep LFA-3 unless the cDNA is sequenced. A mixed DNA probe is not appropriate for detecting for the sheep LFA-3 gene. A DNA probe which is designed and prepared according to the Lathe et al.'s method (refer to Lathe et al., J. Molec. Biol. 183, 1, 1985) is not always useful to clone a gene.

A monoclonal antibody for a sheep receptor for CD2 antigen has been obtained. It may be possible that

cloning of the desired gene can be attained by using the antibody labeled by a radioactive substance to screen the gene from a gene expression library. However, there is no report wherein the monoclonal antibody can be really used for cloning.

Therefore, a sure means by which the sheep LFA-3 gene can be cloned has not been accomplished at present. A DNA probe which is useful for sure cloning of the sheep LFA-3 gene is a DNA probe which has the sequence of LFA-3 gene as it is. Such a DNA probe can selectively hybridize with the sheep LFA-3 gene or mRNA, therefore it is very useful to detect the LFA-3 gene or mRNA.

On the other hand, until now, there have not been known the existence of a LFA-3 like protein deficient in D2 region and a LFA-3 like protein deficient in TM region in sheep and also in humans. Such proteins have been first found in the present invention.

If these proteins have high affinity for CD2 antigen of human T-cells, it is considered that they are useful as a detecting reagent for human T-cells, as a ligand for separating T-cells of humans and the other animals, as an immunoregulative agent or as a therapeutic agent which targets tumors of T-cell family. For these uses, it is necessary to make a mass production of the protein possible. Although a process by genetic engineering techniques is appropriate for a mass production of such a protein, a gene coding for the protein has to be cloned and has to be analyzed in order to perform the process. Further, it is also necessary to search a protein which is more suitable for a process by genetic engineering techniques and to clone a gene coding for such a protein and to analyze the structure of the protein.

An object of the present invention is to provide a protein, which is appropriate for a process by genetic engineering techniques, having high affinity for

CD2 antigen on human T-cells.

Another object of the present invention is to provide a gene coding for such a protein.

A further object of the present invention is to provide a process for preparing such a protein by genetic engineering techniques.

A still further object of the present invention is to provide a carrier onto which such a protein is immobilized.

These and the other objects of the present invention will become apparent from the description hereinafter.

SUMMARY OF THE INVENTION

In accordance with the present invention, there are provided a LFA-3 like protein deficient in TM region, a LFA-3 like protein deficient in D2 region and a derivative of these proteins, a gene coding for the above-mentioned protein, a process for preparing the protein and a carrier onto which the protein is immobilized.

BRIEF EXPLANATION OF THE DRAWINGS

Fig. 1 is a figure in which the amino acid sequence of human LFA-3 (SEQ ID NO:8) is compared with the amino acid sequence of the sheep LFA-3 like protein deficient in TM region (SEQ ID NO:36). Number in the figure represents number of amino acid from the N-terminus of human LFA-3. A symbol "-" represents deficiency of amino acid. A colon is shown between homologous amino acids. TM region is underlined. As is clear from the figure, the sheep LFA-3 like protein deficient in TM region does not have the underlined sequence.

Fig. 2 is a figure in which the amino acid sequence of human LFA-3 (SEQ ID NO:8) is compared with the amino acid sequence of the sheep LFA-3 like protein

deficient in D2 region (SEQ ID NO:1). Number in the figure represents number of amino acid from the N-terminus of human LFA-3. A symbol "-" represents deficiency of amino acid. A colon is shown between homologous amino acids. D2 region is underlined. As is clear from the figure, the sheep LFA-3 like protein deficient in D2 region does not have the underlined sequence.

Fig. 3 is a graph showing rosette formation inhibition activity of the proteins of the present invention obtained in Example 4-r and Example 5-z respectively, that is, sheep D1HC protein and human D1HC protein.

DETAILED DESCRIPTION

A sheep LFA-3 protein is one of proteins of the present invention. The sheep LFA-3 protein is a CD2 antigen receptor derived from sheep having a structure of, from N-terminus, D1 region - D2 region -TM region - C region. More particularly, the sheep LFA-3 protein comprises the amino acid sequence of SEQ ID NO: 31.

A gene coding for a sheep LFA-3 protein is one of genes of the present invention. More particularly, the sheep LFA-3 gene of the present invention codes for the amino acid sequence of SEQ ID NO:31. The sheep LFA-3 gene of the present invention includes a gene which comprises the base sequence of SEQ ID NO:30.

The sheep LFA-3 gene of the present invention can be obtained as described below.

First, mRNA of sheep LFA-3 is prepared. The mRNA of sheep LFA-3 can be extracted from sheep cells or a sheep organ wherein a sheep LFA-3 gene has been expressed, such as leukocytes and hepatic cells. Firstly, RNA is extracted from such a material by means of a conventional method such as guanidine thiocyanate method or hot phenol method. From the extracted RNA, mRNA of sheep LFA-3 can be prepared as poly(A)$^+$RNA by means of an oligo(dT)-cellulose column

(Rabomanyuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, (1988)).

Second, cDNA of sheep LFA-3 is prepared from the obtained mRNA by means of a conventional method or a commercially available kit.

There are synthesized primers each of which is deduced from amino terminal sequence and carboxyl terminal sequence of the known partial amino acid sequence of a sheep receptor for CD2 antigen (SEQ ID NO: 3). With the synthesized primers, the above obtained cDNA is amplified by PCR (polymerase chain reaction) method (refer to Molecular Cloning 2nd edition, edited by J. Sambrook et al., Cold Spring Harbor Laboratory (1989); K. Knoth et al. (1988), Nucleic Acids Research, vol. 16, page 10932). The amplification by PCR method may be cycled suitable times to obtain a sufficient amount of cDNA for the following cloning. The amplified cDNA is detected by conventional gel electrophoresis, e.g., polyacrylamide gel electrophoresis, and extracted.

Nucleotide sequence of the extracted DNA can be determined by inserting the DNA into a suitable vector such as pBR322, pUC18 or M13mp19, cloning the recombinant vector in a suitable host such as E. coli JM109, and utilizing a conventional method, e.g. Sanger method or the like.

There can be used DNA having the determined DNA sequence of sheep LFA-3 as a probe useful for detection of the sheep LFA-3 gene and mRNA thereof. That is, a full-length cDNA of sheep LFA-3 can be easily detected from the sheep cDNA library by using the probe. Also, a full-length cDNA of sheep LFA-3 can be amplified by PCR method with the primers having thus determined sequence, and cloned. The cloned cDNA of sheep LFA-3 is characterized by analyzing with restriction enzymes or sequencing thereof.

Cloning of cDNA of sheep LFA-3 enables to produce sheep LFA-3 by genetic engineering techniques. As host, there can be used a bacterium such as E. coli

or Bacillus, a yeast, a fungus, a cultured cell of animal or plant, or the like.

The sheep LFA-3 protein of the present invention may have modified structure of the above-mentioned structure. Examples of the protein having the modified structure are, for instance, the proteins modified in dependence on a host cell which produces the protein, such as the protein wherein methionine residue is introduced at N-terminus of the protein due to production by using E. coli as host and the protein modified with sugar due to production by using an animal cultured cell as host, and the like.

The LFA-3 like protein deficient in TM region is another protein of the present invention. The LFA-3 like protein deficient in TM region is a single polypeptide having the molecular weight of about 22,000 as protein which has structure of a LFA-3 protein lacking in TM region. "TM region" used herein means a region rich with hydrophobic amino acids contained in a membrane protein. The TM region in human LFA-3 protein is supposed to be the sequence from the 188th Tyr to the 212th Cys of SEQ ID NO:8. Also, the LFA-3 like protein deficient in TM region of the present invention includes polypeptides having a molecular weight higher than 22,000 with sugar chain.

The LFA-3 like protein deficient in TM region of the present invention may have modified structure of the above-mentioned structure. Examples of the protein having the modified structure are, for instance, the proteins modified in dependence on a host cell which produces the protein, such as the protein wherein methionine residue is introduced at N-terminus of the protein due to production by using E. coli as host and the protein modified with sugar due to production by using an animal cultured cell as host, and the like.

The LFA-3 like protein deficient in TM region has low molecular weight in comparison with the molecular weight of LFA-3, and does not have

transmembrane region. Therefore, the protein of the present invention has low antigenicity and is a naturally occuring soluble LFA-3 like protein. The protein of the present invention is very advantageous to the production by genetic engineering techniques in comparison with LFA-3 being a membrane-binding protein by reason that the protein of the present invention can be secreted from animal cultured cells, yeasts and the like after production therein.

Then, there is explained the method for obtaining the gene coding for the LFA-3 like protein deficient in TM region of the present invention and the above-mentioned protein.

First, mRNA of the sheep LFA-3 like protein deficient in TM region is prepared. The mRNA of the sheep LFA-3 like protein deficient in TM region can be extracted from sheep cells or a sheep organ wherein a gene coding for the sheep LFA-3 like protein deficient in TM region has been expressed, such as leukocytes. Firstly, RNA is extracted from such a material by a conventional method such as guanidine thiocyanate method or hot phenol method. Then, from the extracted RNA, mRNA can be prepared as poly(A)$^+$RNA by means of an oligo(dT)-cellulose column (Rabomanyuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, (1988)).

Second, cDNA of the sheep LFA-3 like protein deficient in TM region is prepared from the obtained mRNA by means of a conventional method or a commercially available kit.

There are synthesized mixed primers each of which is deduced from amino terminal sequence and carboxyl terminal sequence of the known partial amino acid sequence of a sheep receptor for CD2 antigen (SEQ ID NO:3). With the synthesized mixed primers, the above obtained cDNA is amplified by PCR method. The amplification by PCR method may be cycled suitable times to obtain a sufficient amount of cDNA for the following cloning. The amplified cDNA is detected by a

conventional gel electrophoresis, e.g., polyacrylamide gel electrophoresis, and extracted.

Nucleotide sequence of the extracted DNA can be determined by inserting the DNA into a suitable vector such as pBR322, pUC18 or M13mp19, cloning the recombinant vector in a suitable host such as E. coli JM109, and utilizing a conventional method, e.g. Sanger method or the like.

There can be used DNA having the DNA sequence of the sheep LFA-3 like protein deficient in TM region determined as described above as a probe useful for detection of the gene coding for the sheep LFA-3 like protein deficient in TM region and mRNA thereof. That is, a full-length cDNA of the sheep LFA-3 like protein deficient in TM region can be easily detected by using the probe from the sheep cDNA library. Nucleotide sequence of the detected cDNA clone is determined. As a result, since the determined sequence has no DNA sequence coding for the sequence of TM region, the cDNA is characterized as the cDNA clone of the sheep LFA-3 like protein deficient in TM region.

The sheep LFA-3 like protein deficient in TM region can be obtained by inserting thus obtained full-length cDNA of the sheep LFA-3 like protein deficient in TM region or a DNA coding for the above-mentioned protein into a suitable expression vector, introducing the recombinant vector into a host cell suitable for the vector, for instance, a bacterium such as E. coli or Bacillus, a yeast, a fungus, a cultured cell of animal or plant or the like and culturing the transformed cell.

The cDNA of the human LFA-3 like protein deficient in TM region is screened as a clone which is selected with a probe having the nucleotide sequence of D1 region and is not selected with a probe having the nucleotide sequence of TM region, from cDNA library containing cDNA of the human LFA-3 like protein deficient in TM region; and cloned. Genes coding for a LFA-3 like protein deficient in TM region of animals

other than human and sheep are selected as a cDNA having no DNA sequence coding for the sequence of TM region from cDNA clones which hybridize to the gene of the sheep LFA-3 like protein deficient in TM region or the gene of the human LFA-3 like protein deficient in TM region.

A LFA-3 like protein deficient in D2 region is a further protein of the present invention. The LFA-3 like protein deficient in D2 region is a single polypeptide having the molecular weight of about 15,000 as protein, which has structure of a LFA-3 protein lacking in D2 region and contains at most one disulfide bonding in the molecule. "D2 region" used herein means a region encoded by DNA which is segmented with introns from DNA encoding other regions on a genomic DNA, being the second immunoglobulin-like domain from N-terminus among two immunoglobulin-like domains of LFA-3 protein. The D2 regions in human and sheep have 6 cystein residues.

According to the present invention, it has been revealed that the LFA-3 like protein having D1 region and no D2 region shows higher affinity for CD2 antigen in comparison with LFA-3. Such fact has demonstrated that capacity for adhere to CD2 antigen in LFA-3 exists in not D2 region but D1 region. Therefore, it is considered that LFA-3 protein wherein there is (are) introduced variation(s) such as partial deficiency and/or substitution in D2 region, has the similar affinity to that of the LFA-3 like protein deficient in D2 region. Thus, the above-mentioned proteins having deficient D2 region are also included in the LFA-3 like protein deficient in D2 region of the present invention.

The LFA-3 like protein deficient in D2 region of the present invention may have modified structure of the above-mentioned structure. Examples of the protein having the modified structure are, for instance, the proteins modified in dependence on a host cell which produces the protein, such as the protein wherein

methionine residue is introduced at N-terminus of the protein due to production by using E. coli as host and the protein modified with sugar due to production by using an animal cultured cell as host, and the like.

The LFA-3 like protein deficient in D2 region of the present invention has low molecular weight in comparison with the molecular weight of LFA-3. The protein of the present invention has at most one disulfide bonding whereas LFA-3 has plural disulfide bondings in the molecule. Therefore, the protein of the present invention is very advantageous by reasons of the low antigenecity and no possibility of forming a wrong disulfide bonding in production by genetic engineering techniques.

Hereinafter there is explained a derivative of the above-mentioned proteins deficient in D2 region, which is a still further protein produced according to the present invention. The derivative of the present invention includes soluble derivatives of the LFA-3 like protein deficient in D2 region which are proteins wherein variation(s) such as deficiency and/or substitution is (are) introduced in TM region rich with hydrophobic amino acids and/or C region thereof. The soluble derivative of the LFA-3 like protein deficient in D2 region is very advantageous to production by genetic engineering techniques by reason that the derivative can be secreted from an animal cultured cell, a yeast or the like after production therein. Also, because D1 region of human and sheep LFA-3 has no cysteine residue, there can be produced the derivative having no cysteine residue or the derivative having at most two cysteine residues according to the present invention. Such derivatives are very advantageous to production by genetic engineering techniques by reason of no possibility that a wrong disulfide bonding is formed.

From the derivative of the present invention wherein a few cysteine residues are introduced can be

produced a carrier onto which the derivative is immobilized through the cysteine residue, a multimer and the derivative covalently bound with other substance.

"D1 region" used herein means a region encoded by DNA which is segmented with an intron from the DNA encoding D2 region on a genomic DNA, being the immunoglobulin-like domain of N-terminus side among two immunoglobulin-like domains of LFA-3 protein.

Also, the derivative of the LFA-3 like protein deficient in D2 region of the present invention may have modified structure of the above-mentioned structure. Examples of the derivative having the modified structure are, for instance, the derivatives modified in dependence on a host cell which produces the derivative such as the derivative wherein methionine residue is introduced at N-terminus of the derivative due to production by using E. coli as host and the derivative modified with sugar due to production by using an animal cultured cell as host, and the like.

The gene coding for the LFA-3 like protein deficient in D2 region and the above-mentioned protein are prepared as described below.

First, mRNA of the sheep LFA-3 like protein deficient in D2 region is prepared. The mRNA of the sheep LFA-3 like protein deficient in D2 region can be extracted from sheep cells or a sheep organ wherein the gene of the sheep LFA-3 like protein deficient in D2 region has been expressed, such as leukocytes. Firstly, RNA is extracted from such a material by means of a conventional method such as guanidine thiocyanate method or hot phenol method. Then, from the extracted RNA, mRNA can be prepared as poly(A)$^+$RNA by means of an oligo(dT)-cellulose column (refer to Rabomanyuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, (1988)).

Second, cDNA of the sheep LFA-3 like protein deficient in D2 region is prepared from the obtained mRNA by means of a conventional method or a commercially

available kit.

There are synthesized mixed primers each of which is deduced from amino terminal sequence and carboxyl terminal sequence of the known N-terminal 29 amino acid residues of the CD2 antigen receptor of sheep enythrocytes (SEQ ID NO:3). With the synthesized mixed primers, the above obtained cDNA is amplified by PCR method. The amplification by PCR method may be cycled suitable times to obtain a sufficient amount of cDNA for the following cloning. Thereby is amplified cDNA coding for N-terminal portion of the sheep LFA-3 like protein deficient in D2 region. The amplified cDNA is detected by a conventional gel electrophoresis, e.g., polyacrylamide gel electrophoresis, and extracted.

Nucleotide sequence of the extracted DNA can be determined by using a suitable vector such as pBR322, pUC18 or pUC19, cloning the vector in a suitable host such as E. coli and utilizing a conventional method, e.g., Sanger method or the like.

There can be used DNA having the determined DNA sequence of the sheep LFA-3 like protein deficient in D2 region as a probe useful for detection of the gene of the sheep LFA-3 like protein deficient in D2 region and mRNA thereof. That is, a full-length cDNA of the sheep LFA-3 like protein deficient in D2 region can be easily detected by using this probe from the cDNA library. The cloned full-length cDNA of the sheep LFA-3 like protein deficient in D2 region is characterized by analyzing with restriction enzymes or sequencing thereof. On the basis of the obtained nucleotide sequence of the gene, amino acid sequence of the sheep LFA-3 like protein deficient in D2 region is determined.

The sheep LFA-3 like protein deficient in D2 region can be obtained by inserting thus obtained cDNA of the sheep LFA-3 like protein deficient in D2 region into a suitable expression vector, introducing the recombinant vector into a host cell suitable for the vector, for instance, a bacterium such as E. coli or

Bacillus, a yeast, a fungus, a cultured cell of animal or plant or the like and culturing the transformed cell.

Also, a soluble derivative of the sheep LFA-3 like protein deficient in D2 region can be prepared as a variant wherein variation(s) such as deficiency and/or substitution is (are) provided in the transmembrane region and/or the cytoplasm region whereas the amino acid sequence of D1 region is maintained, for instance, the derivative of the sheep LFA-3 like protein deficient in D2 region having an amino acid sequence wherein the sequence from the 1st amino acid to the 94th amino acid in SEQ ID NO: 1 is maintained and, in the sequence from the 95th amino acid to the 131st amino acid in SEQ ID NO: 1, a deficiency of at least one amino acid and/or a substitution of sequence exist(s).

The human LFA-3 like protein deficient in D2 region is obtained described below.

First, mRNA of the human LFA-3 like protein deficient in D2 region is prepared. The mRNA of the human LFA-3 like protein deficient in D2 region can be extracted from human cells or a human organ wherein the gene of the human LFA-3 like protein deficient in D2 region has been expressed, such as leukocytes or an established strain of human T-cells. Firstly, RNA is extracted from such a material by means of a conventional method such as guanidine thiocyanate method or hot phenol method. Then, from the extracted RNA, mRNA can be prepared as poly(A)$^+$RNA by means of an oligo(dT)-cellulose column (refer to Rabomanyuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, (1988)).

Second, cDNA of the human LFA-3 like protein deficient in D2 region is prepared from the obtained mRNA by means of a conventional method or a commercially available kit.

There are synthesized 5' primer and 3' primer coding for amino terminal sequence or carboxyl terminal sequence of the known amino acid sequence of human LFA-3

(SEQ ID NO:8). With the synthesized primers, the above obtained cDNA is amplified by PCR method. The amplification by PCR method may be cycled suitable times to obtain a sufficient amount of cDNA for the following cloning. The amplified cDNA is detected by a conventional gel electrophoresis, e.g., polyacrylamide gel eletrophoresis, and extracted from the gel.

Nucleotide sequence of the extracted DNA can be determined by using a suitable vector such as pBR322, pUC18 or M13mp19, cloning the recombinant vector in a suitable host such as E. coli JM109, and using a conventional method, e.g. Sanger method or the like.

Also, the DNA of the human LFA-3 like protein deficient in D2 region is screened from the human cDNA library as a clone which is selected with a probe having the nucleotide sequence of D1 region and is not selected with a probe having the nucleotide sequence of D2 region; and cloned.

The cloned cDNA of the human LFA-3 like protein deficient in D2 region is characterized by analyzing with restriction enzymes or sequencing thereof.

The human LFA-3 like protein deficient in D2 region can be obtained by using a suitable expression vector and a host cell suitable for the vector, for instance, a bacterium such as E. coli or Bacillus, a yeast, a fungus, a cultured cell of animal or plant or the like and culturing the transformed cell.

A soluble derivative of the human LFA-3 like protein deficient in D2 region is prepared as a variant wherein variation(s) such as deficiency and/or substitution is (are) provided in the transmembrane region and/or the cytoplasm region whereas the amino acid sequence of D1 region is maintained, for instance, the derivative of the human LFA-3 like protein deficient in D2 region having an amino acid sequence wherein the sequence from the 1st amino acid to the 94th amino acid in SEQ ID NO: 13 is maintained and, in the sequence from the 95th amino acid to the 134th amino acid in SEQ ID

NO: 13, a deficiency of at least one amino acid and/or a substitution exits(s); or the derivative of the human LFA-3 like protein deficient in D2 region having an amino acid sequence wherein the sequence from the 1st amino acid to the 93rd amino acid in SEQ ID NO: 13 is maintained and, in the sequence from the 94th amino acid to the 134th amino acid in SEQ ID NO:13, a deficiency of at least one amino acid and/or a substitution exist(s).

Genes coding for the LFA-3 like protein deficient in D2 region of animals other than human and sheep are selected from cDNA clones which hybridize to the gene of the sheep LFA-3 like protein deficient in D2 region or the gene of the human LFA-3 like protein deficient in D2 region, as cDNA having no DNA sequence coding for the sequence of D2 region.

By using thus obtained cDNA of the LFA-3 like protein deficient in D2 region of animals other than human and sheep, the protein corresponding to the selected cDNA and a derivative thereof can be obtained in the same manner as described above.

Further, the present invention has first revealed that a carrier onto which the LFA-3 like protein deficient in D2 region or a derivative thereof is immobilized can adsorb cells having CD2 antigen. It is found that the carrier onto which the LFA-3 like protein deficeint in D2 region or the derivative thereof can selectively adsorb cells having CD2 antigen such as human T-cells and other animals T-cells. Also, adsorbed cells can be easily separated from the carrier with trypsin. Therefore, there can be utilized the carrier onto which the LFA-3 like protein deficient in D2 region or the derivative thereof for selective adsorption or separation of the T-cells.

As a carrier to be used for immobilization in the present invention, there can be used any of carriers onto which protein can be immobilized. Examples of the carrier to be used in the present invention are, for instance, plastic beads, plastic plates, plastic Schales

and the like. The LFA-3 like protein deficient in D2 region or the derivative thereof can be immobilized by hydrophobic bonding, covalent bonding, ionic bonding or the like onto the carrier.

The whole amino acid sequence of sheep LFA-3 has been revealed by obtaining the cDNA of sheep LFA-3 of the present invention and determining the full-length base sequence thereof. According to the present invention, analysis and cloning of a sheep genomic LFA-3 gene are enabled. The sheep genomic LFA-3 gene is useful for genetic engineering production of sheep LFA-3 in an animal cultured cell as host whereas genomic DNA may contain introns.

In the genetic engineering production of sheep LFA-3, there can be produced not only molecules having the amino acid sequence of sheep LFA-3 which naturally occures, but also variants artificially bearing substitution, insertion and/or deficiency of at least one amino acid and proteins combined with other protein. Also, chemically or enzymatically modified derivatives can be produced therefrom. Among the derivatives, there are contained derivatives having different affinity for CD2 antigen being a physiological ligand for LFA-3 from that of sheep LFA-3.

There can be used the sheep LFA-3 and variants thereof, or derivatives thereof produced by genetic engineering techniques which have affinity to human T-cells as diagnostic agents for T-cell detection. As the diagnostic agents can be utilized the above proteins labeled with an enzyme, a fluorescent agent or an isotope. Also, the produced sheep LFA-3, the variants and the derivatives which have affinity to T-cells, can be chemically or physically immobilized onto the carrier and subjected to separation (isolation or removal) of T-cells. Sheep LFA-3, the derivatives thereof and the proteins combined other protein are used as therapeutic agents which target a tumor of T-cell family or a leucemia cell by utilizing affinity thereof

for T-cell. For instance, sheep LFA-3, the derivative thereof or the protein combined other protein, which is conjugated with a toxin such as ricin can be used as a therapeutic agent.

It is known that CD2 antigen being a natural ligand of LFA-3 participates in various immune responses as functions of T-cells. Therefore, sheep LFA-3, the variant thereof or the derivative thereof which has affinity for CD2 antigen can be utilized as an agent for inhibiting or activating immune response(s), i.e. an immunoregulative agent.

Further, according to the present invention there can be obtained the LFA-3 like protein deficient in TM region and the LFA-3 like protein deficient in D2 region, which are proteins having high affinity for CD2 antigen of human T-cells and are the LFA-3 like proteins suitable for production by genetic engineering techniques.

According to the present invention DNA coding for the above-mentioned proteins can be obtained. Therefore, the present invention enables to produce the LFA-3 like protein deficient in TM region and the LFA-3 like protein deficient in D2 region by genetic engineering techniques. Also, the present invention enables to produce not only the molecules having the amino acid sequence of naturally occuring LFA-3, but also variants bearing artificial substitution, insertion and/or deficiency of at least one amino acid in the amino acid sequence thereof and proteins combined with other protein. Also, the above-mentioned molecules, variants and the combined protein can be chemically or enzymatically modified to give derivatives. Thus obtained derivatives include the derivatives having different affinity for CD2 antigen being a natural ligand of LFA-3 from that of LFA-3.

There can be used as diagnostic agents T-cell detection the sheep LFA-3 protein, the LFA-3 like protein deficient in TM region, the LFA-3 like protein

deficient in D2 region and derivatives thereof which are produced by genetic engineering techniques and have affinity to human T-cells. As the diagnostic agents, there can be utilized the above-mentioned proteins and derivatives thereof which are labeled with an enzyme, a fluorescent agent or an isotope. Also, the carrier of the present invention onto which at least one of them is chemically or physically immobilized can be subjected to separation (isolation or removal) of T-cells. There can be used the sheep LFA-3 protein, the LFA-3 like protein deficient in TM region, the LFA-3 like protein deficient in D2 region and derivatives thereof, and the proteins combined with other protein as therapeutic agents which target a tumor of T-cell family or a leucemia cell by utilizing affinity thereof for T-cells. For instance, the above-mentioned proteins, derivatives and the combined proteins which are conjugated with a toxin such as ricin, can be used as therapeutic agents.

It is known that CD2 antigen being a natural ligand of LFA-3 participates in various immune response as function of T-cell. Therefore, there can be utilized the sheep LFA-3 protein, the LFA-3 like protein deficient in D2 region, the LFA-3 like protein deficient in TM region, a variant thereof, or a derivative thereof which has affinity to CD2 antigen as an agent for inhibiting or activating immune response(s), i.e. an immunoregulative agent.

Also, comparing the amino acid sequence of the sheep LFA-3 like protein deficient in TM region with that of human LFA-3 (Fig. 1), it can be supposed that the human LFA-3 like protein deficient in TM region is a protein having a sequence wherein the sequence from the 188th amino acid to the 212th amino acid lacks in human LFA-3 of SEQ ID NO: 8, or a protein having a sequence wherein the sequence from the 188th amino acid to the 212th amino acid of human LFA-3 of SEQ ID NO: 8 lacks and further, in the sequence between the 213th amino acid to the 222nd amino acid in SEQ ID NO: 8,

at least one amino acid is substituted.

The present invention is more specifically described and explained by means of the following Examples in which all percents are by weight unless otherwise noted. It is to be understood that the present invention is not limited to the Examples, and various changes and modifications may be made in the invention without departing from the spirit and scope thereof.

Example 1

a. Synthesis of primers used in polymerase chain reaction (PCR) for preparing cDNA of sheep LFA-3

As a partial amino acid sequence of a sheep receptor for CD2 antigen, has been disclosed the N-terminal amino acid sequence consisting of 29 amino acid residues shown in SEQ ID NO:3 (refer to Japanese Unexamined Patent Publication No. 150228/1988). In order to use in PCR for preparing cDNA coding for sheep LFA-3, following two kinds of mixed primers were synthesized by means of a DNA synthesizer (made by Applied Biosystems, Model 381A). One mixed primer is shown in SEQ ID NO: 4, consisting of a restriction enzyme BamHI recognition sequence and a following nucleotide sequence deduced from the sequence of the 1st-7th amino acids in SEQ ID NO:3. The other is shown in SEQ ID NO: 5, consisting of a sequence containing a restriction enzyme PstI recognition sequence and a following nucleotide sequence deduced from the sequence of the 27th-22nd amino acids in SEQ ID NO:3.

b. Preparation of double strand cDNA from sheep cells

From sheep was collected 100 mℓ of blood with heparin. The collected blood was centrifuged at 350G, 10 minutes to give a buffy coat fraction. After lysis of erythrocytes in the fraction by using erythrocyte lysing buffer, lysate was washed twice with PBS (phosphate buffered saline) to give sheep

leukocytes. Then, from the obtained sheep leukocytes was extracted RNA by guanidine thiocyanate method. Further poly(A)$^+$RNA was purified by means of an oligo(dT)-cellulose column (refer to Rabomanyuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, 1988). Double strand cDNA was synthesized from the poly(A)$^+$RNA by means of a commercially available kit (You-Prime cDNA Synthesis Kit #27-9260-01, made by Pharmacia). Similarly, another double strand cDNA was synthesized from a commercially available mRNA of sheep liver (made by Clontech).

c. Amplification by PCR method and cloning of cDNA coding for sheep LFA-3

By PCR method were amplified cDNA fragments coding for sheep LFA-3 in vitro. That is, using 100 $\mu\ell$ of a reaction mixture containing 10 mM Tris-HCℓ (pH 8.3), 100 mM potassium chloride, 1.5 mM magnesium chloride, 0.01 % gelatin, 10 μM of each of 2 kinds of the mixed primers synthesized in the above-mentioned a, 10 ng of cDNA of the sheep leukocytes prepared in the above-mentioned b or cDNA of sheep liver, 0.2 mM of 4 kinds of deoxyribonucleic triphosphates (4 dNTP) and 2.5 units of Taq DNA polymerase, 35 cycles of PCR were carried out under the reaction condition per cycle of 94℃, 1 minute; 37℃, 2 minutes; and 72℃, 2 minutes. After completing the reactions, size of PCR products was measured by a polyacrylamide gel electrophoresis, and then it was found that DNA fragments of about 100 base pairs were amplified in both cases of using cDNA of sheep leukocyte and using cDNA of sheep liver. The DNA fragments of about 100 base pairs were extracted from the gel and treated with restriction enzymes BamHI and PstI. Then the treated DNA was inserted into BamHI-PstI site of M13mp19 phage vector (made by TAKARA SHUZO Co.) and cloned by using E. coli JM109 as host.

d. Sequencing of DNA amplified by PCR

There was determined sequence of the DNA of about 100 base pairs prepared from a positive clone obtained in the above-mentioned c by a conventional method using dideoxynucleotide triphosphates. As a result, both sequences between 2 kinds of the mixed primers used in PCR of PCR products from the leukocytes cDNA and the liver cDNA were determined to be the nucleotide sequence of the 19th-66th DNA sequence shown in SEQ ID NO: 30 (2 and 35). The sequence corresponds to the sequence from the 7th amino acid of Gly to the 22nd amino acid of Pro in the known partial amino acid sequence of a sheep receptor for CD2 antigen shown in SEQ ID NO: 3. It is shown that cDNA of sheep LFA-3 contains the above-mentioned nucleotide sequence. The DNA sequence codes the 7th-22nd amino acids in SEQ ID NO: 31 (1 and 36).

e. Isolation of cDNA of sheep LFA-3 and analogues thereof

In order to clone a full-length cDNA of sheep LFA-3, sheep cDNA library was screened by using N-terminal cDNA sequence of sheep LFA-3 determined in the above-mentioned d as a probe. That is, the double strand cDNA from sheep leukocytes prepared in the above-mentioned b was treated with DNA polymerase to give DNA fragments with blunt ends to which EcoRI linker (made by Pharmacia) was connected. Further, the linked DNA fragment was cut with EcoRI, and thereto were connected right and left arms of λ gt11 (made by Stratagene) treated with alkaline phosphatase. Packaging in vitro was carried out to prepare cDNA library. There were synthesized probes having cDNA sequence adjacent N-terminus of sheep LFA-3, i.e., the probe having the nucleotide sequence of SEQ ID NO: 6 and that of SEQ ID NO: 7. By using these probes were screened about 2 x 10^5 recombinant phages. As a result, 3 positive clone (SL-6, SL-40 and SL-43) containing 1.0 kb (kilonucleotide)-1.2 kb of cDNA insert were obtained.

Example 2

f. Sequencing of cDNA of sheep LFA-3

Among the positive clones obtained in Example 1, cDNA contained in SL-6 was sequenced by dideoxy method using M13 phage. As a result, sequence shown in the nucleotide sequence of SEQ ID NO: 30 was found. Thus obtained nucleotide sequence corresponds to, and amino acid sequence determined from the nucleotide sequence is shown as, the amino acid sequence of sheep LFA-3 being the sequence from Val at N-terminus to Pro at C-terminus shown in SEQ ID NO:31. Comparison of the amino acid sequence of SEQ ID NO: 31 with that of human LFA-3 reveals that the determined amino acid sequence has regions corresponding to D1 region, D2 region, TM region and C region of human LFA-3, respectively. Therefore, it is confirmed that the protein encoded by thus obtained DNA is sheep LFA-3.

g. Construction of an expression vector for sheep LFA-3 protein in E. coli

In order to make cDNA coding for sheep LFA-3 protein express in E. coli, an expression vector was constructed. The DNA insert contained in the cDNA clone SL-6 obtained in Example 1 was taken out by cleaving with restriction enzyme EcoRI. The DNA insert was subcloned into EcoRI site of plasmid pUC18. Successively PCR was carried out by using the plasmid as a template. Used 5' primer is shown in SEQ ID NO: 37. This primer is comprised of BamHI recognition sequence, NcoI recognition sequence and the DNA sequence designed according to the sequence of the 1st-7th amino acids in SEQ ID NO: 31. Used 3' primer is shown in SEQ ID NO: 38. The primer is comprised of PstI recognition sequence, sequence of termination codon and the DNA sequence designed according to the sequence of the 219th-225th amino acids in SEQ ID NO: 31. PCR was carried out by using the primers of SEQ ID NO: 37 and SEQ ID NO: 38 to amplify DNA fragments. Thus amplified

DNA fragments were cleaved with restriction eyzymes BamHI and PstI, and thus obtained fragments were inserted into BamHI-PstI site of M13mp19 phage vectors. Nucleotide sequence of the inserted DNA was confirmed by dideoxy method. Then, DNA coding for sheep LFA-3 protein was taken out from the M13mp19 phage vector by cleaving with restriction enzymes NcoI and PstI. Thus obtained DNA was connected to NcoI-PstI site of vector pKK233-2 (made by Pharmacia) for expression to give an expression vector. Thus obtained expression vector is referred to as "pKSL".

h. Production of sheep LFA-3 protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSL of sheep LFA-3 protein. Then, the precultured E. coli was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until absorbance at 600 nm (hereinafter referred as "A_{600}") of the medium containing E. coli became 0.3. Successively thereto was added IPTG (isopropyl-β-D-thio-galactopyranoside, made by Wako Pure Chemical Industries, Ltd.) so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100 and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated and centrifuged to give an insoluble precipitation fraction. The obtained precipitation was washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced sheep LFA-3 protein. The inclusion bodies were dissolved with SDS (sodium dodecyl sulfate) and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight 25,000-27,000.

Example 3

i. Sequencing of cDNA of the sheep LFA-3 like protein deficient in TM region (hereinafter referred as to "sheep ΔTM protein")

Among the positive clones obtained in Example 1, cDNA contained in SL-43 was sequenced by dideoxy method using M13 phage. As a result, sequence shown in the nucleotide sequence of SEQ ID NO: 35. Thus obtained nucleotide sequence corresponds to, and amino acid sequence determined from the nucleotide sequence is shown as, the amino acid sequence of sheep ΔTM protein being the sequence from Val at N-terminus to Ser at C-terminus shown in SEQ ID NO: 36. The sequencing also revealed that sheep ΔTM protein has a signal peptide having 28 amino acid residues which starts from methionine. On the basis of thus obtained information of cDNA and nucleotide sequence of sheep ΔTM protein, natural type sheep ΔTM protein and a derivative thereof can be produced with a recombinant by genetic engineering techniques. Fig. 1 shows comparison of the amino acid sequence of sheep ΔTM protein shown in SEQ ID NO: 13 to that of human LFA-3 shown in SEQ ID NO: 8. Fig. 1 reveals that sheep ΔTM protein lacks TM region (underlined portion) existing in human LFA-3.

j. Preparation of an expression vector of sheep ΔTM protein in E. coli

In order to make cDNA coding for sheep ΔTM protein express in E. coli, an expression vector was constructed. The DNA insert contained in the cDNA clone SL-43 obtained in Example 1 was taken out by cleaving with restriction enzyme EcoRI. The DNA insert was subcloned into EcoRI site of plasmid pUC18. Successively such plasmid was subjected to PCR. Used 5' primers are shown in SEQ ID NO: 32 and 33. These primers are comprised of BamHI recognition sequence, NcoI recognition sequence and the DNA sequence designed according to the sequence of the 1st-7th amino acids

in SEQ ID NO: 36. Used 3' primer is shown in SEQ ID NO: 34. The primer is comprised of SalI recognition sequence, PstI recognition sequence, sequence of termination codon and the DNA sequence designed according to the sequence of the 199th-193rd amino acids in SEQ ID NO: 36. PCR was carried out by using the primers of SEQ ID NO: 32 and 34, or the primers of SEQ ID NO: 33 and 34 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction eyzymes BamHI and SalI, and thus obtained fragments were inserted into each BamHI-SalI site of M13mp19 phage vector. Nucleotide sequence of the inserted DNA was confirmed to have the desired sequence by dideoxy method. Then, DNA of sheep ΔTM protein was taken out from the M13mp19 phage vectors by cleaving with restriction enzymes NcoI and PstI. Thus obtained DNAs were connected to NcoI-PstI site of vector pKK 233-2 (made by Pharmacia) for expression to give two expression vectors. One of thus obtained expression vector is referred to as "pKSLΔTM-0", which was prepared by using the primers of SEQ ID NO: 32 and 34. The other is referred to as "pKSLΔTM-1", which was prepared by using the primers of SEQ ID NO: 33 and 34.

k. Production of sheep ΔTM protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSLΔTM-0 or pKSLΔTM-1 of sheep ΔTM protein. Then, the precultured E. coli was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0) containing 50 mM EDTA, 5

% Triton X-100 and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give an inscluble precipitation fraction. The obtained precipitation was washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced sheep ΔTM protein. The expression vector pKSLΔTM-1 was superior to the expression vector pKSLΔTM-0 in productivity. The inclusion bodies were dissolved with SDS (sodium dodecyl sulfate) and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of apparent molecular weight 23,000-25,000.

ℓ. Solubilization and renaturation of inclusion bodies containing sheep ΔTM protein

All the inclusion bodies obtained in the above-mentioned k were dissolved in 10 mℓ of a buffer (pH 9.5) containing 8M urea, 20 mM ammonium acetate, 0.4 mM cystein and 0.04 mM cystein and centrifuged to give a supernatant. The supernatant was diluted with 8M urea so as to become A_{280} 0.1, and dialyzed against ten-fold amount of the same buffer and successively against PBS to give a solution containing soluble sheep ΔTM protein. Thus obtained protein was subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight 23,000-25,000. The molecular weight also confirmed that the obtained protein is sheep ΔTM protein. Thus prepared sheep ΔTM protein in an amount ranging from 25-0 μg was mixed with 1 x 10^5 Jurkat cells which were washed with the PBS containing 5 % bovine serum albumin (BSA) and 1 % glucose, and contained in 50 μℓ of the same buffer. Thereafter there was observed effect on rosette formation by adding 1 x 10^7 sheep erythrocytes. Sheep ΔTM protein dose-dependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes.

With respect to sheep ΔTM protein, the

concentration for inhibiting 50 % rosette formation was about 20 μ g/mℓ .

Example 4

m. Sequencing of cDNA of the sheep LFA-3 like protein deficient in D2 region (hereinafter referred to as "sheep ΔD2 protein")

Among the positive clones obtained in Example 1, cDNA sequence contained in SL-40 was determined by dideoxy method using M13 phage. As a result, it is revealed that sheep ΔD2 protein is encoded by the nucleotide sequence of SEQ ID NO: 2 and has the amino acid sequence of SEQ ID NO: 1. It is also revealed that sheep ΔD2 protein has a signal peptide having 28 amino acid residues which starts from methionine. On the basis of thus obtained information of cDNA of sheep ΔD2 protein, natural type sheep ΔD2 protein and derivatives thereof can be produced by genetic engineering techniques. The amino acid sequence of SEQ ID NO: 8 is that of human LFA-3 which has been reported by Wallner et al (B. P. Wallner et al., Journal of Experimental Medicine, vol. 166, p 923, (1987)), and SEQ ID NO: 16 shows the DNA sequence thereof. Also, Fig. 2 shows correspondence of the amino acid sequence of sheep ΔD2 protein shown in SEQ ID NO: 1 to that of human LFA-3 shown in SEQ ID NO: 8. Fig. 2 reveals that sheep ΔD2 protein lacks D2 region (underlined portion) existing in human LFA-3.

n. Preparation of an expression vector of sheep ΔD2 protein in E. coli

In order to make cDNA coding for sheep ΔD2 protein express in E. coli, an expression vector was constructed. The DNA insert contained in the cDNA clone SL-40 obtained in Example 1 was taken out by cleaving with restriction enzyme EcoRI. The DNA fragment was subcloned into EcoRI site of plasmid pUC18. Successively such plasmid was subjected to PCR. Used

5' primer is shown in SEQ ID NO: 9. This primer is comprised of BamHI recognition sequence, NcoI recognition sequence and the DNA sequence designed according to the sequence of the 1st-7th amino acids of SEQ ID NO: 2. Used 3' primer is shown in SEQ ID NO: 10. The primer is comprised of SalI recognition sequence, PstI recognition sequence, sequence of termination codon and the DNA sequence designed according to the sequence of the 131st-126th amino acids of SEQ ID NO: 2. PCR was carried out by using the primers of SEQ ID NO: 9 and SEQ ID NO: 10 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction eyzymes BamHI and SalI, and thus obtained fragments were inserted into BamHI-SalI site of M13mp19 phage vector. Nucleotide sequence of the inserted DNA was confirmed by dideoxy method. Then, DNA coding for sheep ΔD2 protein was taken out from the M13mp19 phage vector by cleaving with restriction enzymes NcoI and PstI. Thus obtained DNA was connected to NcoI-PstI site of vector pKK233-2 (made by Pharmacia) for expression to give an expression vector. Thus obtained expression vector is referred to as "pKSLΔD2". SEQ ID NO: 20 shows nucleotide sequence from initiation codon to termination codon coding for sheep ΔD2 protein contained in pKSLΔD2.

o. Production of sheep ΔD2 protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSLΔD2 of sheep ΔD2 protein. Then, the precultured E. coli was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0)

containing 50 mM EDTA, 5 % Triton X-100 and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give an insoluble precipitation fraction. The obtained precipitation was washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced sheep ΔD2 protein. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight 15,000-16,000.

p. Preparation of an expression vector of a soluble derivative of sheep ΔD2 protein (hereinafter referred to as "sheep D1HC protein") in E. coli

In order to make cDNA of sheep D1HC protein express in E. coli, an expression vector was constructed.

Namely, the DNA insert contained in the cDNA clone SL-40 obtained in Example 1 was taken out by cleaving with restriction enzyme EcoRI. The DNA insert was subcloned into EcoRI site of plasmid pUC18. Successively such plasmid was subjected to PCR. Used 5' primer is shown in SEQ ID NO: 9. This primer is comprised of BamHI recognition sequence, NcoI recognition sequence and the DNA sequence designed according to the sequence of the 1st-7th amino acids of SEQ ID NO: 2. Used 3' primer is shown in SEQ ID NO: 11. The primer is comprised of PstI recognition sequence, sequence of termination codon, the 371st-358th nucleotide sequence and the 301st-277th nucleotide sequence of SEQ ID NO: 2. PCR was carried out by using the primers of SEQ ID NO: 9 and SEQ ID NO: 11 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction eyzymes BamHI and PstI, and thus obtained fragments were inserted into BamHI-PstI site of M13mp19 phage vectors. Nucleotide sequence of the inserted DNA was confirmed by dideoxy method. Then, DNA of sheep D1HC protein was taken out from the M13mp19

phage vector by cleaving with restriction enzymes NcoI and PstI. Thus obtained DNA was connected to NcoI-PstI site of vector pKK233-2 (made by Pharmacia) for expression to give an expression vector. Thus obtained expression vector is referred to as "pKSLD1HC". SEQ ID NO: 21 shows nucleotide sequence from initiation codon to termination codon coding for sheep D1HC protein contained in pKSLD1HC.

q. Production of sheep D1HC protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSLD1HC of sheep D1HC protein. Then, the precultured E. coli was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100, 2 mM dithiothreitol and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give insoluble inclusion bodies. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 12,000.

r. Solubilization and renaturation of inclusion bodies containing sheep D1HC protein

All the inclusion bodies obtained in the above-mentioned q were dissolved in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 7.4) containing 6M guanidine chloride and 2 mM EDTA, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution

containing sheep D1HC protein. Thus prepared sheep D1HC protein was mixed with 1 x 10^5 Jurkat cells which were washed with the PBS containing 5 % bovine serum albumin (BSA) and 1 % glucose, and contained in 50 $\mu\ell$ of the same buffer. Thereafter there was observed effect on rosette formation by adding 1 x 10^7 sheep erythrocytes. Sheep D1HC protein dose-dependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes. The results are shown in Fig. 3.

Rosette formation inhibition rate was calculated by the following formula;

$$\text{Inhibition rate (\%)} = 1 - \frac{\text{Rosette positive cells (\%) in sample}}{\text{Rosette positive cells (\%) in control}} \times 100$$

"Rosette positive cells" are cells to which not less than 5 sheep erythrocytes per cell adhere to form rosette.

Example 5

s. Synthesis of primers used in PCR for preparing cDNA of the human LFA-3 like protein deficient in D2 region (hereinafter referred to as "human ΔD2 protein")

There was cloned cDNA of human LFA-3, and the nucleotide sequence thereof has been known (B. P. Wallner et al., Journal of Experimental Medicine, vol. 166, p 923, (1987)). The nucleotide sequence is shown in SEQ ID NO: 16. In order to use in PCR for preparing cDNA coding for human ΔD2 protein, following 2 kinds of primers were synthesized by means of a DNA synthesizer (made by Applied Biosystems, Model 381A). One was a primer, shown in SEQ ID NO: 14, consisting of a sequence containing the recognition sequences of restriction enzymes PstI and NcoI and the 1st-24th nucleotide sequence of SEQ ID NO: 16. The other was a primer, shown in SEQ ID NO: 15, consisting of a sequence containing

the recognition sequences of restriction enzymes PstI and EcoRI and the 753rd-730th nucleotide sequence of SEQ ID NO:16.

t. Preparation of cDNA of human ΔD2 protein

Human T-cell line MOLT-4 (ATCC CRL-1582) was cultured in RPMI1640 medium containing 10 % fetal calf serum (FCS) to give 5 x 10^8 cells. The cells were washed twice with PBS. Successively RNA was extracted from the cells by guanidine thiocyanate method. Further poly(A)$^+$RNA was purified by means of an oligo(dT)-cellulose column (refer to Rabomanyuaruidenshikogaku, edited by Masami Muramatsu, Maruzen, (1988)). Double strand cDNA was synthesized from the poly(A)$^+$RNA by means of a commercially available kit (cDNA synthesis kit # 27-9260-01, made by Pharmacia).

u. Amplification by PCR method and cloning of cDNA coding for human ΔD2 protein, and sequencing thereof

By PCR method were amplified cDNA fragments coding for human ΔD2 protein in vitro. That is, using 100 μℓ of a reaction mixture containing 10 mM Tris-HCℓ (pH 8.3), 100 mM potassium chloride, 1.5 mM magnesium chloride, 0.01 % gelatin, 10 μM of each of 2 kinds of primers synthesized in the above-mentioned s, 10 ng of human double strand cDNA prepared in the above-mentioned t, 0.2 mM of 4 kinds of deoxyribonucleic triphosphates (4 dNTP) and 2.5 units of Taq DNA polymerase, 35 cycles of PCR were carried out under the reaction condition per cycle of 94℃, 1 minute; 37℃, 2 minutes; and 72℃, 2 minutes. After completing the reactions, size of PCR products was measured by a polyacrylamide gel electrophoresis, and then it was found that DNA fragments of about 500 base pairs were amplified. The DNA fragments of about 500 base pairs were extracted from the gel and treated with restriction enzyme PstI. Then the treated DNA was inserted into PstI site of pUC19 vector (made by TAKARA

SHUZO Co.) and cloned by using E. coli JM109 as host. The prepared plasmid is referred to as "pHLΔD2".

Then, in order to sequence cDNA amplified by PCR, the DNA fragment of about 500 base pairs cleaved with PstI was inserted into PstI site of M13mp19 phage vector (made by TAKARA SHUZO Co.) and sequenced by a conventional method using dideoxynucleotide triphosphates. As a result, cDNA sequence of human ΔD2 protein shown in SEQ ID NO: 12 was found among about 500 base pairs of DNA fragments. Such cDNA sequence codes for the protein having the amino acid sequence shown in SEQ ID NO: 13. Comparing human ΔD2 protein shown in SEQ ID NO: 13 with human LFA-3 shown in SEQ ID NO: 8, it is found that human ΔD2 protein lacks the amino acids from the 94th amino acid of Glu to the 181st amino acid of Ser in SEQ ID No: 8, i.e., D2 region.

v. Preparation of an expression vector of human ΔD2 protein in E. coli

In order to make cDNA of human ΔD2 protein express in E. coli, an expression vector was constructed. The DNA of plasmid pHLΔD2 having cDNA of human ΔD2 protain obtained in the above-mentioned u was used as a template to carry out PCR. Used 5' primer is shown in SEQ ID NO: 17. This primer is comprised of KpnI recognition sequence, GG, sequence of initiation codon and the 1st-24th nucleotide sequence of SEQ ID No: 12. Used 3' primer is shown in SEQ ID NO: 18. The primer is comprised of HindIII recognition sequence, NheI recognition sequence, sequence of termination codon and the 402nd-379th nucleotide sequence of SEQ ID NO: 12. PCR was carried out by using the primers of SEQ ID NO: 17 and SEQ ID NO: 18 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes KpnI and HindIII. The cleaved fragments were connected to KpnI-HindIII site of vector pKK233Kpn to give an expression vector. Thus obtained expression vector is referred to as "pKHLΔD2". The

vector pKK233Kpn is a plasmid wherein the sequence from SD sequence to initiation codon AGGAAACAGACCATG of pKK233-2 (made by Pharmacia) has been varied to the sequence AGGAGGTACCGGATG containing the recognition sequence of restriction enzyme KpnI by site directed mutagenesis method. SEQ ID NO: 22 shows the nucleotide sequence from initiation codon to termination codon conding for human ΔD2 protein contained in pKHLΔD2.

w. Production of human ΔD2 protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLΔD2 of human ΔD2 protein. Then, the precultured E. coli was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100 and ⌣ % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give an insoluble precipitation fraction. The obtained precipitation was washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced human ΔD2 protein. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight 15,000-16,000.

x. Preparation of an expression vector of a soluble derivative of human ΔD2 protein (hereinafter referred to as "human D1HC protein") in E. coli

In order to make cDNA of human D1HC protein express in E. coli, an expression vector was

constructed.

That is, the DNA of plasmid pHLΔD2 having cDNA of human ΔD2 protein obtained in the above-mentioned u was used as a template to carry out PCR. Used 5' primer is shown in SEQ ID NO: 17. This primer is comprised of KpnI recognition sequence, GG, sequence of initiation codon and the 1st-24th nucleotide sequence of SEQ ID No: 12. Used 3' primer is shown in SEQ ID NO: 19. The primer is comprised of HindIII recognition sequence, sequence of termination codon and the 402nd-373rd nucleotide sequence and the 297th-268th nucleotide sequence of SEQ ID NO: 12. PCR was carried out by using the primers of SEQ ID NO: 17 and SEQ ID NO: 19 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes KpnI and HindIII. The cleaved fragments were connected to KpnI-HindIII site of vector pKK233Kpn to give an expression vector. Thus obtained expression vector is referred to as "pKHLD1HC". SEQ ID NO: 23 shows the nucleotide sequence from initiation codon to termination codon coding for human D1HC protein contained in pKHLD1HC.

y. Production of human D1HC protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLD1HC of human D1HC protein. Then, the precultured E. coli was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100, 2 mM dithiothreitol and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %.

The cell suspension was sonicated, and centrifuged to give insoluble inclusion bodies. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 13,000.

z. Solubilization and renaturation of inclusion bodies containing human D1HC protein

All the inclusion bodies obtained in the above-mentioned y were dissolved in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 7.4) containing 6M guanidine hydrochloride and 2 mM EDTA, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing human D1HC protein. Thus prepared human D1HC protein in an amount ranging from 2.5-0 μg was mixed with 1 x 10^5 Jurkat cells which were washed with the PBS containing 5 % BSA and 1 % glucose, and contained in 50 μℓ of the same buffer. Thereafter there was observed effect on rosette formation by adding 1 x 10^7 sheep erythrocytes. Human D1HC protein dose-dependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes. The results are shown in Fig. 3.

Example 6

α. Immobilization of sheep or human D1HC protein onto carrier

In 0.1 M glycine buffer (pH 8.2) containing 15 mM NaCℓ was dissolved sheep D1HC protein prepared in the above-mentioned r or human D1HC protein prepared in the above-mentioned z so as to give 100 μg/mℓ of final concentration thereof. Thus obtained solution was added in an amount of 100 μℓ per well to 96-wells microtiter plate made by Costar (catalog number 3590), and incubated at 37℃ for 1 hour to coat bottom of wells in plate with the protein. Then, the plate was treatd with 1 % solution of BSA. After washing the plate thereto were added 2 x 10^5 Jurkat cells per well which were

washed with the PBS containing 5 % BSA and 1 % glucose and suspended in 100 $\mu\ell$ of the same buffer. The plate was allowed to stand for 30 minutes at 4℃. The plate was washed 3 times with PBS, then cells adhered to the bottom of wells were observed. As a result, it is found that the cells adhered over the bottom of wells coated with sheep D1HC protein or human D1HC protein. In contrast, the cells hardly adhered to the bottom of wells coated with no D1HC protein.

Example 7

β. Preparation of an expression vector of sheep D1HC protein containing cysteine residue (hereinafter referred to as "sheep D1HCcys protein") in E. coli

In order to make cDNA of sheep D1HC protein having cysteine residue at carboxyl terminal express in E. coli, an expression vector was constructed. The DNA of plasmid pKSLD1HC having cDNA of sheep D1HC protain obtained in the above-mentioned p was used as a template to carry out PCR. Used 5' primer is the primer used in the above-mentioned p and shown in SEQ ID NO: 9. Used 3' primer is shown in SEQ ID NO: 24. The primer is comprised of HindIII recognition sequence, PstI recognition sequence, sequence of termination codon, sequence coding for cysteine residue and then the following 318th-295th nucleotide sequence of SEQ ID NO: 21. PCR was carried out by using the primers of SEQ ID NO: 9 and SEQ ID NO: 24 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes BamHI and PstI. The cleaved fragment was inserted into BamHI-PstI site of M13mp19 phage vector to give a recombinant vector. Nucleotide sequence of DNA introduced in the recombinant vector was confirmed by dideoxy method. Then, cDNA coding for sheep D1HCcys protein was taken out from the M13mp19 phage vector by cleaving with restriction enzymes NcoI and PstI. Thus obtained DNA was connected to NcoI-PstI site of vector pKK233-2 (made by Pharmacia) to give an expression

vector pKSLD1HCcys. SEQ ID NO: 25 shows the nucleotide sequence from initiation codon to termination codon coding for sheep D1HCcys protein contained in pKSLD1HCcys.

γ. Production of sheep D1HCcys protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSLD1HCcys of sheep D1HCcys protein. Then, the precultured E. coli was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100, 2mM dithiothreitol and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give insoluble inclusion bodies. The obtained inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 13,000.

δ. Solubilization and renaturation of inclusion bodies containing sheep D1HCcys protein

All the inclusion bodies obtained in the above-mentioned γ were dissolved in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 7.4) containing 6M guanidine hydrochloride, 2 mM EDTA and 5mM 2-mercaptoethanol, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing sheep D1HCcys protein. Thus prepared sheep D1HCcys protein in an amount ranging from 2.5-0 μg was mixed with 1 x 10^5 Jurkat cells which were washed with the PBS containing 5

% BSA and 1 % glucose, and contained in 50 $\mu\ell$ of the same buffer. Thereafter there was observed effect on rosette formation by adding 1 x 10^7 sheep erythrocytes. Sheep D1HCcys protein dose-dependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes.

Example 8

ε. Preparation of an expression vector of human D1HC protein containing cysteine residue (hereinafter referred to as "human D1HCcys protein") in E. coli

In order to make cDNA of human D1HC protein having cysteine residue at carboxyl terminal express in E. coli, an expression vector was constructed. The DNA of plasmid pKHLD1HC having cDNA of human D1HC protein obtained in the above-mentioned x, was used as a template to carry out PCR. Used 5' primer is the primer used in the above-mentioned x and shown in SEQ ID NO: 17. Used 3' primer is shown in SEQ ID NO: 26. The primer is comprised of HindIII recognition sequence, sequence of termination codon, sequence coding for cysteine residue and the DNA sequence designed according to the sequence of the 330th-310th amino acids of SEQ ID NO: 23. PCR was carried out by using the primers of SEQ ID NO: 17 and SEQ ID NO: 26 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes KpnI and HindIII. The cleaved fragments was connected to KpnI-HindIII site of vector pKK233Kpn to give the expression vector pKHLD1HCcys. SEQ ID No: 27 shows the nucleotide sequence from initiation codon to termination codon coding for human D1HCcys protein contained in pKHLD1HCcys.

ζ. Production of human D1HCcys protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLD1HCcys of human D1HCcys protein. Then, the precultured E. coli

was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100, 2 mM dithiothreitol and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 0.1 %. The cell suspension was sonicated, and centrifuged to give insoluble inclusion bodies. The obtained inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 13,000.

η. Solubilization and renaturation of inclusion bodies containing human D1HCcys protein

All the inclusion bodies obtained in the above-mentioned ξ were dissolved in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 7.4) containing 6M guanidine hydrochloride, 2 mM EDTA and 5 mM 2-mercaptoethanol, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing human D1HCcys protein. Thus prepared human D1HCcys protein in an amount ranging from 2.5-0 μg was mixed with 1 x 10^5 Jurkat cells washed with the PBS containing 5 % BSA and 1 % glucose, and contained in 50 μℓ of the same buffer. Thereafter there was observed effect on rosette formation by adding 1 x 10^7 sheep erythrocytes. Human D1HCcys protein dose-dependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes.

Example 9

θ. Cloning of cDNA coding for human LFA-3

There was carried out cloning of cDNA coding

for a full-length protein of human LFA-3. In the same manner as in the above-mentioned t, poly(A)$^+$RNA from human T-cell line MOLT-4 was purified. Further the purified poly(A)$^+$RNA was used to synthesize double strand cDNA.

From the double strand cDNA was amplified cDNA coding for human LFA-3 protein by using 2 kinds of primers synthesized in the above-mentioned s being shown in SEQ ID No: 14 and SEQ ID NO: 15 by PCR method in vitro under the same condition as in the above-mentioned u. After completing the reaction, size of PCR products was measured by a polyacrylamide gel electrophoresis, and then it was found that DNA fragments of about 800 base pairs were amplified. The DNA fragments of about 800 base pairs were extracted from the gel and treated with restriction enzyme PstI. Then, the treated DNA was inserted into PstI site of pUC19 vector (made by TAKARA SHUZO Co.) and cloned by using E. coli JM 109 as host.

Then, in order to sequence the DNA amplified by PCR, DNA fragment of about 800 base pairs cleaved with PstI was inserted into PstI site of M13mp19 phage vector (made by TAKARA SHUZO Co.) and determined by a conventional method using dideoxynucleotide triphosphates. As a result, cDNA sequence of human LFA-3 protein shown in SEQ ID NO: 16 was found among DNA fragments of about 800 base pairs. The amino acid sequence of human LFA-3 protein encoded by such cDNA sequence completely coincided with that of human LFA-3 (SEQ ID NO: 8) previously reported by B. P. Wallner et al. (Journal of Experimental Medicine, vol. 166, p 923, 1987).

t. Preparation of an expression vector of soluble human D1 protein having a part of amino acid sequence of D2 region in E. coli

In order to make DNA coding for soluble human D1 protein having the sequence from N-terminus to the first cysteine residue of D2 region (hereinafter

referred to as "human D1cys protein") express in E. coli, an expression vector was constructed. As a template was used cDNA of human LFA-3 protein obtained in the above-mentioned θ to carry out PCR. Used 5' primer is the primer used in the above-mentioned x and shown in SEQ ID NO: 17. Used 3' primer is shown in SEQ ID NO: 28. The primer is comprised of HindIII recognition sequence, sequence of termination codon, sequence coding for cysteine residue and the DNA sequence designed according to the sequence of the 102nd-96th amino acids of SEQ ID NO: 8. PCR was carried out by using the primers of SEQ ID NO: 17 and SEQ ID NO: 28 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction enzymes KpnI and HindIII.

The cleaved fragment was connected to KpnI-HindIII site of vector pKK233Kpn to give an expression vector pKHLD1cys. SEQ ID NO: 29 shows the nucleotide sequence from initiation codon to termination codon coding for human D1cys protein contained in pKHLD1cys.

κ. Production of human D1cys protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLD1cys of human D1cys protein. Then, the precultured E. coli was inoculated in 100 mℓ of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37℃ in 500 mℓ of Sakaguchi flask until A_{600} of the medium containing E. coli became 0.3. Successively thereto was added IPTG so as to give a final concentration of 1 mM and further culture was continued for 6 hours. The cultured cells were collected by centrifugation, and suspended in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100 and 8 % sucrose. Thereto was added lysozyme so as to give a final concentration of 1 %. The cell suspension was

sonicated, and centrifuged to give an insoluble precipation fraction. The obtained precipitation was washed with 50 % glycerol and sucessively with ethanol to give inclusion bodies containing produced human Dlcys protein. The inclusion bodies were dissolved with SDS and subjected to SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 12,000.

λ. Solubilization and renaturation of inclusion bodies containing human Dlcys protein

All the inclusion bodies obtained in the above-mentioned β were dissolved in 10 mℓ of 50 mM Tris-HCℓ buffer (pH 7.4) containing 6M guanidine hydrochloride, 2 mM EDTA, and 5 mM 2-mercaptoethanol, and centrifuged to give a supernatant. The supernatant was dialyzed against PBS to give a solution containing human Dlcys protein.

In addition to the ingredients used in the Examples, other ingredients can be used in the Examples as set forth in the specification to obtain substantially the same results.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) TITLE OF THE INVENTION: CELL ADHESION PROTEIN, GENE CODING FOR THE SAME, PROCESS FOR RREPARING THE SAME AND CARRIER ONTO WHICH THE SAME IS IMMOBILIZED

(ii) NUMBER OF SEQUENCES: 38

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acids

(B) TYPE: amino acid

(vi) ORIGINAL SOURCE

(A) ORGANISM: Ovis

(G) CELL TYPE: leukocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr
 1               5                  10                  15
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys
            20                  25                  30
Asp Lys Val Val Glu Trp Asp Glu Thr Ser Gly Leu Glu Ala Phe Gln
        35                  40                  45
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
    50                  55                  60
Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
65                  70                  75                  80
Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Asp Tyr
                85                  90                  95
```

Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys
100 105 110
Gly Leu Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg Asn
115 120 125
Ser Gly Pro
130

(2) INFORMATION FOR SEQ ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 393 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE
(A) ORGANISM: Ovis
(G) CELL TYPE: leukocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTT TCC CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT TAC 48
Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr
1 5 10 15
GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG AAG 96
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys
20 25 30
GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT CAG 144
Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln
35 40 45

```
TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC ACC     192
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
    50                  55                  60
ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA TCC     240
Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
65                  70                  75                  80
CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAT TAT     288
Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Asp Tyr
                85                  90                  95
GCA AGG CAT AGG TAT GTG CTT TTT GCC ATA CTG CCA GCA GTA ATA TGT     336
Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys
            100                 105                 110
GGC TTG CTG TTT TTA AAA TGT TTT CTG GGA CGT CGT AGC CAA CGA AAC     384
Gly Leu Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg Asn
        115                 120                 125
TCA GGG CCC                                                     393
Ser Gly Pro
    130
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 amino acids

(B) TYPE: amino acids

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: yes

(v) FRAGMENT TYPE: N-terminal peptide

(ix) FEATURE: "Xaa" represents one of natural amino acids. The 12th is preferably Ser. The first is Val or Phe. The third is Glu or Ser.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Val/Phe Ser Gln/Ser Asp Ile Tyr Gly Ala Met Asn Gly Xaa Val Thr Phe Tyr
1 5 10 15

Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Xaa Lys
20 25

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTTGGATCCT TYWSNCARGA YATHTAYGG 29

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ACACTGCAGC ATDATYTCNG TRAANGG 27

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AGCTATGAAC GGGAATGTAA CCTT 24

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ACCTTTTACG TTTCAGAGTC TCAA 24

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 222 amino acids

(B) TYPE: amino acid

(iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE

(A) ORGANISM: Homo sapiens

(G) CELL TYPE: Peripheral Blood Lymphocytes

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Wallner, Barbara P.
Frey, Alexis Z.

(B) TITLE: Primary Structure of Lymphocyte Function-Associated Antigen 3 (LFA-3)

(C) JOURNAL: J. Exp. Med.

(D) VOLUME: 166

(F) PAGE: 923-932

(G) DATE: Oct-1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Phe	Ser	Glu	Gln	Ile	Tyr	Gly	Val	Val	Tyr	Gly	Asn	Val	Thr	Phe	His
1				5				10						15	
Val	Pro	Ser	Asn	Val	Pro	Leu	Lys	Glu	Val	Leu	Trp	Lys	Lys	Gln	Lys
			20					25					30		
Asp	Lys	Val	Ala	Glu	Leu	Glu	Asn	Ser	Glu	Phe	Arg	Ala	Phe	Ser	Ser
		35					40					45			
Phe	Lys	Asn	Arg	Val	Tyr	Leu	Asp	Thr	Val	Ser	Gly	Ser	Leu	Thr	Ile
	50					55					60				
Tyr	Asn	Leu	Thr	Ser	Ser	Asp	Glu	Asp	Glu	Tyr	Glu	Met	Glu	Ser	Pro
65					70					75					80
Asn	Ile	Thr	Asp	Thr	Met	Lys	Phe	Phe	Leu	Tyr	Val	Leu	Glu	Ser	Leu
				85					90					95	

Pro Ser Pro Thr Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val
100 105 110
Gln Cys Met Ile Pro Glu His Tyr Asn Ser His Arg Gly Leu Ile Met
115 120 125
Tyr Ser Trp Asp Cys Pro Met Glu Gln Cys Lys Arg Asn Ser Thr Ser
130 135 140
Ile Tyr Phe Lys Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys Thr
145 150 155 160
Leu Ser Asn Pro Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr
165 170 175
Cys Ile Pro Ser Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro
180 185 190
Ile Pro Leu Ala Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly
195 200 205
Ile Leu Lys Cys Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn
210 215 220

(2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TGGGGATCCA TGGTAAGTCA AGATATTTAT GG 32

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTTGTCGACC TGCAGCTAGG GCCCTGAGTT TCGTTG 36

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 51 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CAACTGCAGC TACGACGTCC CAGAAAACCT ATGCCTTGCA TAATCAATCA C 51

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 402 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(iv) ORIGINAL SOURCE

(A) ORGANISM: Homo sapiens

(H) CELL LINE: T cell line

(vii) IMMEDIATE SOURCE:

(B) CLONE: MOLT-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC CAT      48
Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His
 1               5                  10                  15

GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA AAG      96
Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys
            20                  25                  30

GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA TCT     144
Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser
        35                  40                  45

TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT ATC     192
Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile
    50                  55                  60
```

```
TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG CCA     240
Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro
 65                  70                  75                  80
AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GGT CAT TCA     288
Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His Ser
                 85                  90                  95
AGA CAC AGA TAT GCA CTT ATA CCC ATA CCA TTA GCA GTA ATT ACA ACA     336
Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr Thr
            100                 105                 110
TGT ATT GTG CTG TAT ATG AAT GGT ATT CTG AAA TGT GAC AGA AAA CCA     384
Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys Asp Arg Lys Pro
        115                 120                 125
GAC AGA ACC AAC TCC AAT                                            402
Asp Arg Thr Asn Ser Asn
    130
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 134 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Phe Ser Glu Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His
 1               5                  10                  15
Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys
            20                  25                  30
Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser
        35                  40                  45
```

Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile
50 55 60
Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro
65 70 75 80
Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His Ser
85 90 95
Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr Thr
100 105 110
Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys Asp Arg Lys Pro
115 120 125
Asp Arg Thr Asn Ser Asn
130

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CATCACCTGC AGCCATGGAT GGTTGCTGGG AGCGACGCGG GC 42

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA
(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:
TTCACACTGC AGAATTCTCA ATTGGAGTTG GTTCTGTCTG G 41

(2) INFORMATION FOR SEQ ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 753 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE
(A) ORGANISM: Homo sapiens

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Wallner, Barbara P.
Frey, Alexis Z.
(B) TITLE: Primary Structure of Lymphocyte
Function-Associated Antigen 3 (LFA-3)
(C) JOURNAL: J. Exp. Med.
(D) VOLUME: 166
(F) PAGE: 923-932
(G) DATE: Oct-1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
ATG GTT GCT GGG AGC GAC GCG GGG CGG GCC CTG GGG GTC CTC AGC GTG      48
Met Val Ala Gly Ser Asp Ala Gly Arg Ala Leu Gly Val Leu Ser Val
-28         -25                 -20                 -15

GTC TGC CTG CTG CAC TGC TTT GGT TTC ATC AGC TGT TTT TCC CAA CAA      96
Val Cys Leu Leu His Cys Phe Gly Phe Ile Ser Cys Phe Ser Gln Gln
        -10                 -5                  1

ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC CAT GTA CCA AGC AAT     144
Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
  5                 10                  15                  20

GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA AAG GAT AAA GTT GCA     192
Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala
                  25                  30                  35

GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA TCT TTT AAA AAT AGG     240
Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg
              40                  45                  50

GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT ATC TAC AAC TTA ACA     288
Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr Ile Tyr Asn Leu Thr
          55                  60                  65

TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG CCA AAT ATT ACT GAT     336
Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro Asn Ile Thr Asp
      70                  75                  80

ACC ATG AAG TTC TTT CTT TAT GTG CTT GAG TCT CTT CCA TCT CCC ACA     384
Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro Thr
  85                  90                  95                  100

CTA ACT TGT GCA TTG ACT AAT GGA AGC ATT GAA GTC CAA TGC ATG ATA     432
Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val Gln Cys Met Ile
                  105                 110                 115

CCA GAG CAT TAC AAC AGC CAT CGA GGA CTT ATA ATG TAC TCA TGG GAT     480
Pro Glu His Tyr Asn Ser His Arg Gly Leu Ile Met Tyr Ser Trp Asp
              120                 125                 130
```

```
TGT CCT ATG GAG CAA TGT AAA CGT AAC TCA ACC AGT ATA TAT TTT AAG     528
Cys Pro Met Glu Gln Cys Lys Arg Asn Ser Thr Ser Ile Tyr Phe Lys
        135                 140                 145
ATG GAA AAT GAT CTT CCA CAA AAA ATA CAG TGT ACT CTT AGC AAT CCA     576
Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys Thr Leu Ser Asn Pro
    150                 155                 160
TTA TTT AAT ACA ACA TCA TCA ATC ATT TTG ACA ACC TGT ATC CCA AGC     624
Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr Cys Ile Pro Ser
165                 170                 175                 180
AGC GGT CAT TCA AGA CAC AGA TAT GCA CTT ATA CCC ATA CCA TTA GCA     672
Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala
                185                 190                 195
GTA ATT ACA ACA TGT ATT GTG CTG TAT ATG AAT GGT ATT CTG AAA TGT     720
Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys
            200                 205                 210
GAC AGA AAA CCA GAC AGA ACC AAC TCC AAT TGA                        753
Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn
        215                 220
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TTTGGTACCG GATGTTTTCC CAACAAATAT ATGGTGTT 38

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

TTTAAGCTTG CTAGCTCAAT TGGAGTTGGT TCTGTCTGGT TT 42

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 72 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATCAAGCTTT CAATTGGAGT TGGTTCTGTC TGGTTTTCTG TCTCTGTGTC TTGAATGACC 60

AAGCACATAA AG 72

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE

(A) ORGANISM: Ovis

(G) CELL TYPE: leukocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```
ATG GTA AGT CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT      48
Met Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe
 1               5                  10                  15

TAC GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG      96
Tyr Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly
            20                  25                  30

AAG GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT     144
Lys Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe
        35                  40                  45

CAG TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC     192
Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu
    50                  55                  60
```

```
ACC ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA   240
Thr Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu
 65                  70                  75                  80
TCC CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAT   288
Ser Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Asp
                 85                  90                  95
TAT GCA AGG CAT AGG TAT GTG CTT TTT GCC ATA CTG CCA GCA GTA ATA   336
Tyr Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile
            100                 105                 110
TGT GGC TTG CTG TTT TTA AAA TGT TTT CTG GGA CGT CGT AGC CAA CGA   384
Cys Gly Leu Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg
        115                 120                 115
AAC TCA GGG CCC TAG                                              399
Asn Ser Gly Pro
    130
```

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 321 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
ATG GTA AGT CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT      48
Met Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe
 1               5                  10                  15

TAC GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG      96
Tyr Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly
            20                  25                  30

AAG GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT     144
Lys Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe
        35                  40                  45

CAG TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC     192
Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu
    50                  55                  60

ACC ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA     240
Thr Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu
65                  70                  75                  80

TCC CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAT     288
Ser Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Asp
                85                  90                  95
```

TAT GCA AGG CAT AGG TTT TCT GGG ACG TCG TAG 321
Tyr Ala Arg His Arg Phe Ser Gly Thr Ser
100 105

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE
(A) ORGANISM: Homo sapiens
(G) CELL TYPE: T cell line

(vii) IMMEDIATE SOURCE:
(B) CLONE: MOLT-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

ATG TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC 48
Met Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe
1 5 10 15
CAT GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA 96
His Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln
20 25 30
AAG GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA 144
Lys Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser
35 40 45

```
TCT TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT     192
Ser Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr
     50                  55                  60
ATC TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG     240
Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser
 65                  70                  75                  80
CCA AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GGT CAT     288
Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His
                 85                  90                  95
TCA AGA CAC AGA TAT GCA CTT ATA CCC ATA CCA TTA GCA GTA ATT ACA     336
Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr
            100                 105                 110
ACA TGT ATT GTG CTG TAT ATG AAT GGT ATT CTG AAA TGT GAC AGA AAA     384
Thr Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys Asp Arg Lys
        115                 120                 125
CCA GAC AGA ACC AAC TCC AAT TGA                                    408
Pro Asp Arg Thr Asn Ser Asn
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 333 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
ATG TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC      48
Met Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe
 1               5                  10                  15
CAT GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA      96
His Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln
            20                  25                  30
AAG GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA     144
Lys Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser
        35                  40                  45
TCT TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT     192
Ser Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr
    50                  55                  60
ATC TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG     240
Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser
65                  70                  75                  80
CCA AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GGT CAT     288
Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His
                85                  90                  95
TCA AGA CAC AGA GAC AGA AAA CCA GAC AGA ACC AAC TCC AAT TGA         333
Ser Arg His Arg Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

TTTTTTCGAA CTGCAGCTAA CACGACGTCC CAGAAAACCT ATGCCT 46

(2) INFORMATION FOR SEQ ID NO: 25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 324 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
ATG GTA AGT CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT      48
Met Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe
 1               5                  10                  15

TAC GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG      96
Tyr Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly
            20                  25                  30

AAG GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT     144
Lys Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe
        35                  40                  45

CAG TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC     192
Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu
    50                  55                  60

ACC ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA     240
Thr Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu
65                  70                  75                  80
```

TCC CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAT 288
Ser Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Asp
85 90 95
TAT GCA AGG CAT AGG TTT TCT GGG ACG TCG TGT TAG 324
Tyr Ala Arg His Arg Phe Ser Gly Thr Ser Cys
100 105

(2) INFORMATION FOR SEQ ID NO: 26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:
GGGGAAGCTT TCAACAATTG GAGTTGGTTC TGTCTGG 37

(2) INFORMATION FOR SEQ ID NO: 27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 336 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
ATG TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC      48
Met Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe
 1               5                  10                  15
CAT GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA      96
His Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln
            20                  25                  30
AAG GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA     144
Lys Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser
        35                  40                  45
TCT TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT     192
Ser Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr
    50                  55                  60
ATC TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG     240
Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser
65                  70                  75                  80
CCA AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GGT CAT     288
Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His
                85                  90                  95
TCA AGA CAC AGA GAC AGA AAA CCA GAC AGA ACC AAC TCC AAT TGT TGA     336
Ser Arg His Arg Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn Cys
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TTTAAGCTTC AACAAGTTAG TGTGGGAGAT GGAAG 35

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 315 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

ATG TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG AAT GTA ACT TTC 48
Met Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe
1 5 10 15

CAT GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA 96
His Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln
20 25 30

```
AAG GAT AAA GTT GCA GAA CTG GAA AAT TCT GAA TTC AGA GCT TTC TCA   144
Lys Asp Lys Val Ala Glu Leu Glu Asn Ser Glu Phe Arg Ala Phe Ser
         35                  40                  45
TCT TTT AAA AAT AGG GTT TAT TTA GAC ACT GTG TCA GGT AGC CTC ACT   192
Ser Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr
     50                  55                  60
ATC TAC AAC TTA ACA TCA TCA GAT GAA GAT GAG TAT GAA ATG GAA TCG   240
Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser
 65                  70                  75                  80
CCA AAT ATT ACT GAT ACC ATG AAG TTC TTT CTT TAT GTG CTT GAG TCT   288
Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser
                 85                  90                  95
CTT CCA TCT CCC ACA CTA ACT TGT TGA                              315
Leu Pro Ser Pro Thr Leu Thr Cys
            100
```

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 675 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ovis

(G) CELL TYPE: leukocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```
GTT TCC CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT TAC      48
Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr
 1               5                  10                  15
GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG AAG      96
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys
            20                  25                  30
GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT CAG     144
Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln
        35                  40                  45
TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC ACC     192
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
    50                  55                  60
ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA TCC     240
Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
65                  70                  75                  80
CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAA CCT     288
Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro
                85                  90                  95
CCT CCA ACA CCG TCA GCA TCT TGC TTC TTG ACT GAG GGT GGA AAC ATT     336
Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile
            100                 105                 110
ACT CTC ACC TGC TCG ATC CCG GAA GGT GAC CCC AAA GAG CTC GAT GAT     384
Thr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp
        115                 120                 125
AGT GAC CTA ATA CGG TAT TTG TGG GAA TGT CCG CCA ACA ATA CAG TGT     432
Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gln Cys
    130                 135                 140
```

```
CAC CGT GGC TCG ATT TCA TCT GAA GCC TTT GTC TCA GCG GAA AGT GAT      480
His Arg Gly Ser Ile Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp
145                 150                 155                 160
CTT TCA CAG AAT GTT CAG TGT ATC GTT AGC AAT CCA TTG TTC AGA ACA      528
Leu Ser Gln Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr
                165                 170                 175
TCA GCT TCC GTC TCT TTG TCA ACC TGT TTG CCA GAG GAT TAT GCA AGG      576
Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg
            180                 185                 190
CAT AGG TAT GTG CTT TTT GCC ATA CTG CCA GCA GTA ATA TGT GGC TTG      624
His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys Gly Leu
        195                 200                 205
CTG TTT TTA AAA TGT TTT CTG GGA CGT CGT AGC CAA CGA AAC TCA GGG      672
Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg Asn Ser Gly
    210                 215                 220
CCC                                                                 675
Pro
225
```

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 225 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```
Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr
 1               5                  10                  15
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile                 Lys Gly Lys
            20                  25                  30
Asp Lys Val Val Glu Trp Asp Gln Thr                 Leu Glu Ala Phe Gln
        35                  40                  45
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
    50                  55                  60
Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
65                  70                  75                  80
Pro Ser Val Lys Lys Ser Ser Glu Phe His Leu Arg Val Ile Glu Pro
                85                  90                  95
Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile
            100                 105                 110
Thr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp
        115                 120                 125
Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gln Cys
    130                 135                 140
His Arg Gly Ser Ile Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp
145                 150                 155                 160
Leu Ser Gln Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr
                165                 170                 175
Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg
            180                 185                 190
```

His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys Gly Leu
195 200 205

Leu Phe Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln Arg Asn Ser Gly
210 215 220

Pro
225

(2) INFORMATION FOR SEQ ID NO: 32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

TTGGGGATCC ATGGTTTCCC AAGATATTTA TGG 33

(2) INFORMATION FOR SEQ ID NO: 33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA
(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TTGGGGATCC ATGGTAAGTC AAGATATTTA TGG 33

(2) INFORMATION FOR SEQ ID NO: 34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GTCGACCTGC AGCTACGACG TCCCAGAAAA CCTATG 36

(2) INFORMATION FOR SEQ ID NO: 35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 597 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Ovis
(G) CELL TYPE: leukocyte

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

```
GTT TCC CAA GAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT TAC      48
Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr
 1               5                  10                  15

GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG AAG      96
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys
            20                  25                  30

GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT CAG     144
Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln
        35                  40                  45

TCT TTT AAA AAT AGA GTT CAT TTA GAC ATT GTG TCA GGT AAC CTC ACC     192
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
    50                  55                  60

ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA TCC     240
Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
65                  70                  75                  80

CCA AGT GTT AAA AAG AGC TCC CAG TTC CAC CTC AGA GTG ATT GAA CCT     288
Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro
                85                  90                  95

CCT CCA ACA CCG TCA GCA TCT TGC TTC TTG ACT GAG GGT GGA AAC ATT     336
Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile
            100                 105                 110

ACT CTC ACC TGC TCG ATC CCG GAA GGT GAC CCC AAA GAG CTC GAT GAT     384
Thr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp
        115                 120                 125
```

```
AGT GAC CTA ATA CGG TAT TTG TGG GAA TGT CCG CCA ACA ATA CAG TGT      432
Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gln Cys
    130                 135                 140
CAC CGT GGC TCG ATT TCA TCT GAA GCC TTT GTC TCA GCG GAA AGT GAT      480
His Arg Gly Ser Ile Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp
145                 150                 155                 160
CTT TCA CAG AAT GTT CAG TGT ATC GTT AGC AAT CCA TTG TTC AGA ACA      528
Leu Ser Glu Asn Val Glu Cys Ile Val Ser Asn Pro Leu Phe Arg Thr
                165                 170                 175
TCA GCT TCC GTC TCT TTG TCA ACC TGT TTG CCA GAG GAT TAT GCA AGG      576
Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg
            180                 185                 190
CAT AGG TTT TCT GGG ACG TCG                                         597
His Arg Phe Ser Gly Thr Ser
        195
```

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 199 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

```
Val Ser Glu Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr
 1               5                  10                  15
Val Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys
            20                  25                  30
Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln
        35                  40                  45
Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr
    50                  55                  60
Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
65                  70                  75                  80
Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro
                85                  90                  95
Pro Pro Thr Pro Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile
            100                 105                 110
Thr Leu Thr Cys Ser Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp
        115                 120                 125
Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gln Cys
    130                 135                 140
His L   Gly Ser Ile Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp
145                 150                 155                 160
Leu Ser Gln Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr
                165                 170                 175
Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg
            180                 185                 190
His Arg Phe Ser Gly Thr Ser
        195
```

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

TGGGGATCCA TGGTAAGTCA AGATATTTAT GG 32

(2) INFORMATION FOR SEQ ID NO: 38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: synthetic DNA

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CCCCTGCAGC TAGGGCCCTG AGTTTCGTTG GCT 33

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A sheep LFA-3 like protein deficient in TM region which comprises the amino acid sequence of SEQ ID NO: 36.

2. A gene coding for a sheep LFA-3 like protein according to claim 1, which comprises the DNA sequence of SEQ ID NO: 35.

3. A process for preparing a sheep LFA-3 like protein according to claim 1, which comprises culturing a cell which is transformed by a vector including a DNA coding for a LFA-3 like protein deficient in TM region and successively separating the produced sheep LFA-3 like protein deficient in TM region.

4. The process of Claim 3, wherein the transformed cell is E. coli.

5. A sheep LFA-3 like protein deficient in D2 region which comprises the amino acid sequence of SEQ ID NO: 1.

6. A human LFA-3 like protein deficient in D2 region which comprises the amino acid sequence of SEQ ID NO: 13.

7. A gene coding for a sheep LFA-3 like protein deficient in D2 region which comprises the DNA sequence of SEQ ID NO: 2.

8. A gene coding for a human LFA-3 like protein deficient in D2 region which comprises the DNA sequence of SEQ ID NO: 12.

9. A soluble derivative of a sheep LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 1 wherein amino acids 1 to 94 are conserved and wherein at least one of amino acids 95 to 131 may be substituted or deleted.

10. A soluble derivative of a human LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 13 wherein amino acids 1 to 94 are conserved and wherein at least one of amino acids 95 to 134 may be substituted or deleted.

11. A soluble derivative of a human LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 13 wherein amino acids 1 to

93 are conserved and wherein at least one of amino acids 94 to 134 may be substituted or deleted.

12. A soluble derivative of a sheep LFA-3 like protein deficient in D2 region containing at least one cysteine residue which comprises the amino acid sequence of SEQ ID NO: 25.

13. A soluble derivative of a human LFA-3 like protein deficient in D2 region containing at least one cysteine residue which comprises the amino acid sequence of SEQ ID NO: 27.

14. A process for preparing a soluble derivative of a sheep LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 1 wherein amino acids 1 to 94 are conserved and wherein at least one of amino acids 95 to 131 may be substituted or deleted, which comprises culturing a cell which is transformed by a vector including a DNA coding for said protein and successively separating the produced protein.

15. A process for preparing a soluble derivative of a human LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 13 wherein amino acids 1 to 94 are conserved and wherein at least one of amino acids 95 to 134 may be substituted or deleted, which comprises culturing a cell which is transformed by a vector including a DNA coding for said protein and successively separating the produced protein.

16. A process for preparing a soluble derivative of a human LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 13 wherein amino acids 1 to 93 are conserved and wherein at least one of amino acids 94 to 134 may be substituted or deleted, which comprises culturing a cell which is transformed by a vector including a DNA coding for said protein and successively separating the produced protein.

17. A carrier onto which a soluble derivative of a sheep LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 1 wherein

amino acids 1 to 94 are conserved and wherein at least one of amino acids 95 to 131 may be substituted or deleted is immobilized.

18. A carrier onto which a soluble derivative of a human LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 13 wherein amino acids 1 to 94 are conserved and wherein at least one of amino acids 95 to 134 may be substituted or deleted is immobilized.

19. A carrier onto which a soluble derivative of a human LFA-3 like protein deficient in D2 region which comprises an amino acid sequence of SEQ ID NO: 13 wherein amino acids 1 to 93 are conserved and wherein at least one of amino acids 94 to 134 may be substituted or deleted is immobilized.

20. A process for preparing a soluble derivative of a sheep LFA-3 like protein deficient in D2 region containing at least one cysteine residue which comprises the amino acid sequence of SEQ ID NO: 25, which comprises culturing a cell which is transformed by a vector including a DNA coding for said protein and successively separating the protein.

21. A process for preparing a soluble derivative of a human LFA-3 like protein deficient in D2 region containing at least one cysteine residue which comprises the amino acid sequence of SEQ ID NO: 27, which comprises culturing a cell which is transformed by a vector including a DNA coding for said protein and successively separating the protein.

22. A LFA-3 protein, substantially as herein described with reference to any one of the Examples or Figure 1, 2 or 3 but excluding any comparative examples.

23. A process for preparing a LFA-3 protein, substantially as herein described with reference to any one of the Examples but excluding any comparative examples.

DATED this 6th day of APRIL, 1995

KANEGAFUCHI KAGAKU KOGYO KABUSHIKI KAISHA

Attorney: IAN T. ERNST

Fellow Institute of Patent Attorneys of Australia

of SHELSTON WATERS

ABSTRACT OF THE DISCLOSURE

A sheep LFA-3 protein and derivatives thereof, and derivatives of a human LFA-3 protein, gene coding for the proteins, and processes for preparing the proteins. Said proteins have high affinity for human T-cells.

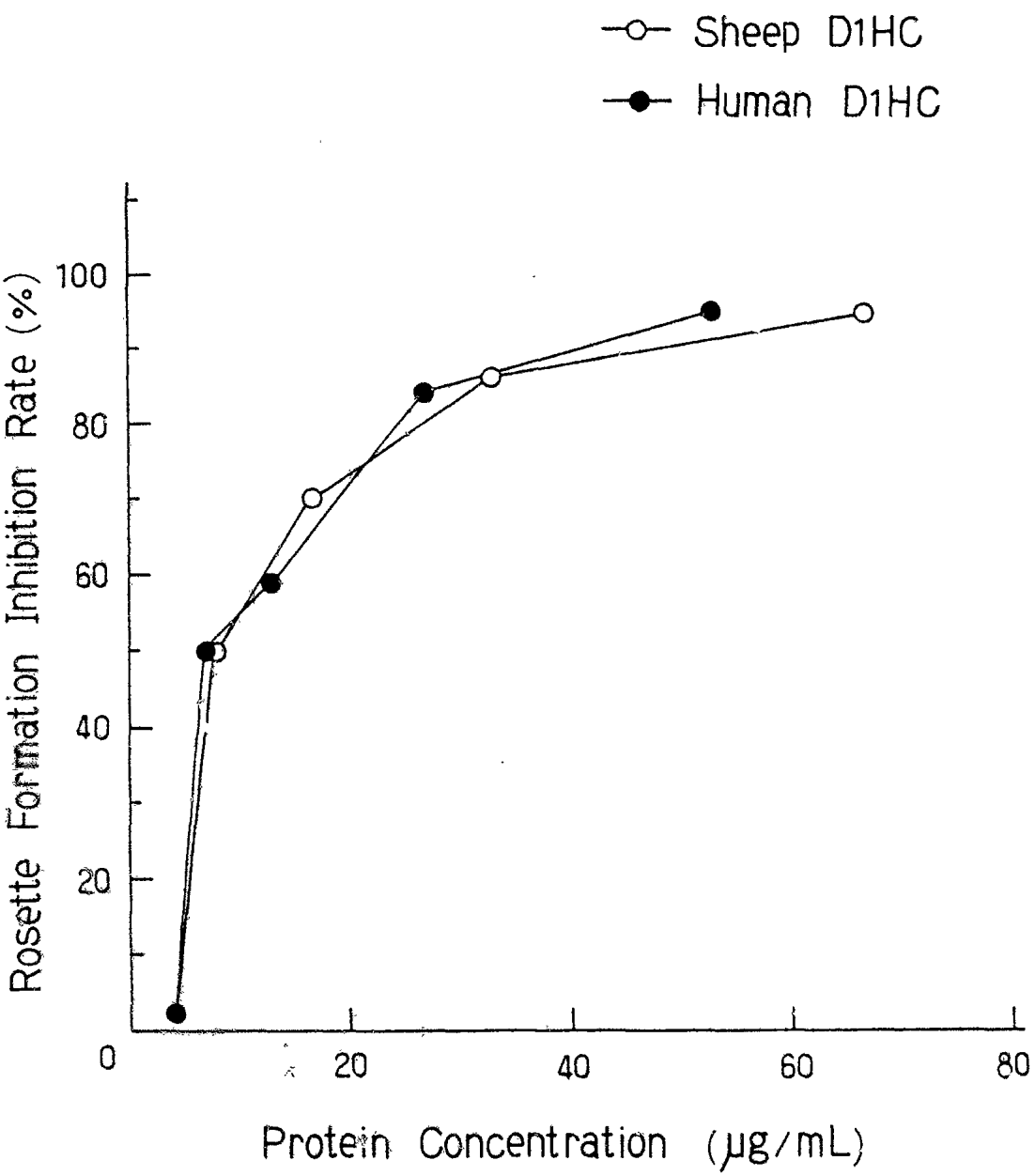
FIG. 1

```
                                                10                                  20
human  Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
          :   :       :   :   :           :   :   :   :   :       :
sheep  Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr Val Ser Glu Ser
                                            30                                  40
human  Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala Glu Leu Glu Asn
          :           :       :   :   :       :   :   :   :       :
sheep  Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys Asp Lys Val Val Glu Trp Asp Gln
                                                50
human  -- Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg Val Tyr Ser Asp Thr Val Ser
          :           :   :       :   :   :   :   :   :       :   :       :   :
sheep  Thr Ser Gly Leu Glu Ala Phe Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser
       60                                       70
human  Gly Ser Leu Thr Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser
          :       :   :   :           :   :           :   :   :       :   :       :   :
sheep  Gly Asn Leu Thr Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
       80                                       90
human  Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro
          :                           :   :   :   :   :   :   :   :   :   :   :   :
sheep  Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile Glu Pro Pro Pro Thr Pro
       100                                     110
human  Thr Leu Thr Cys Ala Leu Thr Asn Gly----Ser Ile Glu Val Gln Cys Met Ile Pro Glu
                  :   :   :       :           :           :   :   :   :
sheep  Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile Thr Leu Thr Cys Ser Ile Pro Glu
          120                                     130
human  His Tyr Asn Ser His Arg Gly----------Leu Ile Met Tyr Ser Trp Asp Cys Pro Met
                                          :   :       :       :   :   :
sheep  Gly Asp Pro Lys Glu Leu Asp Asp Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro
                                  140                                         150
human  ---Glu Gln Cys Lys Arg--------Asn Ser Thr Ser Ile Tyr Phe----Lys Met Glu Asn
             :   :   :               :       :           :               :
sheep  Thr Ile Gln Cys His Arg Gly Ser Ile Ser-----Ser Glu Ala Phe Val Ser Ala Glu Ser
                                            160                                 170
human  Asp Leu Pro Gln Lys Ile Gln Cys Thr Leu Ser Asn Pro Leu Phe Asn Thr Thr Ser Ser
          :   :       :           :   :           :   :   :   :   :       :           :
sheep  Asp Leu Ser Gln Asn Val Gln Cys Ile Val Ser Asn Pro Leu Phe Arg Thr Ser Ala Ser
                                            180                                 190
human  Ile Ile Leu Thr Thr Cys Ile Pro Ser Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile
                  :       :   :       :                       :   :   :
sheep  Val Ser Leu Ser Thr Cys Leu Pro----Glu Asp Tyr Ala Arg His Arg----------
                                            200                                 210
human  Pro Ile Pro Leu Ala Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys
sheep  ------------------------------------------------------------------------------
                                            220
human  Cys Asp Arg Lys Pro Asp Arg Thr Asn Ser Asn
sheep  ---Phe Ser Gly Thr Ser
```

FIG. 2

```
                                                  10                                  20
human  Phe Ser Gln Gln Ile Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His Val Pro Ser Asn
sheep  Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr Val Ser Glu Ser
                                                  30                                  40
human  Val Pro Leu Lys Glu Val Leu Trp Lys Lys Gln Lys Asp Lys Val Ala Glu Leu Glu Asn
sheep  Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys Asp Lys Val Val Glu Trp Asp Gln
                                                  50
human  -- Ser Glu Phe Arg Ala Phe Ser Ser Phe Lys Asn Arg Val Tyr Ler Asp Thr Val Ser
sheep  Thr Ser Gly Leu Glu Ala Phe Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser
       60                                         70
human  Gly Ser Leu Thr Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser
sheep  Gly Asn Leu Thr Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
       80                                         90
human  Pro Asn Ile Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Glu Ser Leu Pro Ser Pro
sheep  Pro Ser Val Lys Lys Ser Ser Gln Phe His Leu Arg Val Ile----------
       100                                        110
human  Thr Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val Gln Cys Met Ile Pro Glu His
sheep  ----------
       120                                        130
human  Tyr Asn Ser His Arg Gly Leu Ile Met Tyr Ser Trp Asp Cys Pro Met Glu Gln Cys Lys
sheep  ----------
       140                                        150
human  Arg Asn Ser Thr Ser Ile Tyr Phe Lys Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys
sheep  ----------
       160                                        170
human  Thr Leu Ser Asn Pro Leu Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr Cys Ile Pro
sheep  ----------
       180                                        190
human  Ser Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr
sheep  ------ Asp Tyr Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile ---
       200                                        210
human  Thr Cys Ile Val Leu Tyr Met Asn Gly Ile Leu Lys Cys ------- Asp Arg Lys Pro Asp
sheep  --- Cys Gly Leu Leu Phe ---------- Leu Lys Cys Phe Leu Gly Arg Arg Ser Gln
                 220
human  Arg Thr Asn Ser Asn
sheep  Arg Asn Ser Gly Pro
```

FIG.3


Sheep D1HC
Human D1HC
Rosette Formation Inhibition Rate (%)
100
80
60
40
20
0
20
40
60
80
Protein Concentration (μg/mL)