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

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- (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:

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Val Ser Gla Asp Ile Tyr Gly Ala Met Asa Gly Asa Val Thr Phe Tyr 5 Val Ser Glu Ser Gla Pro Phe Tar Glu Ile Met Trp L7s L7s Gi7 L7s 25 20 Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln 40 Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr 55 60 the Thr Gly Leu Thr Lys Lau Asp Glu Asp Val Tyr Glu ile Glu Set 15 70 Pro Ser Val Lys Lys Ser Ser Gla Pae His ten Arg Val Ile Glu Pro 8.5 90 Pro Pro The Pro See Ala See Cys Phe Leu The Glu Gly Ash Ile 100 105 The Leu The Cris See Ile Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp 120 125 Ser Asp Leu Ile Arg Tyr Leu Trp Glu Cys Pro Pro Thr Ile Gla Cys 135 His Arg Gly Ser lie Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp 145 150 155 Lew Ser - Ash Val Gln Cys Ile Val Ser Ash Pro Lew Phe Arg Thr 165 170 Ser Ala Ser Val Ser Leu Ser Thr Cys Leu Pro Glu Asp Tyr Ala Arg 180 185 his Arg Phe Ser Gly The Ser 195

- (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:

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Val Ser Gin Asp lie Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr 1 5 Val Ser Glu Ser Gln Pro Phe Thr Glu lle Met Trp Lys Lys Gly Lys 25 Asp Lys Val Val Glu Trp Asp Gin The See Gly Leu Glu Ala Phe Gin 40 Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr 5.5 Ile The Gly Leu The Lys Leu Asp Glu Asp Val Tye Glu Ile Glu See 6.5 70 75 Pro Ser Val Lys Lys Ser Ser Gin Phe His Leu Arg Val Ile Asp Tyr 90 Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys 100 105 Gly Len Len Phe Len Lys Cys Phe Len Gly Arg Arg Ser Gla Arg Ash 120 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 Gin Gin lie Tyr Gly Val Val Tyr Gly Asn Val Thr Phe His

 1 5 10 15

 Val Pro Ser Asn Val Pro Leu Lys Giu Val Leu Trp Lys Lys Gin Lys

 20 25 30

 Asp Lys Val Ala Giu Leu Giu Asn Ser Giu Phe Arg Ala Phe Ser Ser

 35 49 45

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Phe Lys Asn Arg Val Tyr Leu Asp Thr Val Ser Gly Ser Leu Thr lie 50 Tyr Asa Lea The See See Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro 70 15 65 Asn lie Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His Ser 90 85 Arg His Arg Tyr Ala Lew Ile Pro Ile Pro Lew Ala Val Ile The Thr 105 Cys lie Val Leu Tyr Met Asn Gly lie Leu Lys Cys Asp Arg Lys Pro 125 120 Asy Arg The Asa Ser Asa 130

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.

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 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 10 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, 15 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 20 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 25 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, N-terminus, immunoglobulin-like domain immunoglobulin-like domain region), 2 (D2 transmembrane region (TM region) and cytoplasm region (C region) (refer to B. P. Wallner et al., J. Erp. Med.



Further, there is also known human 166, 923, (1987)). molecule which D1 region and D2 has LFA-3 membrane through glycosyl to binds а 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 receptor having D1 region and D2 antigen membrane through glycosyl and binding to а " LFA-3". phosphatidylinositol is referred as 10 to 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 15 mixture of various kinds of cells, because the receptor high affinity for CD2 antigen of human T-cells. it is known that CD2 antigen participates in Further. as functions of T-cells. immune responses Therefore, a sheep receptor for CD2 antigen which has 20 CD2 antigen can used affinity for be as agent and more, a therapeutic immunoregulative as agent which targets a tumor of T-cell family cell by utilizing affinity thereof for T-cells. leukemia sheep receptor for CD2 antigen 25 Α can sheep erythrocytes. obtained from As a process preparing a sheep receptor for CD2 antigen derivative of the receptor, there are known a process solubilizing the receptor from comprising by a surfactant and purifying by 30 erythrocytes an affinity-chromatography using antibodies therefor (refer Unexamined Japanese Patent Publication No. to 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 35 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.

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At present, a protein which naturally exists in very small amount can be prepared inexpensively and in large amount by genetic engineering techniques. 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) However, at present, a whole amino acid sequence first. 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 binding a membrane through glycosyl and to phosphatidylinositol, or a receptor of 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 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.

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On the other hand, until now, there have not 15 the existence of a LFA-3 known like deficient in D2region and a LFA-3 like protein TM region in sheep and also in humans. deficient in Such proteins have been first found in the present invention.

If these proteins have high affinity for 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 therapeutic agent which targets tumors of 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 search а protein which is more suitable for 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 5 provide process for preparing such a protein a bу genetic engineering techniques.

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

10 These and the other objects of the invention will become apparent from the description b einafter.

SUMMARY OF THE INVENTION

15 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 20 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 "-" represents terminus of human LFA-3. A symbol deficiency of amino acid. A colon is shown between homologous amino acids. TM region is underlined. is clear from the figure, the sheep LFA-3 like protein deficient in TM region does not have the underlined 35 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



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deficient in D2 region (SEQ ID NO:1). Number in the figure represents number of amino acid from the Nterminus of human LFA-3. A symbol "-" represents A colon is shown between deficiency of amino acid. 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 10 Example 4-r and Example invention obtained in respectively, that is, sheep D1HC protein and human D1HC protein.

DETAILED DESCRIPTION

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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. \mathbf{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 35 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 of oligo(dT)-cellulose column means an

(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.

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There are synthesized primers each of which is sequence deduced from amino terminal and carboxyl of the known partial amino terminal sequence sequence of a sheep receptor for CD2 antigen (SEQ ID NO: With the synthesized primers, the above obtained 10 3). cDNA is amplified by PCR (polymerase chain reaction) method (refer to Molecular Cloning 2nd edition. Sambrook et al., Cold Spring Harbor Laboratory J. (1989); K. Knoth et al. (1988), Nucleic Acids Research, vol. 16, page 10932). The amplification by PCR method 15 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, polyacrylamide gel electrophoresis, and e.g., extracted. 20 Nucleotide sequence of the extracted DNA can be determined by inserting the DNA into a suitable vector pBR322, pUC18 M13mp19, such or cloning the as recombinant vector in a suitable host such as E. coli JM109, and utilizing a conventional method, e.g. Sanger 25 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, cloned. cDNA The cloned 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 LFA-3 sheep protein of the present may have modified structure of the aboveinvention mentioned structure. Examples of the protein having the for instance. modified structure are, the modified in dependence on a host cell which produces the protein, such as the protein wherein methionine residue introduced at N-terminus of the protein due 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.

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The LFA-3 like protein deficient in TM region is another protein of the present invention. The LFA-3 protein deficient in TM like region is à polypeptide having molecular the weight of 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 present invention has low antigenicity and is naturally occuring soluble LFA-3 like protein. protein of the present invention is very advantageous to the production by genetic engineering techniques 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.

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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.

20 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,

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.

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

There are synthesized mixed primers each is deduced from amino terminal which sequence carboxyl terminal sequence of the known partial amino acid sequence of a sheep receptor for CD2 antigen (SEQ With the synthesized mixed primers, the above .D NO:3). obtained cDNA is amplified PCR by method. 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 10 determined as described above as a probe useful detection of the gene coding for the sheep LFA-3 like protein deficient in TM region and mRNA thereof. a full-length cDNA of the sheep LFA-3 like protein deficient in TM region can be easily detected by using 15 probe from the sheep cDNA library. sequence of the detected cDNA clone is determined. As a determined sequence has result. since the no DNA sequence coding for the sequence of TM region, the cDNA 20 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 switable 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.

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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.

LFA-3 like protein deficient in D2 region further protein of the present invention. LFA-3 like protein deficient in D2 region is a single polypeptide having the molecular weight of about 15,000 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 15 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.

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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 not D2 region but D1 region. Therefore. considered that LFA-3 protein wherein there is (are) deficiency variation(s) such partial introduced as in 'D2 region, has the and/or substitution affinity to that of the LFA-3 like protein deficient in 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 5 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 10 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.

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Hereinafter there is explained a derivative of above-mentioned proteins deficient in D2 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 deficient protein in D2 region which are proteins such wherein variation(s) deficiency as 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 D2 region is very advantageous to production by genetic engineering techniques by reason that derivative can be secreted from an animal cultured cell, a yeast or the like after production therein. 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 production by genetic engineering techniques by reason of no possibility that a wrong disulfide bonding 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 10 modified structure of the above-mentioned Examples of the derivative having the modified structure for instance, the derivatives modified dependence on a host cell which produces the derivative such as the derivative wherein methionine residue is 15 introduced at N-terminus of the derivative due 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.

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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 oligo(dT)-cellulose (refer of column an 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.

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There are synthesized mixed primers which is deduced from amino terminal sequence and carboxyl terminal sequence of the known N-terminal amino acid residues of the CD2 antigen receptor of sheep enythrocytes (SEQ ID NO:3). With the synthesized mixed the above obtained cDNA is amplified by PCR primers. 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 conventional gel electrophoresis, a 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 That is, a full-length cDNA of the sheep mRNA thereof. 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 in deficient D2region is characterized protein with restriction analyzing enzymes or sequencing thereof. 0n 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 SEQ. ID NO: 1 is maintained and, in the in 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).

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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 an established strain of human T-cells. Firstly, RNA is extracted from such material a by means conventional method such as guanidine thiocyanate method or hot phenol method. Then, from the extracted mRNA can be prepared as poly(A)+RNA by means RNA. of oligo(dT)-cellulose an column (refer 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 amplified by obtained cDNA is PCR method. amplification by PCR method may be cycled suitable times to obtain a sufficient amount of cDNA for the following is cloning. The amplified cDNA detected bv gel conventional 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.

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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 20 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 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.

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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 carrier onto which the LFA-3 revealed that a like protein deficient in D2 region or a derivative thereof is immobilized can adsorb cells having CD2 antigen. 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 be easily separated from the carrier cells can Therefore, there can be utilized the carrier trypsin. onto which the LFA-3 like protein deficient in D2 region the derivative thereof for selective adsorption or separation of the T-cells.

As a carrier to be used for immobilization in 35 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

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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 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 sequence thereof. According to the present base invention, analysis and cloning of a sheep genomic LFA-3 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 also variants artificially occures. but insertion and/or deficiency of substitution. amino acid proteins combined with one and Also, or 20 protein. chemically enzymatically modified produced therefrom. be Among derivatives can 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 derivatives thereof produced by thereof. or engineering techniques which have affinity to T-cells diagnostic agents for T-cell detection. can be utilized the diagnostic agents proteins labeled with an enzyme, a fluorescent agent or isotope. Also, the produced sheep LFA-3, the derivatives which variants and the have affinity T-cells. can be chemically or physically immobilized onto the carrier and subjected to separation (isolation 35 or removal) of T-cells. Sheep LFA-3, the derivatives thereof and the proteins combined other protein are used therapeutic agents which target a tumor of T-cell family or a leucemia cell by utilizing affinity thereof

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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 ther peutic agent.

It is known that CD2 antigen being a natural LFA-3 participates in various offunctions of T-cells. Therefore, responses as the variant thereof or the derivative thereof which has affinity for CD2 antigen can be utilized as an for inhibiting or activating immune response(s), agent 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 production suitable for by genetic engineering techniques.

According to the present invention DNA coding 20 the above-mentioned proteins can be Therefore, the present invention enables to produce the LFA-3 like protein deficient in TM region and the LFA-3 protein deficient in D2 region like bv genetic Also, the invention engineering techniques. present enables to produce not only the molecules having the 25 amino acid sequence of naturally occurring LFA-3, variants bearing artificial substitution. and/or deficiency of at least one amino acid in the amino acid sequence thereof and proteins combined with 30 other protein. Also. the above-mentioned molecules. variants and the combined protein can be chemically or enzymatically modified give derivatives. to Thus obtained derivatives include the derivatives having affinity for CD2 antigen being natural a 35 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 genetic engineering techniques produced by 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, above-mentioned proteins. derivatives and combined proteins which are conjugated with a toxin such as ricin, can be used as therapeutic agents.

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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 LFA-3 the sheep LFA-3 protein. like 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 activating immune response(s), inhibiting or 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 of described and explained by means the following Examples in which all percents are by weight unless It is otherwise noted. to be understood that present invention is limited to the Examples. not and various changes and modifications may be made in the invention without departing from the spirit and scope thereof.

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Example 1

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

Âs a partial amino acid sequence of a sheep CD2 for antigen, has been disclosed 15 receptor N-terminal amino acid sequence consisting of 29 acid residues shown in SEQ ID NO:3 (refer to Japanese 150228/1988). Patent Publication No. Unexamined order to use in PCR for preparing cDNA coding for sheep 20 following two kinds of mixed primers synthesized by means of a DNA synthesizer (made by Model 381A). One mixed primer is Applied Biosystems, shown in SEQ ID NO: 4, consisting of restriction a enzyme BamHI recognition sequence and a following 25 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 following nucleotide sequence deduced from the sequence of the 27th-22nd amino acids in SEQ ID NO:3. 30

b. Preparation of double strand cDNA from sheep cells

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

from the obtained sheep leukocytes leukocytes. Then, RNA by guanidine thiocyanate extracted was poly(A)*RNA Further was purified by method. of oligo(dT)-cellulose column (refer to means an 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 Similarly, another double strand cDNA Pharmacia). 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 15 coding for sheep LFA-3 in vitro. That is, using 100 u l 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 μ M of each of 2 kinds of the mixed primers synthesized in the above-mentioned a, 20 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 Tag DNA polymerase, 35 cycles of PCR were units of carried out under the reaction condition per cycle of 25 94°C, 1 minute; 37°C, 2 minutes; and 72°C, 2 minutes. completing the reactions, size of PCR products polyacrylamide gel electrophoresis, measured by a and then it was found that DNA fragments of about 100 base pairs were amplified in both cases of using cDNA of 30 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 Then the treated DNA was inserted into BamHI-PstI 35 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 100 base pairs prepared from a positive clone obtained in the above-mentioned c by a conventional method using dideoxynucleotide triphosphates. 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 10 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. DNA sequence codes the 7th-22nd amino acids in SEQ ID 15 NO: 31 (1 and 36).

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

20 In order to clone a full-length cDNA of sheep sheep cDNA library was screened by using 15terminal cDNA sequence of sheep LFA-3 determined in the above-mentioned d a probe. That is, the ลร in cDNA from sheep leukocytes prepared 25 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 with EcoRI, DNA fragment was cut and thereto were right and left connected arms of λgt11 (made with 30 Stratagene) treated alkaline phosphatase. Packaging in vitro was carried out prepare cDNA to There were synthesized probes library. having sequence adjacent N-terminus of sheep LFA-3, i.e., probe having the nucleotide sequence of SEQ ID NO: 6 and 35 that of SEQ ID NO: 7. By using these probes were screened about 2 x 105 recombinant phages. As a result, positive clone (SL-6, SL-40 and SL-43) containing 1.0 3 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. obtained nucleotide to. sequence corresponds amino acid sequence determined from the nucleotide sequence is shown as, the amino acid sequence of sheep 10 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, and C region of human LFA-3, 15 region 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 20 protein in E. coli

In order to make cDNA coding for sheep LFA-3 protein express in E. coli. an expression vector The DNA insert contained in the cDNA clone constructed. SL-6 obtained in Example 1 was taken out by cleaving with restriction enzyme EcoRI. The DNA 25 insert 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, 30 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 öf Pst is comprised recognition sequence, sequence of termination codon and the DNA the 35 sequence designed according to sequence of 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

fragments were cleaved with restriction eyzymes DNA and thus obtained fragments BamHI and PstI. inserted into BamHI-PstI site of M13mp19 phage vectors. Nucleotide sequence of the inserted DNA was confirmed by DNA coding for sheep LFA-3 dideoxy method. Then, protein was taken out from the M13mp19 phage vector by cleaving with restriction enzymes NcoI and PstI. 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".

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

E. coli JM 109 (made by TAKARA SHUZO Co.) was 15 precultured which has the expression vector pKSL 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°C in 500 ml of Sakaguchi flask until absorbance at 600 nm 20 (hereinafter referred A_{600} ") as of the medium containing E. coli became 0.3. Successively thereto was IPTG (isopropyl- β -D-thio-galactopyranoside, by Wako Pure Chemical Industries, Ltd.) so as to give a final concentration of 1 mM and further culture 25 The continued for 6 hours. cultured cells were collected by centrifugation, and suspended in 10 ml 50 mM Tris-HC \ell 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 %. 30 The cell suspension was sonicated and centrifuged insoluble precipitation fraction. The give an obtained precipitation was washed with 50 % glycerol successively with ethanol to give inclusion containing produced sheep LFA-3 protein. The inclusion 35 bodies were dissolved with SDS (sodium dodecyl sulfate) 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 Δ TM 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 and amino to, sequence determined from the nucleotide sequence shown as, the amino acid sequence of sheep ATM 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 ATM protein has a signal peptide 28 amino acid residues which starts having methionine. On the basis of thus obtained information of cDNA and nucleotide sequence of sheep ATM protein. natural type sheep ΔTM protein and a derivative thereof produced with а recombinant engineering techniques. Fig. 1 shows comparison of the amino acid sequence of sheep ATM protein shown in SEQ ID NO: 13 to that of human LFA-3 shown in SEQ ID NO: 8. Fig. 1 reveals that sheep ATM protein lacks TM region (underlined portion) existing in human LFA-3.

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

In order to make cDNA coding for sheep ATM protein express in E. coli. an expression vector The DNA insert contained in the cDNA clone constructed. 30 SL-43 obtained in Example 1 was taken out by cleaving with restriction enzyme EcoRI. The DNA insert subcloned into EcoRI site of plasmid pUC18. Successively such plasmid was subjected to PCR. Used 35 5' primers are shown in SEQ ID NO: 32 and 33. These are comprised of BamHI recognition sequence. Ncol recognition sequence and the DNA sequence designed according to the sequence of the 1st-7th amino acids

Used 3' primer is shown in SEQ ID NO: in SEQ ID NO: 36. The primer is comprised of SalI recognition sequence, PstI recognition sequence, sequence codon and the DNA termination 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 NO: 33 and 34 to amplify DNA fragments. fragments were cleaved with restriction amplified DNA eyzymes BamHI and SalI, and thus obtained fragments were 10 each BamHI-SalI site of M13mp19 inserted into Nucleotide sequence of the inserted vector. DNA have the desired confirmed to sequence by dideoxy Then, DNA of sheep ΔTM protein was taken out method. vectors 15 from the M13mp19 phage by cleaving with restriction enzymes NcoI and PstI. Thus obtained DNAs were connected to NcoI-PstI site of vector pKK 233-2 Pharmacia) for expression give by to expression vectors. One of thus obtained expression "pKSL∆TM-0", which 20 vector ទែ referred to as prepared by using the primers of SEQ ID NO: 32 and The other is referred to as "pKSLATM-1", which was prepared by using the primers of SEQ ID NO: 33 and 34.

25 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 pKSLATM-0 or pKSL\Delta TM-1 of sheep \Delta TM protein. Then, the precultured 30 coli was inoculated in 100 m l cf LB containing 10 mg of ampicilline, and shaking culture was carried out at 37°C in 500 ml of Sakaguchi flask until E. of the medium containing coli became Successively thereto was added IPTG so as to give a 35 final concentration of 1 mM and further culture for The continued hours. cultured cells were collected by centrifugation, and suspended in 10 ml 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 50 was washed with % precipitation glycerol successively with ethanol to give inclusion bodies containing produced sheep ATM protein. The expression vector pKSLATM-1 was superior to the expression vector pKSLATM-O in productivity. The inclusion bodies were dissolved with (sodium dodecyl sulfate) SDS and to SDS-polyacrylamide gel electrophoresis to subjected detect a band of apparent molecular weight 23,000-25,000.

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

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All the inclusion bodies obtained in the abovementioned 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 20 supernatant. The supernatant was diluted with 8M urea so as to become A280 0.1, and dialyzed against ten-fold of the same buffer and successively against PBS to give a solution containing soluble sheep ATM Thus obtained protein was subjected 25 protein. 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 ATM Thus prepared sheep ATM protein in an amount protein. ranging from 25-0 μ g was mixed with 1 x 10⁵ Jurkat 30 cells which were washed with the PBS containing 5 % albumin (BSA) and 1 % glucose. bovine serum contained in 50 μ ℓ of the same buffer. Thereafter there was observed effect on rosette formation by adding 35 x 10° sheep erythrocytes. Sheep ATM protein dosedependently inhibited the rosette formation of Jurkat cells with sheep erythrocytes.

With respect to sheep ATM protein, the

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

Example 4

5 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 10 dideoxy method using M13 phage. As a result, it is revealed that sheep $\Delta D2$ protein is encoded by nucleotide sequence of SEQ ID NO: 2 and has the amino acid sequence of SEQ ID NO: 1. It is also revealed that sheep AD2 protein has a signal peptide having 15 amino acid residues which starts from methionine. the basis of thus obtained information of cDNA of sheep protein. natural type sheep △D2 protein thereof derivatives can be produced by genetic engineering techniques. The amino acid sequence of SEQ 20 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 25 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 $\Delta D2$ protein lacks D2 region (underlined portion) existing in human LFA-3.

30 n. Preparation of an expression vector of sheep $\Delta D2$ protein in E. coli

In order to make cDNA coding for sheep protein express in E. coli, expression vector an The DNA insert contained in the cDNA clone SL-40 obtained in Example 1 was taken out by cleaving 35 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 of BamHI recognition comprised sequence, 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. primer is comprised of SalI recognition sequence, The 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 10 ID NO: 10 to amplify DNA fragments. Thus amplified DNA fragments were cleaved with restriction eyzymes BamHI and Sall, and thus obtained fragments were inserted into BamHI-SalI site of M13mp19 phage vector. Nucleotide sequence of the inserted DNA was confirmed by dideoxy 15 Then, DNA coding for sheep $\triangle D2$ 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 20 by Pharmacia) for expression to give an expression Thus obtained expression vector is referred to vector. "pKSLAD2". SEQ ID NO: 20 shows nucleotide sequence from initiation codon to termination codon coding for sheep $\Delta D2$ protein contained in pKSL $\Delta D2$.

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o. Production of sheep $\Delta D2$ protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKSLDD2 of sheep $\Delta D2$ 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°C in 500 ml of Sakaguchi flask until Ago 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 The 6 hours. cultured cells were collected by centrifugation, suspended in 10 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton X-100 and 8 % sucrose. was added lysozyme so as to give a final of 0.1 %. The concentration cell suspension and centrifuged give an insoluble sonicated. to fraction. The obtained precipitation precipitation washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced sheep protein. The inclusion bodies were dissolved with 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 $\Delta D2$ protein (hereinafter referred to as "sheep D1HC protein") in E. coli

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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 The DNA insert cleaving with restriction enzyme EcoRL 20 EcoRI site of plasmid pUC18. subcloned into Successively such plasmid was subjected to PCR. 5' primer is shown in SEQ ID NO: 9. This primer is BamHI recognition comprised of sequence. the DNA 25 recognition sequence and 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: The primer is comprised of PstI recognition sequence, sequence of termination codon, the 371st-358th 301st-277th 30 nucleotide sequence and the 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 fragments. Thus amplified DNA fragments cleaved with restriction eyzymes BamHI and PstI, thus obtained fragments were inserted into BamHI-PstI 35 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 Thus obtained DNA was connected to NcoI-PstI and PstI. pKK233-2 (made bv of vector Pharmacia) expression to give an expression vector. Thus obtained expression vector is referred to as "pKSLD1HC". SEQ ID shows nucleotide sequence from initiation codon NO: termination codon coding for sheep D1HC protein contained in pKSLD1HC.

q. Production of sheep D1HC protein by using E. coli as 10 nost

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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°C in 500 ml of Sakaguchi flask until A600 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 collected centrifugation, cultured cells were bv and suspended in 10 m \ell of 50 mM Tris-HC \ell buffer (pH 8.0) 5 % Triton X-100, containing 50 mM EDTA, dithiothreitol and 8 % sucrose. Thereto was as to give a final concentration of 25 lysozyme so %. The cell suspension was sonicated, and centrifuged insoluble inclusion bodies. The to give inclusion dissolved with SDS bodies were and subjected SDS-polyacrylamide gel electrophoresis to detect a band of molecular weight about 12,000. 30

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

All the inclusion bodies obtained in the abovementioned q were dissolved in 10 ml of 50 mM Tris-HCl 35 buffer (pH 7.4) containing 6M guanidine chloride and 2 mM EDTA, and centrifuged to give a supernatant. supernatant was dialyzed against PBS to give a solution containing sheep D1HC protein. Thus prepared sheep D1HC protein was mixed with 1 x 10⁵ Jurkat cells which were washed with the PBS containing 5 % bovine serum albumin (BSA) and 1 % glucose, and contained in 50 μ ℓ of the Thereafter there was observed effect on same buffer. rosette formation by adding 1 x 107 sheep erythrocytes. D1HC protein dose-dependently inhibited Sheep formation of Iurkat cells with rosette sheep erythrocytes. The results are shown in Fig. 3.

10 Rosette formation inhibition rate was calculated by the following formula;

Rosette positive cells (%) in sample

15 Inhibition rate = 1 - x 100

(%) Rosette positive cells (%)

in control

"Rosette positive cells" are cells to which not less than 5 sheep erythrocytes per cell adhere to form 20 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 \D2 protein")

There was closed cDNA of human LFA-3, and the Sequence thereof has been known (B. nucleotide Wallner et al., Journal of Experimental Medicine, 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 AD2 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

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

5 t. Preparation of cDNA of human ΔD2 protein

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Human T-cell line MOLT-4 (ATCC CRL-1582) was cultured in RPMI1640 medium containing 10 % fetal calf serum (FCS) to give 5 x 10⁸ cells. The cells were washed twice with PBS. Successively RNA was extracted from the cells by guanidine thiocyanate method. means poly(A)*RNA was purified by of an oligo(dT)-Rabomanyuaruidenshikogaku, cellulose column (refer to 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 $\Delta D2$ protein, and sequencing thereof

20 By PCR method were amplified cDNA fragments coding for human \D2 protein in vitro. That is, using 100 μ ℓ of a reaction mixture containing 10 mM Tris-(pH 8.3), 100 mM potassium chloride, magnesium chloride, 0.01 % gelatin, 10 μ M of each of 2 kinds of primers synthesized in the above-mentioned s, 25 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 of units polymerase, 35 cycles of PCR were carried out under the reaction condition per cycle of 94°C, 1 minute; 37°C, 2 30 After completing the minutes: and 72℃. 2 minutes. reactions. size of PCR products was measured by polyacrylamide gel electrophoresis, then and was found that DNA fragments of about 500 base pairs were 35 amplified. The DNA fragments of about 500 base pairs gel extracted from the treated with and restriction enzyme PstI. Then the treated DNA was inserted into Pstl 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 "pHL \triangle D2".

in order to sequence cDNA amplified by PCR, the DNA fragment of about 500 base pairs with PstI was inserted into PstI site of M13mp19 phage vector (made by TAKARA SHUZO Co.) and sequenced dideoxynucleotide conventional method using As a result, cDNA sequence of human $\Delta D2$ triphosphates. protein shown in SEQ ID NO: 12 was found among about 500 base pairs of DNA fragments. Such cDNA sequence codes 10 for the protein having the amino acid sequence shown in SEQ ID NO: 13. Comparing human $\Delta D2$ protein shown in SEQ ID NO: 13 with human LFA-3 shown in SEQ ID NO: 8, it is found that human AD2 protein lacks the amino acids from 15 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 △D2 protein in E. coli

20 In order to make cDNA of human \(\DD2 \) protein in E. coli, expression vector express an The DNA of plasmid pHLAD2 having cDNA of constructed. human AD2 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 25 KonI recognition sequence, GG, sequence of initiation codon and the 1st-24th nucleotide sequence of SEQ ID No: Used 3' primer is shown in SEQ ID NO: 18. primer is comprised of HindIII recognition sequence. 30 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 cleaved were with 35 restriction enzymes KpnI and HindIII. The cleaved fragments were connected to KpnI-Hindli site of vector pKK233Kpn to give an expression vector. Thus obtained expression vector is referred to as "pKHL∆D2". 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 $\Delta D2$ protein contained in pKHL $\Delta D2$.

10 w. Production of human △D2 protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was precultured which has the expression vector pKHLDD2 of human $\Delta D2$ protein. Then, the precultured E. coli was inoculated in 100 ml of LB medium containing 10 mg of 15 ampicilline, and shaking culture was carried out at 37°C in 500 ml of Sakaguchi flask until A₆₀₀ of the medium containing E. coli became 0.3. Successively thereto added IPTG so as to give a final concentration of 1 mM 20 and further culture was continued for 6 hours. cultured cells collected by centrifugation, and were suspended in 10 m l of 50 mM Tris-HC l buffer (pH 8.0) 50 mM EDTA, 5 % Triton X-100 and \sim % containing Thereto was added lysozyme so as to give a sucrose. final concentration of 0.1 %. The cell suspension was 25 sonicated. and centrifuged to give an insoluble precipitation fraction. The obtained precipitation washed with 50 % glycerol and successively with ethanol to give inclusion bodies containing produced human $\Delta D2$ 30 protein. The inclusion bodies were dissolved with 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 35 derivative of human $\Delta D2$ 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 pHLAD2 having cDNA of human \(\D2 \) 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 of GG, recognition sequence, sequence codon and the 1st-24th nucleotide sequence of SEQ ID No: Used 3' primer is shown in SEQ ID NO: 19. primer is comprised of HindIII recognition sequence. sequence of termination codon the 402nd-373rd 10 and 297th-268th nucleotide and the sequence 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. 15 The cleaved fragments were connected to KpnI-HindIII of vector pKK233Kpn to give an expression referred Thus obtained expression vector is to as " pKHLD1HC". SEQ NO: 23 shows ID the nucleotide 20 sequence from initiation codon to termination coding for human D1HC protein contained in pKHLD1HC.

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

25 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°C in 500 ml of Sakaguchi flask until Agoo of the medium 30 containing E. coli became 0.3. Successively thereto added IPTG so as to give a final concentration of 1 mM and further culture was continued for The 6 cultured cells centrifugation, collected were by and 35 suspended in 10 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 50 $\mathbf{m}\mathbf{M}$ ELTA. 5 % Triton X-100. mM dithiothreitol % sucrose. and 8 added Thereto was 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 abovementioned y were dissolved in 10 m l of 50 mM Tris-HCl 10 7.4) containing 6M guanidine hydrochloride Hq) 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 μ g was mixed with 1 x 105 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 20 1 x 10⁷ sheep erythrocytes. Human D1HC protein dosedependently inhibited the rosette formation cells with sheep erythrocytes. The results are shown in Fig. 3.

25 Example 6

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 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 $100~\mu$ g/ml of final concentration thereof. Thus obtained solution was added in an amount of $100~\mu$ l per well to 96-wells microtiter plate made by Costar (catalog number 3590), and incubated at 37°C 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 μ ℓ of the same buffer. The plate was allowed to stand for 30 minutes at 4°C. 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.

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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 CMA of sheep D1HC protein 15 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 20 used in the above-mentioned p and shown in SEQ ID NO: 9 3' primer is shown in SEQ ID NO: 24. The primer is comprised of HindIII recognition sequence, PstI sequence, sequence of termination recognition codon. 25 sequence coding for cysteine residue and then 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. cleaved with restriction amplified DNA fragments were enzymes BamHI and PstI. The cleaved fragment was 30 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 35 cleaving with restriction enzymes NcoI and PstI. 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.

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 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 D1HCcvs protein. Then, the precultured E. coli 10 was inoculated in 100 ml of LB medium containing 10 mg of ampicilline, and shaking culture was carried out at 37°C in 500 mℓ of Sakaguchi flask until A₆₀₀ of the E. 0.3. coli became Successively medium containing **IPTG** 15 thereto was added SO as to give concentration of 1 mM and further culture was continued The cultured cells were collected by for hours. centrifugation, and suspended in 10 m & of 50 mM Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, 5 % Triton dithiothreitol and 8 % sucrose. 20 X-100. 2mM was added lysozyme so as to give a final concentration suspension was \mathbf{of} 0.1 %. The cell sonicated. and give insoluble inclusion centrifuged to bodies. The obtained inclusion bodies were dissolved with SDS and SDS-polyacrylamide gel electrophoresis subjected to 25 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 r were dissolved in 10 m ℓ of 50 mM Tris-HC ℓ 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 μ g was mixed with 1 x 10⁵ Jurkat cells which were washed with the PBS containing 5

% BSA and 1 % glucose, and contained in 50 μ ℓ of the same buffer. Thereafter there was observed effect on rosette formation by adding 1 x 10⁷ 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 10 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 15 obtained in the above-mentioned x, was used template to carry out PCR. Used 5' primer is the primer used in the above-mentioned x and shown in SEQ ID NO: Used 3' primer is shown in SEQ ID NO: 26. is comprised of HindIII 20 primer recognition sequence, termination codon. sequence of sequence coding 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 SEQ ID NO: ID 25 NO: 17 and 26 to amplify Thus amplified DNA fragments were cleaved fragments. with restriction enzymes KpnI and Hind III. The cleaved fragments was connected to KpnI-HindIII site of vector pKK233Kpn to give the expression vector pKHLD1HCcys. 30 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 35 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°C in 500 mℓ of Sakaguchi flask until A₆₀₀ of the 0.3. containing E. coli became Successively medium **IPTG** give was added so as to а thereto 5 concentration of 1 mM and further culture was continued cultured cells were collected hours. The and suspended in 10 m l of 50 mM centrifugation. Tris-HCl buffer (pH 8.0) containing 50 mM EDTA, % mM dithiothreitol and 8 Triton X-100, 2 10 Thereto was added lysozyme so as to give The suspension concentration of 0.1 %. cell to give insoluble inclusion sonicated, and centrifuged bodies. The obtained inclusion bodies were dissolved SDS-polyacrylamide with SDS and subjected to 15 electrophoresis to detect a band of molecular weight about 13,000.

 η . Solubilization and renaturation of inclusion bodies 20 containing human D1HCcys protein

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the bodies obtained in the All inclusion were dissolved in 10 m l of 50 mM above-mentioned & (pH 7.4) containing 6M buffer 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 μ g was mixed with 1 x 10⁵ Jurkat cells washed with the PBS containing 5 % BSA and 1 % glucose, and contained in 50 μ ℓ of the same Thereafter there was observed effect on rosette buffer. formation by adding 1 x 107 sheep erythrocytes. Human D1HCcys protein dose-dependently inhibited the 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.

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Then, in order to sequence the DNA amplified by PCR, DNA fragment of about 800 base pairs cleaved with 20 PstI was inserted into PstI site of M13mp19 phage vector (made by TAKARA SHUZO Co.) and determined by a conventional method using dideoxynucleotide a result, cDNA As triphosphates. sequence of human LFA-3 protein shown in SEQ ID NO: 16 was found among DNA 25 fragments of about 800 base pairs. The amino 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, 30 1987).

c. 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 Dlcys protein") express E. an expression vector was constructed. template was used cDNA of human LFA-3 protein obtained in the above-mentioned θ to carry out PCR. 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 of termination recognition sequence, sequence codon. sequence coding for cysteine residue and the DNA sequence designed according to the sequence of 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 amplify DNA fragments. Thus amplified DNA to fragments were cleaved with restriction enzymes KpnI and HindIII.

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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 Dlcys protein by using E. coli as host

E. coli JM 109 (made by TAKARA SHUZO Co.) was 25 precultured which has the expression vector pKHLD1cys of human Dlcys 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°C in 500 ml of Sakaguchi flask until Agoo of the medium 30 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 The cultured cells collected were centrifugation. by 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 successively 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 10 containing human Dlcys protein

All the inclusion bodies obtained in the above-mentioned β were dissolved in 10 m ℓ of 50 mM Tris-HC ℓ 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 D1cys protein.

In addition to the ingredients used in the Examples, other ingredients can be used in the Examples 20 as set forth in the specification to obtain substantially the same results.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(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

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	Pro	Ser	V a 1	Lys	Lys	Ser	Ser	Gln	Phe	His	Leu	A e g	Val	Hé	A s p	Tyr
20					8 5					90					95	

(2) INFORMATION FOR SEQ ID NO: 1: (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 amino acids

Ala Arg His Arg Tyr Val Leu Phe Ala Ile Leu Pro Ala Val Ile Cys 100 105 110 Gly Lew Lew Phe Lew Lys Cys Phe Lew Gly Arg Arg Ser Gln Arg Asn 115 120 125 Ser Gly Pro 5 130 (2) INFORMATION FOR SEQ ID NO: 2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 393 base pairs 10 (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: cDNA to mRNA (iii) HYPOTHETICAL: no 15 (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 Gla Asp Ile Tyr Gly Ala Met Asa Gly Asa Val Thr Phe Tyr 20 1 10 GTT TCA GAG TCT CAA CCG TTT ACA GAG ATT ATG TGG AAG AAG GGG AAG 96 Val Ser Glu Ser Glu Pro Phe Thr Glu ile Met Trp Lys Lys Gly Lys 25 20 30 25 GAT AAA GTT GTA GAA TGG GAT CAA ACA TCT GGA CTC GAA GCT TTT CAG 144 Asp Lys Val Val Glu Tip Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln 15

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	TCT	TTT	AAA	AAT	AGA	GTT	CAT	TTA	GAC	ATT	GTG	TCA	GGT	AAC	CTC	Y C C	192
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		50					5 5					60.					
	ATC	ACC	GGĠ	TTA	ACA	**	TTA	GAT	GAA	GAT	GTG	TAT	GAA	ATT	GAA	TĆC	240
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10	GCA	AGG	CAT	AGG	TAT	GTG	CTT	TTT	GCC	ATA	CTG	CCV	GCA	GTA	ATA	TGT	336
	Ala	Arg	His	Årg	Tyr	V a l	Leu	P h e	Ala	He	Leu	Pro	Ala	V a 1	He	Cys	
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	GGC	TTG	CTG	TTT	ATT	**	TGT	TTT	CTG	GGA	CGT	CGT	AGC	CAA	CGA	AAC	384
	Gly	Leu	Leu	P h e	Leu	Lys	Cys	Phe	Leu	Gly	Arg	Arg	Ser	Gln	λrg	A s n	
15			115					120					125				
	TCA	GGG	CCC														393
	Set	Gly	Pro														
		130															

(2) INFORMATION FOR SEQ ID NO: 3:

- 20 (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 amino acids
 - (B) TYPE: amino acids
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
- 25 (iii) HYPOTHETICAL: yes
 - (v) FRAGMENT TYPE: N-terminal peptide

	(' > PEATURE: "Vee" represents are of natural among said	
	(ix) FEATURE: "Xaa" represents or 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:	
5	Val/Phe Ser Gin/Ser Asp Ile Tyr Gly Ala Met Asn Gly Xaa Val Thr Phe	Tyr
	1 5 10 15	
	Val Ser Gl⊗ Ser Gin Pro Phe Thr Glu Ile Met Xaa Lyš	
	20 25	
	(2) INFORMATION FOR SEQ ID NO: 4:	
10	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 29 base pairs	
	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	:
٦.	(ii) MOLECULE TYPE: other nucleic acid	
15	(A) DESCRIPTION: synthetic DNA	
	(A) DESCRITION. Synthetic DW	
	(iii) HYPOTHETICAL: no	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:	
	GTTGGATCCT TYWSNCARGA YATHTAYGG	2.9
20	(2) INFORMATION FOR SEQ ID NO: 5:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 27 base pairs	
	(B) TYPE: nucleic acid	
	(a) 111 a: 11001010 0010	

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

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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		(iii) HYPOTHETICAL: no	
		(iv) ANTI-SENSE: yes	
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:	
•		ACACTGCAGC ATDATYTCNG TRAANGG	27
	5	(2) INFORMATION FOR SEQ ID NO: 6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base pairs	
		(B) TYPE: nucleic acid	
		(C) STRANDEDNESS: single	
	10	(D) TOPOLOGY: linear	
		(ii) MOLECULE TYPE: other nucleic acid (A) DESCRIPTION: synthetic DNA	•
		(iii) HYPOTHETICAL: no	
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:	
	15	AGCTATGAAC GGGAATGTAA CCTT	2 4
		(2) INFORMATION FOR SEQ ID NO: 7: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base pairs	
	20	(B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
		(ii) MOLECULE TYPE: other nucleic acid (A) DESCRIPTION: synthetic DNA	
		(iii) HYPOTHETICAL: no	
	25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:	
		ACCTÍTTACG TTTCAGASTC TCAA	2 4

	(MATION F QUENCE C (A) LENGT (B) TYPE:	HARACT TH: 222	rerist amino	ICS:	s				
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15	,	(B) TITI (C) JOURI (D) VOLU (F) PAGE: (G) DATE	Fun NAL: J. ME: 166 : 923-9 : Oct-1	ction- Exp. 32 987	Associ Med.	ated An	tigen		A-3)	
	(xi) S	EQUENCE	DESCRI	PTION	SEQ	ÎD NO:	8:			
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20	1		5			10			15	
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		20				٠.		• •		
	ASP LYS	Val Ala	Glu Leu			Glu Phe			Ser	Ser
25	Dha I we	35 Asa Arg	Val Tak		(O	Val Car		45	71.	112
	50		V & L I Y I	55	9 P 1 II 1	741 001	60	er Pea	107	116
		Leu Thr	Ser Ser		lu Asp	Glu Tyr		et Glu	Ser	Pro
	6 5		70			7 5				8 0
	Asn Ilë	The Asp	The Met	Lys P	he Phe	Leu Tyr	Val L	eu Glu	Ser	Leu
30			85			90			9 5	

Pro Ser Pro Thr Leu Thr Cys Ala Leu Thr Asn Gly Ser Ile Glu Val _100 105 Gla Cys Met Ile Pro Glu His Tyr Asa Ser His Arg Gly Leu Ile Met 120 Tyr Ser Trp Asp Cys Pro Met Glu Gla Cys Lys Arg Asa Ser Thr Ser 135 140 lie Tyr Phe Lys Met Glu Asn Asp Leu Pro Gin Lys lie Gin Cys Thr 145 Leu Ser Asn Pro Lee Phe Asn Thr Thr Ser Ser Ile Ile Leu Thr Thr 10 170 165 Cys lie Pro Ser Ser Gly His Ser Arg His Arg Tyr Ala Leu Ile Pro 185 lle Pro Leu Ala Val Ile Thr Thr Cys Ile Val Leu Tyr Met Asn Gly 200 205 195 lle Lea Lys Cys Asp Arg Lys Pro Asp Arg The Asn Ser Asn 15 220 210 215

(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	3 2
	5.	(2) INFORMATION FOR SEQ ID NO: 10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 36 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single	
1	10	(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:	
: :	15	TTTGTCGACC TGCAGCTAGG GCCCTGAGTT TCGTTG	3 6
, ,	20	(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	
	25	(iv) ANTI-SENSE: yes	

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

		CAACTGCAGC TACGACGTCC CAGAAAACCT ATGCCTTGCA TAATCAATCA C 51
	5	(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
;	10	(iv) ORIGINAL SOURCE (A) ORGANISM: Homo sapiens (H) CELL LINE: T cell line
		(vii) IMMEDIATE SOURCE: (B) CLONE: MOLT-4
•	15	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
		TTT TCC CAA CAA ATA TAT GGT GTT GTG TAT GGG ART GTA ACT TTC CAT 48
		Phe Ser Gin Gin lie 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
•	20	Val Pro Ser Asn Val Pro Leu Lys Glu Val Leu Trp Lys Lys Glu 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
	25	TIT 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 lle
		50 55 60

	TAC	AAC	TTA	ACA	TCA	TCA	GAT	GAA	GAT	GAG	TAT	GAA	ATG	GAA	TCG	CCA	240
	Tyr	A s a	Leu	Thr	Ser	Set	Asp	Giu	A s p	Glū	Tyr	Glu	Ne t	Glu	Ser	Pro	
	65					70					7 5					80	
•	AAT	ATT	ACT	GAT	ACC	ATG	AAG	TTC	TTT	CTT	TAT	GTG	CTT	GGT	CAT	TCA	288
5	A s n	He	Thr	As p	Th r	M e t	Lys	P h e	Phe	Len	Tyr	Val	Len	Gly	H i s	Ser	
					85					9.0		•			9 5		
	AGA	CAC	AGA	TAT	GCA	CTT	ATA	CCC	ATA	CCA	TTX	GCA	GTA	ATŢ	A C A	ACA	336
	Arg	H'i s	Arg	Tyr	A i a	Leu	lie	Pro	He	Pro	Leu	Ala	V a 1	I I e	Thr	Thr .	1
				100					105			-		110			
10	TGT	ATT	GTG	CTG	TAT	ATG	AAT	GGT	ATT	CTG	A A A	TGT	GAC	AGA	AAA	CCA	384
	Cys	l l e	V a l	Leu	Tyr	M e .t	A s n	Gly	l i e	Leu	Lys	C y .s	Asp	Å r g	Lys	Pro	
			115					120					125				
	G A (AGA	ACC	AAC	TCC	AAT											402
	ÁSJ			Asn	S.e.r	A s n	l										
15		130															
	(2) IN (i)	SE	QUEI (A)	ION NCE LEN(TYPE	CHA 5TH:	RAC 13	TER 4 an	ISTI nino	CS:								
20	(i	i) M	OLEC	CULE	TY	PE:	pro	tein									
	(i	ii) H	YPO	THE	TICA	L: 3	vės										
	. (х	i) S	EQUI	ENCE	DE	SCR	IPTI	ON:	SEQ	ID	NO:	13:					
	Ph		r Gl	n Gli	n II	e Ty	T G1	y V a	i Ka	[Ty	r Gl	y As	n Ye	t Th	r P'h	e His	
		1	_			5				1					1		
25	V a	I Pr	o Se			l Pr	o Le	u Ly			l Le	n Tr	p Ly			n Lys	
	A c	n Læ	s V 2	21 : 41:		ni La	្រ ដែ	n 1 -	2		n Dh	.a. 1 -	a 11		0	r Ser	
	31.4	, ,,					u U I		<i>11</i> 0 0	1201	لليس	as n	* (1)	a []]		, 45	

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4 5

Phe Lys Asm Arg Val Tyr Len Asp Thr Val Ser Gly Ser Len Thr Ile 55 Tyr Asa Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser Pro 70 75 Asa lie Thr Asp Thr Met Lys Phe Phe Leu Tyr Val Leu Gly His Ser 5 90 Arg His Arg Tyr Ala Leu Ile Pro Ile Pro Leu Ala Val Ile Thr Thr 100 105 110 Cys lie Val Leu Tyr Met Asn Gly Ile Leu Lys Cys Asp Arg Lys Pro . 115 120 10 Asp Arg The Asa See Asa 130

(2) INFORMATION FOR SEQ ID NO: 14:

15

20

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(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:
- (2) INFORMATION FOR SEQ ID NO: 15:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 41 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

41

	(D) TOPOLOGY: linear
5	(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
10	 (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
15	(iii) HYPOTHETICAL: no (vi) ORIGINAL SOURCE (A) ORGANISM: Homo sapiens
20	 (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

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

(G) DATE: Oct-1987

	186			000	100	010	000	000	000	000	040	000	0 77 0	0.77.0		000	4.5
				GGG													4 8
	Met	Val	Ala	Gly	Set	Λsp	Ala	Gly		A l.a	Leu	Gly	Val	Let	Ser	Val	
	-28			-25					-20					-15			
	GTC	TGC	CTG	CTG	CAC	TGC	TTT	GGT	TTC	ATÇ	AGC	TGT	TTT	TÇC	CAA	CYY	96
5	Val	Cys	Leu	Leu	H i s	Cys	P h e	Gly	Phe	He	Ser	Cys	Phe	Ser	Gla	Gln	
			-10					-5					1				
	ATA	TAT	GGT	GTT	GTG	TAT	GGG	AAT	GTA	ACT	TTC	CAT	GTA	CCA	AGC	AAT	1 4 4
-	He	Tyr	Gly	Ϋal	Val	Tyr	Gly	A s n	V a l	Thr	Phe.	His	Ÿ a l	Pro	Ser	A s a	
	5					10					15					20 -	
10	GTG	CCT	TTA	AAA	GAG	GTÇ	CTA	TGG	AAA	KAA	CAA	AAG	GAT	**	GTT	GCA	192
	V a l	Pro	Leu	Lys	Gia	Va l	Leu	Trp	Lys	Lys	Gln	Lys	Asp	Lys	V a l	Ala	•
					2 5					30					3,5		
	GAA	CTG	GAA	AAT	TCT	GAA	TTC	A G.A	GCT	TTC	TCA	TCT	TTT	**	ÄÄT	AGG	240
	Glu	Leu	Glu	A s n	Ser	Glu	Phe	Årg.	Ala	P h e	S-e-r	Ser	Phe	Lys	A s n	Arg	•
15				40					4 5					50			
	GTT	TAT	TTA	GAC	ACT	GTG	TCA	GGT	AGC	CTC	ACT	ATC	TAC	AAC	TTA	ACA	288
	Val	Tyr	Leu	Ásp	Thr	Val	Ser	Gly	Ser	Ļeu	Thr	lle	Tyr	A s n	Leu	Thr	
			5.5					60					65				
	TCA	TCA	GAT	GAA	GAT	GAG	TAT	GAA	ATG	GAA	TCG	CCA	AAT	ATT	ACT	GAT	336
20	Ser	Ser	Åsp	Glu	A s p	Gla	Tyt	Glu	M e. t	Glu	Ser	Pro	Asa	11e	The	Asp	
		70					7 5					80					
	A C C	ATG	AAG	TTC	TTT	CTT	TAT	GTG	CTT	GAG	TCT	CTT	CCA	TCT	CCC	ACA	384
	The	Met	Lys	Phe	Phe	Lea	Tyr	Val	Leu	Gla	Ser	Leu	Pro	Sér	Pro	Thr	
	8 5	i				9 0					9 5	i				100	
25	CTA	ACT	TGT	GCA	TTG	ACT	AAT	GGA	AGC	ATT	GAA	GTC	CAA	TGC	ATG	ATA	432
	Leo	i The	Cys	Ala	Lea	The	Asa	Gly	Ser	Ile	Gla	Ya-l	Gla	Cys	Met	He	
					105					110					115		
	CCA	GAG	C A T	ŤAC	AAC	AGO	CAT	CGA	GGA	CTT	' ATA	ATG	TAC	TCA	TGG	GAT	480
	Pro	ı Gin	His	Tyr	Asn	Set	His	Arg	Gly	Leu	ile	. Met	Tyt	Ser	Trp	A s p	
30				120				•	1 2 5					130		·	
30														•			

••••

	TGŤ	CCT	ATG	GYC	CYY	TGT	AAA	CGT	AAC	TCA	A C C	AGT	ATA	TAT	TTT	AAG	528
	Cys	Pro	Met	Glu	Gla	Cys	Lys	A t g	A a s	Ser	Thr	Ser	He	Tyr	P h e	Lys	
			135					140					145				
	ATG	GAA	AAT	GAT	CTT	CCA	CAA	* * *	ATA	CAG	TGT	ACT	CTT	AGC	AAT	CCA	576
5	Met	Glu	A s n	A s p	Leu	Pro	Gln	Lys	I l e	G 1 n	Cys	Thr	Leu	Ser	Asa	Pro	
		150					155					160					
	TTA	TTT	AAT	Y C Y	ACA	TCA	TCA	ATC	ATT	TTG	ACA	ACC	TGT	ATC	CCA	A G.C	624
	Leu	Phe	A s n	Thr	Thr	Ser	S e r	lle	I i e	Leu	Thr	Thr	Cys	lle	Pro	Ser	
	165					170					175					180	
10	AGC	GGT	CAT	TCA	AGA	CAC	AGA	TAT	GCA	CTT	ATA	CCC	ATA	CCY	TTA	GCA	672
	S e t	.G l y	His	S e t	Arg	His	Åtg	.Tyr	Ala	Len	lle	Pro	He	Pro	Leu	Ala	
					185					190					195		•
	GTÄ	ATT	ACA	ACA	TGT	ATT	GTG	CTG	TAT	ATG	AAT	GGT	ATT	CTG	AAA	TGT	720
	Vai	He	Thr	Th t	Cys	He	Val	Legu	Tyt	Met	A s n	Gly	I i e	Leu	Ly s	Cys	
15				200					2.05					210			
	GAC	AGA	AAA	CCA	GAC	AGA	ACC	AAC	TCC	AAT	TGA						753
	λsp	Arg	Lys	Pro	A:s p	Arg	Thr	A s n	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
- 25 (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
 - (iii) HYPOTHETICAL: no
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

38

TTTGGTACCG GATGTTTTCC CAACAAATAT ATGGTGTT

5	(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	
10	(iii) HYPOTHETICAL: no	
	(iv) ANTI-SENSE: yes	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:	
	TTTAAGCTTG CTAGCTCAAT TGGAGTTGGT TCTGTCTGGT TT	42
15	(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	
20	(ii) MOLECULE TYPE: other nucleic acid (A) DESCRIPTION: synthetic DNA	
	(iii) HYPOTHETICAL: no	
	(iv) ANTI-SENSE: yes	
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19: ATCAAGCTIT CAATTGGAGT TGGTTCTGTC TGGTTTTCTG TCTCTGTGTC TTGAATGACC	6 0 7 2
	AAGCACATAA AG	16

(2) INFORMATION FOR SEQ ID NO: 20:

	(1) SEQUENCE	CHARACTER	121172;			
	(A) LEN	NGTH: 399 ba	se pairs			
	(B) TYF	PE: nucleic a	cid			
5	(C) STR	RANDEDNESS:	single			
	(D) TOP	POLOGY: linea	ar			
	(ii) MOLECUL	LE TYPE: cDN	A to mRN	A		·
	(iii) HYPOTHE	ETICAL: no				
	(vi) ORIGINAL					•
10		GANISM: Ovis				
	(G) CEI	LL TYPE: leu	kocyte			
	(xi) SEQUENC	CE DESCRIPTI	ON: SEQ I	D NO: 20:		
	ATG GTA AGT CA	AA GAT ATT TAT	r GGA GCT	ATG ANT GGG	AAT GTA RCC	TTT 48
	Met Val Ser Gl	ln Asp The Ty	r Gly Alai	Met Asn Gly	Asn Val Thr	Phe
15	1	5		t 6	15	
	TAC GTT TCA GA	AG TCT CAA CC	G TTT ACA	GAG ATT ATG	TGG AAG AAG	G G G 9 6
	Tyr Val Ser Gl	lu Ser Gla Pr	o Phe Thr	Glu lle Met	Trp Lys Lys	Gly
	2	2 0	25	•	30	•
	AAG GAT AAA GT	TT GTA GAA TG	G GAT CAA	ACA TCT GGA	CTC GAA GCT	TTT 144
20	Lys Asp Lys Va	al Val Glu Tr	p Asp Gla	The See Gly	Leu Glu Ala	Phe
	3 5		40		45	
•	CAG TCT TTT A	AA AAT AGA GT	T CAT TTA	GAC ATT GTG	TCA GGT AAC	CTC 192
	Gla Ser Phe Ly	ys Asn Arg Va	l His Leu	Asp Ile Val	Ser Gly Asn	Leu
	50	5	5	60		•

	VCC	ATC	ACC	GGG	TTA	ACA	888	TTA	GAT	GAA	GAT	GTG	TAT	GAA	ATT	GAA	240
	Thr	He	Tht	Gly	Leu	Thr	Lys	Leu	A s p	Glu	Asp	V a l	Tyr	Glu	He	Glu	
	65					70					75					80	
	TCC	CCA	AGT	GTT	**	AAG	AGC	TCC	CAG	TTC	CAC	CTC	AGA	GTG	ATT	GAT	288
5	Ser	Pro	Ser	V a l	Lys	Lys	Ser	Ser	Gln	Phe	His	Leu	Årg	V a l	He	A s p	
					85					98			•		95		
	TAT	GCA	AGG	CAT	AGG	TAT	GTG	CTT	TTT	GCC	ATA	CTG	CCA	GCA	GTA	ATA	336
	Tyr	Ala	Arg	H i s	h r g	Tyr	V a l	Leu	Phe	ÁÌR	11 e	Leu	Pro	λla	Val	I l e	
				100					105					110			
10	TGT	GGC	TTG	CTG	TTT	TTA	λλλ	TGT	TTT	CTG	GGX	CGT	CGT	AGC	CAA	CGA	384
	Cys	Gly	Leu	Leu	Phe	Leu	Lys	Cys	Phe	Leu	Gly	Å r g	Yıg	Ser	Gla	Årg	
			115					120					115				
	AAC	TCA	GGG	CCC	TAG												3.9 9
	Asa	Ser	Gly	Pro													
15		130															

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(2) INFORMATION FOR SEQ ID NO: 21:

	(i) SEQ	UENCE (CHARACT	ERISTI	CS:						
	(1	A) LENG	TH: 321	base p	pairs						
	(]	B) TYPE	: nucleio	acid							
5	,		NDEDNES		gle						
	(1	D) TOPO	LOGY: li	near							
	(iii) HY	РОТНЕТ	ICAL: no	0							
	(xi) SE	QUENCE	DESCRI	PTION:	SEQ I	D NO:	21:				
	ATG GTA	AGT CAA	GAT ATT	TAT GG	A GCT	ATG AAT	GGG	AAT GTA	A C C	TTT	4 8
10	Met Val	Ser Gla	Asp Ile	Tyr Gl	y Ala	Met Asi	Gly	Asa Val	Thr	Phe	
	1		5			10			15		
	TAC GTT	TCA GAG	TCT CAA	CCG TT	T ACA	GAG AT	TATG	TGG AAC	AAG	GGG	9 6
	Tyr Val	Ser Glu	Ser Gin	Pro Ph	e The	Glu II	e Met	Trp Lys	Lys	Gly	
		20			25			3 ()		
15	AAG GAT	AAA GTT	GTA GAA	TGG GA	T CAA	ACA TC	T GGA	CTC GA	GCT	TIT	144
	Lys Asp	Lys Val	Val Glu	Ttp As	p Gin	The Se	r Gly	Len Gl	ala i	Phe	
		35		4	0			4 5			
	CAG TCT	TTT AAA	AAT AGA	GTT CA	T TTK	GAC AT	T GTG	TCA GG	TAAC	CTC	192
	Gln Ser	Phe Lys	Asn Arg	Val Hi	s leu	Asp II	e Val	Ser Gl	y Asn	Lev	
20	5 0			5 5			60				
•	ACC ATC	ACC GGG	TTA ACA	AAA TT	A GAT	GAA GA	T GTG	TAT GA	A ATT	GAA	240
	Thr ile	The Gly	Leu Thr	Lys Le	u Asp	Glu As	p Val	Tyr Gl	a lle	Gia	
	6.5	ĺ	7 0			7	5			80	
	TCG CCA	AGT GT1	C AAA AAG	AGC TO	CC CAG	TTC CX	C CTC	AGA GT	G ATT	GAT	288
25	Ser Pro	Ser Val	l Lys Lys	Ser Se	er Glø	Phe Hi	s Leu	Arg Va	l II e	Asp	
			8 5			90			9 5		

TAT GCA AGG CAT AGG TTT TCT GGG ACG TCG TAG

	Tyr Ala Arg His Arg Phe Ser Gly Thr Ser	
	100 105	
5	(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	
10	(ii) MOLECULE TYPE: cDNA to mRNA	
	(iii) HYPOTHETICAL: no	
	(vi) ORIGINAL SOURCE (A) ORGANISM: Homo sapiens (G) CELL TYPE: T cell line	
15	(vii) IMMEDIATE SOURCE: (B) CLONE: MOLT-4	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:	
	ATG TIT TCC CAA CAA ATA TAT GGT GIT GIG TAT GGG AAT GTA ÂCT TTC	48
20	Mer Phe Ser Gin Gin Ile Tyr Gly Vai Val Tyr Gly Asn Val Thr Phe 1 5 10 15	
20	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 Glu	• •
	20 25 30	
	AAG GAT AAA GTT GCA GAA CTG GAA AAT TET GAA TTC AGA GCT TTC TCA	144
25	Lys Asp Lys Val Ala Glo Leo Giw Asn Ser Glo Phe Arg Ala Phe Ser	
	75 // // // // //	

	ፕሮፕ	ተተተ	4 4 4	AAT	ACC	CTT	TIT	TTI	010	ACT	CTC	TCI	GGT	100	CTC	ACT	192
											•						134
	Ser	Phe	Lys	A s n	Årg	V a l	Tyr	Leu	A s p	Thr	Val	Ser	Gly	Ser	Leu	Thr	
		50					5 5					60					
	ATC	TAC	AAC	ŤTÅ	ACA	TCA	TCA	GAT	GAA	GAT	GAG	TAT	G A Å	ATG	GAA	TCG	240
5	lle	Tyr	A s n	Leu	Thr	Ser	Set	A s p	Glu	Àsp	Glu	Tyr	Glu	Met	Glu	S e r	
	6 5					70					75					80	
	CCA	AAT	ATT	ACT	GAT	ACC	ÁTG	AAG	TTC	TTT	CTT	TÁT	GTG	CTT	GGT	CAT	288
	Pro	A s n	He	Thr	As p	Thr	M e-t	L y s	P h e	P h e	Leu	Tyr	V a i	Leu	Gly	His	
					85					90				٥,	9 5		
10	TCA	AGA	CAC	AGA	TAT	GCA	CTT	ATA	ccc	ATA	C.C.A	TTA	GCA	GTA	ATT	ACA	336
	Ser	Arg	His	Arg	Tyr	Ala	Leu	He	Pro	He	Pro	Leu	Ala	V a i	He	Thr	
				100					105					110			
	ACA	TGT	ATT	GTG	CTG	TAT	ATG	AAT	GGT	ATT	CTG	AAA	TGT	GYC	AGA	* * *	384
	Thr	Cys	He	V à l	Leu	Tyr	Met	À s n	Gly	He	Leu	Lys	Cys	Ásр	Άrg	Lys	
15			115					120					125				
	CCA	GAC	A G A	ACC	AAC	TCC	AAT	T G A			}						408
	Pro	Asp	å.r g	Thr	A s n	Ser	A s n			•							
		130					135										

(2) INFORMATION FOR SEQ ID NO: 23:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear
- 25 (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	4 8
	Met	Phe	Ser	Gla	Gla	I l e	Tyr	Gly	V a l	V a I	Tyr	Gly	A s n	V a l	Thr	Phe	
	1				5					10		-			15		
	CAT	GTA	CCA	AGC	AAT	GTG	CCT	TTA	AAA	GAG	GTC	CTA	TGG	AAA	**	CAA	96
5	His	V a i	Pro	Ser	A s n	Yal	Pro	Leu	Lys	Glu	V a l	Leu	Trp	Lys	Lys	Gln	
				20		·			25					3 0			
	AAG	GAT	AAA	GTT	GCA	G A A	CTG	GAA	AAT	TCT	GAA	TTC	AGA	GCT	TTC	TCA	144
	Lys	Asp	Lys	V a l	λla	Glu	Leu	Głų	A s n	S e r	Glu	Phe	Arg	Ala	Phe	Ser	
			3 5					4 0					4 5				
10	TCT	TTT	AAA	AAT	AGG	GTT	TAT	TTA	GAC	ACT	GTG	T C A	GGT	AGC	CTC	ACT	192
	Ser	P h e	Lys	A s n	Arg	V a l	Tyr	Leu	Asp	Thr	V a l	Set	Gly	S e t	Lea	Thr	
	•	50	,				5 5					60					
	ATC	TAC	AAC	TTA	ACA	TCA	TCA	GAT	GÅA	GAT	GAG	TAT	GAA	ATG	GAA	TCG	240
	lie	Tyr	A s n	Leu	Thr	S e t	S è r	Ásp	Glu	A s p	Glu	Typ	GIn	Me t	Glu	Ser	
15	6 5					70					7.5					80	
	CCA	AAT	TTA	ACT	GAT	ACC	ATG	AAG	TTC	TTT	CTT	TAT	GTG	CTT	GGT	CAT	288
	Pro	Asn	I I e	Thr	A s ^a p	The	Met	Lys	P h e	Phe	Leu	Tyr	V a l	Leu	Gly	His	
					85					90					9 5		
	TCA	AGA	CÁC	AGA	GAC	AGA	AAA	CCA	GAC	AGA	ACC	AAC	TCC	AAT	TGA		333
20	Ser	Arg	H i s	y i &	Ásp	Arg	Lys	Pro	Å s p	Arg	Thr	A s n	Ser	Asn			
				1 0 0					105					1 1.0			

(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	
5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:	
	TTTTTTCGAA CTGCAGCTAA CACGACGTCC CAGAAAACCT ATGCCT	4 6
	(2) INFORMATION FOR SEQ ID NO: 25: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 324 base pairs	
10	(B) TYPE: nucleic acid (C) STRANDEDNESS: both (D) TOPOLOGY: linear	
	(iii) HYPOTHETICAL: no	
•	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:	4.0
15	ATG GTA AGT CAA CAT ATT TAT GGA GCT ATG AAT GGG AAT GTA ACC TTT Met Val Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Val Thr Phe	4,8
	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 Gla Pro Phe Thr Glu Ile Met Trp Lys Lys Gly	
20	20 25 30	
	ANG GAT ANA GTT GTA GAN TGG GAT CAN ACA TCT GGA CTC GAN GCT TTT	144
	Lys Asp Lys Val Val Giu Trp Asp Gin Thr Ser Giy Leu Giu Ala Phe 35 40 45	
•	CAG TOT TIT ARA RAT AGA GIT CAT TIA GAC ATT GIG TOA GGT AAG CTC	192
25	Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu	
	50 ACC ATC ACC GGG TTA ACA AAA TTA GAT GAA GAT GTG TAT GAA ATT GAA	2 4 0,
	The Ite The Gly Leu The 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													
5	Tyr Ala Arg His Arg Phe Ser Gly Thr Ser Cys														
	100 105														
10	(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														
15	(iii) HYPOTHETICAL: no														
	(iv) ANTI-SENSE: yes														
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26: GGGGAAGGTT TCAACAATTG GAGTTGGTTC TGTCTGG	37													
20	(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	•													
25	(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	4.8
	Met	Phe	Set	Gln	Gln	He	Tyr	Gly	V a l	V a l	Tyr	Giy	À s n	V a l	1 d T	Phe	
	1				5					10					15		
	CAT	GTA	CCA	AGC	AAT	GTG	CCT	TTA	AAA	GAG	GTC	CTA	TGG	**	AAA	CAA	9 6
5	His	V a l	Pro.	Ser	k s n	Val	Pro	Leu	Lys	Gln	V a i	Lev	Trp	Lys	L y s	Gla	
	AAG	GAT	AAA	20 GTT	GCA	GAA	CTG	GAA	25 AAT	TCT	GAA	TTC	AGÄ	30 GCT	<u>የ</u> ተር	TCA	144
							Leu										
			35					40					45				
10	TCT	TTT	λλλ	AAT	AGG	GTT	TAT	TTA	GAC	ACT	GTG	TCA	GGT	AGC	CTC	ACT	192
	S.e r	Phe	Lys	Asn	Arg	V a l	Tyr	Leu	À s.p	T.h.r	Val	Ser	Gly	Ser	Leu	Thr	
		5.0					5 5					60					-
	ATC	TAC	AAC	TTA	ACA	TCA	TCA	GAT	GAA	GAT	GAG	TAT	GAA	ATG	GAA	TÇG	240
							Ser										
15	65					70		·			75	Ť				80	
	CCA	AAT	ATT	ACT	GAT	ACC	ATG	AAG	TTC	TTT	CŤŦ	TAT	GTG	ETT	GGT	CAT	288
	Pro	Asa	Πē	The	A s p	The	Met	Lys	Phe	Phe	Lew	Ťýr	Val	Leu	Giy	His	
					85					90					9 5		
	TCA	AGA	CAC	AGA	GAC	AGA	AAA	CCA	GAC	A G A	ĄCC	AAC	TCC	AAŤ	TGT	TGA	336
20	Ser	Årg	His	Arg	Asp	Y t &	Lys	Pro	Ásp	Arg	Thr	Asn	1 9.8	Asn	Cys		
				100					105					110			

5	(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	
10	(iv) ANTI-SENSE: yes	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28: TITALGCTTC LACALGTTAG TGTGGGLGAT GGLLG	35
15	(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	
20	(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 Gla Gla fle Tyr Gly Val Val Tyr Gly Asa Val Thr Phe 1 5 10 15	
	CAT GTA CCA AGC AAT GTG CCT TTA AAA GAG GTC CTA TGG AAA AAA CAA	9 6
25	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	TTÇ	AGA	GCT	TTC	TCA	144
	Lys	Asp	Lys	¥a l	Ala	Glu	Leu	Glu	A s a	Ser	Glu	Phe	Å t g	Ala	P h e	Ser	
			35					40		•			45				
	TCT	TTT	AAA	AAT	AGG	GTT	TAT	ATT	GAC	ACT	GTG	TCA	GGT	AGC	CTC	ACT	192
5	Set	Phe	Lys	A s a	Arg	V a l	Tyr	Leu	A s p	Thr	Val	Set	Gly	Ser	Len	Thr	
		50					5 5			1		60					
	ATC.	TAC	KAC	TTA	ACA	TCA	T'C A	GAT	GÀA	GAT	GÁG	TAT	GAA	ATG	GAA	TCG	240
	He	Tyt	Asa	Leu	Thr	Ser	S e r	A s p	Glu	Asp	Gla	Tyr	Glu	Met	Glu	Seir	
	65					70					7.5					80	
10	CCA	AAT	ATT	ACT	GAT	ACC	ATG	AAG	TTC	TTT	CTT	TAT	GTG	CTT	GAG	TCT	288
	Pro	Å s n	He	Thr	Ås p	Thr	Me t	Lys	Phe-	Phe	Leu	Tyr	V a l	Leu	Glu	Ser	
					8 5					90					9.5		
	CTT	CCA	TET	CCC	ALA	CTA	ACT	TGT	TGA								315
	Leu	Pro	Ser	Pro	Thr	Leu	Thr	Cys									
15				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

20

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Ovis
- 25 (G) CELL TYPE: leukocyte
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

	GTT TC	CAA	GAT	ATT	TAT	GGA	GCT	ATG	AAT	GGG	AAT	GTA	ACC	TTT	TAC	48
	Val Se	r Gla	Åsp	He	Tyr	Gly	Ala	Met	A s a	Gly	A s a	V a l	Jhe	Phe	Tyr	
	1			5					10					15		
	GTT TC	A GAG	TCT	CAA	CCG	TTT	ACA	GAG	ATT	ATG	TGG	AAG	AAG	GGG	AAG	96
5	Val Se	t Glu	Set	Gln	Pro	Phe	Thr	Glu	ll e	Met	Trp	Lys	Lys	Gly	Lys	
			20					25					3 Q			
	GAT AA	A GTT	GTA	GAA	TGG	GAT	CAA	ACA	TCT	GGA	CTC	GAA	GÇŤ	TTT	CAG	144
	Asp Ly	s Val	Val	Gin	Trp	A s p	Gln	Thr	S e r	Gly	Leu	Glu	Ala	Phe	Gln	
		35					40					45				
10	TÇT TT	T AAA	AAT	AGA	GTT	CAT	ATT	GAC	ATT	ĠTG	TCA	GGT	AAC	CTC	ACC	192
•	Ser Ph	e Lys	A s n	Arg	V a l	His	Leu	λsp	11 e	V a l	Ser	Gly	A s a	Leu	Thr	
	50					5 5					60					
	ATC AC	c GGG	ATT	A C A	AAA	TTA	GAT	GAA	GAT	GTG	TAT	GAA	ATT	GAA	TCC	240
	lle Th	r Gly	Leu	Thr	Lys	Leu	Asp	Glu	A s p	V a-1	Tyr	Glu	lle	Gla	Ser	
15	6.5				70					75					80	
	CCA AG	T GTT	AAA	AAG	AGC	TCC	CAG	TTC	CAC	CTC	AGA	GTG	ATT	GAA	CCT	288
	Pro Se	r Val	675	Lys	Ser	Ser	Gin	Phe	His	Leu	Åιg	V a l	He	Gla	Pro	
				8.5					90					95		
	CCT CC	A ACA	CCG	TCA	GCA	TCT	TGC	TTC	TTG	ACT	GAG	G G.T	GGA	VVC	TTK	336
20	Pro Pr	o Thr	Pro	Ser	Alá	Ser	Cys	Phe	Leu	Thr	Glu	Gly	Gly	A s a	4 Fe	
			100					105					110			
	ACT CT	C ACC	TGC	TCG	ATC	CCG	GÄA	GGT	GAC	CCC	AAA	GAG	CTC	GAT	GAT	384
	Thr Le	u Thr	Cys	Ser	He	Pro	Giu	Gly	Ásp	Pro	Lys	Giu	Leu	Asp	Ásp	
		115					120					125				
25	AGT GA	C CTA	A.T.A	CGG	TAT	TTG	TGG	GAA	TGT	CCG	CCY	ACA	ATA	CAG	TGT	432
•	Ser As	p Lea	He	Å f g	Týr	Lëu	Trp	Glu	C.y s	orq	Pro	Thr	lie	Gla	Cy \$	
	13	0				135					140					

- - - -

	CAC CGT	GGC TCG	ATT TCA	TCT GAR	GCC TT	T GTC TO	CA GCG GAA	AGT GAT	480
	His Arg	Gly Ser	lle Ser	Ser Glu	Ala Ph	e Val Se	er Ala Gli	ı Ser Asp	
	145		150			155		160	
	CTT TCA	CAG AAT	GTT CAG	TGT ATO	GTT AG	C AAT C	CA TTG TT	C AGA ACA	528
5	Leu Ser	Gin Asn	Val Gln	Cys II	val Se	er Asn P	ro Leu Ph	e Arg Thr	
			165		17	0		175	
	TCA GCT	TCC GTC	TCT TTG	TCA AC	C TGT TT	IG CCA G	AG GAT TA	T GCA AGG	576
	Ser Ala	Ser Val	Ser Leu	Ser Th	r Cys Le	or que	lu Asp Ty	r Ala Arg	
		180		40	185		19	0	
10	CAT AGG	TAT GTG	CTT TTT	GCC AT	CTG CC	CA GCA G	TA ATA TG	T GGC TTG	624
	His Arg	Tyr Val	Leu Phe	Ala II	Leu Pr	ro Ala V	al lie Cy	s Gly Leu	
*	and the	195		20)		205		•
	CTG TTT	TTA AAA	TGT TTT	CTG GG	CGT CG	GT AGC C	AA CGA AA	C TCA GGG	672
	Leu Phe	Leu Lys	Cys Phe	Leu Gl	Arg At	rg Ser G	la årg As	n Ser Gly	•
15	210			215		2	20		
	CÇC								675
	orq								
	225								

5	(i) SEQ (MATION FOR QUENCE CHAR A) LENGTH: B) TYPE: am OLECULE TYP	ACTERIS 225 amin ino acid	TICS: no acids		
	, ,	YPOTHETICAL				
	(xi) SE	EQUENCE DES	CRIPTIO	N: SEQ ID N	0: 31:	
	Val Ser	Gln Asp Ile	Tyr Gly	Ala Met Asn	Gl/y Asn Val	The Phe Tyr
	1	5		10		15
10	Val Ser	Glu Ser Gln	Pro Phe		\$	Lys Gly Lys
		2/0		25	~) s. t	30
	vzh rlz	Val Val Glu 35	ith V2.b	40	45	Ala rue Gin
	Ser Phe	Lys Asn Arg	Val His			Ach len The
15	50	ajv non nig	5 5	noù itab i i o	60	NJA BCC ICI
		Gly Leu Thr		Asp Glu Asp	•	lle Glu Ser
	5 5 ·		70	,	75	80
	Pro Ser	Val Lys Lys	Ser Ser	Gla Phe His	Leu Arg Val	lle Glu Pro
		8.5		90		95
20	Pro Pró	Thr Pro Ser	Ala Ser	Cys Phe Leu	The Glu Gly	Gly Asn Ile
		100		105		110
	Thr Leu	The Cys Sec	Ile Pro	Glu Gly Asp	Pro Lys Gla	ı Leu Asp Asp
	C	115	Trr lan	120 Trn Gin Cre	Pro Pro Th	i r lle Gla Cys
25	3er asp 130		135	ith ata ola	140	i iio oik ojo
				Glu Ala Phe		a Glu Ser Asp
-	145	, , , , , , ,	150		155	160
	Let Ser	r Gla Asa Val	Gln Cys	lle Val Ser	Asn Pro Le	u Phe Atg Tht
		165		170		175
30	Ser Ala	a Ser Val Sei	Leu Ser	The Cys Lau	Pro Gla As	p Tyr Ala Arg
		180		185		190

His Arg Tyr Val Leu Phe Ala lle Leu Pro Ala Val lle 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

10

5

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: synthetic DNA
- 15 (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:

20

- (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:	
5		TTGGGGATCC ATGGTAAGTC AAGATATTTA TGG	33
10	(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	
15		(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:	
		GTCGACCTGC AGETACGACG TCCCAGAAAA CCTATG	36
20	(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	
25		(vi) ORIGINAL SOURCE: (A) ORGANISM: Ovis (G) CELL TYPE: leukocyte	

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

	GTT	TCL	CAA	GAT	ATT	TAT	GGA	GCT	ATG	AAT	$\tt GGG$	AAT	GTA	ACC	TTT	TAC	4 8
	V a t	Ser	Gla	Asp	lle	Tyr	Gly	Ala	Met	A s n	Gly	A s n	Y a l	Thr	P h e	Tyr	
	1				5					10					15		
	GTT	TCA	GAG	TCT	CAA	CCG	TTT	A C A	G A-G	ATT	ATG	TGG	AAG	AAG	GGG	A A G	96
5	Val	Ser	Gla	Ser	Gln	Pro	P h e	T & r	Giu	lle	Met	Trp	Lys	Lys	Gly	Lys	
				20					25					3 0			
	GAT	AAA	GTT	GTA	GAA	TGG	GAT	CAA	ACA	TCT	GGA	CTC	GAA	GCT	TTT	CAG	144
	A s p	Lys	V a l	V a ł	Glu	Trp	Asp	Gln	Thr	Set	Gly	Leu	Glu	Ala	P h e	Gln	
			3 5					4 0					4.5				
10	TCT	TTT	AAA	AAŤ	A G A	GTT	CAT	TTA	GAÇ	ATT	GTG	TCA	GGT	A A C	CTC	ACC	192
	Ser	P h e	Lys	A s n	Årg:	.V a l	His	Leu	A s p	Tle	V a l	Ser	Gly	ksn	Leu	Thr	
		50					5 5					6 0.					
	AŤC	ACC	G.G.G	TTA	ACA	AAA	TTA	GAŢ	GAA	ĜAT	GTG	TAT	G A A	ATT	GÀA	TCC	240
	He	Thr	Gly	Leu	Thr	Lys	Leu	Asp	Glu	A s p	Val	Tyr	Glu	He	Glu	Ser	
15	6 5					70					75					8 0	
	CCA	AGT	GTT	AAA	A A G	ÁGC	TCC	CAG	TTC	CAC	CTC	AGA	GTG	ATT	GAA	CCT	288
	Pro	Ser	V a l	Lys	Lys	Ser	Ser	Gln	Phe	H i s	Leu	Årg	V a l	I I e	Glú	P r o.	
					8 5					90					9 5		
	CCT	CCA	ACA	CCG	TCA	GCA	TCT	TGC	TTC	TTG	ACT	GAG	GGT	GGA	AAC	TTA	336
20	Pro	Pro	Thr	Pro	S e r	Λla	Ser	Cys	Phe	Leu	Thr	G l, u	Gly	Giy	A s n	I I e	
				1.00					105					110			
	ACT	CTC	ACC	TGC	TCG	ATC	CCG	GAA	GGT	GAC	CCC	AAA	GAG	CTC	GAŤ	GAT	384
	The	Lea	The	C y s	S e r	Fle	Pro	Glu	Gly	A s p	Pro	Lys	Glu	Leu	Asp	Åзр	
			115					120					125				

	AGT	GAC	CTA	ATA	CGG	TAT	TTG	TGG	GAA	TGT	CCG	CCA	ACA	ATA	CYC	TGT	432
	Ser	à s p	Leu	[] e	Arg	Tyr	Leu	Trp	Gla	C 7 s	Pro	P r o	Thr	lle	Gln	Cys	
		130					135					140					
	CAC	CGT	GGC	TCG	ATT	TCA	TCT	GAA	GCC	TTT	GTC	TCA	GCG	GAA	AGT	GAT	480
5	a i s	å i g	Gly	Ser	[le	Ser	Ser	Glu	Ala	Phe	V a l	Ser	Ala	Glu	S e.r	Asp	
	145					150					155					160	
	CTT	TCA	CAG	AAT	GTT	CAG	TGT	ATC	GTT	AGC	AAT	CCA	TTG	TTC	AGA	ACA	528
	Leu	Ser	Gla	Asn	V a l	Gla	Cys	He	V a l	S er r-	Å s n	Pro	Leu	Phe	A f g	Thr	
					165					170					175		
10	TCA	GCT	TCC	G o'C	TCT	TTG	TCA	ACC	TGT	TTG	CCA	GAG	GAT	TAT	GCA	AGG	576
	Ser	Ala	Ser	V a l	Ser	Leu	Ser	Thr	Суs	Leu	Pro	Glu	Å s.p	Tyr	A 1 a	Arg	
				180					185					190			
	CAT	A G G	TTT	TCT	GGĞ	ACĞ	TCG										597
	His	Arg	Phe	Ser	Gly	That	Ser										
15			195														

- (2) INFORMATION FOR SEQ ID NO: 36:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 199 amino acids
 - (B) TYPE: amino acid
- 20 (ii) MOLECULE TYPE: protein
 - (iii) HYPOTHETICAL: yes
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Yal Ser Gln Asp Ile Tyr Gly Ala Met Asn Gly Asn Yal Thr Phe Tyr 5 10 Yal Ser Glu Ser Gln Pro Phe Thr Glu Ile Met Trp Lys Lys Gly Lys Asp Lys Val Val Glu Trp Asp Gln Thr Ser Gly Leu Glu Ala Phe Gln Ser Phe Lys Asn Arg Val His Leu Asp Ile Val Ser Gly Asn Leu Thr lle Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu ile Glu Ser Pro Ser Val Lys Lys Ser Ser Gln Phe His Len Arg Val Ile Glu Pro Pro Pro The Pro Ser Ala Ser Cy's Phe Leu The Glu Gly Gly Asn Ile Thr Leu Thr Cys Ser lle Pro Glu Gly Asp Pro Lys Glu Leu Asp Asp Ser Asp Lew He Arg Tyr Lau Tsp Glu Cys Pro Pro Thr He Gla Cys 1.35 His L I'y Ser He Ser Ser Glu Ala Phe Val Ser Ala Glu Ser Asp Leu Ser Gln Asn Val Gin Cys Ile Val Ser Asn Pro Leu Phe Arg Thr Ser Ala Ser Val Ser Len Ser The Cys Leu Pro Glu Asp Tyr Ala Arg His Arg Phe Ser Gly The Ser

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- 30 (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	3 2
(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
- 10 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.
- 15 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
- 20 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.
- 30 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.
- 35 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.

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
- 25 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.
- 35 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



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15.



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
- 20 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.
- 30 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

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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.

F/G./

	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 !le Tyr Gly Ala Met Asn Gly Asn Val Thr Phe Tyr Val Ser Glu Ser
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 [le Val Ser
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
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
human	4
sheep	Ser Ala Ser Cys Phe Leu Thr Glu Gly Gly Asn Ile Thr Leu Thr Cys Ser Ile Pro Glu
human	His Tyr Asn Ser His Arg GlyLeu 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
human	Glu Gln Cys Lys ArgAsn Ser Thr Ser lle Tyr PheLys Met Glu Asn
sheep	Thr [le Gln Cys Wis Arg Gly Ser [le SerSer Glu Ala Phe Val Ser Ala Glu Ser
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 Gin Asn Val Gin Cys lie Val Ser Asn Pro Leu Phe Arg Thr Ser Ala Ser
human	Ile He Leu Thr Thr Cys He Pro Ser Ser Gly His Ser Arg His Arg Tyr Ala Leu He
sheep	idi dor bor oby till old borise and till the till the till
human	200 210 Pro Ile Pro Leu Ala Val Ile Thr Thr Cys Ile Val Leu Tvr Met asn Gly Ile Leu Ly
sheep	
human	220 n <u>Cys</u> Asp Arg Lys Pro Asp Arg Thr Asm Ser Asm
sheep	Phe Ser Gly Thr Ser

F1G.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
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 Vai Ser 60 70
human	Gly Ser Leu Thr Ile Tyr Asn Leu Thr Ser Ser Asp Glu Asp Glu Tyr Glu Met Glu Ser
sr ep	Gly Asn Leu Thr Ile Thr Gly Leu Thr Lys Leu Asp Glu Asp Val Tyr Glu Ile Glu Ser
h wan	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
human	Thr Leu Thr Cys Ala Leu Thr Asn Gly Ser [le Glu Val Gln Cys Met [le Pro Glu His
sheep	
humań	120 130 Tyr Asn Ser His Arg Gly Leu Ile Met Tyr Ser Trp Asp Cys Pro Met Glu Gln Cys Lys
sheep	
human	140 150 Arg Asn Ser Thr Ser Ile Tyr Phe Lys Met Glu Asn Asp Leu Pro Gln Lys Ile Gln Cys
sheep	
human	160 170 Thr Leu Ser Asn Pro Leu Phe Asn Thr Thr Ser Ser Lie Lie Leu Thr Thr Cys Ile Pro
sheep	
human	180 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	: : : : : : : : : : : : : : : : : : :
human	Arg Thr Asn Ser Asn
sheep	Arg Asn Ser Gly Pro

F1G.3

-∽ Sheep D1HC

→ Human D1HC

