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

(57) Abstract: A novel protein Common Lymphatic Endothelial and Vascular 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.

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

## BACKGROUND OF THE INVENTION

### Field of the Invention

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.

### Background Art

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.

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.

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.

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.

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

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, **C**ommon **L**ymphatic **E**ndothelial and **V**ascular **E**ndothelial **R**eceptor-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.

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

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

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.

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.

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.

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.

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.

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.

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

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 vessles (lymphatic sinusoids that belong to the efferent lymphatic system) within the lymph node. In FIGS. 1g and 1h, the arrows point to HEV.

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).

FIGS. 3A and 3B. CLEVER-1 is involved in lymphocyte binding to endothelial ells 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 ± SEM.

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 ± SEM.

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).

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)±SEM.

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 neuraminadase 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 negaive (lane 17). The upper band represents the standard form and the lower one is the splice variant of CLEVER-1.

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).

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 Genebank 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

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.

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

The term "effective amount" refers to that amount of the indicated agent that is sufficient to achieve the desired effect.

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.

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.

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.

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.

By "systemic vasculature" is meant the vascular network of blood vessels throughout the body of an animal or human.

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 axiliary 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.

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).

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.

By "efferent lymphatics" meant the vessels that drain the lymph nodules (nodes).

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.

The lymphocytes then circulate throughout the body in the bloodstream. 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.

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 al-

lows for subsequent emigration of the cells into the surrounding tissues (extravasation).

**The Identification and Purification of CLEVER-1**

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.

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.

Two of the hybridomas produced antibodies (designated 3-266 and 3-372) tat 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 August 21, 2001, with DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig.

In molecular weight determinations by immunoblotting, both antibodies 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).

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.

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.

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).

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 fibornectin. In addition, CLEVER-1 has seven fascicling 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.

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.

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.

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).

**CLEVER-1 Mediated Binding of Leukocytes**

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.

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.

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.

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.

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.

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.

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.

**CLEVER-1 Mediated Cell Binding to Malignant Cells**

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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 establish-

ing 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.

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.

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.

**Agents that Block or Inhibit CLEVER-1 Mediated Cell Binding**

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.

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.

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.

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.

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.

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, non-covalent 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 recog-

nize and bind antigen, although at a lower affinity than the entire binding site. *See*, Ladner *et al.*, U.S. Patent No. 4,946,778, and Bird, R.E. *et al., Science, 242*:423-426 (1988).

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. Patent No. 4,816,567).

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. Patent 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).

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. Patent 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. Patent No. 4,816,567; Cabilly *et al.*, EP 0 125 023 B1; Boss *et al.*, U.S. Patent 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. Patent No. 5,225,539; Winter, EP 0239400 B1; and Queen *et al.*, U.S. Patent 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.

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.

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. Patent No. 4,699,880.

**In Vitro Adhesion Assay and Diagnostic Uses Thereof**

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.

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.

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.

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.

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.

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.

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.

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.

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

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.

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 pre-incubated with lymph node sections (a non-competitive assay) or simultaneously added with lymphocytes to the lymph node sections (a competitive assay).

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.

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,

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.

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.

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 fluoroimager.

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.

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.

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.

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.

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.

**Therapeutic Uses of CLEVER-1 Antagonists**

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.

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, ostheoarthritis), 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.

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.

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.

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.

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.

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

The antagonists can be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

**Formulations of Compounds**

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.

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.

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.

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.

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, bydroxymethylcellulose 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).

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

EXAMPLE 1

**Production of Monoclonal Antibodies**

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 q 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.

Immunoperoxidase stainings were performed as described (Salmi, *Science 257*:1407-1409 (1992)). Briefly, acetone fixed 6 μm 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.

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 Figure 1. Figure 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. Figures 1a-1c are from the skin. Figures 1d-1i are from a lymph node. Figures 1a, 1d and 1g show the staining with monoclonal antibody 3-266, Figures 1b, 1e and 1h show the staining with monoclonal antibody 3-372 and Figures 1c, 1f and 1i show the staining with a negative con-

trol antibody, 3G6. In Figures 1a, 1b and 1c, the arrows point to the epithelium and arrowheads to afferent lymphatics. In Figures 1d and 1e, the arrows point to the lymphatic vessels (lymphatic sinusoids that belong to the efferent lymphatic system) within the lymph node. In Figures 1g and 1h, the arrows point to HEV.

EXAMPLE 2

**Determination of Molecular Weight of CLEVER-1**

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).

Both antibodies recognized a molecule of the same size (about 270-300 kDa; Figure 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

**Purification and Molecular Characterization of CLEVER-1**

The molecule recognized by 3-372 antibody was purified from human lymph node lysate overnight (lysis buffer: 150 mM NaCl, 10 mM Tris-base, 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/ ucsfhtml3.2/msfit.htm) of the University of California, San Francisco mass spectrometry facility.

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 (10% of the 2570 amino acids of stabilin-1) and spanned the amino acids between 53 an 2301.

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.

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 (Figure 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.

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

**In Vitro Adhesion Assay**

Lymph node sections were first incubated with 3-266, 3-372 or control antibodies against human HLA ABC (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).

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 (Figure 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 (Figure 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.

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 (Figure 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 (Figures 4A and 3).

EXAMPLE 5

**CLEVER-1 is Upregulated at Sites of Inflammation on HEV-like Vessels**

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 (Figure 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

**Clever-1 Also Mediates Binding of Monocytes and Granulocytes to HEV-like Vessels**

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 (Figure 6).

EXAMPLE 7

**CLEVER-1 controls lymphocyte trafficking *in vivo***

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 sacrification (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.

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 sinuses when tested at sacrification 3 days af-

ter the final 3-372 dose. No signal was detected in rabbits which received a control antibody (data not shown).

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)SGFSFSR(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)WDAYCFR(V) | |
| 1104.54 | 1104.526 | 12.6406 | 53 | 61 | (K)QTCPSGWLR(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)ILLGPEGVPLQR(V) | |
| 1330.63 | 1330.575 | 41.7401 | 953 | 965 | (R)AGNGGCHGLATCR(A) | |
| 1330.63 | 1330.633 | -1.9754 | 1873 | 1882 | (R)CDHFETRPLR(L) | |
| 1374.66 | 1374.632 | 20.1117 | 62 | 72 | (R)ELPDQITQDCR(Y) | |
| 1456.79 | 1456.776 | 9.6229 | 1069 | 1082 | (R)LGGQEVATLNPTTR(W) | |
| 1493.8 | 1493.796 | 2.4215 | 508 | 521 | (R)TIGQILASTEAFSR(F) | |
| 1555.7 | 1555.663 | 23.5678 | 219 | 231 | (R)CLPGYTQQGSECR(A) | |
| 1678.94 | 1678.913 | 16.1953 | 1802 | 1817 | (R)NVEALASDLPNLGPLR(T) | |
| 1730.89 | 1730.887 | 1.9674 | 1054 | 1068 | (R)AHFLQGALFEEELAR(L) | |
| 1912.82 | 1912.832 | -6.3742 | 936 | 952 | (K)LGFAGDGYQCSPIDPCR(A) | |
| 2057.05 | 2057.03 | 9.5484 | 1655 | 1673 | (R)SEDLLEQGYATALSGHPLR(F) | |
| 2165.11 | 2165.14 | -13.6320 | 1707 | 1725 | (R)VLLPPEALHWEPDDAPIPR(R) | |
| 2295.22 | 2295.224 | -1.5853 | 389 | 410 | (R)EILTTAGPFTVLVPSVSSFSSR(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

**Table 2. CLEVER-1 Expression Is Induced Mainly on Vessels Surrounded by Lymphocytic Infiltrations in Inflamed Synovia**

| | The degree of inflammatory infiltration[2] | |
|---|---|---|
| Expression of CLEVER-1 on HEV-like vessels[1] | 0/1 (n=9) | 2/3 (n=9) |
| -/+ | 100% | 0 |
| ++/+++ | 0 | 100% |

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

[2] Degree 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.

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.

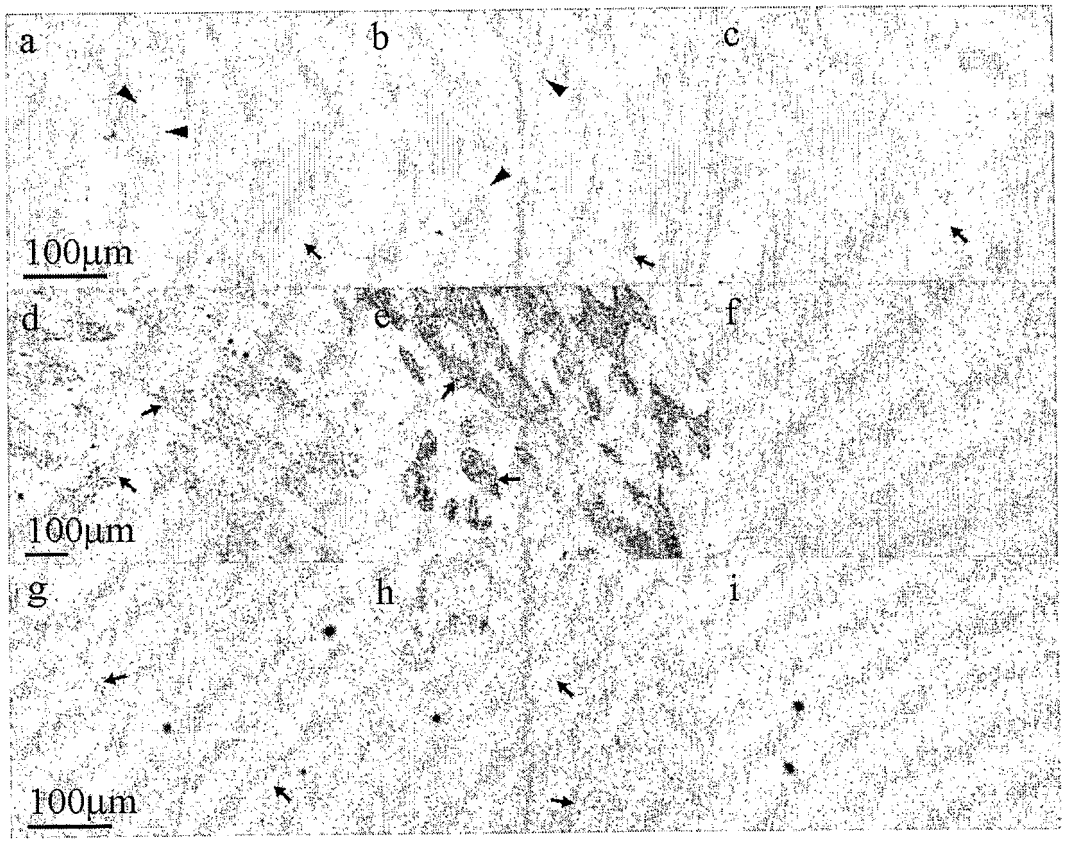



Fig. 1

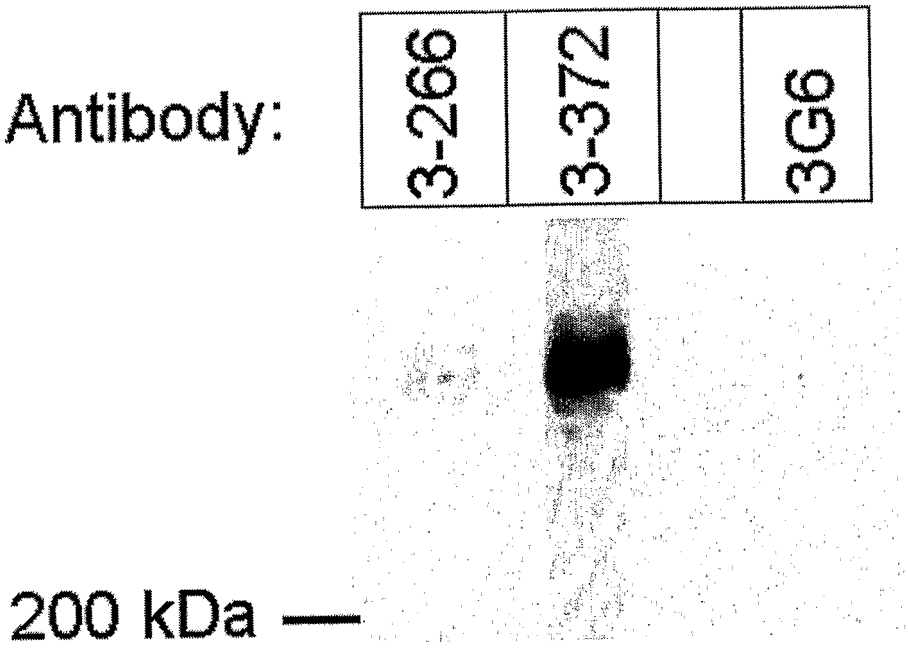
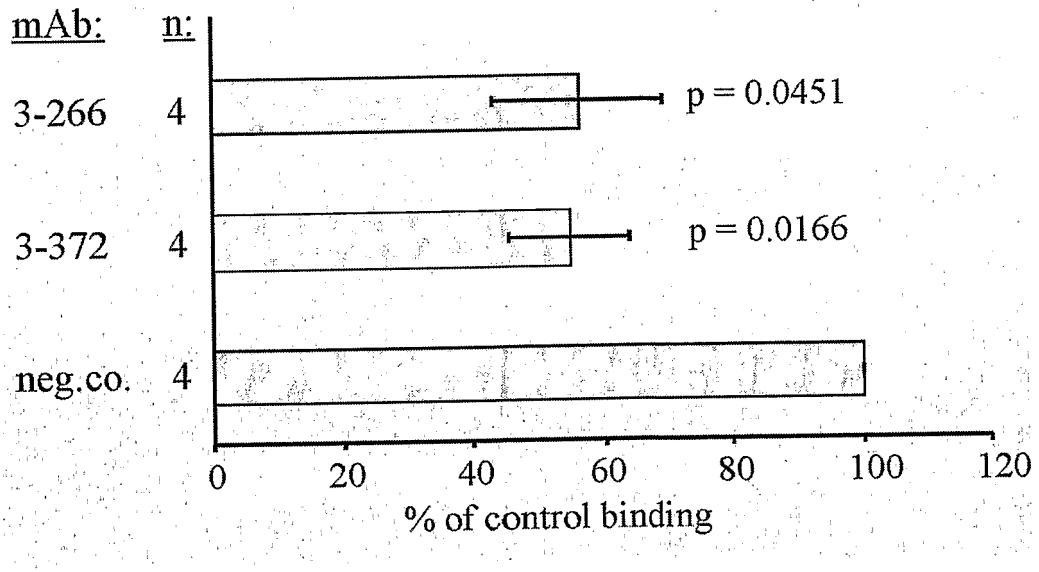



*Fig. 2*

A **Lymphocyte binding to HEVs:**




B **Lymphocyte binding to lymph endothelium:**

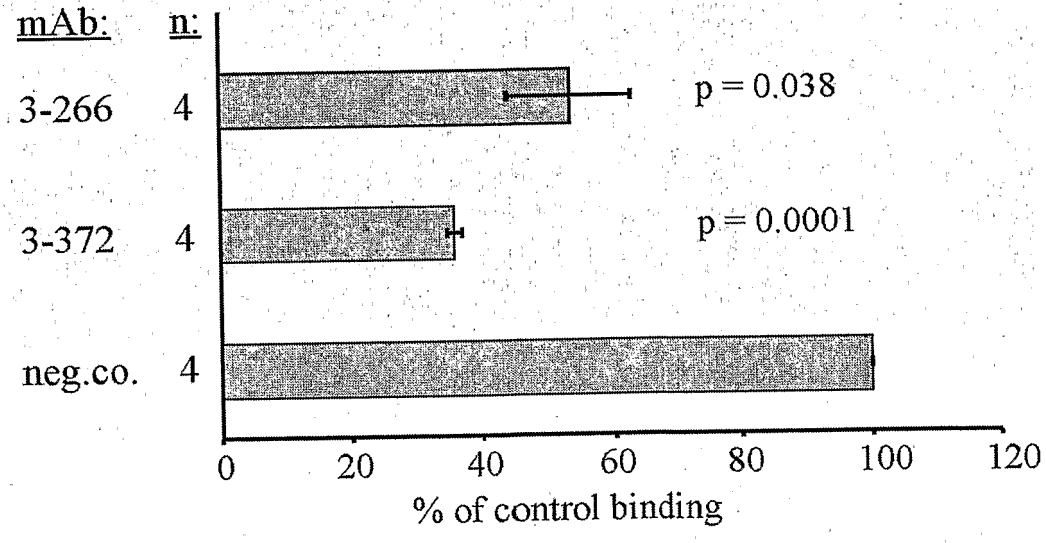
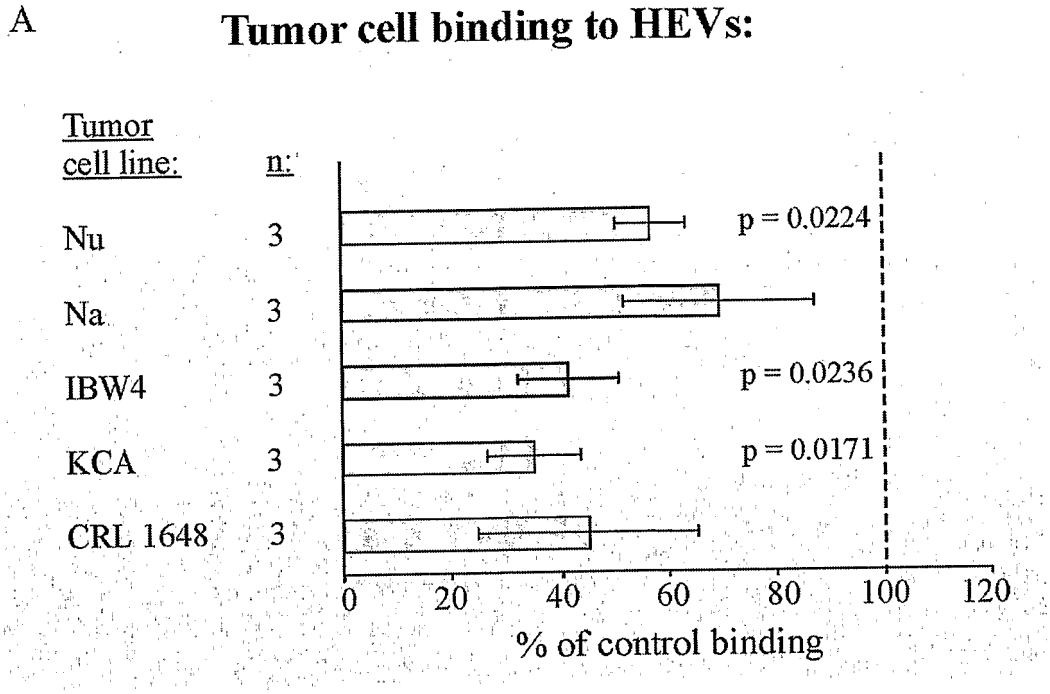



*Fig. 3*

A


Tumor cell binding to HEVs:
Tumor cell line:
n:
Nu 3
Na 3
IBW4 3
KCA 3
CRL 1648 3
p = 0.0224
p = 0.0236
p = 0.0171
0 20 40 60 80 100 120
% of control binding

B

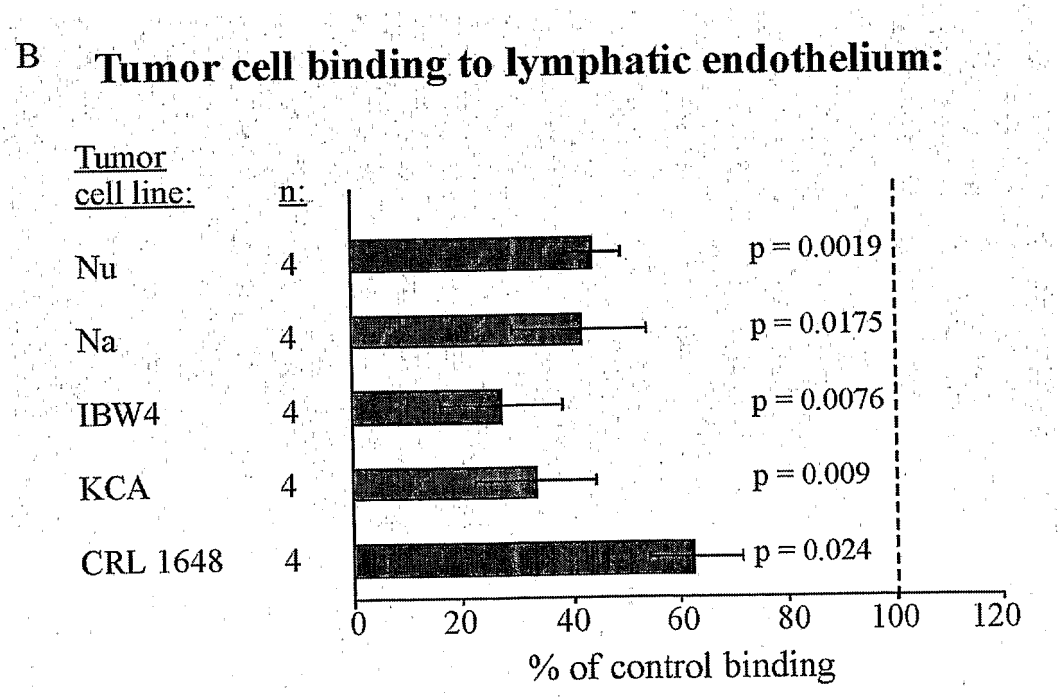

Tumor cell binding to lymphatic endothelium:
Tumor cell line:
n:
Nu 4
Na 4
IBW4 4
KCA 4
CRL 1648 4
p = 0.0019
p = 0.0175
p = 0.0076
p = 0.009
p = 0.024
0 20 40 60 80 100 120
% of control binding

*Fig. 4*

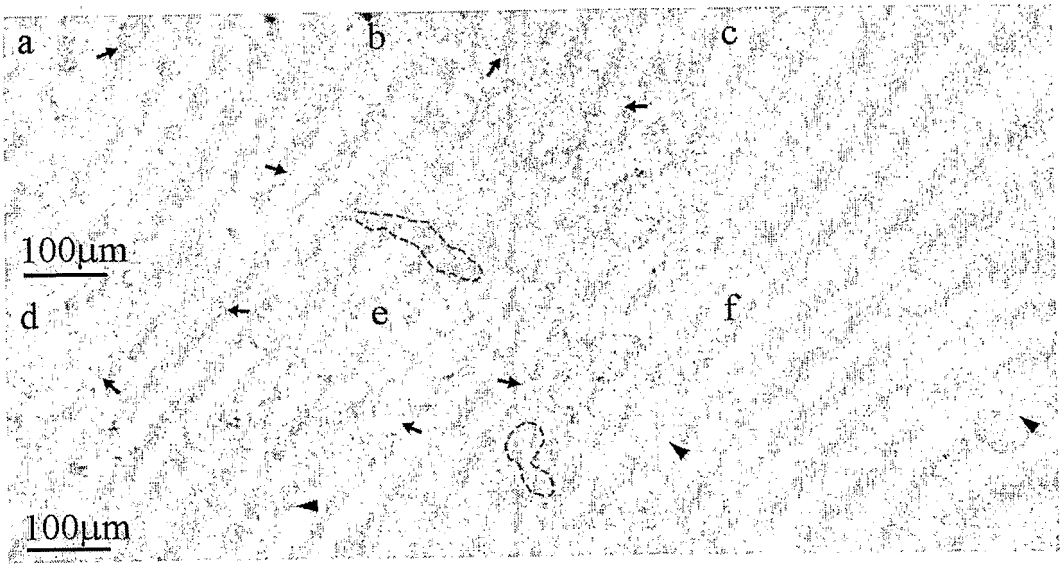



Fig. 5

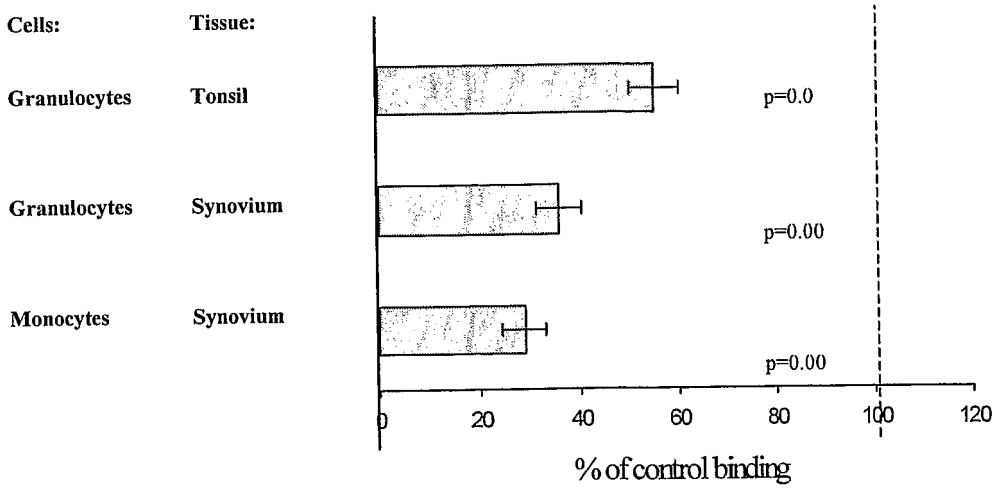

Cells:
Tissue:
Granulocytes
Tonsil
Granulocytes
Synovium
Monocytes
Synovium
p=0.0
p=0.00
p=0.00
0
20
40
60
80
100
120
% of control binding

*Fig. 6*

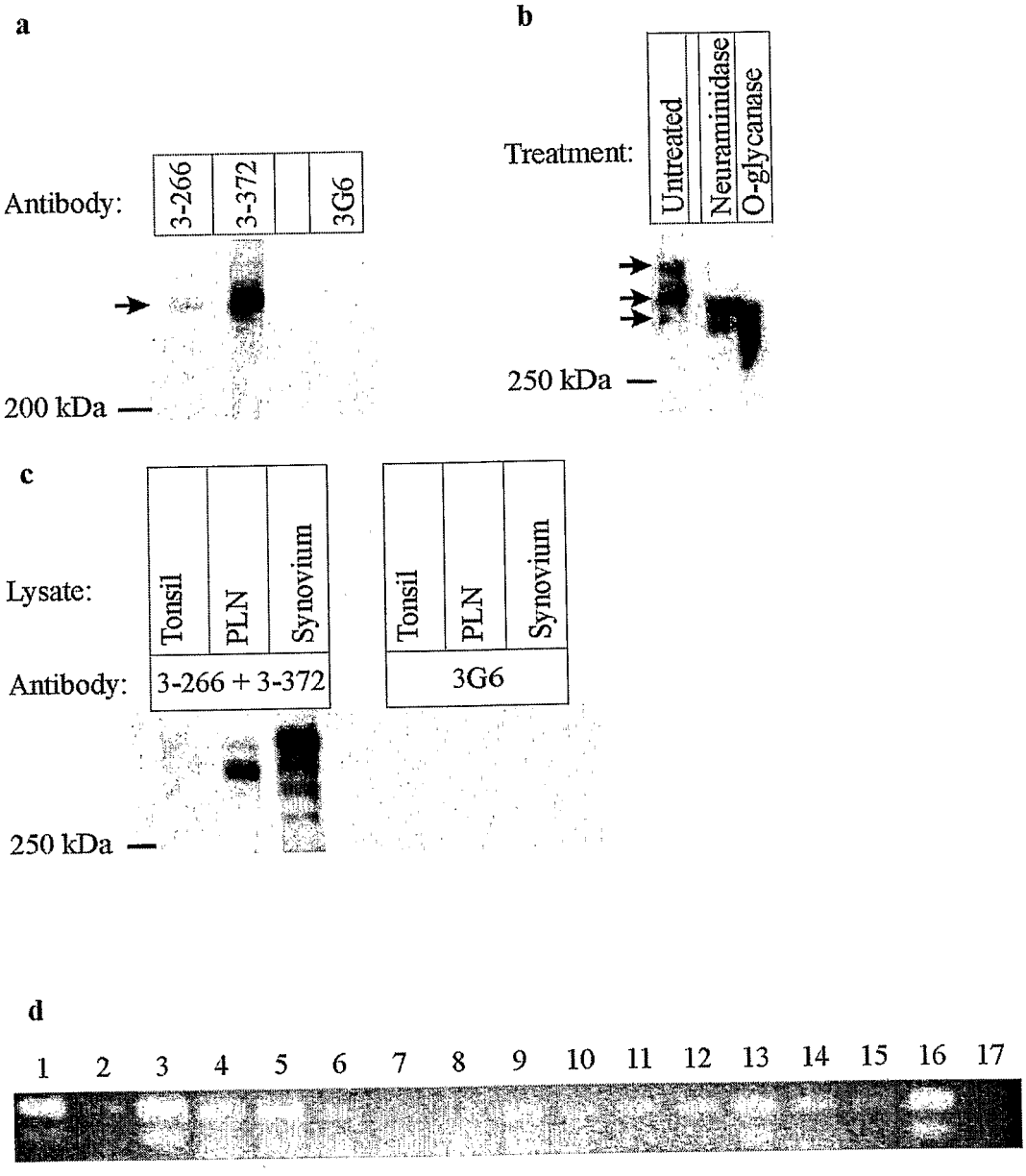
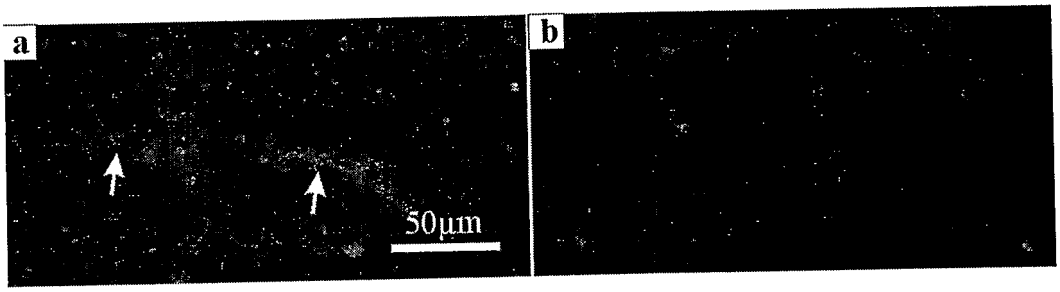
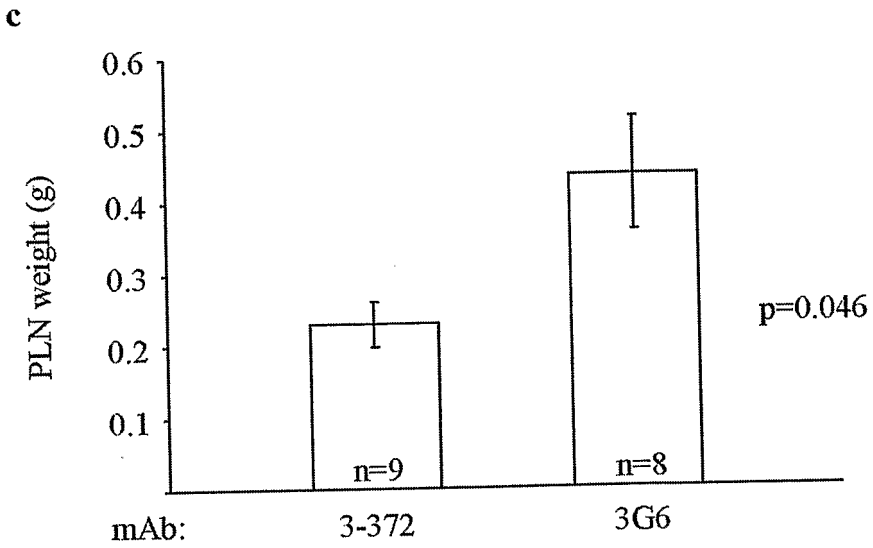

a
Antibody:
3-266
3-372
3G6
200 kDa
b
Treatment:
Untreated
Neuraminidase
O-glycanase
250 kDa
c
Lysate:
Tonsil
PLN
Synovium
Antibody:
3-266 + 3-372
Tonsil
PLN
Synovium
3G6
250 kDa
d
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

*Fig. 7*


a
b
50µm


c
0.6
0.5
0.4
0.3
0.2
0.1
PLN weight (g)
p=0.046
n=9
n=8
mAb:
3-372
3G6

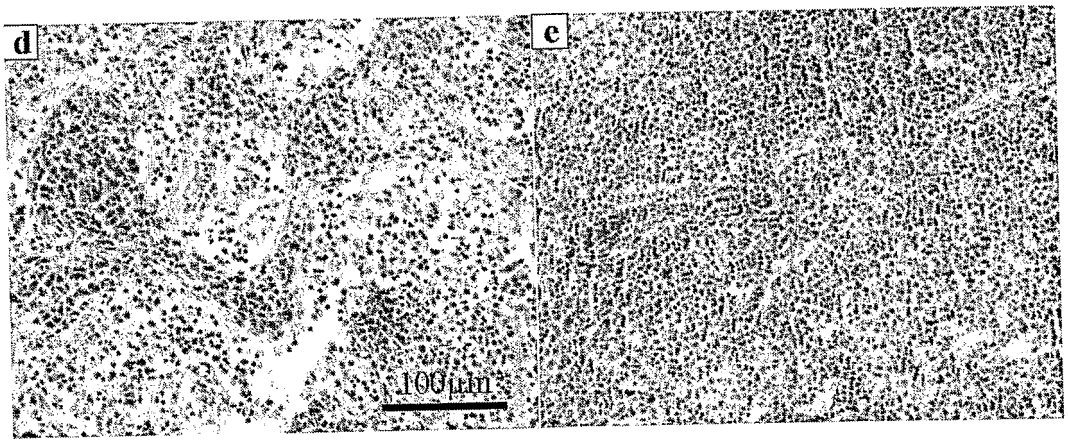

d
e
100µm

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 tcg 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
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tcc agg acc atg aat gca tcc ctt gcc cag cag ctc tgt aga cag cac     1302
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*Fig. 9*

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ttc tcc ttc tcc cgg  ggc tgc tct tac aca  tgt gcc aag aag atc     3975
cag gtg ccg gac tgc  tgc cct ggt ttc ttt  ggc acg ctg tgt gag     4020
cca tgc cca ggg ggt  cta ggg ggg gtg tgc  tca ggc cat ggg cag     4065
tgc cag gac agg ttc  ctg ggc agc ggg gag  tgc cac tgc cac gag     4110
ggc ttc cat gga acg  gcc tgt gag gtg tgt  gag ctg ggc cgc tac     4155
ggg ccc aac tgc acc  gga gtg tgt gac tgt  gcc cat ggg ctg tgc     4200
cag gag ggg ctg caa  ggg gac gga agc tgt  gtc tgt aac gtg ggc     4245
tgg cag ggc ctc cgc  tgt gac cag aaa atc  acc agc cct cag tgc     4290
cct agg aag tgc gac  ccc aat gcc aac tgc  gtg cag gac tcg gcc     4335
gga gcc tcc acc tgc  gcc tgt gct gcg gga  tac tcc ggc aat ggc     4380
atc ttc tgt tca gag  gtg gac ccc tgc gcc  cac ggc cat ggg ggc     4425
tgc tcc cct cat gcc  aac tgt acc aag gtg  gca cct ggg cag cgg     4470
aca tgc acc tgc cag  gat ggc tac atg ggc  gac ggg gag ctg tgc     4515
cag gaa att aac agc  tgt ctc 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 ctc     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
aca gcc cac acc gtg  ggg gac ggc ctc acc  tgc cgt gcc cga gtc      4785
ggc ctg gag ctc ctg  agg gat aag cat gcc  tca ttc ttc agc ctc      4830
cgc ctc ctg gaa tat  aag gag ctc aag ggc  gat ggg cct ttc acc      4875
atc ttc gtg ccg cac  gca gat cta atg agc  aac ctg tcg cag gat      4920
gag ctg gcc cgg att  cgt gcg cat cgc cag  ctg gtg ttt cgc tac      4965
cac gtg gtt ggc tgt  cgg cgg ctg cgg agc  gag gac ctg ctg gag      5010
cag ggg tac gcc acg  gcc ctc tca ggg cac  cca ctg cgc ttc agc      5055
gag agg gag ggc agc  ata tac ctc aat gac  ttc gcg cgc gtg gtg      5100
agc agc gac cat gag  gcc gtg aac ggc atc  ctg cac ttc att gac      5145
cgt gtc ctg ctg ccc  ccc gag gcg ctg cac  tgg gag cct gat gat      5190
gct ccc atc ccg agg  aga aat gtc acc gcc  gcc gcc cag ggc ttc      5235
ggt tac aag atc ttc  agc ggc ctc ctg aag  gtg gcc ggc ctc ctg      5280
ccc ctg ctt cga gag  gca tcc cat agg ccc  ttc aca atg ctg tgg      5325
ccc aca gac gcc gcc  ttt cga gct ctg cct  ccg gat cgc cag gcc      5370
tgg ctg tac cat gag  gac cac cgt gac aag  cta gca gcc att ctg      5415
cgg ggc cac atg att  cgc aat gtc gag gcc  ttg gca tct gac ctg      5460
ccc aac ctg ggc cca  ctt cga acc atg cat  ggg acc ccc atc tct      5505
ttc tcc tgc agc cga  acg cgg ccc ggt gag  ctc atg gtg ggt gag      5550
gat gat gct cgc att  gtg cag cgg cac ttg  ccc ttt gag ggt ggc      5595
ctg gcc tat ggc atc  gac cag ctg ctg gag  cca cct ggc ctt ggt      5640
gct cgc tgt gac cac  ttt gag acc cgg ccc  ctg cga ctg aac acc      5685
tgc agc atc tgt ggg  ctg gag cca ccc tgt  cct gag ggg tca cag      5730
gag cag ggc agc cct  gag gcc tgc tgg cgc  ttc tac ccg aag ttc      5775
tgg acg tcc cct ccg  ctg cac tct ttg gga  tta cgc agc gtc tgg      5820
gtc cac ccc agc ctt  tgg ggt agg ccc caa  ggc ctg ggc agg ggc      5865
tgc cac cgc aat tgt  gtc acc acc acc tgg  aag ccc agc tgc tgc      5910
cct ggt cac tat ggc  agt gag tgc caa gct  tgc cct ggc ggc ccc      5955
agc agc cct tgt agt  gac cgt ggc gtg tgc  atg gac ggc atg agt      6000
ggc agt ggg cag tgt  ctg tgc cgt tca ggt  ttt gct ggg aca gcc      6045
tgt gaa ctc tgt gct  cct ggt gcc ttt ggg  ccc cat tgt caa gcc      6090
tgc cgc tgc act gtg  cat ggc cgc tgt gat  gag ggc ctt ggg ggc      6135
tct ggc tcc tgc ttc  tgt gat gaa ggc tgg  act ggg cca cgc tgt      6180
gag gtg caa ctg gag  ctg cag cct gtg tgt  acc cca ccc tgt gca      6225
ccc gag gct gtg tgc  cgt gca ggc aac agc  tgt gag tgc agc ctg      6270
ggc tat gaa ggg gat  ggc cgc gtg tgt aca  gtg gca gac ctg tgc      6315
cag gac ggg cat ggt  ggc tgc agt gag cac  gcc aac tgt agc cag      6360
gta gga aca atg gtc  act tgt acc tgc ctg  ccc gac tac gag ggt      6405
gat ggc tgg agc tgc  cgg gcc cgc aac ccc  tgc aca gat ggc cac      6450
cgc ggg ggc tgc agc  gag cac gcc aac tgc  ttg agc acc ggc ctg      6495
aac aca cgg cgc tgt  gag tgc cac gca ggc  tac gta ggc gat gga      6540
ctg cag tgt ctg gag  gag tcg gaa cca cct  gtg gac cgc tgc ttg      6585
ggc cag cca ccg ccc  tgc cac tca gat gcc  atg tgc act gac ctg      6630
cac ttc cag gag aaa  cgg gct ggc gtt ttc  cac ctc cag gcc acc      6675
agc ggc cct tat ggt  ctg aac ttt tcg gag  gct gag gcg gca tgc      6720
gaa gca cag gga gcc  gtc ctt gct tca ttc  cct cag ctc tct gct      6765
gcc cag cag ctg ggc  ttc cac ctg tgc ctc  atg ggc tgg ctg gcc      6810
aat ggc tcc act gcc  cac cct gtg gtt ttc  cct gtg gcg gac tgt      6855
ggc aat ggt cgg gtg  ggc gta gtc agc ctg  ggt gcc cgc aag aac      6900
ctc tca gaa cgc tgg  gat gcc tac tgc ttc  cgt gtg caa gat gtg      6945
gcc tgc cga tgc cga  aat ggc ttc gtg ggt  gac ggg atc agc acg      6990
```

*Fig. 9 (cont.)*

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tgc 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
cgg ggt ctc gac ttc  ctg gac ttc ctg gat  gat gag ctc acg tat      7125
aag aca ctc ttc gtc  cct gtc aat gaa ggc  ttt gtg gac aac atg      7170
acg ctg agt ggc cca  gac ttg gag ctg cat  gcc tcc aac gcc acc      7215
ctc cta agt gcc aac  gcc agc cag ggg aag  ttg ctt ccg gcc cac      7260
tca ggc ctc agc ctc  atc atc agt gac gca  ggc cct gac aac agt      7305
tcc tgg gcc cct gtg  gcc cca ggg aca gtt  gtg gtt agc cgt atc      7350
att gtg tgg gac atc  atg gcc ttc aat ggc  atc atc cat gct ctg      7395
gcc agc ccc ctc ctg  gca ccc cca cag ccc  cag gca gtg ctg gcg      7440
cct gaa gcc cca cct  gtg gcg gca ggc gtg  ggg gct gtg ctt gcc      7485
gct gga gca ctg ctt  ggc ttg gtg gcc gga  gct ctc tac ctc cgt      7530
gcc cga ggc aag ccc  acg ggc ttt ggc ttc  tct gcc ttc cag gcg      7575
gaa gat gat gct gac  gac gac ttc tca ccg  tgg caa gaa ggg acc      7620
aac ccc acc ctg gtc  tct gtc ccc aac cct  gtc ttt ggc agc gac      7665
acc ttt tgt gaa ccc  ttc gat gac tca ctg  ctg gag gag gac ttc      7710
cct gac acc cag agg  atc ctc aca gtc aag  tgacgaggct ggggctgaaa    7760
gcagaagcat gcacagggag gagaccactt ttattgcttg tctgggtgga tggggcagga 7820
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*Fig. 9 (cont.)*

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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 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 ggc tca gcc tgc cag gag tgc caa gac      486
Val Cys Gln Glu Asn Phe Arg Gly Ser Ala Cys Gln Glu Cys Gln Asp
            140                 145                 150

ccc aac cgg ttc ggg cct gac tgc caa tcg gtg tgc agc tgt gtg cac      534
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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

ggt 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
Arg Arg Trp Trp Thr Leu Ala Gly Gln Glu Ile Thr Val Thr Phe Asn
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
505 510 515 520

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
585 590 595 600

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
620 625 630

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
635 640 645

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
650 655 660

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
665 670 675 680

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

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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                 720                 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                 735                 740

aat tgc agt gat ggg atc cag ggc aat ggg gcc tgc ctc tgc ttc cca      2310
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      2358
Asp Tyr Lys Gly Ile Ala Cys His Ile Cys Ser Asn Pro Asn Lys His
                765                 770                 775

gga gag caa tgc cag gaa gac tgc ggc tgt gtc cat ggt ctc tgc gac      2406
Gly Glu Gln Cys Gln Glu Asp Cys Gly Cys Val His Gly Leu Cys Asp
            780                 785                 790

aac cgc cca ggc agt ggg ggg gtg tgc cag cag ggc acg tgt gcc cct      2454
Asn Arg Pro Gly Ser Gly Gly Val Cys Gln Gln Gly Thr Cys Ala Pro
        795                 800                 805

ggc ttc agt ggc cgg ttc tgc aac gag tcc atg ggg gac tgt ggg ccc      2502
Gly Phe Ser Gly Arg Phe Cys Asn Glu Ser Met Gly Asp Cys Gly Pro
    810                 815                 820

aca ggg ctg gcc cag cac tgc cac ctg cat gcc cgc tgt gtt agc cag      2550
Thr Gly Leu Ala Gln His Cys His Leu His Ala Arg Cys Val Ser Gln
825                 830                 835                 840

gag ggt gtt gcc aga tgt cgc tgt ctt gat ggc ttt gag ggt gat ggc      2598
Glu Gly Val Ala Arg Cys Arg Cys Leu Asp Gly Phe Glu Gly Asp Gly
                845                 850                 855

ttc tcc tgc aca cct agc aac ccc tgc tcc cac ccg gac cgt gga ggc      2646
Phe Ser Cys Thr Pro Ser Asn Pro Cys Ser His Pro Asp Arg Gly Gly
            860                 865                 870

tgc tca gag aat gct gag tgt gtc cct ggg tcc ctg ggc acc cac cac      2694
Cys Ser Glu Asn Ala Glu Cys Val Pro Gly Ser Leu Gly Thr His His
        875                 880                 885

tgc aca tgc cac aaa ggc tgg agt ggg gat ggc cgc gtc tgt gtg gct      2742
Cys Thr Cys His Lys Gly Trp Ser Gly Asp Gly Arg Val Cys Val Ala
    890                 895                 900

att gac gag tgt gag ctg gac gtg aga ggt ggc tgc cac acc gat gcc      2790
Ile Asp Glu Cys Glu Leu Asp Val Arg Gly Gly Cys His Thr Asp Ala
905                 910                 915                 920

ctc tgc agc tat gtg ggc ccc ggg cag agc cga tgc acc tgc aag ctg      2838
Leu Cys Ser Tyr Val Gly Pro Gly Gln Ser Arg Cys Thr Cys Lys Leu
                925                 930                 935

ggc ttt gcc ggg gat ggc tac cag tgc agc ccc atc gac ccc tgc cgg      2886
Gly Phe Ala Gly Asp Gly Tyr Gln Cys Ser Pro Ile Asp Pro Cys Arg
            940                 945                 950

gca ggc aat ggc ggc tgc cac ggc ctg gcc acc tgc cgg gca gtg ggg      2934
Ala Gly Asn Gly Gly Cys His Gly Leu Ala Thr Cys Arg Ala Val Gly
        955                 960                 965

gga ggt cag cgg gtc tgc acg tgc ccc cct ggc ttt ggg ggt gat ggc      2982
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Phe Ser Cys Tyr Gly Asp Ile Phe Arg Glu Leu Glu Ala Asn Ala His
985 990 995 1000

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Phe Ser Ile Phe Tyr Gln Trp Leu Lys Ser Ala Gly Ile Thr Leu
1005 1010 1015

cct gcc gac cgc cga gtc aca gcc ctg gtg ccc tcc gag gct gca 3120
Pro Ala Asp Arg Arg Val Thr Ala Leu Val Pro Ser Glu Ala Ala
1020 1025 1030

gtc cgt cag ctg agc ccc gag gac cga gct ttc tgg ctg cag cca 3165
Val Arg Gln Leu Ser Pro Glu Asp Arg Ala Phe Trp Leu Gln Pro
1035 1040 1045

agg acg ctg ccg aac ctg gtc agg gcc cat ttt ctc cag ggt gcc 3210
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 ctc 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

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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 gct  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 ggc cat ggg cag        4065
Pro Cys Pro Gly Gly  Leu Gly Gly Val Cys  Ser Gly His Gly Gln
                1335                1340                1345

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
                1365                1370                1375

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
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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
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 Gln  Leu Val Phe Arg Tyr
                1635                1640                1645

cac gtg gtt ggc 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 ggc 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 ggc 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 ggc ctc ctg      5280
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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 ggc 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 ggc      5595
Asp Asp Ala Arg Ile  Val Gln Arg His Leu  Pro Phe Glu Gly Gly
                1845                1850                1855

ctg gcc tat ggc atc  gac cag ctg ctg gag  cca cct ggc 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 ggc agc cct  gag gcc tgc tgg cgc  ttc tac ccg aag ttc      5775
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
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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
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
                2205                2210                2215

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
                2220                2225                2230

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
                2235                2240                2245

gcc cag cag ctg ggc  ttc cac ctg tgc ctc  atg ggc tgg ctg gcc      6810
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Ala Gln Gln Leu Gly  Phe His Leu Cys Leu  Met Gly Trp Leu Ala
                2250                2255                2260

aat ggc tcc act gcc  cac cct gtg gtt ttc  cct gtg gcg gac tgt      6855
Asn Gly Ser Thr Ala  His Pro Val Val Phe  Pro Val Ala Asp Cys
                2265                2270                2275

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
                2280                2285                2290

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
                2295                2300                2305

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
                2310                2315                2320

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
                2325                2330                2335

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
                2340                2345                2350

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
                2355                2360                2365

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
                2370                2375                2380

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
                2385                2390                2395

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
                2400                2405                2410

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
                2415                2420                2425

tcc tgg gcc cct gtg  gcc cca ggg aca gtt  gtg gtt agc cgt atc      7350
Ser Trp Ala Pro Val  Ala Pro Gly Thr Val  Val Val Ser Arg Ile
                2430                2435                2440

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
                2445                2450                2455

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
                2460                2465                2470

cct gaa gcc cca cct  gtg gcg gca ggc gtg  ggg gct gtg ctt gcc      7485
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
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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
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| | | |
|---|---|---|
| 0-1 | **Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)** | |
| 0-1-1 | Prepared using | PCT-EASY Version 2.92 (updated 01.10.2002) |
| 0-2 | **International Application No.** | PCT/FI 0 3 / 0 0 0 1 0 |
| 0-3 | **Applicant's or agent's file reference** | 2020166PC/or |

| | | |
|---|---|---|
| 1 | **The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:** | |
| 1-1 | page | 9 |
| 1-2 | line | 23 - 28 |
| 1-3 | **Identification of Deposit** | |
| 1-3-1 | Name of depositary institution | DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH |
| 1-3-2 | Address of depositary institution | Mascheroder Weg 1b, D-38124 Braunschweig, Germany |
| 1-3-3 | Date of deposit | 21 August 2001 (21.08.2001) |
| 1-3-4 | Accession Number | DSMZ ACC2519 |
| 1-4 | **Additional Indications** | According to Rule 13bis.6 PCT the Applicant wishes to make use of the expert provisions in those countries which provide for such |
| 1-5 | **Designated States for Which Indications are Made** | all designated States |
| 1-6 | **Separate Furnishing of Indications** These indications will be submitted to the International Bureau later | NONE |
| 2 | **The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:** | |
| 2-1 | page | 9 |
| 2-2 | line | 23 - 28 |
| 2-3 | **Identification of Deposit** | |
| 2-3-1 | Name of depositary institution | DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH |
| 2-3-2 | Address of depositary institution | Mascheroder Weg 1b, D-38124 Braunschweig, Germany |
| 2-3-3 | Date of deposit | 21 August 2001 (21.08.2001) |
| 2-3-4 | Accession Number | DSMZ ACC2520.> |
| 2-4 | **Additional Indications** | According to Rule 13bis.6 PCT the Applicant wishes to make use of the expert provisions in those countries which provide for such |

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| | | |
|---|---|---|
| 2-5 | **Designated States for Which Indications are Made** | all designated States |
| 2-6 | **Separate Furnishing of Indications**<br>These indications will be submitted to the International Bureau later | NONE |

## FOR RECEIVING OFFICE USE ONLY

| | | |
|---|---|---|
| 0-4 | **This form was received with the international application:** (yes or no) | YES |
| 0-4-1 | Authorized officer | |

## FOR INTERNATIONAL BUREAU USE ONLY

| | | |
|---|---|---|
| 0-5 | **This form was received by the international Bureau on:** | |
| 0-5-1 | Authorized officer | |

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

MediCity Research Laboratory
University of Turku
Tykistökatu 6 A
FIN-20520 Turku

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

| Identification reference given by the DEPOSITOR: | Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: |
|---|---|
| 3-266 | DSM ACC2519 |

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2001-08-21 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

| | |
|---|---|
| Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH | Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiss |
| Address: Mascheroder Weg 1b, D-38124 Braunschweig | Date: 2001-08-29 |

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

MediCity Research Laboratory
University of Turku
Tykistökatu 6 A
FIN-20520 Turku

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

| I. DEPOSITOR | II. IDENTIFICATION OF THE MICROORGANISM |
|---|---|
| Name: MediCity Research Laboratory<br>University of Turku<br>Address: Tykistökatu 6 A<br>FIN-20520 Turku | Accession number given by the<br>INTERNATIONAL DEPOSITARY AUTHORITY:<br>DSM ACC2519<br><br>Date of the deposit or the transfer[1]:<br>2001-08-21 |

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2001-08-21 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

| | |
|---|---|
| Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH<br><br>Address: Mascheroder Weg 1b<br>D-38124 Braunschweig | Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):<br><br>U. Weiß<br><br>Date: 2001-08-29 |

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

MediCity Research Laboratory
University of Turku
Tykistökatu 6 A
FIN-20520 Turku

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

| I. IDENTIFICATION OF THE MICROORGANISM | |
|---|---|
| Identification reference given by the DEPOSITOR:<br>3-372 | Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:<br>DSM ACC2520 |

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
( ) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2001-08-21 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

| | |
|---|---|
| Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH<br>Address: Mascheroder Weg 1b<br>D-38124 Braunschweig | Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):<br>U. Wirth<br>Date: 2001-08-29 |

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

MediCity Research Laboratory
University of Turku
Tykistökatu 6 A
FIN-20520 Turku

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

| I. DEPOSITOR | II. IDENTIFICATION OF THE MICROORGANISM |
|---|---|
| Name: MediCity Research Laboratory<br>University of Turku<br>Address: Tykistökatu 6 A<br>FIN-20520 Turku | Accession number given by the<br>INTERNATIONAL DEPOSITARY AUTHORITY:<br>DSM ACC2520<br><br>Date of the deposit or the transfer[1]:<br>2001-08-21 |

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2001-08-21 [2].
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

| | |
|---|---|
| Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH<br><br>Address: Mascheroder Weg 1b<br>D-38124 Braunschweig | Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):<br><br>V. Weiß<br><br>Date: 2001-08-29 |

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

| | | | |
|---|---|---|---|
| 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 | | |
| 公开(公告)号 | EP1463760A2 | 公开(公告)日 | 2004-10-06 |
| 申请号 | EP2003729265 | 申请日 | 2003-01-08 |
| [标]申请(专利权)人(译) | JALKANEN SIRPA<br>IRJALA HEIKKI<br>萨尔米MARKO | | |
| 申请(专利权)人(译) | JALKANEN，SIRPA<br>IRJALA，HEIKKI<br>萨尔米，MARKO | | |
| 当前申请(专利权)人(译) | JALKANEN，SIRPA<br>IRJALA，HEIKKI<br>萨尔米，MARKO | | |
| [标]发明人 | JALKANEN SIRPA<br>IRJALA HEIKKI<br>SALMI MARKO | | |
| 发明人 | JALKANEN, SIRPA<br>IRJALA, HEIKKI<br>SALMI, MARKO | | |
| IPC分类号 | G01N33/50 A61K39/395 A61K45/00 A61P29/00 A61P35/00 A61P35/04 A61P43/00 C07K14/705 C07K16/28 C12N15/02 C12P21/08 C12Q1/02 G01N33/15 G01N33/53 G01N33/574 A61K38/17 | | |
| CPC分类号 | A61K2039/505 A61P29/00 A61P35/00 A61P35/04 A61P43/00 C07K14/7056 C07K16/28 | | |
| 优先权 | 60/346288 2002-01-09 US | | |
| 其他公开文献 | EP1463760B1 | | |
| 外部链接 | Espacenet | | |


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。