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(54) **COMMON LYMPHATIC ENDOTHELIAL
AND VASCULAR ENDOTHELIAL
RECEPTOR-1 (CLEVER-1) AND USES
THEREOF**

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C07K 16/30
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(57) **ABSTRACT**

A novel protein Common Lymphatic Endothelial and Vas-
cular Endothelial Receptor-1 (CLEVER-1) is described.
CLEVER-1 mediates leukocyte and malignant cell binding
to vascular and lymphoid endothelial cells. CLEVER-1 is
the first protein that has been reported to mediate both influx
into and efflux from the lymph nodes. Also provided are
methods of treating inflammation and preventing metastasis
of malignant cells by providing an inhibitor of CLEVER-1
binding.

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Jan. 9, 2002 (US)..... 60346288

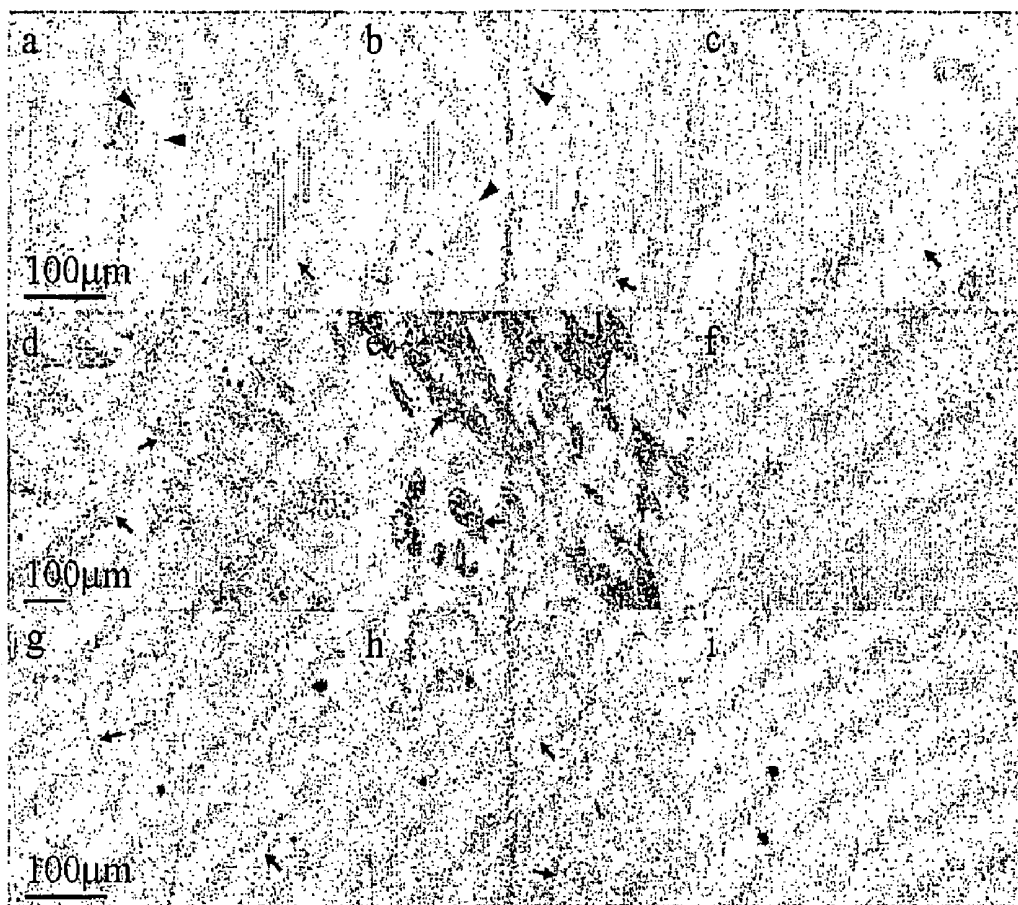


Fig. 1

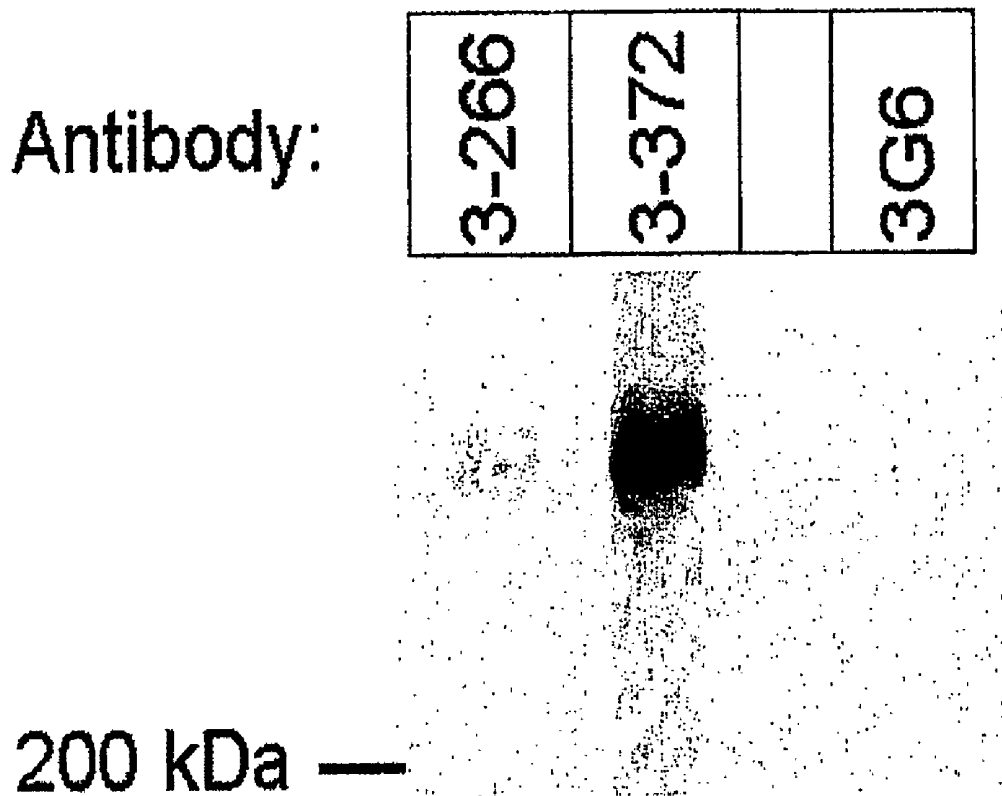
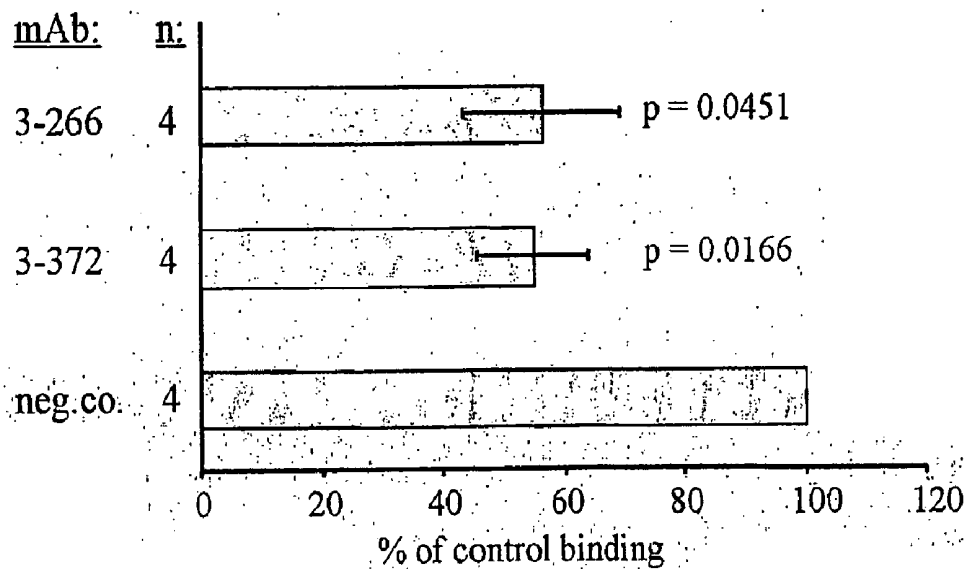


Fig. 2

A Lymphocyte binding to HEVs:



B Lymphocyte binding to lymph endothelium:

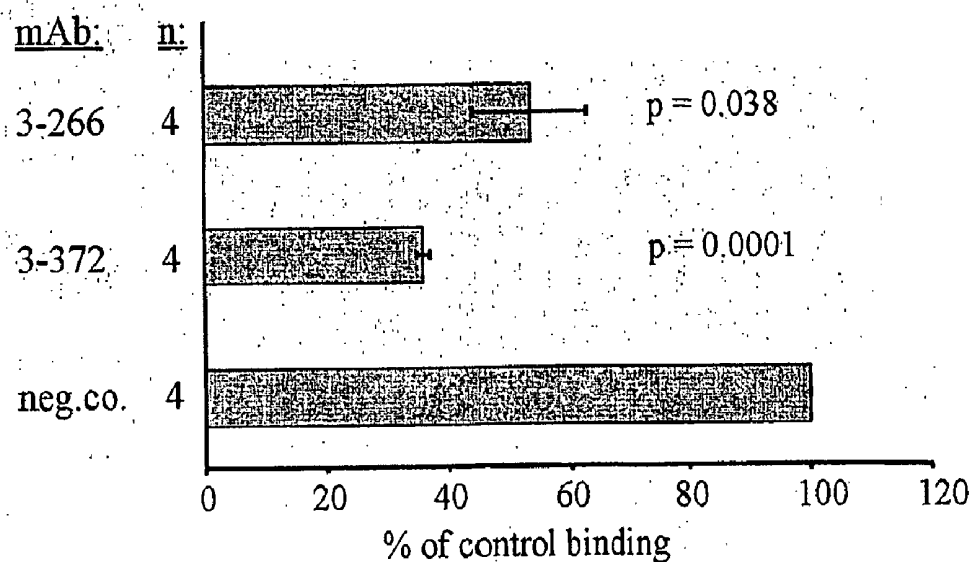
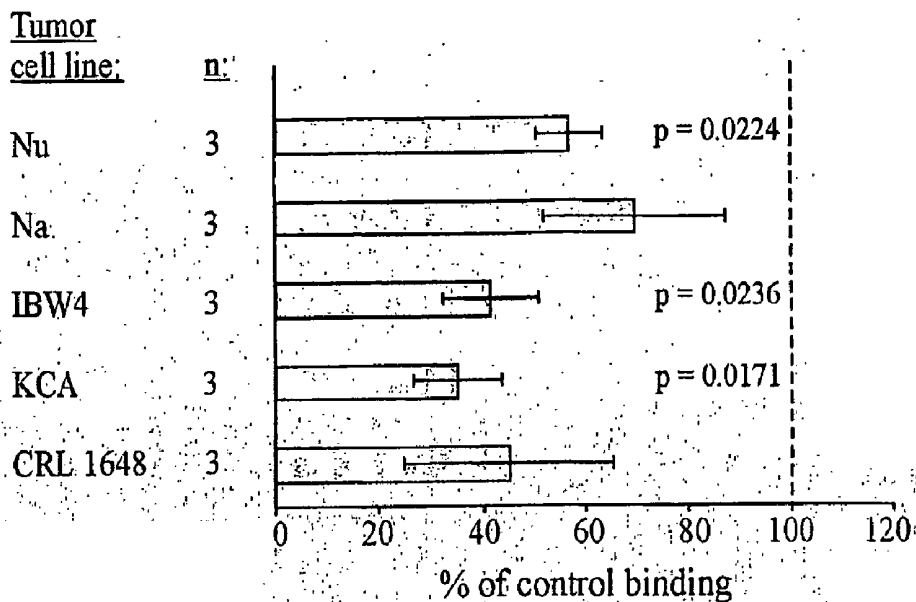


Fig. 3

A Tumor cell binding to HEVs:



B Tumor cell binding to lymphatic endothelium:

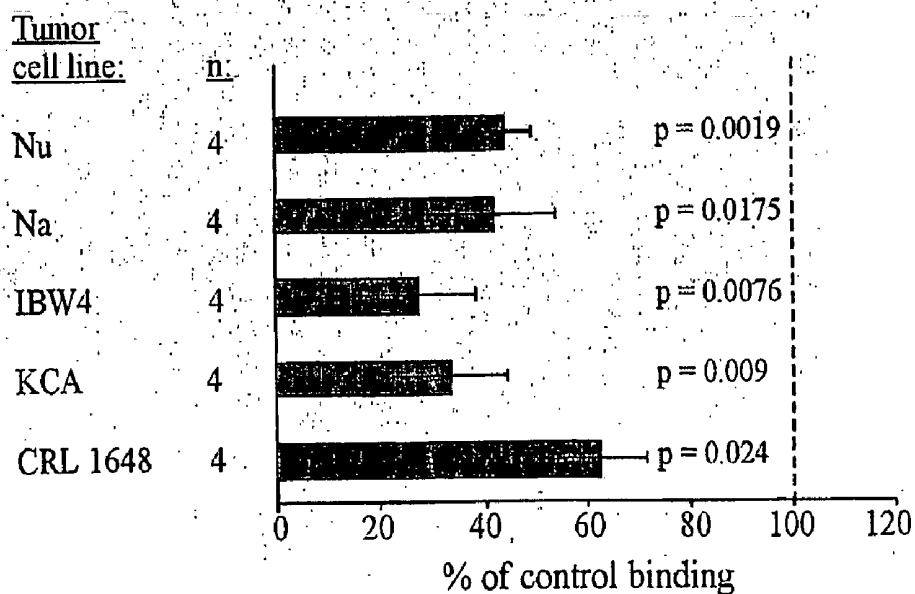


Fig. 4

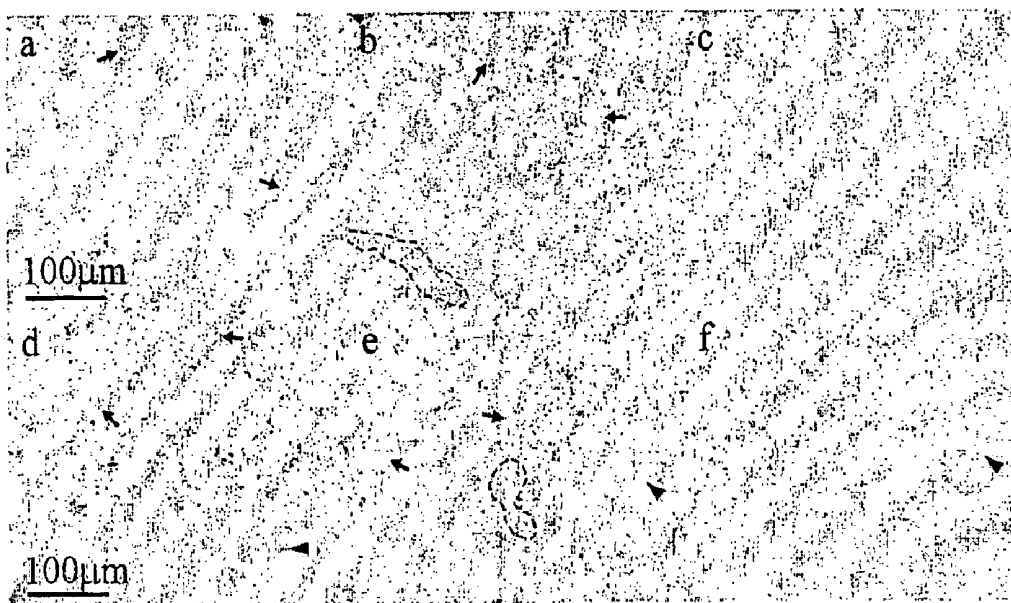


Fig. 5

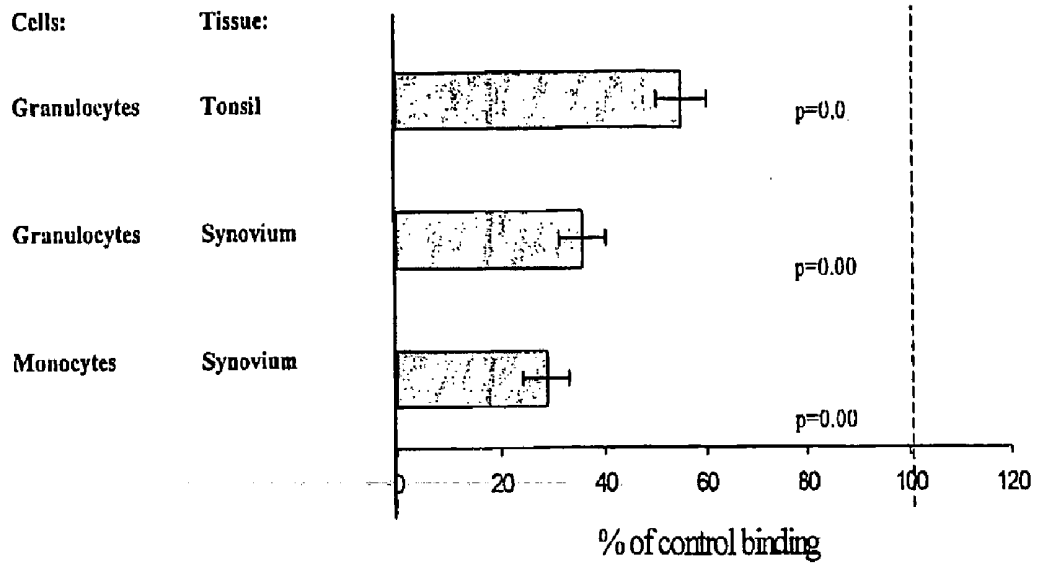


Fig. 6

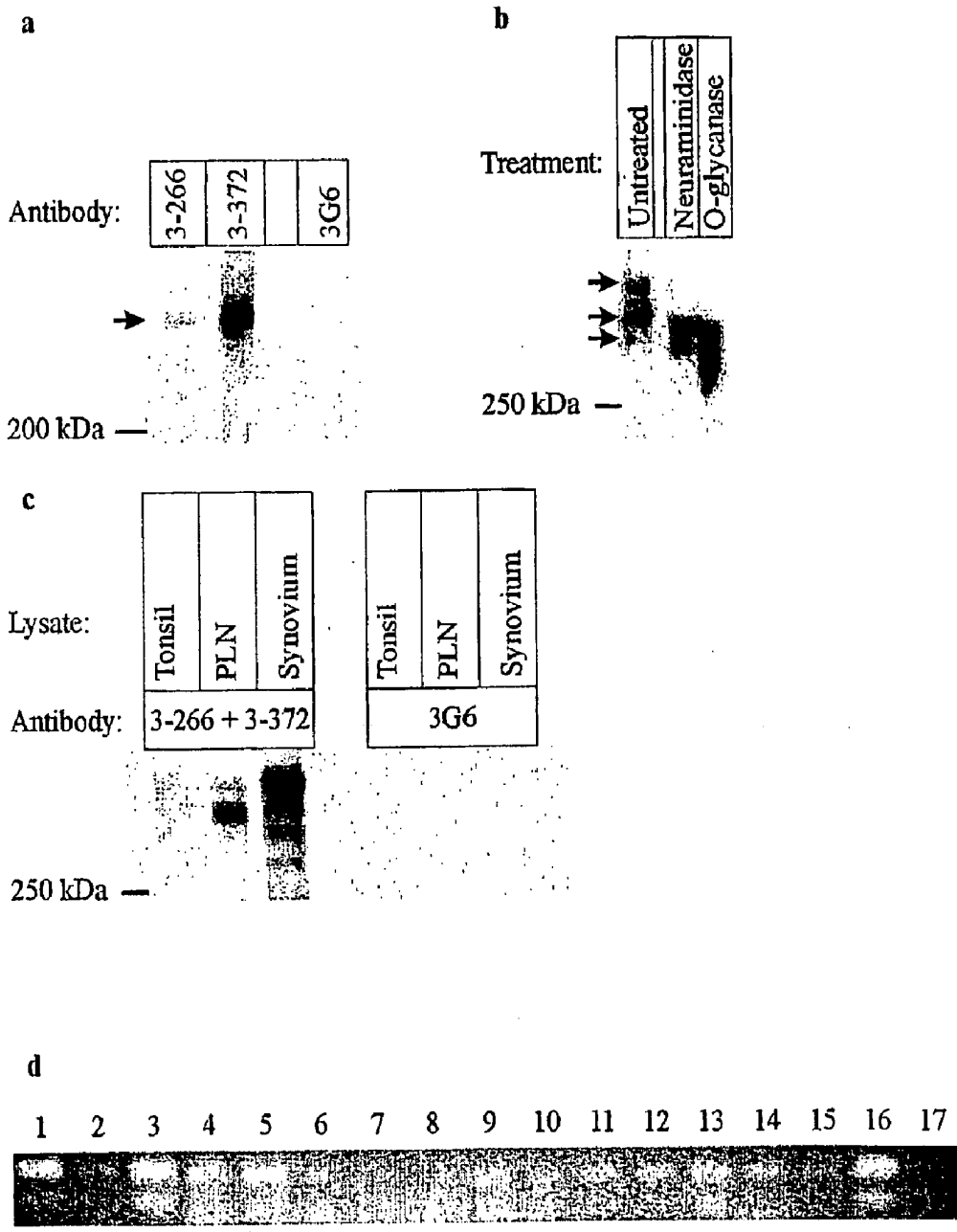


Fig. 7

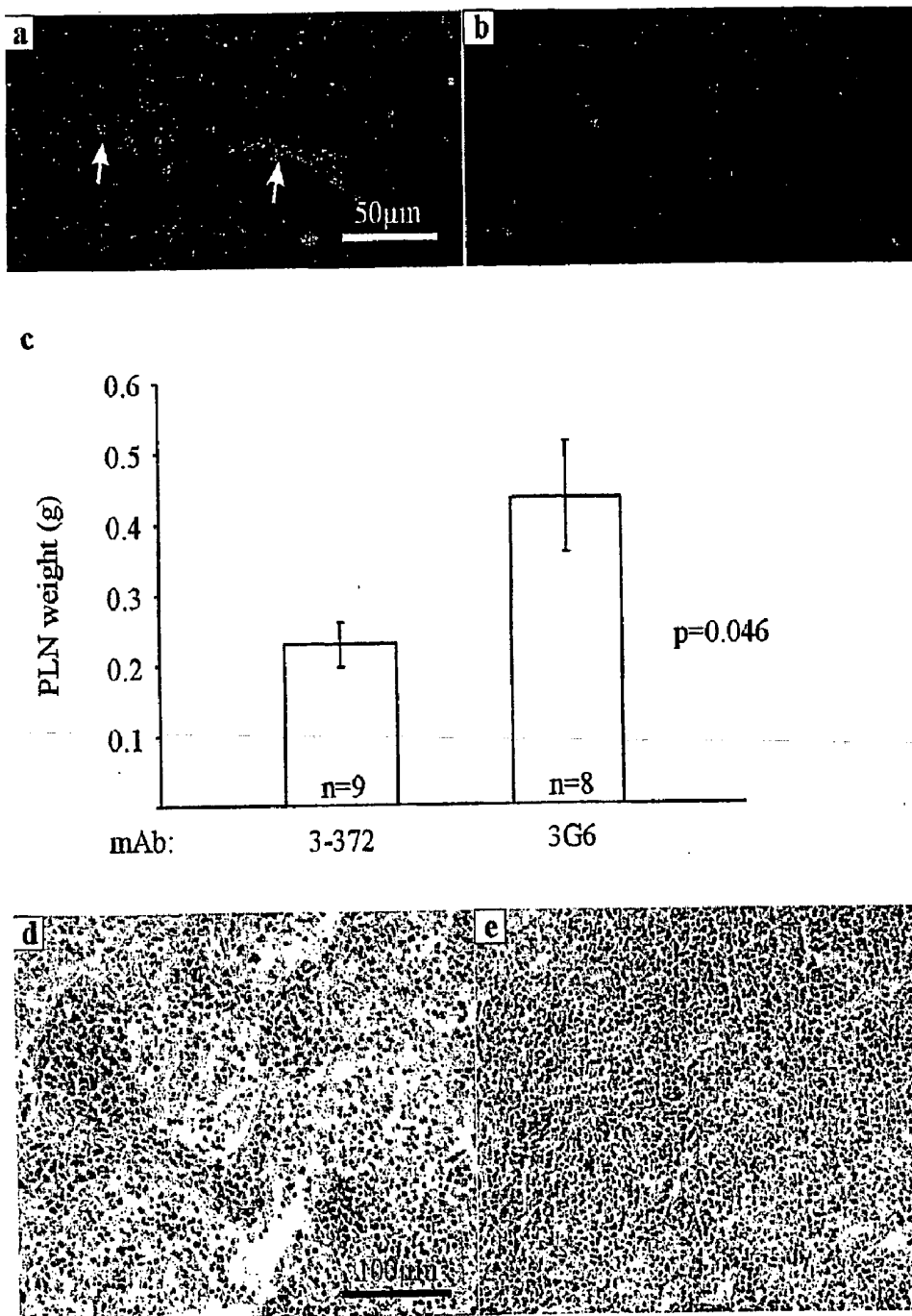


Fig. 8

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ggg cag gtg ctg ttc aaa ggc tgt gat gtg aaa acc acg ttt gtc act	150
cat gta ccc tgc acc tgc tgc gcg gcc atc aag aag cag acg tgt ccc	198
tca ggc tgg ctg cgg gag ctc ccg gat cag ata acc cag gac tgc cgc	246
tac gaa gta cag ctg ggg ggc tct atg gtg tcc atg agc ggc tgc aga	294
cgg aag tgc cgg aag caa gtg gtg cag aag gcc tgc tgc cct ggc tac	342
tgg ggt tcc cgg tgc cat gaa tgc cct ggg ggc gct gag acc cca tgc	390
aat ggc cac ggg acc tgc ttg gat ggc atg gac agg aat ggg acc tgt	438
gtg tgc cag gaa aac ttc cgc ggc tca gcc tgc cag gag tgc caa gac	486
ccc aac cgg ttc ggg cct gac tgc caa tcc gtg tgc agc tgt gtg cac	534
gga gtg tgc aac cat ggg cca ggt ggg gat gga agc tgc ctg tgc ttt	582
gct gga tac act ggc ccc cac tgt gat caa gag ctg ccc gtc tgc cag	630
gag ctg cgc tgt ccc cag aac acc cag tgc tcc gca gag gct ccc agc	678
tgc agg tgc ctg ccc ggc tac aca cag cag ggc agt gaa tgc cga gcc	726
ccc aac ccc tgc tgg cca tca ccc tgc tca ctg ctg gcc cag tgc tcc	774
gtg agc ccc aag ggg cag gct cag tgt cac tgc cct gag aac tac cat	822
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ggt ggc tgc ccc agc aac tct act ttg tgt gtg tac cag aag ccg ggc	918
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gct tct gcg ggc tgc ttc gcc ttc tgc tcc ccc ttc tcc tgc gac cgg	1014
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cac gag gtg cag aag gcc acg cag aca ggc cgg gtg ttc ctg cag ctg	1158
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aca gcg ggc cct ttc acc gtg ctg gtg cca tcc gtc tcc tcc ttc tcc	1254
tcc agg acc atg aat gca tcc ctt gcc cag cag ctc tgt aga cag cac	1302
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cga agg tgg tgg acg ctg gcc ggg cag gag atc acc gtc acc ttt aac	1398
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acc gtt gag aag ctc atc tcc aag ggt cgg atc ctc acc atg gcg aac	1830
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caa acc atc atg gaa caa ggc tgc tgc aaa ggt ttt ttc ggg cct gac	2214
tgc acg cag tgt cct ggg ggc ttc tcc aac ccc tgc tat ggc aaa ggc	2262
aat tgc agt gat ggg atc cag ggc aat ggg gcc tgc ctc tgc ttc cca	2310
gac tac aag ggc atc gcc tgc cac atc tgc tcc aac cca aac aag cat	2358

Fig. 9

<u>gga gag caa tgc cag gaa gac tgc ggc tgt gtc cat ggt ctc tgc gac</u>	2406
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<u>ggc ttc agt ggc cgg ttc tgc aac gag tcc atg ggg gac tgt ggg ccc</u>	2502
aca ggg ctg gcc cag cac tgc cac ctg cat gcc cgc tgt gtt agc cag	2550
gag ggt gtt gcc aga tgt cgc tgt ctt gat ggc ttc gag ggt gat ggc	2598
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tgc aca tgc cac aaa ggc tgg agt ggg gat ggc cgc gtc tgt gtg gct	2742
att gac gag tgt gag ctg gac gtg aga ggt ggc tgc cac acc gat gcc	2790
ctc tgc agc tat gtg ggc ccc ggg cag agc cga tgc acc tgc aag ctg	2838
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gcc acc aac ggt gtc cta cac atc etc agc cag gtc tta ctg ccc	3390
ccc cya ggg gat gtg ccc ggt ggg cag ggg ttg ctg cag cag ctg	3435
gac ttg gtg cct gcc ttc agc etc ttc cgg gaa ttg ctg cag cac	3480
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cac ctg gac gca gac aca gtg cgg cac cat gtg gtc ctg ggg gag	3615
gcc etc tcc atg gaa acc ctg cgg aag ggt gga cac cgc aac tcc	3660
ctc ctg ggc cct gcc cac tgg abc gtc ttc tac aac cac agt ggc	3705
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gga gcc tcc acc tgc gcc tgt gct gcg gga tac tcc ggc aat ggc	4380
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tgc tcc cct cat gcc aac tgt acc aag gtg gca cct ggg cag cgg	4470
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cag gaa att aac agc tgt etc atc cac cac ggg ggc tgc cac att	4560
cac gcc gag tgc atc ccc act ggc ccc cag cag gtc tcc tgc agc	4605
tgc cgt gag ggt tac agc ggg gat ggc atc cgg acc tgc gag etc	4650
ctg gac ccc tgc tct aag aac aat gga gga tgc agc cca tat gcc	4695

Fig. 9 (cont.)

acc tgc aaa agc aca	ggg gat ggc cag agg	aca tgt acc tgc gac	4740
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ctc tca gaa cgc tgg	gat gcc tac tgc ttc	cgt gtg caa gat gtg	6945
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Fig. 9 (cont.)

tyc aat ggg aag ctg ctg gat gtg ctg gct gcc act gcc aac ttc	7035
tcc acc ttc tat ggg atg cta ttg ggc tat gcc aat gcc acc cag	7080
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gcc agc ccc ctc ctg gca ccc cca cag ccc cag gca gtg ctg gcg	7440
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Fig. 9 (cont.)

**COMMON LYMPHATIC ENDOTHELIAL AND
VASCULAR ENDOTHELIAL RECEPTOR-1
(CLEVER-1) AND USES THEREOF**

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The invention is in the field of cell adhesion proteins. Specifically, the invention is in the field of CLEVER-1, a novel protein that facilitates the influx of leukocytes and malignant cells into the lymphatic system, and also the efflux of the same out of the lymph nodes.

[0003] 2. Background Art

[0004] Leukocytes are the major cellular components of inflammatory and immune responses. Leukocytes include lymphocytes, natural killer (NK) cells, monocytes, dendritic cells and granulocytes (neutrophils, eosinophils and basophils). See, HARRISON'S PRINCIPLES OF INTERNAL MEDICINE, Fauci, A. S. et al. eds. (14th ed. 1998). Lymphocytes are composed of B cells and T cells. B cells provide humoral immunity and are the precursors of plasma cells. T cells provide cell mediated immunity. In tissues monocytes differentiate further into macrophages. At sites of inflammation, blood monocytes can attach to inflamed endothelia. Macrophages recognize and take up a wide range of exogenous materials such as bacteria. Granulocytes also have critical roles in inflammation. They are needed to clear infections with extracellular bacteria. The immune response has an important role in the growth, differentiation, and mobilization of granulocytes.

[0005] Continuous lymphocyte recirculation between blood and lymphoid tissues forms a basis for the function of the immune system. However such lymphocyte recirculation inadvertently also facilitates at least two medical conditions: inflammation and metastasis.

[0006] Lymphocytes enter the lymphoid tissues by binding to vascular endothelial cells. Lymphocyte adherence to endothelial cells is mediated by complementary, surface expressed molecules on both cell types. The adhesion molecules and mechanisms of lymphocyte entrance into the tissues from the blood have been thoroughly characterized, but mechanisms controlling lymphocyte exit from the non-lymphoid and lymphoid tissues via lymphatics have remained unknown.

[0007] The majority of lymphocytes extravasate into the lymph nodes via specialized vessels called high endothelial venules, or HEV. The rest of the incoming lymphocytes enter the nodes via afferent lymphatics together with antigens and other types of hematopoietic cells such as dendritic cells, macrophages and granulocytes. However, only lymphocytes are able to leave the nodes via the efferent lymphatic system by first traversing the sinusoidal endothelium and then entering the efferent lymphatic vessel. To maintain the homeostasis in the lymph node the numbers of entering and exiting lymphocytes need to be well balanced. The molecular mechanisms involved in lymphocyte exit are unknown.

[0008] In addition to being of fundamental importance in normal lymphocyte recirculation, the lymphatics also regulate seeding of metastasizing cells in approximately 50% of cancers that use this type of vessel for spreading. Lymph

nodes are often the first organ to develop metastases, especially in the case of carcinomas. The design of the lymphatic system makes it relatively easy for malignant tumor cells to enter (Sleeman, J. P., *Recent Results Cancer Res.* 157:55-81 (2000)), and thus compounds that prevent the entry and exit of malignant tumor cells from the lymphatics have tremendous therapeutic potential.

BRIEF SUMMARY OF THE INVENTION

[0009] Recognizing the need to control lymphocyte recirculation, the inventors initiated a study of the proteins of the efferent lymphatic vessels. These studies have culminated in the discovery of a novel protein, Common Lymphatic Endothelial and Vascular Endothelial Receptor-1 (CLEVER-1), a binding protein that mediates adhesion of lymphocytes (and malignant tumor cells) to endothelium in both the systemic vasculature and in the lymphatics. The inventors have discovered that by blocking the interaction of CLEVER-1 and its lymphocyte substrate, the artisan can, for the first time simultaneously, control lymphocyte recirculation and lymphocyte migration, and related conditions such as inflammation, at the site of lymphocyte influx into, and efflux from, the tissues. The inventors have also discovered that CLEVER-1 also mediates binding of other types of leukocytes such as monocytes and granulocytes to HEV-like vessels. Further, by blocking the interaction of CLEVER-1 and malignant tumor cells, the artisan can also, for the first time, control metastasis by preventing malignant cells that bind to CLEVER-1 from being taken up by the lymphatic vessels, and thus preventing spread of the malignancy into the lymph nodes.

[0010] Accordingly, in a first embodiment, the invention is directed to cellular and subcellular extracts that contain CLEVER-1.

[0011] In a further embodiment, the invention is directed to purified or isolated CLEVER-1.

[0012] The invention further provides a method for inhibiting CLEVER-1 mediated leukocyte (such as lymphocyte, monocyte, and granulocyte) adhesion in a subject in need of the same, such method comprising administering a CLEVER-1 binding agent, or soluble CLEVER-1, to such subject.

[0013] The invention further provides a method for preventing or reducing CLEVER-1 mediated inflammation in a subject in need of the same, such method comprising administering a CLEVER-1 binding agent, or soluble CLEVER-1, to such subject.

[0014] The invention further provides a method for inhibiting CLEVER-1 mediated metastasis of malignant cells in a subject in need of the same, especially metastasis into the lymph nodes, such method comprising blocking the interaction of CLEVER-1 and the metastasizing cell in such subject with a CLEVER-1 binding agent, or by administering soluble CLEVER-1, to such subject.

[0015] The invention further provides a method for extracting cells that bind to CLEVER-1 from a population of cells, such method comprising mixing a population of cells with a preparation that contains CLEVER-1 and purifying the CLEVER-1-cell complex away from the rest of the non-binding cells in the population. Such method can be used to enrich for a subpopulation of CLEVER-1 binding cells.

[0016] The invention further provides a method for identifying an agent that binds to CLEVER-1, such method comprising determining whether a substance will successfully or unsuccessfully compete with leukocytes, malignant cells or with CLEVER-1 antibodies for binding to CLEVER-1.

[0017] The invention further provides a method for identifying an agent that is capable of inhibiting CLEVER-1 mediated cell migration, such method comprising assaying CLEVER-1 trafficking of lymphocytes into the afferent lymphatics or HEV, and/or out of the lymph nodes, in the presence of such agent, and identifying such agent on the basis of its ability to inhibit such trafficking. In another embodiment, CLEVER-1 trafficking of malignant cells is assayed.

[0018] The present invention also provides a method of stimulating CLEVER-1 binding, for example, in immunocompromised hosts to facilitate leukocyte (such as lymphocyte, monocyte and granulocyte) trafficking and the function of immune defense systems.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIGS. 1a-1i. Indirect immunoperoxidase staining showing that monoclonal antibodies 3-266 and 3-372 recognize endothelium both in afferent and efferent lymphatic systems and on HEV. FIGS. 1a-1c are from the skin, FIGS. 1d-1i are from a lymph node. FIGS. 1a, 1d and 1g show the staining with monoclonal antibody 3-266, FIGS. 1b, 1e and 1h show the staining with monoclonal antibody 3-372 and FIGS. 1c, 1f and 1i show the staining with a negative control antibody, 3G6. In FIGS. 1a, 1b and 1c, the arrows point to the epithelium and arrowheads to afferent lymphatics. In FIGS. 1d and 1e, the arrows point to the lymphatic vessels (lymphatic sinusoids that belong to the efferent lymphatic system) within the lymph node. In FIGS. 1g and 1h, the arrows point to HEV.

[0020] FIG. 2. Monoclonal antibodies 3-266 and 3-372 recognize an about 270-300 kDa molecule. Molecules in lymph node lysates were separated by SDS-PAGE, blotted to nitrocellulose sheets and probed with monoclonal antibody 3-266 and 3-372 or with a negative control antibody (3G6).

[0021] FIGS. 3A and 3B. CLEVER-1 is involved in lymphocyte binding to endothelial cells both in HEV and lymphatics. An adhesion assay was performed to measure lymphocyte binding to HEV (FIG. 3A) and to lymphatic endothelium (FIG. 3B). The sections were pre-incubated with monoclonal antibody 3-266 or 3-372, or negative control antibody anti-HLA ABC or 3G6 ("neg co") after which the sections were overlaid with normal lymphocytes. The results of three to four independent inhibition experiments are shown as mean percentage of maximal binding \pm SEM.

[0022] FIGS. 4A and 4B. CLEVER-1 is involved in binding of tumor cells to endothelial cells both in HEV and lymphatics. An adhesion assay was performed to measure binding of different tumor cell lines to HEV (FIG. 4A) and to lymphatic endothelium (FIG. 4B). The sections were pre-incubated with 3-372 or negative control antibody (anti-HLA ABC) after which the sections were overlaid with different tumor cells: Nu, NA, IBW4, KCA and CRL 1648.

The results of three to four independent inhibition experiments are shown as mean percentage of maximal binding \pm SEM.

[0023] FIG. 5. CLEVER-1 is induced on HEV-like vessels at sites of inflammation in connection to infiltrations of inflammatory cells (FIGS. 5a-5c, synovium; FIGS. 5d-5f, skin). FIG. 5a. Fibrotic type of inflamed synovium without any marked infiltrations of inflammatory cells. Only the afferent lymphatics expressed CLEVER-1 (arrows). FIG. 5b. CLEVER-1 was upregulated on a HEV-like vessel (marked by a dashed line) within a heavy lymphocytic infiltration. FIG. 5c. Staining with a negative control antibody (3G6). FIG. 5d. In normal skin afferent lymphatics expressed CLEVER-1 (arrows), but in inflamed skin HEV-like vessels (dashed line) also expressed CLEVER-1 (FIG. 5e). Negative control staining (FIG. 5f). Arrowheads point to epidermis (FIGS. 5d-5f).

[0024] FIG. 6. CLEVER-1 mediates binding of monocytes and granulocytes to HEV-like vessels at sites of inflammation. Contribution of CLEVER-1 in binding of monocytes and granulocytes to inflamed synovial vessels and binding of granulocytes to tonsil was tested using Stamper-Woodruff type of binding assay. 3-372 and 3-266 (pooled) but not the class-matched control antibody (3G6) significantly inhibited binding of granulocytes and monocytes to HEV-like vessels in the organs tested. The results of four independent assays are shown as mean percentage of maximal binding (=100% in the presence of the control antibody) \pm SEM.

[0025] FIG. 7. Molecular characterization of CLEVER-1. (a) Antibodies 3-266 and 3-372 recognize a 270-300 kDa molecule in immunoblotting. 3G6 is a negative control antibody. (b) In gels run 48 hr for better resolution, at least three different isoforms of CLEVER-1 are seen, and enzymatic digestions with neuraminidase and O-glycans reveal the sialoglycoprotein nature of CLEVER-1. (c) Relative contribution of different isoforms of CLEVER-1 is different in tonsil, lymph nodes and synovium. (d) An alternative spliced form missing exon 27 is present in 1. lung, 2. brain, 3. placenta, 4. heart, 5. liver, 6. skeletal muscle, 7. kidney, 8. pancreas, 9. spleen, 10. thymus, 11. prostata, 12. testis, 13. ovary, 14. small testine, 15. colon, 16. lymph nodes. Water control negative (lane 17). The upper band represents the standard form and the lower one is the splice variant of CLEVER-1.

[0026] FIG. 8. CLEVER-1 is expressed on the surface of endothelium in vivo and inhibition of its function blocks lymphocyte trafficking. Intravenously given 3-372 antibody (a) but not a negative class-matched control antibody (b) localized on the surface of HEV in lymph nodes after a 5 min circulation. HEV is pointed out by arrows in a. (c) Anti-CLEVER antibody treatment significantly inhibits the increase of the size of the lymph nodes draining the footpads. (One lymph node of a 3-372 treated rabbit was not found). (d) Lymphatic sinusoids of 3-372 treated animals contained less lymphocytes than those of control treated rabbits (e).

[0027] FIG. 9. The nucleotide sequence (7879 nt) of CLEVER-1. Boxed in grey are the translation initiation codon, translation stop codon, the two RGDs, the potential polyadenylation signal and the four nucleotide differences compared to Genbank entry AJ 2752213 (stabilin-1), i.e.,

nucleotides 1131, 2767, 6629 and 6969. Underlined are the nucleotides corresponding to the alternatively spliced exons.

DETAILED DESCRIPTION OF THE INVENTION

[0028] The term “ameliorate” denotes a lessening of an effect. To ameliorate a condition or disease refers to a lessening of the symptoms of the condition or disease.

[0029] The term “modulate” means to control in a predictable fashion, either by increasing or by decreasing the targeted parameter, as indicated from the context.

[0030] The term “effective amount” refers to that amount of the indicated agent that is sufficient to achieve the desired effect.

[0031] The term “inflammatory condition” refers to a physiological or pathological condition that is accompanied by an inflammatory response in a subject, which includes, inter alia, an undesired accumulation of leukocytes at one or more sites in such subject. The inflammatory condition can be hyperacute, acute, subacute or chronic. The inflammatory condition can be localized at the site of the inflammatory lesion or diffuse throughout the subject.

[0032] The term “drug” denotes any pharmaceutical or physiological agent, composition, bioactive compound, or combinations thereof, useful in the diagnosis, cure, mitigation, treatment, or prevention of a disease, or for any other medical purpose. The term “drug” is intended to be interpreted broadly and is not limited in terms of chemical composition or biological activity.

[0033] The term “essentially free of contaminants” refers to a substance that is of, undesired or unnecessary substances that had been present during the in vitro or in vivo synthesis of the desired substance.

[0034] The term “treatment” or “treating” refers to the administration of an agent to a subject for purposes which can include prophylaxis, amelioration, prevention or cure of an undesired disorder. Such treatment need not necessarily completely ameliorate the disorder, for example, inflammation; it is sufficient that such treatment ameliorates the disorder to a degree that is beneficial to the subject to which it is administered. Further, such treatment can be used in conjunction with other traditional treatments, for example, alternative treatments for reducing the inflammatory condition, known to those of skill in the art and as desired by the practitioner.

[0035] By “systemic vasculature” is meant the vascular network of blood vessels throughout the body of an animal or human.

[0036] By “lymphatic system” is meant the specialized part of the circulatory system that consists of lymph, the lymphatics, and the lymph nodes. The lymph nodes are located along the paths of the lymph collecting vessels and in isolated nodules of lymphatic patches in the intestinal wall. Additionally, there are specialized lymphatic organs such as the tonsils, thymus and spleen. B lymphocytes begin their final stages of maturation within the germinal centers of the lymph nodes' cortical nodules. Maturing lymphocytes are then pushed to the more densely packed outer layers as they mature, before being released into the efferent lymphatics. The lymph nodes that are located in the floor of the

mouth are called the submental and submaxillary lymph nodes. The superficial cervical lymph nodes are located in the neck. The superficial cubital or supratroclear lymph nodes are located just above the bend in the elbow. The axillary lymph nodes are clustered deep within the underarm and upper chest region. Inguinal lymph nodes are located in the groin. By “lymphatics” is meant the vessels that return lymph to the blood. Lymph is the clear fluid that flow in the lymphatics. Lymph arises from plasma that filters into the interstitial spaces from blood flowing through the capillaries. Although most of this plasma is taken up and absorbed by cells or the blood, a small amount is not absorbed. The lymphatics act as drains to collect this excess fluid and return it to the venous blood just before it reaches the heart. The lymph nodes act as filters that collect the lymph from several different lymphatics and “percolate” the lymph through spaces termed sinuses before draining into a single efferent draining vessel.

[0037] By “afferent lymphatics” is meant the vessels through which antigens enter the lymph nodes. Lymphocytes can enter the lymph nodes via the afferent lymphatics or via the high endothelial venules (HEV).

[0038] By “high endothelial venules” (HEV) is meant a specialized cortical postcapillary venules whose endothelium is simple cuboidal to columnar instead of simple squamous. HEVs are located mainly in the paracortex of the lymph nodes. Lymphocytes cross the HEV, and thus “traffic” into the lymph nodes by diapedesis, that is, the lymphocytes stick to the luminal surface of the HEV, and then squeeze into the space between two or more HEV cells.

[0039] By “efferent lymphatics” meant the vessels that drain the lymph nodules (nodes).

[0040] By “lymphocyte recirculation” is meant the continuous movement of lymphocytes throughout the circulatory and lymph system. Lymphocytes leave the lymph node and are first delivered via the lymph to venous system draining into the heart.

[0041] The lymphocytes then circulate throughout the body in the blood-stream. Most of the lymphocytes are redelivered to the spleen or to another lymph node. About 10% go to non-lymphoid organs. Lymphocytes that have never been activated cannot enter non-lymphoid organs.

[0042] Lymphocyte “trafficking” refers to lymphocyte cell movement to specific locations. Outside of the lymph nodes, the trafficking of circulating lymphocytes allows the lymphocyte to accumulate at sites of inflammation. Activated effector lymphocytes tend to home to areas of inflammation, resulting in a large influx of lymphocytes in areas of inflammation. At the inflamed site, lymphocytes attach to the endothelial cells that line the blood vessels. This attachment localizes the lymphocyte at the site of inflammation and allows for subsequent emigration of the cells into the surrounding tissues (extravasation).

[0043] The Identification and Purification of CLEVER-1

[0044] The basis of the invention is the discovery of a new molecule, a novel protein herein designated “Common Lymphatic Endothelial and Vascular Endothelial Receptor-1 in the systemic vasculature, and in the afferent and efferent lymphatics. It has been found that leukocytes such as lymphocytes, monocytes, and granulocytes, and malignant

cells specifically bind to this protein. It has also been found that this protein acts as a receptor that facilitates entry of bound leukocytes and malignant cells through the walls of the systemic vasculature, into the lymph nodes and out of the lymph nodes.

[0045] To search for a protein that played a role in lymphocyte lymphatic efflux, the inventors first identified cell migration-associated lymphatic structures from isolated efferent lymphatic vessels of human lymph nodes. These structures were used to produce monoclonal antibodies. Hybridomas were screened on frozen sections of human lymph nodes using immunoperoxidase staining.

[0046] Two of the hybridomas produced antibodies (designated 3-266 and 3-372) that clearly stained lymphatic endothelium both in afferent and efferent lymphatic systems and vascular endothelium on HEV, while other structures remained unstained. This is consistent with the expected pattern for an antibody that recognizes a lymphocyte migration-associated structure. Cell culture of 3-266 (DSM ACC2519) and cell culture of 3-372 (DSM ACC2590) were both deposited under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the Purposes of Patent Procedure on Aug. 21, 2001, with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig.

[0047] In molecular weight determinations by immunoblotting, both anti-bodies recognized a molecule of the same size, about 270-300 kDa. Due to this and an identical staining pattern, these antibodies were considered to recognize the same antigen; that antigen was named Common Lymphatic Endothelial and Vascular Endothelial Receptor-1 (CLEVER-1).

[0048] CLEVER-1 was purified from CLEVER-1 containing lymph node preparations using affinity chromatography with the 3-372 antibody. The eluted material was subjected to SDS-PAGE analysis and silver staining. The specific band was excised, reduced, alkylated and digested with trypsin.

[0049] After cleavage with trypsin, mass spectrometric analyses yielded 27 peptides. Twenty-one (77%) of those had identical sequences with stabilin. These sequences covered altogether 268 amino acids (10% of the 2570 amino acids of stabilin-1) and spanned the amino acids between 53 and 2301 (Table 1). The peptide data suggest that CLEVER-1 has some homology with stabilin-1 at the structural level. No functional information regarding stabilin-1 can be found in the literature.

[0050] Peptide analysis of CLEVER-1 indicates no significant homology with any of the known endothelial homing-associated molecules, such as ICAM-1 (Intercellular Adhesion Molecule), ICAM-2, or VAP-1 (Vascular Adhesion Protein).

[0051] CLEVER-1 has several structural motifs that are associated with adhesive functions in other molecules. They include a proteoglycan link homology region important in CD44 for hyaluronan binding and two RGD motifs known to serve as integrin ligands in certain molecules, such as in fibronectin. In addition, CLEVER-1 has seven fasciclin domains also present in several molecules such as priostin, fasciclin and transforming growth factor- β -induced gene, big-h3 and in all of these cases it is essential for adhesive

function of these molecules. Interestingly, twenty-two epidermal growth factor (EGF) repeats are also found in CLEVER-1. This structural domain is also present in all members of the selectin family. Although the lectin domains of selectins are of utmost importance for the transient interaction with their sialomucin ligands, EGF-repeats have been reported to functionally contribute with lectin domains to binding between leukocytes and endothelium. Based on the structural complexity of CLEVER-1 it may turn out have several ligand molecules and be multifunctional in its nature.

[0052] It has been discovered that CLEVER-1 is involved in the process of lymphocyte recirculation. CLEVER-1 is present on the endothelium of the systemic vasculature, especially on the HEV and also on the endothelium of both afferent and efferent lymphatic systems. CLEVER-1 is a protein adhesion molecule, and especially, a cell adhesion molecule (CAM), that mediates adhesion of lymphocytes and of malignant tumor cells to CLEVER-1 in the systemic vasculature and the lymphatic system. These sites are of utmost importance as control points in lymphocyte trafficking.

[0053] CLEVER-1 is the first molecule that has been identified to facilitate lymphocyte and malignant cell exit from the lymph nodes. Additionally, CLEVER-1 is the first molecule that has been identified that regulates both entrance of lymphocytes and tumor cells into the lymph nodes and exit of lymphocytes and tumor cells from the lymph nodes. CLEVER-1 has been found to also mediate binding of other leukocytes such as monocytes and granulocytes to HEV-like vessels.

[0054] By "CLEVER-1 mediated cell binding" is meant the specific association of CLEVER-1 with either a leukocyte, such as a lymphocyte, monocyte, or granulocyte, or a CLEVER-1-binding malignant cell. CLEVER-1 mediated cell binding can occur with CLEVER-1 in a soluble form or in a particulate form (for example, when CLEVER-1 is present in a form that is membrane associated).

[0055] CLEVER-1 Mediated Binding of Leukocytes

[0056] According to the invention, adhesion of leukocytes, such as lymphocytes, monocytes, and granulocytes, to the endothelium (that is, to an endothelial cell(s)) in the systemic vasculature, especially the HEV, and to the endothelium in the afferent and efferent lymphatics can be blocked by blocking the binding between such endothelial cell's CLEVER-1 and leukocyte.

[0057] In the systemic vasculature, inhibiting or preventing endothelial cell CLEVER-1 mediated lymphocyte binding will inhibit or prevent lymphocytes, especially activated lymphocytes, from accumulating at such sites, and thus prevent or lessen inflammation at such sites. Thus, the invention provides a method of treating inflammation, by administering an agent that inhibits or prevents CLEVER-1 mediated endothelial cell binding to lymphocytes.

[0058] In the afferent lymphatics, inhibiting or preventing lymphocytes from binding to endothelial cell CLEVER-1 will inhibit or prevent such lymphocytes from entering the afferent lymphatics and thus the lymph nodes. Thus, the invention provides a method of treating inflammation, by administering an agent that inhibits or prevents CLEVER-1 mediated endothelial cell binding to lymphocytes in the

afferent lymphatics and especially at HEV in lymph nodes or HEV-like venules at sites of inflammation. The invention also provides a method of inhibiting lymphocyte trafficking into the lymph nodes, by administering an agent that inhibits or prevents afferent lymphatic CLEVER-1 mediated endothelial cell binding, and especially HEV binding, to lymphocytes and other leukocytes.

[0059] In the efferent lymphatics, inhibiting or preventing lymphocytes from binding to endothelial cell CLEVER-1 will prevent the lymphocytes from exiting the lymph node and entering the blood. Thus, the invention provides a method of treating inflammation, by administering an agent that inhibits or prevents CLEVER-1 mediated endothelial cell binding to lymphocytes in the efferent lymphatics. The invention also provides a method of inhibiting lymphocyte trafficking out of the lymph nodes, by administering an agent that inhibits or prevents efferent lymphatic CLEVER-1 mediated endothelial cell binding to lymphocytes.

[0060] Therefore, CLEVER-1 binding with lymphocytes presents a unique, three-prong approach to treat diseases or conditions characterized by an undesired lymphocyte accumulation or trafficking in which the artisan can target lymphocyte entry into the lymph nodes, lymphocyte exit from the lymph nodes, and lymphocyte binding to the systemic vasculature, with the same agent.

[0061] The discovery of CLEVER-1 and its role has thus resulted in a new method to control lymphocyte migration by inhibiting CLEVER-1 mediated cell binding to such cells. Thus, the present invention provides a method of inhibiting undesired CLEVER-1 mediated lymphocyte trafficking, and thus blocking harmful or otherwise undesired lymphocyte migration, by preventing the association of CLEVER-1 with lymphocytes. Similarly, the invention provides a method of inhibiting undesired CLEVER-1 mediated binding of other leukocytes by preventing association of CLEVER-1 with the leukocytes.

[0062] The present invention also provides a method of stimulating CLEVER-1 binding, for example, in immunocompromised hosts to facilitate lymphocyte trafficking and other leukocyte binding and the function of immune defense systems.

[0063] CLEVER-1 Mediated Cell Binding to Malignant Cells

[0064] Because cancer cells often break away from a malignant tumor and enter the lymphatics, cancer cells travel to and establish themselves in the lymph nodes. According to the invention, the ability of a malignant tumor cell to establish itself in a lymph node can be inhibited or prevented by inhibiting or preventing CLEVER-1 binding to such malignant tumor cell.

[0065] The term "tumor" refers to a neoplasm, a tissue mass that is characteristic of a neoplasia. Neoplasia is distinguished from other forms of tissue growth, first, by the formation of a tissue mass, a neoplasm, or tumor. Second, neoplasia is considered to be an irreversible process. Third, neoplastic tissue tends to morphologically resemble its tissue of origin. Fourth, neoplastic tissue tends to functionally resemble its tissue of origin. Fifth, neoplasms grow and function somewhat independently of the homeostatic mechanisms that control normal tissue growth and function.

[0066] A neoplasm can be benign or malignant. A benign neoplasm consists of a discrete tissue mass that continues to grow. A benign neoplasm will simply push adjacent tissues out of its way as it grows.

[0067] The definitive features of a malignant neoplasm, a malignancy, are invasion and metastasis, that is, the spread of the neoplasm to a distant site. A malignant neoplasm will grow into the adjacent tissue, rather than pushing it away. The terms "malignant neoplasm," "malignant tumor," and "cancer" are synonymous.

[0068] Cancer cells typically invade thin-walled vessels such as small veins, venules, capillaries and lymphatics. The passage of cancer cells via lymphatics to lymph nodes, and via blood vessels to other organs and structures, and the subsequent implantation and growth of the cancer cells in those sites is referred to as "metastasis." The lymph nodes are common sites for metastasis.

[0069] Cancer cells can also spread by seeding—shedding into, for example, the peritoneal fluid. The cells can be carried by the fluid to a distant site on the peritoneal surface where they can implant and form new foci of cancer growth.

[0070] Most neoplasms are one of four types: epithelial, non-epithelial, blastomas and teratomas. Malignant epithelial neoplasms are termed carcinomas. An adenocarcinoma is a carcinoma in which gland-like structures are present. Carcinomas can be papillary or cystic. Benign epithelial neoplasms are generally adenomas, polyps or papillomas.

[0071] Non-epithelial tumors can also be benign or malignant. They are generally named by a prefix that indicates the histologic type and a suffix. The suffix -oma generally means benign while the suffix -sarcoma means malignant. However, several malignant neoplasms have traditional names ending in -oma: for example, melanoma, hepatoma, and lymphoma.

[0072] Lymphomas are malignant neoplasms arising from cells of the lymphoid series. Blastomas and teratomas contain more than one type of tissue. Malignant teratomas are often termed teratocarcinomas.

[0073] A "leukemia" is a tumor of white blood cells that is present in the bone marrow and blood. A "lymphoma" is a tumor of white blood cells that is present in the lymph nodes and tissues.

[0074] According to the invention, the binding of CLEVER-1-binding malignant cells to the endothelium in the systemic vasculature, especially the HEV, and to the endothelium in the afferent and efferent lymphatics can be inhibited or prevented by inhibiting or preventing the binding between such endothelial cell's CLEVER-1 and such malignant cell. Thus, the invention provides a method of treating cancer, and especially, a method of preventing metastasis, by administration of an agent that inhibits or prevents CLEVER-1 mediated malignant cell binding to the endothelium.

[0075] In the systemic vasculature, inhibiting or preventing CLEVER-1 mediated cell binding will inhibit or prevent the establishment of CLEVER-1 binding malignant cells at such sites, and thus lessen or prevent metastasis of such malignant cells. Thus, the invention provides a method of treating cancer, and especially, a method for preventing metastasis of a CLEVER-1 binding malignant cell, by

administering an agent that inhibits or prevents CLEVER-1 mediated endothelial cell binding to CLEVER-1-binding tumor cells in the systemic vasculature. The invention also provides a method of inhibiting metastasis, by administering an agent that inhibits or prevents systemic vasculature CLEVER-1 mediated endothelial cell binding to such malignant cells.

[0076] In the afferent lymphatics, inhibiting or preventing CLEVER-1 binding malignant cells from binding to endothelial cell CLEVER-1 will inhibit or prevent such CLEVER-1 binding malignant cell from entering and establishing in the lymph node, and thus lessen or prevent metastasis of such cell to the lymph node or thus to other sites in the body. In this context it is worth to note that a metastasizing malignant cell cannot survive long times without matrix support—a condition present for example in blood. Thus, the invention provides a method of treating cancer, and especially, a method for preventing metastasis of a CLEVER-1 binding malignant cell, by administering an agent that inhibits or prevents CLEVER-1 mediated endothelial cell binding to CLEVER-1-binding malignant cells in the afferent lymphatics and at HEV in systemic vasculature. The invention also provides a method of inhibiting metastasis, by administering an agent that inhibits or prevents afferent lymphatic CLEVER-1 mediated endothelial cell binding, and especially HEV binding, to such malignant cells.

[0077] In the efferent lymphatics, inhibiting or preventing CLEVER-1 binding malignant cells from binding to endothelial cell CLEVER-1 will inhibit or prevent such CLEVER-1 binding malignant cell from leaving the lymph node, and thus lessen or prevent metastasis of such cell from the lymph node to other sites in the body. Thus, the invention provides a method of treating cancer, and especially, a method for preventing metastasis of a CLEVER-1 binding malignant cell, by administering an agent that inhibits or prevents CLEVER-1 mediated endothelial cell binding to CLEVER-1-binding malignant cells in the efferent lymphatics. The invention also provides a method of inhibiting metastasis, by administering an agent that inhibits or prevents efferent lymphatic CLEVER-1 mediated endothelial cell binding to such malignant cells.

[0078] CLEVER-1 interaction with CLEVER-1 binding malignant cells thus presents a unique, three-prong approach to inhibit or prevent metastasis in which not only can the artisan block such malignant cells from entering into and exiting from the lymph system, but also, the artisan can block association of such malignant cell with CLEVER-1 in the vascular endothelium.

[0079] Agents that Block or Inhibit CLEVER-1 Mediated Cell Binding

[0080] Soluble CLEVER-1 and antibodies to CLEVER-1 can be provided to the host cell to block or inhibit CLEVER-1 mediated cell binding. Soluble CLEVER-1 can be used to “coat” the CLEVER-1 binding sites on the leukocyte, such as lymphocyte, monocyte, or granulocyte, or tumor cell and thus prevent the coated cell from association with the native CLEVER-1 on the HEV or afferent or efferent lymphatics.

[0081] CLEVER-1 antibodies can be administered to a patient in need of the same to coat CLEVER-1 that is present

on the vascular endothelium or lymphatics, of such patient, especially the afferent lymphatics so as to prevent leukocyte or malignant cell binding to such CLEVER-1 in the patient. CLEVER-1 antibody producing cells can be administered directly to the patient so as to provide a source of the same.

[0082] Moreover, the present invention provides a method of identifying an agent that inhibits the binding of CLEVER-1 to cells by providing an agent to cells in the presence of CLEVER-1 and comparing the binding of CLEVER-1 to cells provided with the agent to binding of CLEVER-1 in the absence of the agent. Similarly, the invention provides a method of identifying an agent that stimulates the binding of CLEVER-1 to cells by providing an agent to cells in the presence of CLEVER-1 and comparing the binding of CLEVER-1 to cells provided with the agent to binding of CLEVER-1 in the absence of said agent.

[0083] The term “antibody” is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies), polyclonal antibodies, as well as antibody fragments and single chain antibodies (e.g., Fab, F(ab')₂, Fv), so long as they exhibit the desired biological activity.

[0084] Papain digestion of antibodies produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

[0085] Single chain “Fv” is the minimum antibody fragment which contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in tight, noncovalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site. See, Ladner et al., U.S. Pat. No. 4,946,778, and Bird, R. E. et al., *Science*, 242:423-426 (1988).

[0086] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by a hybridoma culture, uncontaminated by other immunoglobulins. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used

in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (e.g., Cabilly et al., U.S. Pat. No. 4,816,567).

[0087] Preparation of immunizing antigen, and polyclonal and monoclonal antibody production can be performed as described herein, or using other suitable techniques. A variety of methods have been described (see e.g., Kohler et al., *Nature* 256:495-497 (1975), and *Eur. J. Immunol.* 6:511-519 (1976); Milstein et al., *Nature* 266:550-552 (1977); Koprowski et al., U.S. Pat. No. 4,172,124; Harlow, E. and D. Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y., 1988); *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, Vol. 2 (Supplement 27, 1994), Ausubel, F. M. et al., John Wiley & Sons, eds., New York, N.Y.), Chapter 11, (1991)). Generally, a hybridoma can be produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as SP2/0) with antibody producing cells. The antibody producing cell, preferably those of the spleen or lymph nodes, are obtained from animals immunized with the antigen of interest. The fused cells (hybridomas) can be isolated using selective culture conditions, and cloned by limiting dilution. Cells which produce antibodies with the desired binding properties can be selected by a suitable assay (e.g., ELISA).

[0088] The term "antibody" also includes chimeric, humanized or primatized (CDR-grafted) antibodies, as well as chimeric or CDR-grafted single chain antibodies, and the like, comprising portions derived from different species. "Chimeric" antibodies (immunoglobulins) have a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly et al., U.S. Pat. No. 4,816,567; Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). The various portions of these antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. For example, nucleic acids encoding a chimeric or humanized chain can be expressed to produce a contiguous protein. See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; Cabilly et al., EP 0 125 023 B1; Boss et al., U.S. Pat. No. 4,816,397; Boss et al., EP 0120694 B1; Neuberger, M. S. et al., WO 86/01533; Neuberger, M. S. et al., EP 0194276 B1; Winter, U.S. Pat. No. 5,225,539; Winter, EP 0239400 B1; and Queen et al., U.S. Pat. Nos. 5,585,089, 5,698,761 and 5,698,762. See also, Newman, R. et al., *Bio-Technology* 10: 1455-1460 (1992), regarding primatized antibody.

[0089] By "agonist antibody" is meant an antibody which is able to bind to CLEVER-1 and facilitate adhesion of lymphocytes (and malignant tumor cells) to endothelium. By "antagonist antibody" is meant an antibody that is able to bind to CLEVER-1 and inhibit adhesion of lymphocytes (and malignant tumor cells) to endothelium.

[0090] Anti-idiotypic antibodies are also provided. Anti-idiotypic antibodies recognize antigenic determinants associated with the antigen-binding site of another antibody.

Anti-idiotypic antibodies can be prepared against second antibody by immunizing an animal of the same species, and preferably of the same strain, as the animal used to produce the second antibody. See e.g., U.S. Pat. No. 4,699,880.

[0091] In Vitro Adhesion Assay and Diagnostic Uses Thereof

[0092] In a further embodiment, the present invention is directed to an adhesion assay in which CLEVER-1 binding is used to assay for the presence of leukocytes or malignant cells that bind to HEV and lymphatic endothelium. Both static and non-static assays are possible. The adhesion assay is exemplified in Example 4. Both static and non-static assays can be used to study leukocyte binding to systemic vasculature.

[0093] In the static assay, a tissue section is exposed to leukocytes or malignant cells for a desired period of time, without continuous agitation or rotation of the preparation during the exposure. Static assays are preferred for examining the ability of leukocytes and malignant cells to bind to lymphatic endothelium, especially the efferent lymph vessels.

[0094] In the non-static assay, the CLEVER-1 containing tissue sample and leukocytes are constantly rotated during the time period in which the leukocytes are given to adhere to the CLEVER-1. Non-static assays are preferred for studying leukocyte and malignant cell binding to CLEVER-1 in the HEV. The non-static assay mimics adhesion to the systemic vasculature.

[0095] In another embodiment, the present invention relates to a method for detection of malignant tumor cells. As explained with detail in Example 1, CLEVER-1 antibodies reduce the binding of malignant tumor cells to the vascular and lymphoid endothelium, demonstrating CLEVER-1 is a receptor for such malignant tumor cells. CLEVER-1 protein, or fragments thereof, or CLEVER-1 binding compounds, including but not restricted to, antibodies against CLEVER-1, both monoclonal and polyclonal, antibodies against antigenic fragments of CLEVER-1, both monoclonal and polyclonal, antigenic polypeptides, small molecule inhibitors or drugs, can be used in both quantitative and qualitative assays to detect the presence of malignant tumor cells in a sample, said sample being tissue or blood from a human or animal.

[0096] CLEVER-1 protein, or fragments thereof, or the above-mentioned CLEVER-1 binding compounds can be attached to a solid support matrix, including but not limited to microtiter plates, agarose columns, or magnetic beads. The above-mentioned sample can then be exposed to said solid support matrix, and the percentage of cells retained by said solid support matrix determined. For example, a sample from a normal, healthy individual, said individual being either a human or an animal, would have a statistically predicted number of leukocytes that bind to CLEVER-1. A sample that contains both leukocytes and malignant tumor cells would have a detectably higher number of cells that bind CLEVER-1.

[0097] In a preferred embodiment, a blood or tissue sample that has been taken from a patient who is in need of treatment, especially treatment for cancer, is used as the source of the CLEVER-1 binding cells in the in vitro adhesion assay. Such patient can be a patient being treated

for a previously diagnosed malignancy, or a patient suspected of having a malignant tumor, or a patient who appears to be cured of such malignant tumor but is in need of monitoring for the reoccurrence of the same. Preferably, such blood or tissue sample is from a patient who is to be tested for the presence of malignant cells that bind to CLEVER-1 in such sample.

[0098] The blood or tissue sample that is to be examined in the *in vitro* assay of the invention can be processed, if desired, by methods known in the art so as to further extract or concentrate any CLEVER-1 binding cells that might be present in the sample, prior to the sample's being used in the *in vitro* adhesion assay of the invention.

[0099] Additionally, once the *in vitro* adhesion assay is complete, the adherent cells can be studied using other methods. For example, in the static assay, where the bound cells have been fixed, ability of the adherent cells to be recognized by a monoclonal antibody that is diagnostic for the type of tumor can be performed.

[0100] Detecting the binding of malignant cells to the CLEVER-1 containing lymphatic endothelium indicates that the patient is in need of treatment for such malignant cells, and especially to prevent the metastasis of such malignant cells

[0101] The present invention provides in this aspect a novel, efficient, and convenient assay for identifying antagonists, including but not limited to, monoclonal and polyclonal antibodies, peptides, protein fragments, small molecular inhibitors, drugs, and other agents, which can inhibit the adhesion of leukocytes and malignant tumor cells to the vascular and lymphatic endothelium.

[0102] For example, CLEVER-1 containing samples of lymph node sections can be incubated with and without the agent, and the number of bound lymphocytes and/or malignant cells determined. The antagonists can be preincubated with lymph node sections (a non-competitive assay) or simultaneously added with lymphocytes to the lymph node sections (a competitive assay).

[0103] Such a screen can also be used to customize an anti-metastasis treatment to an individual patient, and allows the practitioner to identify and select those agents or combinations thereof that have the best ability to inhibit CLEVER-1 malignant cell binding to vascular and/or lymphatic endothelium in such patient, and thus maximize the benefit of the treatment with such agents for such patient.

[0104] Additionally, such *in vitro* assay allows the practitioner to select for agents that provide a beneficial effect on disrupting malignant cell: CLEVER-1 containing endothelium interactions, nevertheless, minimize, if possible, the effect of such treatment on CLEVER-1 mediated leukocyte binding,

[0105] An antagonist can inhibit malignant cell or lymphocyte cell migration into or out of the lymph nodes. In a preferred embodiment, antagonists would inhibit both entrance and exit of an undesired cell into and out of the lymph nodes, respectively. As such, malignant tumor cells would preferably be prevented from entering a lymph node, and establishing there, and any that did enter the lymph nodes via an afferent lymph vessel independent mechanisms would be contained, thus slowing metastasis.

[0106] This assay can be used further to monitor the efficacy of chemotherapy treatments administered to an individual, said individual being a human or an animal, in need thereof. Samples can be analyzed before, during, and after chemotherapy for the presence of malignant tumor cells that bind to CLEVER-1 or antigenic fragments thereof, or CLEVER-1 binding compounds.

[0107] In a further embodiment, purified CLEVER-1 protein, or fragments thereof can be used for high volume screening of antagonists that are capable of preventing or lowering the ability of a leukocyte or malignant cell to adhere to endothelial cell CLEVER-1. CLEVER-1 protein, or fragments thereof can be attached to a solid support matrix, including but not limited to a microtiter plate, an agarose column, or magnetic beads, using standard methods well known in the art. Antagonists can be screened for interaction with CLEVER-1 or fragments thereof, either in the absence or presence of leukocytes. Leukocytes or malignant cells can be labeled with fluorescent dyes such as, for example, bis-carboxyethyl carboxyfluorescein or fluorescein isothiocyanate and the number of bound cells in presence or absence of the antagonists can be analyzed by a fluorimeter.

[0108] The high volume screen assay of this aspect of the invention can be used to screen combinatorial libraries for molecules that inhibit the binding of leukocytes and malignant tumor cells to CLEVER-1 or a fragments thereof. Antagonists that show strong affinity for purified CLEVER-1 protein or fragments thereof can be screened further using the *in vitro* adhesion assay described above.

[0109] Antibodies used in the methods of the invention as CLEVER-1 binding compounds are preferably antibodies with a specificity against CLEVER-1, or an antigenic fragment thereof. Such antibodies can be polyclonal or monoclonal.

[0110] Another potential CLEVER-1 antagonist is a peptide derivative of the CLEVER-1 polypeptide that are naturally or synthetically modified analogs of the polypeptides that have lost biological function yet still recognize and bind to the ligand of the polypeptides to thereby effectively block the interaction of said ligand with CLEVER-1. Examples of peptide derivatives include, but are not limited to, small peptides or peptide-like molecules.

[0111] Another potential human CLEVER-1 antagonist is a peptide derivative of the ligand polypeptides which are naturally or synthetically modified analogs of the polypeptides that have lost biological function yet still recognize and bind to CLEVER-1 to thereby effectively block CLEVER-1. Examples of peptide derivatives include, but are not limited to, small peptides or peptide-like molecules.

[0112] The present invention relates to a diagnostic method for the detection of cells that contain CLEVER-1, that is, CLEVER-1 positive cells, in samples taken from the human or animal body. Such a method would involve the use of CLEVER-1 binding compounds, including but not limited to, monoclonal and polyclonal antibodies, with specificity for CLEVER-1. Such compounds can be labeled with a substance, such as a colorimetric dye or radioactive molecule, to permit rapid and easy detection of binding of the compound to cells that express CLEVER-1.

[0113] Therapeutic Uses of CLEVER-1 Antagonists

[0114] In another embodiment, the present invention relates to a method of treating malignant carcinomas. It is common for carcinomas to metastasize first to the regional lymph nodes (Sleeman, J. P., *Recent Results Cancer Res.* 157:55-81 (2000)). As described herein, CLEVER-1 is involved in the entrance and exit of malignant tumor cells to and from the lymph nodes. As such, antagonists that inhibit malignant tumor cell binding to CLEVER-1, including but not limited to, monoclonal and polyclonal antibodies, peptides, small molecule inhibitors, drugs, and other such agents can reduce metastasis and serve as effective chemotherapeutic agents.

[0115] In another aspect, the present invention relates to a method of treating disorders where the leukocyte-endothelial cell adhesion reaction is associated with acute or chronic inflammatory diseases such as skin inflammations, diabetes, connective tissue diseases (such as lupus, rheumatoid arthritis, osteoarthritis), obstructive and restrictive lung diseases (such as asthma, ARDS, sarcoidosis, idiopathic pulmonary fibrosis), inflammatory bowel diseases (such as ulcerative colitis and Crohn's disease), various nephritides, non-viral hepatitis, cirrhosis, cholangitis, atherosclerosis, vasculitis, thyroiditis, multiple sclerosis, myositis, ischemia reperfusion injury, transplantation rejection.

[0116] The antagonists can also be employed to treat histamine-mediated allergic reactions and immunological disorders including late phase allergic reactions, chronic urticaria, and atopic dermatitis. IgE-mediated allergic reactions such as allergic asthma, rhinitis, and eczema can also be treated.

[0117] The antagonists can also be employed to treat chronic and acute inflammation by preventing the extravasation of leukocytes to a wound area. They can also be employed to regulate normal pulmonary macrophage populations, since chronic and acute inflammatory pulmonary diseases are associated with sequestration of mononuclear phagocytes in the lung.

[0118] Antagonists can also be employed to treat rheumatoid arthritis by preventing the extravasation of leukocytes into synovial fluid in the joints of patients. Monocyte influx and activation plays a significant role in the pathogenesis of both degenerative and inflammatory arthropathies.

[0119] The antagonists can also be employed to treat asthma and allergy by preventing eosinophil accumulation in the lung. The antagonists can also be employed to treat subepithelial basement membrane fibrosis which is a prominent feature of the asthmatic lung.

[0120] The antagonists can also be employed for treating atherosclerosis, by preventing monocyte infiltration in the artery wall.

[0121] The antagonists can be employed in a composition with a pharmaceutically acceptable carrier, e.g., as herein-after described.

[0122] Formulations of Compounds

[0123] The antagonists of CLEVER-1 can be used as therapeutic compositions. The antagonists of CLEVER-1 can be administered as a single dose or in multiple doses. The antagonists of the present invention can be administered

either as an independent therapeutic regime or in combination with other therapeutic agents. The antagonists can be combined with conventional therapies, which can be administered simultaneously or sequentially.

[0124] Such therapeutic compositions can consist solely of the antagonist of CLEVER-1 although, preferably, the compositions will contain the antagonist of CLEVER-1 combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described for example in Remington: The Science and Practice of Pharmacy, Gennaro, Alfonso, 20th ed. (2000). In order to form a pharmaceutically acceptable composition that is suitable for effective administration to a patient in need of such composition, such compositions will contain an effective amount of the antagonist of CLEVER-1 together with a suitable amount of carrier vehicle.

[0125] Compositions containing antagonists of CLEVER-1 can be administered perorally, intravenously, intramuscularly, or sub-cutaneously at the appropriate dosages, which will depend upon the severity of the condition of the patient and upon such criteria as the patient's height, weight, sex, age, and medical history. The dose will also depend upon whether the compound of the invention is being administered to a human patient or in a veterinary setting to an animal, in need thereof.

[0126] For the purpose of parenteral administration, compositions containing the antagonists of CLEVER-1 are preferably dissolved in distilled water and the pH-value is preferably adjusted to about 6 to 8. In order to facilitate the lyophilization process resulting in a suitable product, lactose can be added to the solution. Preferably, the solution is then filtered sterilized, introduced into vials, and lyophilized. In a preferred embodiment, the compound of the invention is administered orally to a patient, at the time of eating or shortly thereafter. The concentration of the antagonists of CLEVER-1 in these composition, whether oral or parenteral, can vary, e.g., from 10^{-12} M to 10^{-3} M.

[0127] Additional pharmaceutical methods can be employed to control the duration of action. Controlled release preparations can be achieved by the use of polymers to complex or adsorb the antagonists of CLEVER-1. The controlled delivery can be exercised by selecting appropriate macromolecules (for example, polyesters, polyamino acids, polyvinyl pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, and protamine sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate the antagonists of CLEVER-1 into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly (lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating the CLEVER-1 antagonists into these polymeric particles, it is possible to entrap these derivatives in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly (methylmethacrylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such teachings are

disclosed in Remington: The Science and Practice of Pharmacy, Gennaro, Alfonso, 20th ed. (2000).

[0128] The following Example serves only to illustrate the invention, and is not to be construed as in any way limiting the invention.

EXAMPLE 1

[0129] Production of Monoclonal Antibodies

[0130] Balb/c mice were immunized to footpads four times at one week intervals, with incomplete Freund's adjuvant containing suspension made from lymphatic vessels excised from human lymph nodes under stereo microscope. The suspension was made by cutting the vessels to small pieces by scissors and the pieces in phosphate buffered saline were then drawn back and forth into a syringe connected to a 21 g needle. The popliteal lymph node lymphocytes from the immunized mice were isolated by a glass homogenizer. The popliteal lymph node lymphocytes of the immunized mice were fused with Sp2/0 myeloma cells. Hybridoma supernatants were primarily tested on frozen sections of human lymph nodes using immunoperoxidase staining. The testing conditions were the same for antibodies 3-266 and 3-372 generated by two of the hybridomas.

[0131] Immunoperoxidase stainings were performed as described (Salmi, *Science* 257:1407-1409 (1992)). Briefly, acetone fixed 6 μ m frozen sections from different human tissues (lymph nodes, appendix, bronchus, cerebellum, epididymis, esophagus, heart, small and large intestine, kidney, liver, lung, normal and psoriatic skin, synovium, testis and tonsil) were stained with antibody 3-266, 3-372 or 3G6, a negative class matched control antibody for 3-266 and 3-372 (mouse IgG1) and 3,3'-diaminobenzidine was used as a substrate. Procedures for tissue collection were approved by the Local and National Boards of Medicolegal Affairs in Finland.

[0132] Two of the hybridomas produced antibodies (3-266 (DSM ACC2519) and 3-372 (DSM ACC2590)) that clearly stained lymphatic endothelium both in afferent and efferent lymphatic systems and vascular endothelium on HEV, while the other structures remained unstained. The staining of the lymphatic endothelium is shown in FIG. 1. FIG. 1 is an indirect immunoperoxidase staining that shows that monoclonal antibodies 3-266 and 3-372 recognize endothelium both in afferent and efferent lymphatic systems and on HEV. FIGS. 1a-1c are from the skin. FIGS. 1d-1i are from a lymph node. FIGS. 1a, 1d and 1g show the staining with monoclonal antibody 3-266, FIGS. 1b, 1e and 1h show the staining with monoclonal antibody 3-372 and FIGS. 1c, 1f and 1i show the staining with a negative control antibody, 3G6. In FIGS. 1a, 1b and 1c, the arrows point to the epithelium and arrowheads to afferent lymphatics. In FIGS. 1d and 1e, the arrows point to the lymphatic vessels (lymphatic sinusoids that belong to the efferent lymphatic system) within the lymph node. In FIGS. 1g and 1h, the arrows point to HEV.

EXAMPLE 2

[0133] Determination of Molecular Weight of CLEVER-1

[0134] Molecular weight determination was performed by immunoblotting. One percent NP-40 lysates containing of

human lymph nodes was analyzed using 5-12.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was run in non-reducing conditions. The molecules in the gel were blotted overnight to nitrocellulose sheets and probed with 3-266, 3-372 or a negative control antibody (3G6) (Salmi, M. et al., *J. Exp. Med.* 183:569-579 (1996)). Peroxidase conjugated rabbit anti-mouse Ig was used as the second stage reagent. Detection was performed using enhanced chemiluminescence system according to the instructions of the manufacturer (Amersham).

[0135] Both antibodies recognized a molecule of the same size (about 270-300 kDa; FIG. 2). Due to this and an identical staining pattern, these antibodies were assumed to recognize the same antigen and this antigen was named CLEVER-1.

EXAMPLE 3

[0136] Purification and Molecular Characterization of CLEVER-1

[0137] The molecule recognized by 3-372 antibody was purified from human lymph node lysate overnight (lysis buffer: 150 mM NaCl, 10 mM Trisbase, pH 7.2, 1.5 mM MgCl₂, 1% NP-40, 1% aprotinin, and 1 mM PMSF) as described in Smith, D. J. et al., *J. Exp. Med.* 188:17-27 (1998). After centrifugation, the lysate supernatant was applied sequentially to immunoaffinity columns containing CnBr-activated Sepharose beads armed with irrelevant mAbs and 3-372 (3 mg/ml beads). After washing with lysis buffer, the antigens recognized by 3-372 were eluted with 50 mM triethylamine, frozen and subsequently lyophilized. The eluted material was then subjected to SDS-PAGE analysis and silver staining (O'Connell, K. L. and Stults, J. T., *Electrophoresis* 18:349-359 (1997)). The specific band was excised, reduced, alkylated and digested with trypsin (Promega) overnight at +37° C. as described (Shevchenko, A. et al., *Anal. Chem.* 68:850-855 (1996); O'Connell, K. L. and Stults, J. T., *Electrophoresis* 18:349-359 (1997)). The peptides were analyzed using PerSeptive BioSystems Voyager DE-PRO mass spectrometer operated in the reflectron delayed-extraction mode. Calibration of the spectrum was performed internally by using autolysis products of trypsin or with added calibration mixture 2 (PerSeptive BioSystems). Database search was performed by MS-Fit algorithm (<http://prospector.ucsf.edu/ucshtml3.2/msfit.htm>) of the University of California, San Francisco mass spectrometry facility.

[0138] After cleavage with trypsin, mass spectrometric analyses yielded 27 peptides. 21 (77%) of those had identical nucleotide sequences with two Genebank entries: AJ 275213, a submission for a cDNA clone called stabilin-1, and D87433, a cDAA clone KIAA0246 isolated from the cell line KG-1. The peptide sequences covered altogether 268 amino acids (100% of the 2570 amino acids of stabilin-1) and spanned the amino acids between 53 and 2301.

[0139] Next we designed primers based on the peptide sequences, the 5' end of the cDNA for stabilin-1 and the 3' end of the cDNA of KIAA0246 and used them to make several RT-PCR fragments that were then ligated together to clone the full-length 7879 bp cDNA (SEQ ID NO:1). Sequencing of the whole construct revealed a high homology with the existing 3' end KIAA0246 sequence available

in the data bank. However, it contained 4 nucleotide differences, when compared to the Stabilin sequence. They all cause a change at the amino acid level. Two of these changes are identical with the genomic sequence data available from the HUGO project (AC 006208), but since the genomic clone only covers about half of the gene for this cDNA, the nature of the two other changes remains to be determined.

[0140] Sequencing of several different CLEVER-1 cDNA-clones also revealed the existence of at least two alternatively spliced isoforms of the molecule: the regions covered by exons 23 (nucleotides 2377-2562) and 27 (nucleotides 2914-3009) can be spliced out. We could confirm the existence of one of those splice variants (lacking exon 27) also at the mRNA level (FIG. 7) but the second one (lacking exon 23) that we cloned from a human peripheral lymph node library was not visible in the system suggesting a low abundance/turnover of the mRNA encoding this form.

[0141] The sequence comparisons revealed significant homologies to proteoglycan link protein-like sequence, epidermal growth factor-like repeats and two RGD motifs being well in line with the adhesive properties of CLEVER-1.

EXAMPLE 4

[0142] In Vitro Adhesion Assay

[0143] Lymph node sections were first incubated with 3-266, 3-372 or control antibodies against human HLAABC (HB-95, ATCC and 3G6 (against chicken T cells) and then overlaid with Ficoll gradient (Pharmacia) purified peripheral blood mononuclear cells or different human tumor cell lines (lymphoblastoid cell lines, KCA and IBW4; a Burkitt lymphoma CRL-1648; squamocellular carcinoma lines NA and NU). Thereafter, the sections were subjected to two different types of assays: 1. A non-static assay, which optimally measures binding of cells to HEV and is performed under rotatory conditions (60 rpm on orbital shaker for 30 min at +7° C). 2. A static assay, in which the sections overlaid with cells are let to stay in static conditions for 15 min, followed by 5 min of rotation at 60 rpm and then again 15 min without rotation at 7° C. (Static conditions were needed to optimal binding to lymphatic endothelium). The adherent cells were fixed in 1% glutaraldehyde. The number of lymphocytes bound to HEV and to sinusoidal (lymphatic) endothelium was counted single blind under dark-field illumination in which setting the sinusoidal vessels are easily recognizable. The results of the inhibition assays are presented as percentage of control binding (the number of adherent cells/vessel in the presence of control mAb defines 100% adherence).

[0144] When the assay was performed in non-static conditions, mimicking the blood flow, lymphocyte binding to HEV was reduced 43.6% and 45.2% by 3-266 and 3-372, respectively (FIG. 3A). To mimic the conditions at sites of lymphocyte exit, the assay was performed in static conditions. In these assays lymphocyte binding to lymphatic endothelium was decreased 46.4% and 64% by 3-266 and 3-372, respectively (FIG. 3B). These data indicate that the molecule recognized by 3-266 and 3-372 indeed mediate lymphocyte binding both to HEV and to lymphatic endothelium.

[0145] To study the role of this molecule in migration of malignant cells, the assays were performed using three

lymphoma cell lines (CRL 1648, KCA and IBW4) and two squamocellular carcinoma cell lines (NA and NU). For these assays 3-372 antibody was chosen because of its higher inhibitory capacity (FIG. 3). The results of these experiments clearly demonstrated that CLEVER-1 is also involved in binding of malignant cells to endothelium both at entrance and exit sites within the lymph nodes (FIGS. 4A and 3).

EXAMPLE 5

[0146] CLEVER-1 is Upregulated at Sites of Inflammation on HEV-Like Vessels

[0147] Synovial samples from 18 patients suffering from chronic arthritis and undergoing synovectomies, skin samples from diseased skin of patients suffering from psoriasis (n=5), lichen (n=1), mycosis fungoides (n=1), erythrodermia (n=2), exanthema (n=1), folliculitis (n=3) and normal skin samples from 15 individuals were studied for expression of CLEVER-1 using immunoperoxidase method as described above. Like in normal non-lymphoid tissues CLEVER-1 was present in afferent lymphatic vessels in inflamed synovial and both in normal and diseased skin samples. In addition, CLEVER-1 expression was induced on HEV-like vessels that appear at sites of inflammation and are surrounded by heavy infiltrations of inflammatory cells (FIG. 5). Table 2 illustrates complete correlation between the extent of inflammatory infiltration and upregulation of CLEVER-1 expression in synovial samples. The same phenomenon was observed in skin samples: all diseased skin samples had inflammatory infiltrations that contained CLEVER-1 positive HEV-like vessels. Those vessels were absent in normal skin samples.

EXAMPLE 6

[0148] Clever-1 Also Mediates Binding of Monocytes and Granulocytes to HEV-Like Vessels

[0149] Human monocytes from peripheral blood were purified from Ficoll-gradient (Pharmacia) isolated mononuclear cells by letting them to adhere to plastic surfaces for an hour at +37° C. Granulocytes were purified from leukocyte rich buffy coats from human blood using Percoll-gradient (Pharmacia) centrifugation. Their binding was tested to HEV-like vessels in inflamed synovium. In addition, granulocyte binding was tested to tonsil HEV that brightly express CLEVER-1. (When tonsils are removed they always have variable extent of inflammation, although they as a lymphoid organ have HEV without any inflammation). Both granulocytes and monocytes bound efficiently to HEV-like vessels in inflamed synovium and granulocytes adhered avidly to HEV in tonsils. Their binding to these organs was significantly inhibited by the antibody pool containing 3-372 and 3-266 but not with the control antibody (FIG. 6).

EXAMPLE 7

[0150] CLEVER-1 Controls Lymphocyte Trafficking in vivo

[0151] In order to verify that CLEVER-1 has a functional role in vivo, it was at first confirmed by intravenous injection of 3-372 antibody that rabbits express CLEVER-1 on the surface of endothelium in vivo. The presence of CLEVER-1 on HEV was detected after the 3-372 antibody had circulated

5 minutes in vivo using frozen sections and FITC labelled anti-mouse IgG second stage antibody after sacrifice (FIG. 8a). In this time frame the intravenously given 180 kDa immunoglobulin molecule does not have a possibility to leak and diffuse into the tissue. Based on these results antibody 3-372 (and a class-matched negative control antibody) was given to the rabbits immunized with keyhole limpet hemocyanin to footpads and the effects of the antibody treatment on the size and cellularity of the lymph nodes draining the footpads was analyzed.

[0152] Antibody treatment against CLEVER-1 significantly prevented increase of the size of the popliteal lymph nodes (FIG. 8c) indicating that CLEVER-1 has a functional role in lymphocyte traffic in vivo. Most likely it exerts its effects both at lymphocyte entrance in HEV and their exit in lymphatic sinusoids, because the rabbits treated with 3-372

antibody had only few lymphocytes in their lymphatic sinusoids when analyzed using histological section (FIG. 8d) Intravenously given 3-372 antibody was also detected to bind CLEVER-1 on lymphatic sinusoids when tested at sacrifice 3 days after the final 3-372 dose. No signal was detected in rabbits which received a control antibody (data not shown).

[0153] All documents, e.g., scientific publications, patents and patent publications recited herein are hereby incorporated by reference in their entirety to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference in its entirety. Where the document cited only provides the first page of the document, the entire document is intended, including the remaining pages of the document.

TABLE 1

Matches with CLEVER-1 and stabilin-1						
1. 21/27 matches (77%). 275350.0 Da, pI = 6.04. Acc. #6469374. HOMO						
SAPIENS. (AJ275213) stabilin-1.						
m/z submitted	MH ⁺ matched	Delta ppm	start	end	Peptide Sequence (Click for Fragment Ions)	Modifications
775.488	775.483	6.4034	372	377	(R)VFLQLR(V)	
787.36	787.3739	-17.6253	1299	1305	(R)SGFSPSR(G)	
799.495	799.5042	-11.4617	1585	1591	(R)VGLELLR(D)	
812.495	812.4994	-5.4309	1047	1053	(R)TLPNLVR(A)	
917.502	917.4997	2.4556	1040	1046	(R)AFWLQPR(T)	
1017.44	1017.425	14.4862	2295	2301	(R)WDAVCFR(V)	
1104.54	1104.526	12.6406	53	61	(K)OTCPGWLRL(E)	
1212.7	1212.695	3.9482	1021	1032	(R)VTALVPSEAAVR(Q)	
1284.65	1284.622	21.4530	1678	1688	(R)EGSIYLNDFAR(V)	
1291.79	1291.774	12.5434	613	624	(R)ILLGPEGVPLOR(V)	
1330.63	1330.575	41.7401	953	965	(R)AGNGGCHGLATCR(A)	
1330.63	1330.633	-1.9754	1873	1812	(R)CDHFETPLR(L)	
1374.66	1374.632	20.1117	62	72	(R)ELPDQITQDCR(Y)	
1456.79	1456.776	9.6229	1069	1082	(R)LGGQEVATLNPTR(W)	
1493.8	1493.796	2.4215	508	521	(R)TIGQILASTEAPSR(F)	
1555.7	1555.663	23.5678	219	231	(R)CLPGYTOOGSECR(A)	
1678.94	1678.913	16.1953	1802	1817	(R)NVEALASDLNGLPLR(T)	
1730.89	1730.887	1.9674	1054	1068	(R)AHPLOGALFEEELAR(L)	
1912.82	1912.832	-6.3742	936	952	(K)LGEAGDGYQCSPIDPCR(A)	
2057.05	2057.03	9.5484	1655	1673	(R)SEDLLEQYATALSGHPLR(F)	
2165.11	2165.14	-13.6320	1707	1725	(R)VLLPPEALHWPDDAPIPR(R)	
2295.22	2295.224	-1.5853	389	410	(R)EILTLAGPFTVLVPSVSSFSSR(T)	

6 unmatched masses: 871.5410 949.4800 1360.6500 1538.6900 1787.9200 2008.1400
 The matched peptides cover 10% (268/2570 AA's) of the protein.
 Coverage Map for This Hit (MS-Digest index #): 427477

[0154]

TABLE 2

CLEVER-1 Expression Is Induced Mainly on Vessels Surrounded by Lymphocytic Infiltrations in Inflamed Synovia		
Expression of CLEVER-1	The degree of inflammatory infiltration ²	
	0/1 (n = 9)	2/3 (n = 9)
on HEV-like vessels ¹		
-/+	100%	0
++/+++	0	100%

¹Intensity was scored as -, ±, + negative or weak, ++, +++ moderate or strong.

²Degree of the inflammatory cell infiltration in 18 synovial samples was scored as: 0/1, none or few lymphocytes around the vessels, 2/3 marked or massive lymphocytic infiltrations.

[0155]

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 1

<210> SEQ ID NO 1

<211> LENGTH: 7879

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: exon

<222> LOCATION: (31)..(7740)

<400> SEQUENCE: 1

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cca ctc tgc ctc ctg gcc ttc tgc ctg gca ggc ttc agc ttc gtc agg      102
Pro Leu Cys Leu Leu Ala Phe Cys Leu Ala Gly Phe Ser Phe Val Arg
  10                15                20

ggg cag gtg ctg ttc aaa ggc tgt gat gtg aaa acc acg ttt gtc act      150
Gly Gln Val Leu Phe Lys Gly Cys Asp Val Lys Thr Thr Phe Val Thr
  25                30                35                40

cat gta ccc tgc acc tcg tgc gcg gcc atc aag aag cag acg tgt ccc      198
His Val Pro Cys Thr Ser Cys Ala Ala Ile Lys Lys Gln Thr Cys Pro
                45                50                55

tca ggc tgg ctg cgg gag ctc ccg gat cag ata acc cag gac tgc cgc      246
Ser Gly Trp Leu Arg Glu Leu Pro Asp Gln Ile Thr Gln Asp Cys Arg
  60                65                70

tac gaa gta cag ctg ggg ggc tct atg gtg tcc atg agc ggc tgc aga      294
Tyr Glu Val Gln Leu Gly Gly Ser Met Val Ser Met Ser Gly Cys Arg
  75                80                85

cgg aag tgc cgg aag caa gtg gtg cag aag gcc tgc tgc cct ggc tac      342
Arg Lys Cys Arg Lys Gln Val Gln Lys Ala Cys Cys Pro Gly Tyr
  90                95                100

tgg ggt tcc cgg tgc cat gaa tgc cct ggg ggc gct gag acc cca tgc      390
Trp Gly Ser Arg Cys His Glu Cys Pro Gly Gly Ala Glu Thr Pro Cys
  105                110                115                120

aat ggc cac ggg acc tgc ttg gat ggc atg gac agg aat ggg acc tgt      438
Asn Gly His Gly Thr Cys Leu Asp Gly Met Asp Arg Asn Gly Thr Cys
  125                130                135

gtg tgc cag gaa aac ttc cgc gcc tca gcc tgc cag gag tgc caa gac      486

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Val	Cys	Gln	Glu	Asn	Phe	Arg	Gly	Ser	Ala	Cys	Gln	Glu	Cys	Gln	Asp	
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ccc	aac	cgg	ttc	ggg	cct	gac	tgc	caa	tcg	gtg	tgc	agc	tgt	gtg	cac	534
Pro	Asn	Arg	Phe	Gly	Pro	Asp	Cys	Gln	Ser	Val	Cys	Ser	Cys	Val	His	
		155					160					165				
gga	gtg	tgc	aac	cat	ggg	cca	cgt	ggg	gat	gga	agc	tgc	ctg	tgc	ttt	582
Gly	Val	Cys	Asn	His	Gly	Pro	Arg	Gly	Asp	Gly	Ser	Cys	Leu	Cys	Phe	
	170					175					180					
gct	gga	tac	act	ggc	ccc	cac	tgt	gat	caa	gag	ctg	ccc	gtc	tgc	cag	630
Ala	Gly	Tyr	Thr	Gly	Pro	His	Cys	Asp	Gln	Glu	Leu	Pro	Val	Cys	Gln	
	185					190				195					200	
gag	ctg	cgc	tgt	ccc	cag	aac	acc	cag	tgc	tcc	gca	gag	gct	ccc	agc	678
Glu	Leu	Arg	Cys	Pro	Gln	Asn	Thr	Gln	Cys	Ser	Ala	Glu	Ala	Pro	Ser	
			205						210					215		
tgc	agg	tgc	ctg	ccc	ggc	tac	aca	cag	cag	ggc	agt	gaa	tgc	cga	gcc	726
Cys	Arg	Cys	Leu	Pro	Gly	Tyr	Thr	Gln	Gln	Gly	Ser	Glu	Cys	Arg	Ala	
			220					225					230			
ccc	aac	ccc	tgc	tgg	cca	tca	ccc	tgc	tca	ctg	ctg	gcc	cag	tgc	tcg	774
Pro	Asn	Pro	Cys	Trp	Pro	Ser	Pro	Cys	Ser	Leu	Leu	Ala	Gln	Cys	Ser	
		235				240						245				
gtg	agc	ccc	aag	ggg	cag	gct	cag	tgt	cac	tgc	cct	gag	aac	tac	cat	822
Val	Ser	Pro	Lys	Gly	Gln	Ala	Gln	Cys	His	Cys	Pro	Glu	Asn	Tyr	His	
	250					255					260					
ggc	gat	ggg	atg	gtg	tgt	ctg	ccc	aag	gac	cca	tgc	act	gac	aac	ctt	870
Gly	Asp	Gly	Met	Val	Cys	Leu	Pro	Lys	Asp	Pro	Cys	Thr	Asp	Asn	Leu	
	265				270				275						280	
ggg	ggc	tgc	ccc	agc	aac	tct	act	ttg	tgt	gtg	tac	cag	aag	ccg	ggc	918
Gly	Gly	Cys	Pro	Ser	Asn	Ser	Thr	Leu	Cys	Val	Tyr	Gln	Lys	Pro	Gly	
			285						290					295		
cag	gcc	ttc	tgc	acc	tgc	cgg	cca	ggc	ctg	gtc	agc	atc	aac	agc	aac	966
Gln	Ala	Phe	Cys	Thr	Cys	Arg	Pro	Gly	Leu	Val	Ser	Ile	Asn	Ser	Asn	
		300						305					310			
gct	tct	gcg	ggc	tgc	ttc	gcc	ttc	tgc	tcc	ccc	ttc	tcc	tgc	gac	cgg	1014
Ala	Ser	Ala	Gly	Cys	Phe	Ala	Phe	Cys	Ser	Pro	Phe	Ser	Cys	Asp	Arg	
		315				320							325			
tct	gcc	act	tgc	cag	gtg	acc	gct	gat	ggg	aag	acc	agc	tgt	gtg	tgc	1062
Ser	Ala	Thr	Cys	Gln	Val	Thr	Ala	Asp	Gly	Lys	Thr	Ser	Cys	Val	Cys	
	330					335					340					
agg	gaa	agc	gag	gtg	ggg	gat	ggg	cgt	gcc	tgc	tac	gga	cac	ctg	ctc	1110
Arg	Glu	Ser	Glu	Val	Gly	Asp	Gly	Arg	Ala	Cys	Tyr	Gly	His	Leu	Leu	
	345				350					355					360	
cac	gag	gtg	cag	aag	gcc	acg	cag	aca	ggc	cgg	gtg	ttc	ctg	cag	ctg	1158
His	Glu	Val	Gln	Lys	Ala	Thr	Gln	Thr	Gly	Arg	Val	Phe	Leu	Gln	Leu	
			365						370					375		
agg	gtc	gcc	gtg	gcc	atg	atg	gac	cag	ggc	tgc	cgg	gaa	atc	ctt	acc	1206
Arg	Val	Ala	Val	Ala	Met	Met	Asp	Gln	Gly	Cys	Arg	Glu	Ile	Leu	Thr	
		380						385					390			
aca	gcg	ggc	cct	ttc	acc	gtg	ctg	gtg	cca	tcc	gtc	tcc	tcc	ttc	tcc	1254
Thr	Ala	Gly	Pro	Phe	Thr	Val	Leu	Val	Pro	Ser	Val	Ser	Ser	Phe	Ser	
		395				400						405				
tcc	agg	acc	atg	aat	gca	tcc	ctt	gcc	cag	cag	ctc	tgt	aga	cag	cac	1302
Ser	Arg	Thr	Met	Asn	Ala	Ser	Leu	Ala	Gln	Gln	Leu	Cys	Arg	Gln	His	
	410					415					420					
atc	atc	gca	ggg	cag	cac	atc	ctg	gag	gac	aca	agg	acc	caa	caa	aca	1350
Ile	Ile	Ala	Gly	Gln	His	Ile	Leu	Glu	Asp	Thr	Arg	Thr	Gln	Gln	Thr	
	425				430				435						440	
cga	agg	tgg	tgg	acg	ctg	gcc	ggg	cag	gag	atc	acc	gtc	acc	ttt	aac	1398

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				445					450						455	
caa	ttc	acg	aaa	tac	tcc	tac	aag	tac	aaa	gac	cag	ccc	cag	cag	acg	1446
Gln	Phe	Thr	Lys	Tyr	Ser	Tyr	Lys	Tyr	Lys	Asp	Gln	Pro	Gln	Gln	Thr	
			460					465					470			
ttc	aac	atc	tac	aag	gcc	aac	aac	ata	gca	gct	aat	ggc	gtc	ttc	cac	1494
Phe	Asn	Ile	Tyr	Lys	Ala	Asn	Asn	Ile	Ala	Ala	Asn	Gly	Val	Phe	His	
			475				480					485				
gtg	gtc	act	ggc	ctg	cgg	tgg	cag	gcc	ccc	tct	ggg	acc	cct	ggg	gat	1542
Val	Val	Thr	Gly	Leu	Arg	Trp	Gln	Ala	Pro	Ser	Gly	Thr	Pro	Gly	Asp	
			490			495					500					
ccc	aag	aga	act	atc	gga	cag	atc	ctc	gcc	tct	acc	gag	gcc	ttc	agc	1590
Pro	Lys	Arg	Thr	Ile	Gly	Gln	Ile	Leu	Ala	Ser	Thr	Glu	Ala	Phe	Ser	
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cgc	ttt	gaa	acc	atc	ctg	gag	aac	tgt	ggg	ctg	ccc	tcc	atc	ctg	gac	1638
Arg	Phe	Glu	Thr	Ile	Leu	Glu	Asn	Cys	Gly	Leu	Pro	Ser	Ile	Leu	Asp	
				525					530						535	
gga	cct	ggg	ccc	ttc	aca	gtc	ttt	gcc	cca	agc	aat	gag	gct	gtg	gac	1686
Gly	Pro	Gly	Pro	Phe	Thr	Val	Phe	Ala	Pro	Ser	Asn	Glu	Ala	Val	Asp	
				540				545							550	
agc	ttg	cgt	gac	ggc	cgc	ctg	atc	tac	ctc	ttc	aca	gcg	ggt	ctc	tct	1734
Ser	Leu	Arg	Asp	Gly	Arg	Leu	Ile	Tyr	Leu	Phe	Thr	Ala	Gly	Leu	Ser	
				555			560						565			
aaa	ctg	cag	gag	ttg	gtg	cgg	tac	cac	atc	tac	aac	cac	ggc	cag	ctg	1782
Lys	Leu	Gln	Glu	Leu	Val	Arg	Tyr	His	Ile	Tyr	Asn	His	Gly	Gln	Leu	
				570			575						580			
acc	gtt	gag	aag	ctc	atc	tcc	aag	ggt	cgg	atc	ctc	acc	atg	gcg	aac	1830
Thr	Val	Glu	Lys	Leu	Ile	Ser	Lys	Gly	Arg	Ile	Leu	Thr	Met	Ala	Asn	
						590					595				600	
cag	gtc	ctg	gct	gtg	aac	att	tct	gag	gag	ggg	cgc	atc	ctg	ctg	gga	1878
Gln	Val	Leu	Ala	Val	Asn	Ile	Ser	Glu	Glu	Gly	Arg	Ile	Leu	Leu	Gly	
					605					610					615	
ccc	gag	ggg	gtc	ccg	ctg	cag	agg	gta	gac	gtg	atg	gcc	gcc	aat	ggt	1926
Pro	Glu	Gly	Val	Pro	Leu	Gln	Arg	Val	Asp	Val	Met	Ala	Ala	Asn	Gly	
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gtg	atc	cac	atg	ctg	gac	ggc	atc	ctg	ctg	ccc	ccg	acc	atc	ctg	ccc	1974
Val	Ile	His	Met	Leu	Asp	Gly	Ile	Leu	Leu	Pro	Pro	Thr	Ile	Leu	Pro	
						635							645			
atc	ctg	ccc	aag	cac	tgc	agc	gag	gag	cag	cac	aag	att	gtg	gcg	ggc	2022
Ile	Leu	Pro	Lys	His	Cys	Ser	Glu	Glu	Gln	His	Lys	Ile	Val	Ala	Gly	
						655						660				
tcc	tgt	gtg	gac	tgc	caa	gcc	ctg	aac	acc	agc	acg	tgt	ccc	ccc	aac	2070
Ser	Cys	Val	Asp	Cys	Gln	Ala	Leu	Asn	Thr	Ser	Thr	Cys	Pro	Pro	Asn	
						670					675				680	
agt	gtg	aag	ctg	gac	atc	ttc	ccc	aag	gag	tgt	gtc	tac	atc	cat	gac	2118
Ser	Val	Lys	Leu	Asp	Ile	Phe	Pro	Lys	Glu	Cys	Val	Tyr	Ile	His	Asp	
						685				690					695	
cca	acg	ggg	ctc	aat	gtg	cta	aag	aag	ggc	tgt	gcc	agc	tac	tgc	aac	2166
Pro	Thr	Gly	Leu	Asn	Val	Leu	Lys	Lys	Gly	Cys	Ala	Ser	Tyr	Cys	Asn	
						700				705					710	
caa	acc	atc	atg	gaa	caa	ggc	tgc	tgc	aaa	ggt	ttt	ttc	ggg	cct	gac	2214
Gln	Thr	Ile	Met	Glu	Gln	Gly	Cys	Cys	Lys	Gly	Phe	Phe	Gly	Pro	Asp	
						715									725	
tgc	acg	cag	tgt	cct	ggg	ggc	ttc	tcc	aac	ccc	tgc	tat	ggc	aaa	ggc	2262
Cys	Thr	Gln	Cys	Pro	Gly	Gly	Phe	Ser	Asn	Pro	Cys	Tyr	Gly	Lys	Gly	
						730									740	
aat	tgc	agt	gat	ggg	atc	cag	ggc	aat	ggg	gcc	tgc	ctc	tgc	ttc	cca	2310

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Asn Cys Ser Asp Gly Ile Gln Gly Asn Gly Ala Cys Leu Cys Phe Pro 745 750 755 760	
gac tac aag ggc atc gcc tgc cac atc tgc tcg aac cca aac aag cat Asp Tyr Lys Gly Ile Ala Cys His Ile Cys Ser Asn Pro Asn Lys His 765 770 775	2358
gga gag caa tgc cag gaa gac tgc ggc tgt gtc cat ggt ctc tgc gac Gly Glu Gln Cys Gln Glu Asp Cys Gly Cys Val His Gly Leu Cys Asp 780 785 790	2406
aac cgc cca ggc agt ggg ggg gtg tgc cag cag ggc acg tgt gcc cct Asn Arg Pro Gly Ser Gly Gly Val Cys Gln Gln Gly Thr Cys Ala Pro 795 800 805	2454
ggc ttc agt ggc cgg ttc tgc aac gag tcc atg ggg gac tgt ggg ccc Gly Phe Ser Gly Arg Phe Cys Asn Glu Ser Met Gly Asp Cys Gly Pro 810 815 820	2502
aca ggg ctg gcc cag cac tgc cac ctg cat gcc cgc tgt gtt agc cag Thr Gly Leu Ala Gln His Cys His Leu His Ala Arg Cys Val Ser Gln 825 830 835 840	2550
gag ggt gtt gcc aga tgt cgc tgt ctt gat ggc ttt gag ggt gat ggc Glu Gly Val Ala Arg Cys Arg Cys Leu Asp Gly Phe Glu Gly Asp Gly 845 850 855	2598
ttc tcc tgc aca cct agc aac ccc tgc tcc cac ccg gac cgt gga ggc Phe Ser Cys Thr Pro Ser Asn Pro Cys Ser His Pro Asp Arg Gly Gly 860 865 870	2646
tgc tca gag aat gct gag tgt gtc cct ggg tcc ctg ggc acc cac cac Cys Ser Glu Asn Ala Glu Cys Val Pro Gly Ser Leu Gly Thr His His 875 880 885	2694
tgc aca tgc cac aaa ggc tgg agt ggg gat ggc cgc gtc tgt gtg gct Cys Thr Cys His Lys Gly Trp Ser Gly Asp Gly Arg Val Cys Val Ala 890 895 900	2742
att gac gag tgt gag ctg gac gtg aga ggt ggc tgc cac acc gat gcc Ile Asp Glu Cys Glu Leu Asp Val Arg Gly Gly Cys His Thr Asp Ala 905 910 915 920	2790
ctc tgc agc tat gtg ggc ccc ggg cag agc cga tgc acc tgc aag ctg Leu Cys Ser Tyr Val Gly Pro Gly Gln Ser Arg Cys Thr Cys Lys Leu 925 930 935	2838
ggc ttt gcc ggg gat ggc tac cag tgc agc ccc atc gac ccc tgc cgg Gly Phe Ala Gly Asp Gly Tyr Gln Cys Ser Pro Ile Asp Pro Cys Arg 940 945 950	2886
gca ggc aat ggc ggc tgc cac ggc ctg gcc acc tgc cgg gca gtg ggg Ala Gly Asn Gly Gly Cys His Gly Leu Ala Thr Cys Arg Ala Val Gly 955 960 965	2934
gga ggt cag cgg gtc tgc acg tgc ccc cct ggc ttt ggg ggt gat ggc Gly Gly Gln Arg Val Cys Thr Cys Pro Pro Gly Phe Gly Gly Asp Gly 970 975 980	2982
ttc agc tgt tat gga gac atc ttc cgg gag ctg gag gca aat gcc cac Phe Ser Cys Tyr Gly Asp Ile Phe Arg Glu Leu Glu Ala Asn Ala His 985 990 995 1000	3030
ttc tcc atc ttc tac caa tgg ctt aag agt gcc ggc atc acg ctt Phe Ser Ile Phe Tyr Gln Trp Leu Lys Ser Ala Gly Ile Thr Leu 1005 1010 1015	3075
cct gcc gac cgc cga gtc aca gcc ctg gtg ccc tcc gag gct gca Pro Ala Asp Arg Arg Val Thr Ala Leu Val Pro Ser Glu Ala Ala 1020 1025 1030	3120
gtc cgt cag ctg agc ccc gag gac cga gct ttc tgg ctg cag cca Val Arg Gln Leu Ser Pro Glu Asp Arg Ala Phe Trp Leu Gln Pro 1035 1040 1045	3165
agg acg ctg ccg aac ctg gtc agg gcc cat ttt ctc cag ggt gcc	3210

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Arg Thr Leu Pro Asn	Leu Val Arg Ala His	Phe Leu Gln Gly Ala	
	1050	1055	1060
ctc ttc gag gag gag	ctg gcc cgg ctg ggt	ggg cag gaa gtg gcc	3255
Leu Phe Glu Glu Glu	Leu Ala Arg Leu Gly	Gly Gln Glu Val Ala	
	1065	1070	1075
acc ctg aac ccc acc	aca cgc tgg gag att	cgc aac att agt ggg	3300
Thr Leu Asn Pro Thr	Thr Arg Trp Glu Ile	Arg Asn Ile Ser Gly	
	1080	1085	1090
agg gtc tgg gtg cag	aat gcc agc gtg gat	gtg gct gac ctc ctt	3345
Arg Val Trp Val Gln	Asn Ala Ser Val Asp	Val Ala Asp Leu Leu	
	1095	1100	1105
gcc acc aac ggt gtc	cta cac atc ctc agc	cag gtc tta ctg ccc	3390
Ala Thr Asn Gly Val	Leu His Ile Leu Ser	Gln Val Leu Leu Pro	
	1110	1115	1120
ccc cga ggg gat gtg	ccc ggt ggg cag ggg	ttg ctg cag cag ctg	3435
Pro Arg Gly Asp Val	Pro Gly Gly Gln Gly	Leu Leu Gln Gln Leu	
	1125	1130	1135
gac ttg gtg cct gcc	ttc agc ctc ttc cgg	gaa ttg ctg cag cac	3480
Asp Leu Val Pro Ala	Phe Ser Leu Phe Arg	Glu Leu Leu Gln His	
	1140	1145	1150
cat ggg ttg gtg ccc	cag att gag gct gcc	act gcc tac acc atc	3525
His Gly Leu Val Pro	Gln Ile Glu Ala Ala	Thr Ala Tyr Thr Ile	
	1155	1160	1165
ttt gtg ccc acc aac	cgc tcc ctg gag gcc	cag ggc aac agc agt	3570
Phe Val Pro Thr Asn	Arg Ser Leu Glu Ala	Gln Gly Asn Ser Ser	
	1170	1175	1180
cac ctg gac gca gac	aca gtg cgg cac cat	gtg gtc ctg ggg gag	3615
His Leu Asp Ala Asp	Thr Val Arg His His	Val Val Leu Gly Glu	
	1185	1190	1195
gcc ctg tcc atg gaa	acc ctg cgg aag ggt	gga cac cgc aac tcc	3660
Ala Leu Ser Met Glu	Thr Leu Arg Lys Gly	Gly His Arg Asn Ser	
	1200	1205	1210
ctc ctg ggc cct gcc	cac tgg atc gtc ttc	tac aac cac agt ggc	3705
Leu Leu Gly Pro Ala	His Trp Ile Val Phe	Tyr Asn His Ser Gly	
	1215	1220	1225
cag cct gag gtg aac	cat gtg cca ctg gaa	ggc ccc atg ctg gag	3750
Gln Pro Glu Val Asn	His Val Pro Leu Glu	Gly Pro Met Leu Glu	
	1230	1235	1240
gcc cct ggc cgc tcg	ctg att ggt ctg tcg	ggg gtc ctg acg gtg	3795
Ala Pro Gly Arg Ser	Leu Ile Gly Leu Ser	Gly Val Leu Thr Val	
	1245	1250	1255
ggc tca agt cgc tgc	ctg cat agc cac get	gag gcc ctg cgg gag	3840
Gly Ser Ser Arg Cys	Leu His Ser His Ala	Glu Ala Leu Arg Glu	
	1260	1265	1270
aaa tgt gta aac tgc	acc agg aga ttc cgc	tgc act cag ggc ttc	3885
Lys Cys Val Asn Cys	Thr Arg Arg Phe Arg	Cys Thr Gln Gly Phe	
	1275	1280	1285
cag ctg cag gac aca	ccc agg aag agc tgt	gtc tac cga tct ggc	3930
Gln Leu Gln Asp Thr	Pro Arg Lys Ser Cys	Val Tyr Arg Ser Gly	
	1290	1295	1300
ttc tcc ttc tcc cgg	ggc tgc tct tac aca	tgt gcc aag aag atc	3975
Phe Ser Phe Ser Arg	Gly Cys Ser Tyr Thr	Cys Ala Lys Lys Ile	
	1305	1310	1315
cag gtg ccg gac tgc	tgc cct ggt ttc ttt	ggc acg ctg tgt gag	4020
Gln Val Pro Asp Cys	Cys Pro Gly Phe Phe	Gly Thr Leu Cys Glu	
	1320	1325	1330
cca tgc cca ggg ggt	cta ggg ggg gtg tgc	tca gcc cat ggg cag	4065

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Pro	Cys	Pro	Gly	Gly	Leu	Gly	Gly	Val	Cys	Ser	Gly	His	Gly	Gln	
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tgc	cag	gac	agg	ttc	ctg	ggc	agc	ggg	gag	tgc	cac	tgc	cac	gag	4110
Cys	Gln	Asp	Arg	Phe	Leu	Gly	Ser	Gly	Glu	Cys	His	Cys	His	Glu	
				1350					1355					1360	
ggc	ttc	cat	gga	acg	gcc	tgt	gag	gtg	tgt	gag	ctg	ggc	cgc	tac	4155
Gly	Phe	His	Gly	Thr	Ala	Cys	Glu	Val	Cys	Glu	Leu	Gly	Arg	Tyr	
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ggg	ccc	aac	tgc	acc	gga	gtg	tgt	gac	tgt	gcc	cat	ggg	ctg	tgc	4200
Gly	Pro	Asn	Cys	Thr	Gly	Val	Cys	Asp	Cys	Ala	His	Gly	Leu	Cys	
				1380					1385					1390	
cag	gag	ggg	ctg	caa	ggg	gac	gga	agc	tgt	gtc	tgt	aac	gtg	ggc	4245
Gln	Glu	Gly	Leu	Gln	Gly	Asp	Gly	Ser	Cys	Val	Cys	Asn	Val	Gly	
				1395					1400					1405	
tgg	cag	ggc	ctc	cgc	tgt	gac	cag	aaa	atc	acc	agc	cct	cag	tgc	4290
Trp	Gln	Gly	Leu	Arg	Cys	Asp	Gln	Lys	Ile	Thr	Ser	Pro	Gln	Cys	
				1410					1415					1420	
cct	agg	aag	tgc	gac	ccc	aat	gcc	aac	tgc	gtg	cag	gac	tcg	gcc	4335
Pro	Arg	Lys	Cys	Asp	Pro	Asn	Ala	Asn	Cys	Val	Gln	Asp	Ser	Ala	
				1425					1430					1435	
gga	gcc	tcc	acc	tgc	gcc	tgt	gct	gcg	gga	tac	tcc	ggc	aat	ggc	4380
Gly	Ala	Ser	Thr	Cys	Ala	Cys	Ala	Ala	Gly	Tyr	Ser	Gly	Asn	Gly	
				1440					1445					1450	
atc	ttc	tgt	tca	gag	gtg	gac	ccc	tgc	gcc	cac	ggc	cat	ggg	ggc	4425
Ile	Phe	Cys	Ser	Glu	Val	Asp	Pro	Cys	Ala	His	Gly	His	Gly	Gly	
				1455					1460					1465	
tgc	tcc	cct	cat	gcc	aac	tgt	acc	aag	gtg	gca	cct	ggg	cag	cgg	4470
Cys	Ser	Pro	His	Ala	Asn	Cys	Thr	Lys	Val	Ala	Pro	Gly	Gln	Arg	
				1470					1475					1480	
aca	tgc	acc	tgc	cag	gat	ggc	tac	atg	ggc	gac	ggg	gag	ctg	tgc	4515
Thr	Cys	Thr	Cys	Gln	Asp	Gly	Tyr	Met	Gly	Asp	Gly	Glu	Leu	Cys	
				1485					1490					1495	
cag	gaa	att	aac	agc	tgt	ctc	atc	cac	cac	ggg	ggc	tgc	cac	att	4560
Gln	Glu	Ile	Asn	Ser	Cys	Leu	Ile	His	His	Gly	Gly	Cys	His	Ile	
				1500					1505					1510	
cac	gcc	gag	tgc	atc	ccc	act	ggc	ccc	cag	cag	gtc	tcc	tgc	agc	4605
His	Ala	Glu	Cys	Ile	Pro	Thr	Gly	Pro	Gln	Gln	Val	Ser	Cys	Ser	
				1515					1520					1525	
tgc	cgt	gag	ggt	tac	agc	ggg	gat	ggc	atc	cgg	acc	tgc	gag	ctc	4650
Cys	Arg	Glu	Gly	Tyr	Ser	Gly	Asp	Gly	Ile	Arg	Thr	Cys	Glu	Leu	
				1530					1535					1540	
ctg	gac	ccc	tgc	tct	aag	aac	aat	gga	gga	tgc	agc	cca	tat	gcc	4695
Leu	Asp	Pro	Cys	Ser	Lys	Asn	Asn	Gly	Gly	Cys	Ser	Pro	Tyr	Ala	
				1545					1550					1555	
acc	tgc	aaa	agc	aca	ggg	gat	ggc	cag	agg	aca	tgt	acc	tgc	gac	4740
Thr	Cys	Lys	Ser	Thr	Gly	Asp	Gly	Gln	Arg	Thr	Cys	Thr	Cys	Asp	
				1560					1565					1570	
aca	gcc	cac	acc	gtg	ggg	gac	ggc	ctc	acc	tgc	cgt	gcc	cga	gtc	4785
Thr	Ala	His	Thr	Val	Gly	Asp	Gly	Leu	Thr	Cys	Arg	Ala	Arg	Val	
				1575					1580					1585	
ggc	ctg	gag	ctc	ctg	agg	gat	aag	cat	gcc	tca	ttc	ttc	agc	ctc	4830
Gly	Leu	Glu	Leu	Leu	Arg	Asp	Lys	His	Ala	Ser	Phe	Phe	Ser	Leu	
				1590					1595					1600	
cgc	ctc	ctg	gaa	tat	aag	gag	ctc	aag	ggc	gat	ggg	cct	ttc	acc	4875
Arg	Leu	Leu	Glu	Tyr	Lys	Glu	Leu	Lys	Gly	Asp	Gly	Pro	Phe	Thr	
				1605					1610					1615	
atc	ttc	gtg	ccg	cac	gca	gat	cta	atg	agc	aac	ctg	tcg	cag	gat	4920

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Ile Phe Val Pro His Ala Asp Leu Met Ser Asn Leu Ser Gln Asp	
1620 1625 1630	
gag ctg gcc cgg att cgt gcg cat cgc cag ctg gtg ttt cgc tac	4965
Glu Leu Ala Arg Ile Arg Ala His Arg Ser Gln Leu Val Phe Arg Tyr	
1635 1640 1645	
cac gtg gtt gcc tgt cgg cgg ctg cgg agc gag gac ctg ctg gag	5010
His Val Val Gly Cys Arg Arg Leu Arg Ser Glu Asp Leu Leu Glu	
1650 1655 1660	
cag ggg tac gcc acg gcc ctc tca ggg cac cca ctg cgc ttc agc	5055
Gln Gly Tyr Ala Thr Ala Leu Ser Gly His Pro Leu Arg Phe Ser	
1665 1670 1675	
gag agg gag gcc agc ata tac ctc aat gac ttc gcg cgc gtg gtg	5100
Glu Arg Glu Gly Ser Ile Tyr Leu Asn Asp Phe Ala Arg Val Val	
1680 1685 1690	
agc agc gac cat gag gcc gtg aac ggc atc ctg cac ttc att gac	5145
Ser Ser Asp His Glu Ala Val Asn Gly Ile Leu His Phe Ile Asp	
1695 1700 1705	
cgt gtc ctg ctg ccc ccc gag gcg ctg cac tgg gag cct gat gat	5190
Arg Val Leu Leu Pro Pro Glu Ala Leu His Trp Glu Pro Asp Asp	
1710 1715 1720	
gct ccc atc ccg agg aga aat gtc acc gcc gcc gcc cag gcc ttc	5235
Ala Pro Ile Pro Arg Arg Asn Val Thr Ala Ala Ala Gln Gly Phe	
1725 1730 1735	
ggt tac aag atc ttc agc ggc ctc ctg aag gtg gcc gcc ctc ctg	5280
Gly Tyr Lys Ile Phe Ser Gly Leu Leu Lys Val Ala Gly Leu Leu	
1740 1745 1750	
ccc ctg ctt cga gag gca tcc cat agg ccc ttc aca atg ctg tgg	5325
Pro Leu Leu Arg Glu Ala Ser His Arg Pro Phe Thr Met Leu Trp	
1755 1760 1765	
ccc aca gac gcc gcc ttt cga gct ctg cct ccg gat cgc cag gcc	5370
Pro Thr Asp Ala Ala Phe Arg Ala Leu Pro Pro Asp Arg Gln Ala	
1770 1775 1780	
tgg ctg tac cat gag gac cac cgt gac aag cta gca gcc att ctg	5415
Trp Leu Tyr His Glu Asp His Arg Asp Lys Leu Ala Ala Ile Leu	
1785 1790 1795	
cgg ggc cac atg att cgc aat gtc gag gcc ttg gca tct gac ctg	5460
Arg Gly His Met Ile Arg Asn Val Glu Ala Leu Ala Ser Asp Leu	
1800 1805 1810	
ccc aac ctg gcc cca ctt cga acc atg cat ggg acc ccc atc tct	5505
Pro Asn Leu Gly Pro Leu Arg Thr Met His Gly Thr Pro Ile Ser	
1815 1820 1825	
ttc tcc tgc agc cga acg cgg ccc ggt gag ctc atg gtg ggt gag	5550
Phe Ser Cys Ser Arg Thr Arg Pro Gly Glu Leu Met Val Gly Glu	
1830 1835 1840	
gat gat gct cgc att gtg cag cgg cac ttg ccc ttt gag ggt gcc	5595
Asp Asp Ala Arg Ile Val Gln Arg His Leu Pro Phe Glu Gly Gly	
1845 1850 1855	
ctg gcc tat gcc atc gac cag ctg ctg gag cca cct gcc ctt ggt	5640
Leu Ala Tyr Gly Ile Asp Gln Leu Leu Glu Pro Pro Gly Leu Gly	
1860 1865 1870	
gct cgc tgt gac cac ttt gag acc cgg ccc ctg cga ctg aac acc	5685
Ala Arg Cys Asp His Phe Glu Thr Arg Pro Leu Arg Leu Asn Thr	
1875 1880 1885	
tgc agc atc tgt ggg ctg gag cca ccc tgt cct gag ggg tca cag	5730
Cys Ser Ile Cys Gly Leu Glu Pro Pro Cys Pro Glu Gly Ser Gln	
1890 1895 1900	
gag cag gcc agc cct gag gcc tgc tgg cgc ttc tac ccg aag ttc	5775

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Glu	Gln	Gly	Ser	Pro	Glu	Ala	Cys	Trp	Arg	Phe	Tyr	Pro	Lys	Phe		
				1905					1910					1915		
tgg	acg	tcc	cct	ccg	ctg	cac	tct	ttg	gga	tta	cgc	agc	gtc	tgg	5820	
Trp	Thr	Ser	Pro	Pro	Leu	His	Ser	Leu	Gly	Leu	Arg	Ser	Val	Trp		
				1920					1925					1930		
gtc	cac	ccc	agc	ctt	tgg	ggt	agg	ccc	caa	ggc	ctg	ggc	agg	ggc	5865	
Val	His	Pro	Ser	Leu	Trp	Gly	Arg	Pro	Gln	Gly	Leu	Gly	Arg	Gly		
				1935					1940					1945		
tgc	cac	cgc	aat	tgt	gtc	acc	acc	acc	tgg	aag	ccc	agc	tgc	tgc	5910	
Cys	His	Arg	Asn	Cys	Val	Thr	Thr	Thr	Trp	Lys	Pro	Ser	Cys	Cys		
				1950					1955					1960		
cct	ggt	cac	tat	ggc	agt	gag	tgc	caa	gct	tgc	cct	ggc	ggc	ccc	5955	
Pro	Gly	His	Tyr	Gly	Ser	Glu	Cys	Gln	Ala	Cys	Pro	Gly	Gly	Pro		
				1965					1970					1975		
agc	agc	cct	tgt	agt	gac	cgt	ggc	gtg	tgc	atg	gac	ggc	atg	agt	6000	
Ser	Ser	Pro	Cys	Ser	Asp	Arg	Gly	Val	Cys	Met	Asp	Gly	Met	Ser		
				1980					1985					1990		
ggc	agt	ggg	cag	tgt	ctg	tgc	cgt	tca	ggt	ttt	gct	ggg	aca	gcc	6045	
Gly	Ser	Gly	Gln	Cys	Leu	Cys	Arg	Ser	Gly	Phe	Ala	Gly	Thr	Ala		
				1995					2000					2005		
tgt	gaa	ctc	tgt	gct	cct	ggt	gcc	ttt	ggg	ccc	cat	tgt	caa	gcc	6090	
Cys	Glu	Leu	Cys	Ala	Pro	Gly	Ala	Phe	Gly	Pro	His	Cys	Gln	Ala		
				2010					2015					2020		
tgc	cgc	tgc	act	gtg	cat	ggc	cgc	tgt	gat	gag	ggc	ctt	ggg	ggc	6135	
Cys	Arg	Cys	Thr	Val	His	Gly	Arg	Cys	Asp	Glu	Gly	Leu	Gly	Gly		
				2025					2030					2035		
tct	ggc	tcc	tgc	ttc	tgt	gat	gaa	ggc	tgg	act	ggg	cca	cgc	tgt	6180	
Ser	Gly	Ser	Cys	Phe	Cys	Asp	Glu	Gly	Trp	Thr	Gly	Pro	Arg	Cys		
				2040					2045					2050		
gag	gtg	caa	ctg	gag	ctg	cag	cct	gtg	tgt	acc	cca	ccc	tgt	gca	6225	
Glu	Val	Gln	Leu	Glu	Leu	Gln	Pro	Val	Cys	Thr	Pro	Pro	Cys	Ala		
				2055					2060					2065		
ccc	gag	gct	gtg	tgc	cgt	gca	ggc	aac	agc	tgt	gag	tgc	agc	ctg	6270	
Pro	Glu	Ala	Val	Cys	Arg	Ala	Gly	Asn	Ser	Cys	Glu	Cys	Ser	Leu		
				2070					2075					2080		
ggc	tat	gaa	ggg	gat	ggc	cgc	gtg	tgt	aca	gtg	gca	gac	ctg	tgc	6315	
Gly	Tyr	Glu	Gly	Asp	Gly	Arg	Val	Cys	Thr	Val	Ala	Asp	Leu	Cys		
				2085					2090					2095		
cag	gac	ggg	cat	ggt	ggc	tgc	agt	gag	cac	gcc	aac	tgt	agc	cag	6360	
Gln	Asp	Gly	His	Gly	Gly	Cys	Ser	Glu	His	Ala	Asn	Cys	Ser	Gln		
				2100					2105					2110		
gta	gga	aca	atg	gtc	act	tgt	acc	tgc	ctg	ccc	gac	tac	gag	ggt	6405	
Val	Gly	Thr	Met	Val	Thr	Cys	Thr	Cys	Leu	Pro	Asp	Tyr	Glu	Gly		
				2115					2120					2125		
gat	ggc	tgg	agc	tgc	cgg	gcc	cgc	aac	ccc	tgc	aca	gat	ggc	cac	6450	
Asp	Gly	Trp	Ser	Cys	Arg	Ala	Arg	Asn	Pro	Cys	Thr	Asp	Gly	His		
				2130					2135					2140		
cgc	ggg	ggc	tgc	agc	gag	cac	gcc	aac	tgc	ttg	agc	acc	ggc	ctg	6495	
Arg	Gly	Gly	Cys	Ser	Glu	His	Ala	Asn	Cys	Leu	Ser	Thr	Gly	Leu		
				2145					2150					2155		
aac	aca	cgg	cgc	tgt	gag	tgc	cac	gca	ggc	tac	gta	ggc	gat	gga	6540	
Asn	Thr	Arg	Arg	Cys	Glu	Cys	His	Ala	Gly	Tyr	Val	Gly	Asp	Gly		
				2160					2165					2170		
ctg	cag	tgt	ctg	gag	gag	tcg	gaa	cca	cct	gtg	gac	cgc	tgc	ttg	6585	
Leu	Gln	Cys	Leu	Glu	Glu	Ser	Glu	Pro	Pro	Val	Asp	Arg	Cys	Leu		
				2175					2180					2185		
ggc	cag	cca	ccg	ccc	tgc	cac	tca	gat	gcc	atg	tgc	act	gac	ctg	6630	

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Gly	Gln	Pro	Pro	Pro	Cys	His	Ser	Asp	Ala	Met	Cys	Thr	Asp	Leu		
				2190					2195					2200		
cac	ttc	cag	gag	aaa	cgg	gct	ggc	gtt	ttc	cac	ctc	cag	gcc	acc	6675	
His	Phe	Gln	Glu	Lys	Arg	Ala	Gly	Val	Phe	His	Leu	Gln	Ala	Thr		2215
				2205					2210							
agc	ggc	cct	tat	ggt	ctg	aac	ttt	tcg	gag	gct	gag	gcg	gca	tgc	6720	
Ser	Gly	Pro	Tyr	Gly	Leu	Asn	Phe	Ser	Glu	Ala	Glu	Ala	Ala	Cys		2230
				2220					2225							
gaa	gca	cag	gga	gcc	gtc	ctt	gct	tca	ttc	cct	cag	ctc	tct	gct	6765	
Glu	Ala	Gln	Gly	Ala	Val	Leu	Ala	Ser	Phe	Pro	Gln	Leu	Ser	Ala		2245
				2235					2240							
gcc	cag	cag	ctg	ggc	ttc	cac	ctg	tgc	ctc	atg	ggc	tgg	ctg	gcc	6810	
Ala	Gln	Gln	Leu	Gly	Phe	His	Leu	Cys	Leu	Met	Gly	Trp	Leu	Ala		2260
				2250					2255							
aat	ggc	tcc	act	gcc	cac	cct	gtg	ggt	ttc	cct	gtg	gcg	gac	tgt	6855	
Asn	Gly	Ser	Thr	Ala	His	Pro	Val	Val	Phe	Pro	Val	Ala	Asp	Cys		2275
				2265					2270							
ggc	aat	ggt	cgg	gtg	ggc	gta	gtc	agc	ctg	ggt	gcc	cgc	aag	aac	6900	
Gly	Asn	Gly	Arg	Val	Gly	Val	Val	Ser	Leu	Gly	Ala	Arg	Lys	Asn		2290
				2280					2285							
ctc	tca	gaa	cgc	tgg	gat	gcc	tac	tgc	ttc	cgt	gtg	caa	gat	gtg	6945	
Leu	Ser	Glu	Arg	Trp	Asp	Ala	Tyr	Cys	Phe	Arg	Val	Gln	Asp	Val		2305
				2295					2300							
gcc	tgc	cga	tgc	cga	aat	ggc	ttc	gtg	ggt	gac	ggg	atc	agc	acg	6990	
Ala	Cys	Arg	Cys	Arg	Asn	Gly	Phe	Val	Gly	Asp	Gly	Ile	Ser	Thr		2320
				2310					2315							
tgc	aat	ggg	aag	ctg	ctg	gat	gtg	ctg	gct	gcc	act	gcc	aac	ttc	7035	
Cys	Asn	Gly	Lys	Leu	Leu	Asp	Val	Leu	Ala	Ala	Thr	Ala	Asn	Phe		2335
				2325					2330							
tcc	acc	ttc	tat	ggg	atg	cta	ttg	ggc	tat	gcc	aat	gcc	acc	cag	7080	
Ser	Thr	Phe	Tyr	Gly	Met	Leu	Leu	Gly	Tyr	Ala	Asn	Ala	Thr	Gln		2350
				2340					2345							
cgg	ggt	ctc	gac	ttc	ctg	gac	ttc	ctg	gat	gat	gag	ctc	acg	tat	7125	
Arg	Gly	Leu	Asp	Phe	Leu	Asp	Phe	Leu	Asp	Asp	Glu	Leu	Thr	Tyr		2365
				2355					2360							
aag	aca	ctc	ttc	gtc	cct	gtc	aat	gaa	ggc	ttt	gtg	gac	aac	atg	7170	
Lys	Thr	Leu	Phe	Val	Pro	Val	Asn	Glu	Gly	Phe	Val	Asp	Asn	Met		2380
				2370					2375							
acg	ctg	agt	ggc	cca	gac	ttg	gag	ctg	cat	gcc	tcc	aac	gcc	acc	7215	
Thr	Leu	Ser	Gly	Pro	Asp	Leu	Glu	Leu	His	Ala	Ser	Asn	Ala	Thr		2395
				2385					2390							
ctc	cta	agt	gcc	aac	gcc	agc	cag	ggg	aag	ttg	ctt	ccg	gcc	cac	7260	
Leu	Leu	Ser	Ala	Asn	Ala	Ser	Gln	Gly	Lys	Leu	Leu	Pro	Ala	His		2410
				2400					2405							
tca	ggc	ctc	agc	ctc	atc	atc	agt	gac	gca	ggc	cct	gac	aac	agt	7305	
Ser	Gly	Leu	Ser	Leu	Ile	Ile	Ser	Asp	Ala	Gly	Pro	Asp	Asn	Ser		2425
				2415					2420							
tcc	tgg	ggc	cct	gtg	gcc	cca	ggg	aca	ggt	gtg	ggt	agc	cg	atc	7350	
Ser	Trp	Ala	Pro	Val	Ala	Pro	Gly	Thr	Val	Val	Val	Ser	Arg	Ile		2440
				2430					2435							
att	gtg	tgg	gac	atc	atg	gcc	ttc	aat	ggc	atc	atc	cat	gct	ctg	7395	
Ile	Val	Trp	Asp	Ile	Met	Ala	Phe	Asn	Gly	Ile	Ile	His	Ala	Leu		2455
				2445					2450							
gcc	agc	ccc	ctc	ctg	gca	ccc	cca	cag	ccc	cag	gca	gtg	ctg	gcg	7440	
Ala	Ser	Pro	Leu	Leu	Ala	Pro	Pro	Gln	Pro	Gln	Ala	Val	Leu	Ala		2470
				2460					2465							
cct	gaa	gcc	cca	cct	gtg	gcg	gca	ggc	gtg	ggg	gct	gtg	ctt	gcc	7485	

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Pro	Glu	Ala	Pro	Pro	Val	Ala	Ala	Gly	Val	Gly	Ala	Val	Leu	Ala	
				2475					2480					2485	
gct	gga	gca	ctg	ctt	ggc	ttg	gtg	gcc	gga	gct	ctc	tac	ctc	cgt	7530
Ala	Gly	Ala	Leu	Leu	Gly	Leu	Val	Ala	Gly	Ala	Leu	Tyr	Leu	Arg	
				2490					2495					2500	
gcc	cga	ggc	aag	ccc	acg	ggc	ttt	ggc	ttc	tct	gcc	ttc	cag	gcg	7575
Ala	Arg	Gly	Lys	Pro	Thr	Gly	Phe	Gly	Phe	Ser	Ala	Phe	Gln	Ala	
				2505					2510					2515	
gaa	gat	gat	gct	gac	gac	gac	ttc	tca	ccg	tgg	caa	gaa	ggg	acc	7620
Glu	Asp	Asp	Ala	Asp	Asp	Asp	Phe	Ser	Pro	Trp	Gln	Glu	Gly	Thr	
				2520					2525					2530	
aac	ccc	acc	ctg	gtc	tct	gtc	ccc	aac	cct	gtc	ttt	ggc	agc	gac	7665
Asn	Pro	Thr	Leu	Val	Ser	Val	Pro	Asn	Pro	Val	Phe	Gly	Ser	Asp	
				2535					2540					2545	
acc	ttt	tgt	gaa	ccc	ttc	gat	gac	tca	ctg	ctg	gag	gag	gac	ttc	7710
Thr	Phe	Cys	Glu	Pro	Phe	Asp	Asp	Ser	Leu	Leu	Glu	Glu	Asp	Phe	
				2550					2555					2560	
cct	gac	acc	cag	agg	atc	ctc	aca	gtc	aag	tgacgaggct	ggggctgaaa				7760
Pro	Asp	Thr	Gln	Arg	Ile	Leu	Thr	Val	Lys						
				2565					2570						
gcagaagcat	gcacagggag	gagaccactt	ttattgcttg	tctgggtgga	tggggcagga										7820
ggggctgagg	gcctgtccca	gacaataaag	tgccctcagc	ggatgtgggc	catgtcacc										7879

What is claimed is:

1. A purified lymphatic endothelial glycoprotein, CLEVER-1, having a molecular weight of 270-300 kD in SDS-PAGE under non-reducing conditions, recognisable by a monoclonal antibody selected from the group consisting of

- (a) monoclonal antibody, DSM ACC 2519; and
- (b) monoclonal antibody, DSM ACC 2590.

2. The glycoprotein according to claim 1, encoded by a nucleic acid selected from the group of

- (a) sequence of SEQ ID NO: 1;
- (b) sequence of SEQ ID NO: 1 lacking nucleotides 2377-2562; and
- (c) sequence of SEQ ID NO: 1 lacking nucleotides 2914-3009.

3. A CLEVER-1 antibody.

4. The CLEVER-1 antibody of claim 3, which is monoclonal antibody 3-266 (DSM ACC2519).

5. The CLEVER-1 antibody of claim 3, which is monoclonal antibody 3-372 (DSM ACC2590).

6. A cell-free preparation comprising CLEVER-1.

7. A method of diagnosing inflammatory diseases in a patient, said method comprising:

- (a) exposing a blood or tissue sample from said patient to CLEVER-1 in vitro for a period of time and under conditions sufficient to allow binding of leukocytes if present in said sample; and
- (b) detecting leukocytes bound to said CLEVER-1 in said blood or tissue sample.

8. A method of detecting malignant cells in a patient, said method comprising:

(a) exposing a blood or tissue sample from a patient to CLEVER-1 in vitro for a period of time and under conditions sufficient to allow binding of said malignant cells if present in said sample; and

(b) detecting whether any malignant cells bound to said CLEVER-1 in said blood or tissue sample.

9. The method of claim 8, wherein said CLEVER-1 is on a solid support.

10. The method of claim 8, wherein said CLEVER-1 is provided on lymphoid tissue.

11. The method of claim 8, wherein said CLEVER-1 is present in the membrane of endothelial cells.

12. The method of claim 8, wherein said CLEVER-1 is in a soluble form.

13. The method of claim 8, wherein said detection step is performed by imaging.

14. A method of identifying an agent that inhibits the binding of CLEVER-1 to cells, said method comprising:

(a) providing an agent to cells in the presence of CLEVER-1; and

(b) comparing the binding of CLEVER-1 to cells in (a) to binding of CLEVER-1 in the absence of said agent.

15. A method of identifying an agent that stimulates the binding of CLEVER-1 to cells, said method comprising:

(a) providing an agent to cells in the presence of CLEVER-1; and

(b) comparing the binding of CLEVER-1 to cells in (A) to binding of CLEVER-1 in the absence of said agent.

16. A method of removing malignant cells from a sample, said method comprising:

(a) exposing said malignant cells to CLEVER-1 in vitro for a period of time and under conditions sufficient to allow binding of said malignant cells if present in said sample, and

(b) separating said CLEVER-1 and the malignant cells that adhere thereto from said sample.

17. A method of treating inflammation in a patient in need of the same, said method comprising administering an agent that inhibits CLEVER-1 mediated leukocyte binding to said patient, wherein said inhibiting agent is selected from the group comprising:

(a) CLEVER-1 antibodies or fragments thereof; and

(b) soluble CLEVER-1 or fragments thereof.

18. A method of preventing metastasis in a patient in need of the same, said method comprising administering an agent that inhibits CLEVER-1 mediated malignant cell binding to said patient, wherein said inhibiting agent is selected from the group comprising:

(a) CLEVER-1 antibodies or fragments thereof; and

(b) soluble CLEVER-1 or fragments thereof.

19. The method of claim 18, wherein said CLEVER-1 mediated cell binding inhibits leukocyte binding.

20. The method of claim 18, wherein said CLEVER-1 mediated cell binding inhibits lymphocyte binding.

21. The method of claim 18, wherein said CLEVER-1 mediated cell binding inhibits monocyte binding.

22. The method of claim 18, wherein said CLEVER-1 mediated cell binding inhibits granulocyte binding.

23. The method of claim 18, wherein said CLEVER-1 mediated cell binding inhibits malignant cell binding.

24. The method of claim 18, wherein said CLEVER-1 binding agent is administered to a patient in need of an inhibition of CLEVER-1 mediated cell binding.

25. The method of claim 24, wherein said patient is in need of treatment of inflammation.

26. The method of claim 24, wherein said patient is in need of treatment for a malignancy or possible malignancy.

27. A method of stimulating CLEVER-1 binding in a patient in need of the same, said method comprising: administering an agent that stimulates CLEVER-1 mediated leukocyte binding to said patient.

* * * * *

专利名称(译)	常见的淋巴管内皮和血管内皮受体-1 (聪明-1) 及其用途		
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摘要(译)

描述了一种新的蛋白质共同淋巴内皮和血管内皮细胞受体-1 (CLEVER-1)。CLEVER-1介导白细胞和恶性细胞与血管和淋巴内皮细胞的结合。CLEVER-1是第一种报道介导淋巴结流入和流出的蛋白质。还提供了通过提供CLEVER-1结合抑制剂来治疗炎症和预防恶性细胞转移的方法。

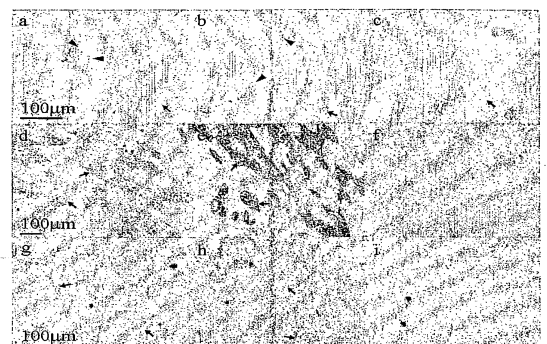


Fig. 1