PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:	WO 92/03473
C07K 7/00, C12P 21/08 A61K 37/02, 39/395	A1	(43) International Publication Date:	5 March 1992 (05.03.92)

(21) International Application Number: PCT/US91/06053

(22) International Filing Date: 23 August 1991 (23.08.91)

(30) Priority data:
573,624 27 August 1990 (27.08.90) US

(71) Applicant: CETUS CORPORATION [US/US]; 1400 Fifty-Third Street, Emeryville, CA 94608 (US).

(72) Inventors: LIU, David, Y. ; 201 Ferne Avenue, Palo Alto, CA 94306 (US). KAYMAKCALAN, Zehra ; 520 Norvell Street, El Cerrito, CA 94530 (US). MUNDY, Kirsten ; 2450 Heatherleaf Lane, Martinez, CA 94553 (US).

(74) Agent: GIOTTA, Gregory, J.; Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CD18 PEPTIDE MEDICAMENTS FOR THE TREATMENT OF DISEASE

(57) Abstract

Peptide medicaments, and antibody thereto, useful for treating or preventing diseases, particularly diseases involving an inflammatory response by a host organism against infection, that are preferably derived from the β subunit, CD18, of the leukocyte integrins, and that have the property of either interfering with, or preventing undesirable leukocyte adhesion to biological materials, particular to endothelial cells, or that interfere with, or prevent the chemotaxis of leukocytes through the endothelial cells monolayer, consequently minimizing undesirable cell/tissue leukocyte binding and thus preventing or minimizing diseases resulting therefrom.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**ES**	Spain	**MG**	Madagascar
AU	Australia	**FI**	Finland	**ML**	Mali
BB	Barbados	**FR**	France	**MN**	Mongolia
BE	Belgium	**GA**	Gabon	**MR**	Mauritania
BF	Burkina Faso	**GB**	United Kingdom	**MW**	Malawi
BG	Bulgaria	**GN**	Guinea	**NL**	Netherlands
BJ	Benin	**GR**	Greece	**NO**	Norway
BR	Brazil	**HU**	Hungary	**PL**	Poland
CA	Canada	**IT**	Italy	**RO**	Romania
CF	Central African Republic	**JP**	Japan	**SD**	Sudan
CG	Congo	**KP**	Democratic People's Republic of Korea	**SE**	Sweden
CH	Switzerland			**SN**	Senegal
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SU+**	Soviet Union
CM	Cameroon	**LI**	Liechtenstein	**TD**	Chad
CS	Czechoslovakia	**LK**	Sri Lanka	**TG**	Togo
DE*	Germany	**LU**	Luxembourg	**US**	United States of America
DK	Denmark	**MC**	Monaco		

+ Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

PEPTIDE MEDICAMENTS FOR THE TREATMENT OF DISEASE

This invention is in the area of molecular biology/biochemistry and presents peptides having defined amino acid sequences that are useful medicaments, particularly when employed as anti-inflammatory prophylactics or therapeutics.

During an inflammatory response peripheral blood leukocytes, consisting of neutrophils and monocytes, bind to and migrate thru the vascular endothelial cell layer and cross the basement membrane in response to chemotactic factors, and enter the infected tissue where they are effective at controlling or ridding the organism of the infection. When a host defense system responds properly to an infection, the inflammatory response is tightly controlled such that leukocytes enter only the infected area, and consequently do not damage healthy tissue. In certain disease conditions, particularly sepsis, leukocyte action is not tightly controlled, and consequently can cause extensive vascular damage arising as a result of the release of oxygen-derived free radicals, as well as proteases and phospholipases from the neutrophils which thus can cause significant cellular and tissue injury. Harlan, J.M., 1987, Acta Med Scand Suppl., 715:123; Weiss, S., 1989, New England J. of Med., 320:365. For example, sepsis associated neutrophil-mediated endothelial injury has been linked to loss of vascular integrity, thrombosis, and tissue necrosis.

The initial event that leads to neutrophil damage of endothelial cells is the adhesion of neutrophils to the endothelial cell surface. In significant part this is medicated by cellular adhesion molecules associated with the neutrophils that cause them to bind to the endothelial cell surface. The neutrophil adhesion molecules bind to a molecule on the surface of endothelial cells termed ICAM-1 (Intercellular Adhesion Molecule 1). To date, a partial list of the adhesion molecules that have been identified that are involved in this reaction are lymphocyte function-associated antigen-1 (LFA-1), macrophage antigen-1 (MAC-1), also termed MO-1, OKM-1 and complement receptor type-3 (CR-3), and p150,95, also termed complement receptor type-4 (CR-4) and Leu M-5. These molecules collectively have been termed the LFA-1 family, leukocyte adhesion proteins, leuCAM, and the leukocytes integrins. All three molecules are α–ß heterodimers. The ß subunit is identical in the three molecules, while the α subunit differs. Kurzinger, K., and Springer, T.A., 1982, J. of Biol. Chem., 257:12412; Sanchez-Madrid, F., et al., 1983, J. Exp. Med., 158:1785; Trowbridge, I.S., and Omary, M.B., 1981, PNAS (USA), 78:3039.

The three leukocyte integrins are predominately expressed by immune cells. For instance, LFA-1 is found on virtually all immune cells. Kurzinger, K. and Springer, T.A., supra. MAC-1 is expressed by monocytes, macrophages,

granulocytes, large granular lymphocytes, and immature and CD-5+ B cells. De La Hera, A., et al. 1988, Eur. J. of Immun., 18:1131. p150,95 protein shares the same cell type distribution as MAC-1, but is further expressed by activated lymphocytes, as well as hairy cell leukemia. It is a marker of the latter disease. Schwarting, R., et al., 1985, Blood, 65:974; Miller, B.A., et al., 1985, J. of Immun., 134:3286.

Studies have implicated the leukocyte integrins in cellular adhesion events. For example, LFA-1 is involved in antigen-dependent and antigen-independent interactions of immune cells. Springer, T.A., et al., 1987, Annual Review Immun., 5:223; Martz, E., 1986, Hum. Immunology, 18:3. Most telling are studies utilizing a monoclonal antibody to LFA-1, which have revealed that binding to LFA-1 by monoclonal antibody partially or totally inhibits T lymphocytes adherence to endothelial cells (Mentzer, S.J., et al. 1986, J. of Cell Physiol., 126:285), fibroblasts (Dustin, N.L., et al., 1986, J. of Immun., 137:245), epidermal keratinocytes (Dustin, N.L., et al., 1988, J. of SubBiology, 107:321), and hepatocytes (Roos, E., and Roossien, F., 1987, J. of SubBiology, 105:553).

The role of MAC-1 in cellular adhesion was initially demonstrated also using monoclonal antibodies. Such studies show that MAC-1 binds to C3bi-coated erythrocytes, and that such binding could be inhibited by monoclonal antibodies to MAC-1. Beller, B.I., et al., 1982, J. of Exp. Med., 156:1000. Additionally, MAC-1 has been shown to be involved in macrophage binding to Leishmania Promastigotes, E. coli, and Histoplasma Capsulatum. Mosser, D. and Edelson, P., 1985, J. of Immun., 135:2785; Wright, S. and Jong, M., 1986, J. of Exp Med., 164:1876; Bullock, W. and Wright, S., 1987, J. of Exp. Med., 165:195. Other studies have shown that MAC-1 is involved in neutrophil and monocyte chemotaxis, as well as adherence to glass and plastic surfaces, and to endothelial and epithelial cell monolayers.

p150,95 is reported to be significantly involved in peripheral blood monocyte adhesion to substrates and endothelial cells, phagocytosis of latex particles, and chemotaxis. Keizer, et al., 1987, Eur. J. of Immun., 17:1317; te Velde, A., et al., 1987, Immunology, 61:261. Further, studies using a monoclonal antibody that is directed to p150,95 have shown it to be utilized in conjugate formation by cytotoxic T lymphocytes.

The studies described above, as well as additional studies suggest that the leukocyte integrins function as general adhesion proteins to effect immune cell function. Further, the studies described above have used monoclonal antibodies directed either to the α or ß subunits of the three integrins. For the most part, these studies have shown the common ß subunit to play the predominant role in the adhesion-related functions of

these molecules. Recently the cDNA clone that encodes the ß subunit of human LFA-1, MAC-1, and p150,95 has been isolated. Kishimoto, T., et al., 1987, Cell, 48:681; and Law, S.K.A. et al., 1987, EMBO J., 6:915-919.

As mentioned above, inflammation is a significant part of an organism's defense to infection and may be a cause of injury of extravascular tissue. Moreover, in certain instances there is an uncontrolled inflammatory response, such as that observed in septic shock, which may contribute to the pathogenesis of the disease. Leukocytes have been implicated as being, at least in part, responsible for the damage associated with acute ischemic shock by releasing reactive oxygen metabolites, proteases, and phospholipases at the disease sites. This is supported by studies which have shown that animals depleted of peripheral blood leukocytes show significantly reduced damage from myocardial ischemia and reperfusion. Further, reperfusion injury can be minimized by in vivo administration of MAC-1 monoclonal antibodies. Finally, a rabbit model of hemorrhagic shock and resuscitation reveals that monoclonal antibodies against the ß subunit of MAC-1 exhibited a protective effect to liver and the asternal intestinal track. Simpson, et al., J. of Clinical Invest., 1988, 81:624; Vedder, N. and Harlan, J., 1988, J. of Clinical Invest., 81:676. Taken together, these results suggest significant therapeutic value for reagents that block the adhesion of leukocytes via the three leukocyte integrins in controlling tissue and organ injury resulting from a number of disease situations including myocardial infarction, hemorrhagic shock, and other events that cause ischemia that are followed by reestablishing normal circulatory blood flow.

Finally, it is noteworthy that ICAM-1, the endothelial cell receptor for integrin binding, is also the receptor for rhinovirus binding. Staunton, D., et al., 1990, Cell, 61:243. Rhinovirus is a member of the picornavirus family and is responsible for about 50% of common colds. Sperber, S. and Hayden, F. (1988) Antimicrob. Agents Chemother. 32, vol. 409, page 32. A prophylactic approach to preventing the common cold is to interfere with the binding of rhinovirus to cell bound ICAM-1. Indeed, a soluble form of ICAM-1 has recently been reported to be effective. Marlin, S.D., et al., 1990, Nature, 344:70.

In one aspect, the invention presented herein describes peptides that prevent or interfere with undesirable binding of cells or virions to cells or tissues that express an appropriate membrane receptor (e.g., ICAM) for the cells or virions and thus prevents or minimizes disease resulting from the binding of the cells or virions.

A second aspect of the invention describes peptides that have sequence homology to particular regions of the ß subunit, CD18, of the leukocyte integrins that compete with the integrins to prevent undesirable tissue binding of leukocytes or

virions thereto, as well as interfere with leukocyte chemo-attractiveness to the tissue, thereby preventing or minimizing disease to the tissue resulting from leukocyte or virion binding.

A third aspect of the invention is the description of peptides that inhibit or prevent leukocyte adhesion, without affecting leukocyte chemotaxis, thus preventing undesirable tissue binding of the leukocytes thereto, and consequently preventing or minimizing disease to the tissue resulting from leukocyte binding.

A fourth aspect of the invention is the description of methods for prophylactically or therapeutically treating patients suffering from various diseases with peptides that interfere with leukocyte or virion cell adhesion and/or leukocyte chemotaxis.

A fifth aspect of the invention is the description of methods for prophylactically or therapeutically treating patients suffering from various diseases with peptides that interfere with leukocyte cell adhesion and/or chemotaxis, particularly diseases caused by rhinovirus infection.

A sixth aspect of the invention is the description of antibody to CD18 peptides, and prophylactic and therapeutic applications of the antibody.

Yet another aspect of the invention is the description of peptides that inhibit the cellular adhesion properties of leukocytes, wherein said peptides are not readily hydrolyzable, and thus exhibit a prolonged in vivo circulation time.

These and other aspects of the invention will be apparent upon a full consideration of the invention as presented below.

Figure 1 shows the amino acid sequence of the ß subunit, CD18, of the leukocyte integrins. Underlined regions correspond to peptides that were synthesized and tested for activity in the leukocyte/endothelial cell adhesion assay. Smaller peptides within a region were also synthesized and tested for activity. Each peptide is denoted by the number in the figure, that is 1 through 6, and a second number that refers to the number of amino acids in the peptide. Thus, 1-26 refers to a peptide from region 1 with 26 amino acids. Since region 1 consists of only 26 amino acids, 1-26 denotes a peptide that spans the entire region. 1-15, however, refers to a peptide from region 1 consisting of 15 amino acids. The peptides are numbered from the carboxyl to the amino terminal end of the molecule.

Table 1 shows the amino acid sequence of various CD18 peptides that were tested for their capacity to inhibit polymorphonuclear leukocyte adhesion to endothelial cells.

Table 2 shows the inhibitory effect of several CD18 peptides on polymorphonuclear leukocyte adhesion over a concentration range of 10^{-4} to 10^{-8} M. It is apparent that 4-29 is most effective at 10^{-5} molar.

Table 3 shows the effects of the peptide 4-29 on polymorphonuclear leukocyte adhesion over a concentration range of 10^{-4} to 10^{-8} M using polymorphonuclear leukocytes from different human donors, and after the peptide had been stored under various conditions.

Table 4 shows the effects of the peptide 4-15 on polymorphonuclear leukocyte adhesion over a concentration range of 10^{-4} to 10^{-6} M, and using polymorphonuclear leukocytes from different donors.

Table 5 shows the chemotaxis inhibitory activity of the CD18 peptides, 1-26, 2-24, 3-29, 4-29, 5-24, and 6-25.

Table 6 shows the chemotaxis inhibitory activity of the CD18 peptide, 4-29.

The invention described herein draws on previously published work. By way of example, such work consists of scientific papers, patents or pending patent applications. All of these publications and applications, cited previously or below, are hereby incorporated by reference.

The instant invention is composed of several unique methods and compositions. Each aspect of the invention will now be discussed separately.

Peptides have been discovered that prevent or interfere with cell-cell or cell-virion adhesion events that are useful medicaments for treating a variety of diseases, preferably diseases resulting from untoward adhesion of leukocytes or rhinovirus. These peptides have amino acid sequence homology to regions of the beta subunit of the leukocyte intergrins, CD18.

Preferred is a peptide that incorporates the following 5 amino acids:

NH_2-Trp-Arg-Asn-Val-Thr-Arg-COOH

More preferred is the above peptide incorporated into a larger peptide that has the following sequence:

NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH

Most preferred is the above peptide incorporated into a larger peptide that has the following sequence:

NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-COOH

Figure 1 shows the amino acid sequence of the beta subunit of the leukocyte integrins, CD18, and those peptides that interfere with leukocyte or virion adhesion are underlined. Specifically, the peptides were tested for their ability to compete with and

prevent adhesion of leukocytes to activated endothelial cells. Additionally, the ability of these peptides to inhibit leukocyte chemotaxis was determined.

The peptides described above can be made by techniques well known in the art, such as, for example, the Merrifield solid-phase method described in Science, 232:341-347 (1985). The procedure may use commercially available synthesizers such as a Biosearch 9500 automated peptide machine, with cleavage of the blocked amino acids being achieved with hydrogen fluoride, and the peptides purified by preparative HPLC using a Waters Delta Prep 3000 instrument, on a 15-20 μm Vydac C4 PrepPAK column.

It will be appreciated by those skilled in the art that although the precise chemical structure of the preferred CD18 peptides are shown herein, that particular alterations to the structure may be desired depending on a number of factors, a key factor being the use to which the peptide is being put to. For example, for convenience of administration to a patient a peptide may be formulated as an acidic or basic salt, or in neutral form. Further, the primary amino acid sequence of the protein may be augmented by derivatization using sugar moieties (glycosylation) or by other supplementary molecules such as lipids, phosphate, acetyl groups and the like, as well as by conjugation with saccharides, polyethylene glycols (PEGs) and polyoxyethylene glycols (POGs). Such modifications are included in the definition of peptide herein so long as the activity of the peptide, as defined above, is not destroyed. It is expected, of course, that such modifications may quantitatively or qualitatively affect the activity, either by enhancing or diminishing the activity of the protein in the various assays.

Furthermore, it will be particularly appreciated by those skilled in this art, that peptide medicaments that have an increased in vivo residence time may be advantageous for particular applications. The in vivo residence time of peptides may be increased using methods known in the art, and two exemplary methods include synthesizing peptides that have substantially non-hydrolyzable peptide bonds, or that are bound to, or associated with bio-compatible polymers. Exemplary polymers are described by Ulbrich, K., et al., 1986, Makromol. Chem., 187:1131; and Rihova, B., 1986, J. of Chromatography, 376:221.

It will be appreciated by those skilled in the art that the peptides described herein can be administered to mammals, including humans, either alone or in combination with other anti-inflammatory agents, or they may be combined with various pharmaceutically acceptable diluents or carriers. Such are widely known to those skilled in the art and are formulated according to standard pharmaceutical practices.

Exemplary diluents include physiologic saline, or buffered saline, as well as Ringer's and dextrose injection fluid, and dextrose saline and lactated Ringer's injection

or diluent solutions containing additional therapeutic agents, preferably antibiotics or antibodies known to be efficacious in the treatment of inflammatory conditions.

Leukocyte adherence can be measured using several assays known in the art, and the preferred assay is described by Charo, et al., 1985, Blood, 65:473. Briefly, the assay consists of labelling leukocytes with an appropriate label, incubating them with endothelial cells and determining the number of leukocytes that adhere. Preferably the cells are labelled with a gamma ray emitting isotope and the preferred labels are ^{111}Indium-oxide or ^{51}chromium.

Leukocytes may be isolated from human donors using standard techniques. This generally consists of isolating blood in a physiologically balanced salt solution containing an appropriate anticoagulant, and separating the leukocytes by an appropriate separation step, preferably on Ficoll-Hypaque gradients. Contaminating erythrocytes can be removed by hypotonic lysis. The resulting leukocytes are suspended in a physiologically buffered solution, pH 7.4. The preferred physiological buffered solution is Hank's balanced salt solution that is calcium and magnesium free.

The isolated leukocytes can then be labelled by incubating them for an appropriate time, generally 15 minutes, with the desired radioisotope at a predetermined concentration. The radiolabelled cells are washed to remove unincorporated label, and then suspended in an appropriate solution to perform the adhesion assay described below.

Endothelial cells can be prepared from a number of sources and by several techniques known in the art. Preferably they are obtained from human umbilical veins using the procedure of Charo et al., above. Generally, endothelial cells are isolated by enzymatic digestion of the umbilical veins using, preferably, collagenase as described by Jaffe, E.A., et al., 1973, J. of Clin. Invest, 52:2745. The cells are grown on an appropriate tissue culture substratum, preferably gelatine-coated surfaces.

The endothelial cells may be grown in a variety of tissue culture media containing appropriate supplements such as an appropriate concentration of fetal calf serum, and other supplements/additives routinely utilized by those skilled in this art that are recognized as being favorable for endothelial cells. The endothelial cells may be passaged with a dilute solution of an appropriate protease, and if desired a metal ion chelator. Preferably a solution consisting of 0.05 to 0.25% trypsin and 0.02% EDTA is used. To ensure that the cells are indeed endothelial cells, they are tested by immunofluorescence for Factor VIII antigen, a known endothelial cell marker.

Leukocyte adherence to endothelial cell monolayers may be determined as follows. Early passage endothelial cells, generally not beyond the fifth passage, are cultured on an appropriate substratum and in a suitable cell culture medium. The

culture substratum is preferably pre-coated with an appropriate substance that enhances the adherence of the endothelial cells. Several such substances are known including fibronectin, poly-L-lysine, gelatin and laminin. Fibronectin is preferred. An appropriate culture substratum is a 96 well micro titer plate, and a suitable medium is Medium 199 containing fetal calf serum and other supplements known to be beneficial for the growth and maintenance of endothelial cells that are well known to those skilled in the art. Prior to adding a predetermined number of labelled leukocytes, the endothelial cell monolayer is washed with a physiologically balanced salt solution containing a reduced amount of fetal calf serum, preferably 1%. The preferred solution is RPMI supplemented with 1% fetal calf serum.

The endothelial cell monolayer containing added leukocytes is incubated for a time sufficient to permit maximum adherence of the leukocytes, and preferably this is conducted at 37°C for 30 minutes in an appropriate cell culture atmosphere. Generally this would consist of growing and incubating the cells for the assay period in 5% CO_2, 95% air, and 95% humidity. Next, non-adherent leukocytes are removed by any number of techniques known in the art, and the number of leukocytes adherent to the endothelial cell monolayers determined by measuring the amount of radioisotope associated with the endothelial cell monolayer. Controls are run that take into account basal binding, i.e., binding to endothelial cells not activated with TNF.

In a typical experiment run in quadruplicate, the assay is highly reliable, giving standard deviations less than 10%, and usually less than 5%, of mean values. Typically the results are expressed as the percent of leukocytes added to the endothelial cells that remain adherent after non-adherent cells have been removed.

Using the above assay, typically peptides to be tested are added over a range of concentrations, preferably from 10^{-4} M to 10^{-7} M. The peptides are synthesized as described above, lyophilized and dissolved in 15-25 µl of dimethylsulfoxide. This volume is then suitably diluted in an appropriate medium, preferably RPMI containing 1% fetal calf serum to give the desired final concentration to be tested.

The endothelial cells were activated with 125 U/ml of TNF having a specific activity of 2×10^7 U/mg for at least 4 hours in RPMI with 1% fetal calf serum prior to the addition of the leukocytes. TNF causes the induction of ICAM expression on endothelial surfaces which is a receptor for leukocyte integrin binding.

The materials and methods for ascertaining the chemotactic inhibitory properties of peptides are generally known in the art, and the preferred procedure is described by Capsoni, et al., 1989, J. of Immunol. Meth., 120:125. Generally, chemotaxis is determined by positioning the leukocytes and a chemotactic substance on opposite sides of a membrane in appropriate culture media. The preferred apparatus for doing a

chemotaxis assay is produced by Costar Corporation, Cambridge, MA, and is termed the trans-well cell culture apparatus. The size of the membrane is selected so that the leukocytes do not have unrestricted access to the substance; rather if a chemotactic response is elicited the leukocytes adhere to, and migrate into and through the filter. If a substance is being tested for inhibitory activity this can be achieved by combining it with the leukocytes or the chemotactic substance.

The procedure of Capsoni, et al., above, was followed with the following modifications. Leukocytes may be isolated and labelled with ^{111}Indium as described for the adhesion assay, above. The cells are resuspended after labelling in an appropriate cell culture medium at about 5 x 10^6 cells/ml. Next, a desired amount of the cell suspension is mixed with a predetermined amount of the peptide(s) to be tested for inhibitory activity, and the mixture added to an appropriate filter device. Three μm - pore membranes are situated in the wells of a 24 trans-well tissue culture plate. The cell/inhibitory peptide mixture is incubated for a short time at 37°C to acclimate the cells to the membrane surface, and to provide sufficient time for them to settle onto the membrane surface. Next, inserts are set in wells containing cell culture media. The media contains zymosan-activated human serum at about 0.5%. Zymosan activation generates complement-derived chemotactic factors which attract the leukocytes through the pores of the membrane. This media was also prewarmed for an appropriate time prior to addition to the cell culture wells. After a 30 minute incubation period at 37°C, the number of leukocytes that have migrated through the membrane filter, in the presence or absence of CD18 peptides, is readily determined by counting the amount of ^{111}Indium present in the media. This may be facilitated by adding an appropriate detergent at an appropriate concentration to the media in the wells prior to removing an aliquot for counting. In this way, the inhibitory activities of the peptide being tested could be determined.

The effect of CD18 peptides on rhinovirus binding can be determined using known methods and materials as described by Abraham, G. and Colonno, R. J. (1984) J. Virol. vol. 51 page 340, with modifications as described by Marlin, S. D. et al., (1990) Nature, vol. 344. page 70. Briefly, the procedure consists of radiolabelling rhinovirus by growing virally infected cells in culture media containing a suitable radioisotope, and isolating the virus from the culture media using methods known in the art. Particularly effective is precipitation of virions from the culture media using polyethylene glycol, followed by pelleting the virions through a 30% sucrose step gradient.

The radiolabelled virions can be employed in a cell adhesion assay, using cells that express ICAM-1. The preferred cells are either endothelial cells prepared as described above or HeLa cells as described by Abraham, G. and Colonno, R. J., above. The assay can be conducted essentially as described for measuring neutrophil adhesion to endothelial cells, with the addition of the appropriate CD18 peptide to the assay mixture. It would be ascertained that those peptides that bind to ICAM-1 and prevent virion binding are readily identified by counting the number of virions that bind to the cell monolayer in the presence of the peptides.

Antibody, either polyclonal or monoclonal or recombinant, the latter preferably humanized, can be generated against the inhibitory peptides. Such antibody would be used for binding to the CD18 molecule present on leukocytes, and thus interfere with, or prevent the adhesion of leukocytes to endothelium.

Monoclonal antibody may be produced using the general procedures described by Kohler, G. and Milstein, C., 1975, Nature, 256:495, which have been modified over the years as is known in the art. These initial studies involved fusing murine lymphocytes and drug selectable plasmacytomas to produce hybridomas. Subsequently, the technique has been applied to produce hybrid cell lines that secrete human monoclonal antibodies. The latter procedures are generally described by Abrams, P., 1986, Methods in Enzymology, 121:107, but other modifications are known to those skilled in the art.

Regardless of whether murine or human antibody is produced, the antibody secreting cells are combined with the fusion partner and the cells fused via electrofusion or with a suitable fusing agent, preferably polyethylene glycol, and more preferably polyethylene glycol 1000. The latter is added to a cell pellet containing the antibody secreting cells and the fusion partner in small amounts over a short period of time accompanied with gentle agitation. After the addition of the fusing agent, the cell mixture is washed to remove the fusing agent and any cellular debris, and the cell mixture consisting of fused and unfused cells seeded into appropriate cell culture chambers containing selective growth media. After a period of one to three weeks, hybrid cells are apparent, and may be identified as to antibody production and subcloned to ensure the availability of a stable monoclonal cell line.

The preferred antibody is human monoclonal antibody which can be prepared from lymphocytes sensitized with a peptide either in vivo or in vitro by immortalization of antibody-producing hybrid cell lines, thereby making available a permanent source of the desired antibody. In vivo immunization techniques are well known in the art, while in vitro techniques are generally described by Luben, R. and Mohler, M., 1980, Molecular Immunology, 17:635, Reading, C. Methods in Enzymology, 121 (Part

One):18, or Voss, B., 1986, Methods in Enzymology, 121:27. A number of in vitro immunization systems have been shown to be effective for sensitizing human B-cells. Reading, C., 1982, J. of Immun. Methods, 53:261.

Sensitized lymphocytes can be immortalized by viral transformation. The preferred viral transformation technique for human lymphocytes involves the use of Epstein-barr virus. The virus is capable of transforming human B-cells, and has been used to generate human monoclonal antibodies. Crawford, D. et al., 1983, J. of General Virology, 64:697; Kozbor, V. and Roder, J., 1983, J. Immun. Today, 4:72.

Another procedure whereby sensitized lymphocytes may be immortalized consist of a combination of the above two techniques, that is viral transformation and cell fusion. The preferred combination consist of transforming antibody secreting cells with Epstein-barr virus, and subsequently fusing the transformed cells to a suitable fusion partner. The fusion partner may be a mouse myeloma cell line, a heteromyeloma line, or a human myeloma line, or other immortalized cell line. PCT Patent Application No. 81/00957; Schlom et al., 1980, PNAS USA, 77:6841; Croce et al., 1980, Nature, 288:488. The preferred fusion partner is a mouse-human hetero-hybrid, and more preferred is the cell line designated F3B6. This cell line is on deposit with the American Type Culture Collection, Accession No. HB8785. It was deposited April 18, 1985. The procedures for generating F3B6 are described in European Patent Application, Publication No. 174,204. Techniques applicable to the use of Epstein-Barr virus transformation and the production of immortal antibody secreting cell lines are presented by Roder, J. et al., 1986, Methods in Enzymology, 121:140. Basically, the procedure consist of isolating Epstein-Barr virus from a suitable source, generally an infected cell line, and exposing the target antibody secreting cells to supernatants containing the virus. The cells are washed, and cultured in an appropriate cell culture medium. Subsequently, virally transformed cells present in the cell culture can be identified by the presence of the Epstein-Barr viral nuclear antigen, and transformed antibody secreting cells can be identified using standard methods known in the art.

Antibodies to peptides are produced using standard coupling procedures to suitable carrier molecules as is known in the art. The preferred procedures and compositions are described in U.S. Patent No. 4,762,706, issued August 9, 1988, to McCormick, et al. For example, antibodies are produced by injecting a host animal such as rabbit, rat, goat, mouse, etc., with the peptide or peptide fragment. Before injection, the peptides are first conjugated with a suitable carrier molecule, preferably keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). The conjugation is preferably achieved via a sulfhydryl group in a cysteine residue on the peptide. If a peptide lacks a cysteine residue it may be added using known methods.

A heterobifunctional crosslinking reagent may be used to couple the carrier protein and the desired peptide. The preferred coupling reagent is N-maleimido-6-amino caproyl ester of 1-hydroxy-2-nitro-benzene-4-sulfonic acid sodium salt, and its preparation and used are known in the art, and is described in U.S. Patent No. 4,762,706.

Having described what the applicants believe their invention to be, the following examples are presented to illustrate the invention, and are not to be construed as limiting the scope of the invention. For example, variation in the source, type, or method of producing antibodies; different labels and/or signals; test supports of different materials and configurations; different immobilization methods may be employed without departing from the scope of the present invention.

Example 1

Affect of CD18 Peptides on Polymorphonuclear Leukocyte Adhesion to Endothelial Cell Monolayers

Those peptides shown in Table 1 were synthesized and tested for their capacity to interfere with, or block adhesion of polymorphonuclear leukocytes to human endothelial cell monolayers. These peptides correspond to those underlined in the structure of CD18 shown in Figure 1. Smaller peptides within a region were also synthesized and tested for activity.

Each peptide is denoted by the number in the figure, that is 1 through 6, and a second number that refers to the number of amino acids in the peptide. Thus, 1-26 refers to a peptide from region 1 with 26 amino acids. Since region 1 consists of only 26 amino acids, 1-26 denotes a peptide that spans the entire region. However, 1-15, refers to a peptide from region 1 consisting of 15 amino acids. The peptides are numbered from the carboxyl to the amino terminal end of the molecule.

Peptides were synthesized using Merrifield's solid-phase method. Merrifield, R.B., 1963, J. of Amer. Chem. Soc., 75:48-79. A Biosearch 9500 automated peptide machine was employed with T-Boc amine protection. Cleavage was performed using hydrogen fluoride, and the resulting peptides were purified by preparative high pressure liquid chromatography using a Walter's Deltaprep 3000 with a PrePak C18 column using an aqueous-acetonitrile-trifluoroacetic acid (TFA) mobile phase.

Table 1

CD18 Peptides

Peptides	
1-26:	NH_2-Try-Pro-Ile-Asp-Leu-Tyr-Tyr-Leu-Met-Asp-Leu-Ser-Tyr-Ser-Met-Leu-Asp-Asp-Leu-Arg-Asn-Val-Lys-Lys-Leu-Gly-COOH
1-15:	NH_2-Ser-Tyr-Ser-Met-Leu-Asp-Asp-Leu-Arg-Asn-Val-Lys-Lys-Leu-Gly-COOH
2-24:	NH_2-Phe-Asp-Tyr-Pro-Ser-Val-Gly-Gln-Leu-Ala-His-Lys-Leu-Ala-Glu-Asn-Asn-Ile-Gln- Pro-Ile-Phe-Ala-Val-Thr-COOH
2-16:	NH_2-Ala-His-Lys-Leu-Ala-Glu-Asn-Asn-Ile-Gln-Pro-Ile-Phe-Ala-Val-Thr-COOH
3-29:	NH_2-Ile-Pro-Lys-Ser-Ala-Val-Gly-Glu-Leu-Ser-Glu-Asp-Ser-Ser-Asn-Val-Val-His-Leu-Ile-Lys-Asn-Ala-Tyr-Asn-Lys-Leu-Ser-Ser-COOH
3-17:	NH_2-Ser-Ser-Asn-Val-Val-His-Leu-Ile-Lys-Asn-Ala-Tyr-Asn-Lys-Leu-Ser-Ser-COOH
4-29:	NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH
4-15:	NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-COOH
4-14:	NH_2-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH
5-24:	NH_2-Val-Gly-Lys-Gln-Leu-Ile-Ser-Gly-Asn-Leu-Asp-Ala-Pro-Glu-Gly-Gly-Leu-Asp-Ala-Met-Met-Gln-Val-Ala-COOH
5-14:	NH_2-Asp-Ala-Pro-Glu-Gly-Gly-Leu-Asp-Ala-Met-Met-Gln-Val-Ala-COOH
6-25:	NH_2-Arg-Ile-Gly-Phe-Gly-Ser-Phe-Val-Asp-Lys-Thr-Val-Leu-Pro-Phe-Val-Asn-Thr-His-Pro-Asp-Lys-Leu-Arg-Asn-COOH
6-16:	NH_2-Lys-Thr-Val-Leu-Pro-Phe-Val-Asn-Thr-His-Pro-Asp-Lys-Leu-Arg-Asn-COOH
WT:	NH_2-Cys-Arg-Ile-Ala-Arg-Leu-Glu-Glu-Lys-Val-Lys-Thr-Leu-Lys-Ala-Gln-Asn-Ser-Glu-Leu-Ala-Ser-Thr-Ala-Asn-Met-Leu-Arg-Glu-Gln-Val-Ala-Gln-Leu-Lys-Gln-Lys-Val-Met-Asn-His-Ala-COOH

JUN-k: NH_2-Arg-Ile-Ala-Arg-Leu-Lys-Glu-Lys-Val-Lys-Thr-Leu-Lys-Ala-Lys-Asn-Ser-Glu-Leu-Ala-Ser-Thr-Ala-Asn-Met-Leu-Arg-Glu-Gln-Val-Ala-Gln-Leu-Lys-Gln-Lys-Val-Met-Asn-His-Ala-COOH

2X: NH_2-Arg-Ile-Ala-Arg-Leu-Glu-Glu-Lys-Val-Lys-Thr-Leu-Lys-Ala-Glu-Asn-Ser-Glu-Leu-Ala-Ser-Thr-Ala-Asn-Met-Leu-Arg-Glu-Glu-Val-Ala-Gln-Leu-Glu-Gln-Glu-Val-Met-Asn-His-Ala-COOH

The peptides were lyophilized and stored dessicated at 4°C until used. At the time a peptide was to be tested for activity, it was weighed out into an Eppendorf tube, and dissolved in about 15-25 µl of dimethyl sulfoxide and then diluted to 250 µl with assay media consisting of RPMI media containing 1% fetal calf serum to give working stock solutions of 4 x 10^{-3} M. If desired, these stock solutions were aliquoted and stored at -70°C. Prior to performing the assay, the 4 x 10^{-3} M stock was diluted in tubes to give final concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M in the assay.

The endothelial cells were isolated from human umbilical cords by mild collagenase digestion. Collagenase was obtained from Worthington Corporation, Freehole, New Jersey, and the general procedure is described by Jaffe, E.A., et al., 1973, J. of Clin. Invest., 52:2745. The cells obtained from collagenase digestion were grown on gelatin-coated flasks in cell culture medium consisting of medium 199 (Gibco,Grand Island, New York) buffered with 25 mM Hepes. The media was supplemented with 20% fetal calf serum. The media also contained 60 µg/ml sodium heparin (Sigma Corporation, St. Louis, MO), 2 mM L-glutamine and 50 µg/ml of bovine hypothalamus extract. The bovine tissue was obtained from Pel Freeze, Rogers, AK. The hypothalamus extract serves as a source of endothelial cell growth factor. The pH of the cell culture media was 7.4.

After the endothelial cells reach confluency, they are passaged with 0.25% trypsin containing 0.02% EDTA, and subsequent subculturing was performed using the same solution. The cells were exposed to this mixture in Hank's balanced salt solution at room temperature for about 1 minute.

Finally, approximately 2 x 10^4 cells/well were seeded in microtiter plates. The endothelial cell nature of the cells was confirmed both by their cobblestone morphology at confluency, and the fact that they stained positive for Factor VIII antigen by indirect immunofluorescence. The latter procedure is well known in the art, and is described by Jaffe, E. A., 1973, J. Clin. Invest., 52:2745.

Monolayers of endothelial cells, prior to the fifth passage, were established on polystyrene, 96-well flat bottom micro titer plates (Corning Corporation) in Medium 199 containing 20% fetal calf serum 25 mM hepes, pH 7.4, and the other supplements described above. The surfaces of the micro titer plates were incubated with 6.4 µg/ml

human plasma fibronectin for 30 minutes at 25°C prior to plating the endothelial cells. The solution of fibronectin was removed before addition of endothelial cells.

The endothelial cell cultures were used when they were confluent. The endothelial monolayers were washed with RPMI plus 1% fetal calf serum and activated with 125 U/ml of TNF, and then incubated with labelled polymorphonuclear leukocytes at a final concentration 5 x 10^5 cells per well. The cells were allowed to settle for 30 minutes onto the endothelial cell monolayers.

Human polymorphonuclear leukocytes were obtained from venus blood from several healthy adult volunteers using an anti-coagulant (10% heparin) followed by centrifugation of the blood on Ficoll-Hypaque gradients. Contaminating erythrocytes were removed by hypotonic lysis. The remaining cell population consisted of 95 to 98% polymorphonuclear leukocytes, and these cells were suspended at a concentration of 50 x 10^6 cells per ml in Hank's balanced salt solution, pH 7.4.

The polymorphonuclear leukocytes were labelled with ^{111}Indium-oxide (100 µCi/ 10^8 PMNs) (10 mCi/mml, Amersham Corp.). Labelling occurred at room temperature in Hank's solution for 15 minutes, after which the labelled cells were isolated by centrifugation for 5 minutes, and to remove residual unincorporated label, washed twice with Hank's balanced salt solution, and then suspended in RPMI supplemented with 1% fetal calf serum.

As mentioned above, 5 x 10^5 of the labelled PMNs cells were added per well in 96-well micro titer plates. Incubations were conducted for 30 minutes at 37°C, in a tissue culture incubator in an atmosphere of 5% CO_2, 95% air.

After the 30 minute incubation period, during which the polymorphonuclear leukocytes adhere to the endothelial cell monolayer, the micro titer plates were filled and sealed with adherent transparent plastic (Dynatech, Inc., Alexander, Virginia), inverted and centrifuged using a micro plate carrier, obtainable from Beckman Instruments Corp. Centrifugation was at 75 x g for 5 minutes at room temperature. This effectively removed nonadherent PMNs from the endothelial cell monolayers. Next, the plates were blotted dry and the number of polymorphonuclear leukocytes that remained adherent to the endothelial cell monolayers was determined using a gamma counter. The results are shown in Table 2, and they are expressed as the percent of polymorphonuclear leukocytes that remained adherent to the endothelial cell monolayers.

The data shown in Table 2 is instructive in several aspects. Firstly, the most active peptide is 4-29. That is, the number 4 peptide derived from CD18 that has 29 amino acids. This peptide shows significant inhibitory activity in the PMN adhesion

assay. The molecule shows a peak activity in the range of 10^{-5} to 10^{-6} M. Secondly, a 14 amino acid peptide of the number 4 peptide shows little activity. The range of activities for the 29 mer varies over the concentrations tested.

Table 2

Summary of Effect of CD18 Peptides on PMN Adhesion to TNF Activated ECs

	Average % Change				
Peptides	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
4-29	-30(10)*	-65(10)	-48(14)	+3(10)	-12(6)
1-26	-41(6)	-33(3)	-6(7)	+24(3)	+9(6)
1-15	-24(4)	(ND)	-9(5)	(ND)	-4(4)
2-24	-79(4)	(ND)	-10(5)	(ND)	-2(4)
2-16	-7(4)	(ND)	-1(5)	(ND)	-2(4)
3-29	-58(4)	+12(2)	-5(6)	+27(2)	-2(4)
3-17	-13(4)	+43(2)	+13(6)	-18(2)	-7(4)
4-14	-9(4)	+28(4)	-4(8)	+12(4)	-6(4)
5-24	-17(4)	(ND)	-13(5)	(ND)	-6(4)
5-14	+9(4)	(ND)	+7(5)	(ND)	0(4)
6-25	-13(4)	(ND)	+8(5)	(ND)	+12(4)
6-16	+6(4)	(ND)	-11(5)	(ND)	-25(4)
WT	-54(2)	0(2)	+22(6)	+8(2)	-15(2)
JUN-k	-25(2)	-15(2)	+3(2)	-7(2)	(ND)
2X	-38(2)	-1(2)	-18(4)	-23(2)	-20(2)

* = (# Donors, Variation Between Different PMN Donors was About ± 20%)

A significant number of the peptides exhibited activity at 10^{-4} M, but little or no activity at lower concentrations, and none of the peptides were as active as 4-29 over the range of 10^{-5} to 10^{-6} M. The 26 amino acid peptide of the number 1 peptide of CD18 (1-26) shows inhibitory activity with an apparent peak in the range of 10^{-4} to 10^{-5} M. Similarly, the 24 amino acid peptide of the number 2 region of CD18 (2-24), and 3-29, show activity at 10^{-4} M. The peptides that showed little inhibitory activity are derived from the fifth and sixth regions of the molecule and contain 14 or 24, or 16 or 25 amino acids, respectively.

Finally, it was observed that the JUN-k, and WT peptides inhibited polymorphonuclear leukocyte adhesion at a concentration range of 10^{-4} M, while the peptide 2X had activity over a concentration range of 10^{-4} to 10^{-8} M. The proteins

that contain these peptides are known in the art or described by Angel, P., et al., 1988, Nature, 332:166.

Studies were conducted using the peptide 4-29 with the intent of determining what effect polymorphonuclear leukocytes from different individuals would have on the effectiveness of the peptide in the adhesion assay. Also the stability of the peptide to various storage conditions was determined. The results are shown in Table III.

Experiments using polymorphonuclear leukocytes isolated from donors 100 and 272 indicate that freezing and thawing the peptide does not significantly effect its activity. Moreover, there is significant variation in the activity of the peptide dependent on the donor from which the polymorphonuclear leukocytes were isolated. Finally, Table III also shows that the activity of peptide is maintained after storage for different times at 4°C.

Table 3

Comprehensive Summary of CD18 Peptide #4-29 Data Showing Percent Change in PMN Adhesion to TNF Activated ECs

	Peptide Concentration (M)					
Donor #	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	Peptide Condition
269	-29	(ND)	-68	(ND)	-13	1 day 4°
314	-32	(ND)	-76	(ND)	-4	" "
178	-64	(ND)	-67	(ND)	+2	fresh
475	-19	(ND)	-76	(ND)	-20	" "
121	-14	-59	-44	+3	-15	frozen
390	-16	-43	-9	0	-22	" "
593	(ND)	-86	-14	+14	(ND)	fresh, or 4° for 2 wks
323	(ND)	-42	-53	+45	(ND)	fresh, or 4° for 2 wks
155*	(ND)	-29	-13	+59	(ND)	1 wk 4°
272*	(ND)	-71	-14	+2	(ND)	1 wk 4°
100	-1	-63	-51	-8	(ND)	fresh
100	-38	-81	-68	-29	(ND)	frozen
272	-36	-62	-59	-13	(ND)	fresh
272	-55	-80	-62	-40	(ND)	frozen
108	-16	-63	-19	-2	-1	frozen
489	-31	-72	-27	-15	-12	frozen
108*	-68	-87	-32	-2	+21	frozen
489*	-37	-79	-37	-7	-3	frozen
155	-7	-57	-41	+1	(ND)	fresh
272	+53	-48	-41	+42	(ND)	fresh

* endothelial cells were activated with IL-1.

Additional studies were conducted with 4-29 with the intent of determining if a shorter peptide within this region would have activity. Since 4-14 was shown to have little activity, 4-15 was tested and the results are shown in Table 4. Clearly, 4-15 is active, with the peak of activity being in the 10^{-4} to 10^{-5} M range. Variability in the assay was observed as a function of the donor from which the PMNs were isolated.

Table 4

Percent Change of Adhesion

	Donor	10^{-4} M	10^{-5} M	10^{-6} M
	155	-74	-53	-23
	272	-73	-70	-19
	279	-45	-26	-12
	279	-63	-21	-20
	499	-36	-36	-9
Average		-58±7	-41±8	-17±2

	Donor	10^{-5} M	10^{-5} M	10^{-6} M
	140	-50	-6	-6
	195	70	0	2
	363	-13	-18	-14
	515	-5	4	-10
	140	-53	0	24
Average		-24±10	-5±3	-6±2

Example 2

Effect of CD18 Peptides on Chemotaxis of Polymorphonuclear Leukocytes

Many of the materials and methods used to test the inhibitory activity of the CD18 peptides on the chemotactic response of the polymorphonuclear leukocytes are similar or identical to those used to perform the adhesion assays described in Example 1. Polymorphonuclear leukocytes were isolated and labelled with ^{111}Indium as described before, and the cells were suspended in RPMI 1640 culture medium at a concentration of 5 x 10^6/ml. Next, 75 µl of the cell suspension and 25 µl of media containing a desired concentration of a CD18 peptide were added to trans-well inserts, and the mixture was incubated at 37°C for 10 minutes.

To the bottom wells of the 24-well plate 0.6 ml of media containing 0.5% zymosan-activated human serum was added. This solution was also warmed to 37°C for 10 minutes prior to use.

Next, the assay was conducted by incubating the trans-well inserts containing the cell suspension with the desired CD18 peptide in the 24-well plates at 37°C for 30 minutes. Subsequently, the inserts were removed, and 60 µl of a 10% sodium dodecyl sulphate solution was added to the wells of the 24-well plate. The plates were incubated with gentle shaking at room temperature for 15 minutes, and 110 µl aliquots were removed and the amount of ^{111}Indium determined using a gamma-counter.

Table 5 shows the results, which are expressed as the percent inhibition or enhancement of migration caused by the peptides over a concentration range of 10^{-4} to 10^{6} M. The data were gathered from polymorphonuclear leukocytes isolated from four different human donors numbered 591,593, 195 and 499. It is immediately apparent upon reviewing the data that there is a marked inhibition of chemotaxis migration at concentrations ranging from 10^{-4}-10^{-5} M for peptides 2-24, 3-29 and 4-29. Maximum inhibition is a function both of the concentration of the peptide tested, as well as the donor from which the polymorphonuclear leukocytes were isolated.

Table 5

Chemotaxis Summary CD18 Peptides*

Peptide[M]		Donors 591	593	195	499
1-26	10^{-4} M	-9	-50	19	8
	10^{-5} M	-36	-19	-41	-3
	10^{-6} M	-39	-14	-37	13
2-24	10^{-4} M	-73	-89	-48	-77
	10^{-5} M	-9	-40	-33	-33
	10^{-6} M	-17	-22	22	0
3-29	10^{-4} M	-57	-85	-7	-33
	10^{-5} M	-6	-17	11	23
	10^{-6} M	11	-6	-26	-10
4-29	10^{-4} M	-74	-88	-93	-87
	10^{-5} M	-88	-88	-93	-79
	10^{-6} M	0	4	11	0
5-24	10^{-4} M	51	-33	-52	0
	10^{-5} M	55	12	-56	-15
	10^{-6} M	52	-4	-22	-26
6-25	10^{-4} M	-16	-51	-4	-3
	10^{-5} M	49	26	11	-13
	10^{-6} M	49	20	-44	-8

* Data are expressed as the per cent inhibition (-) or enhancement of polymorphonuclear leukocyte migration.

Partly because of the variation in the results observed using polymorphonuclear leukocytes from different individuals, additional experiments were done to confirm the inhibitory effects of the most active peptide, 4-29. The experiments were done using polymorphonuclear leukocytes from 6 donors, with one of the donors, 195, being used here and in the previous experiment. Furthermore, the experiments were conducted over a concentration range of 10^{-4}-10^{-7}. Table 6 shows the results. The inhibitory activity of the peptide was confirmed, with significant activity being observed for all donors. The peak of activity is at 10^{-5} M. It should be noted, as was observed in the previous experiment, that there is variation in the per cent inhibition using polymorphonuclear leukocytes from different donors.

Table 6

Effect of CD18 Peptide 4-29 on Neutrophil Chemotaxis

	Peptide Concentration			
Donor	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M
	(% Inhibition of Migration)[a]			
140	83	69	2	0
279	74	49	17	0
169	--	75	--	--
359	--	74	--	--
195	76	87	34	--
363	76	79	34	--

a Measured by total numbers of cell that have migrated through the filter in response to 0.5% zymosan-activated human serum.

Example 3

Inhibition of Rhinovirus Binding to Endothelial Cells

The peptide 4-29 can be tested for the capacity of inhibiting rhinovirus binding to activated endothelial cells using the assay described in Example 1, and additionally having present in the assay mixture varying amounts of the peptide and about 1 x 10^4 counts per minute of labelled rhinovirus. Radiolabelled virus may be produced as described by Abraham, G. and Colonno, R. J., 1984, J. Virol., 51:340, with modifications as described by Marlin, S. D. et al., 1990, Nature, 344:70. The endothelial cells are preincubated with 4-29 for 30 minutes at 4°C to prevent endocytosis of the peptide. It would be determined that a concentration of about 50-100 µg/ml would provide for about 50% inhibition of rhinovirus binding.

Example 4

Prophylactic use of CD18 Peptide (4-29) for Preventing Colds

The CD18 peptide 4-29 can be applied for the efficacious prevention of common colds by formulating it in a pharmaceutically acceptable nasal carrier in an effective amount. The amount can be empirically determined by those skilled in the art, but will generally be in the range of about 50 µg - 1 mg/ml. Nasal pharmaceutical formulations are well known to those skilled in the art, and are detailed in

"Remington's Pharmaceutical Sciences", 14th Edition, 1970. It will be appreciated, however, that the choice of a suitable carrier will depend on the nature of the particular nasal dosage form desired. That is, whether the peptide will be formulated as a nasal solution for use as drops or spray, a nasal suspension, a nasal ointment, or a nasal gel. Preferred is a nasal dosage form consisting of a solution, suspensions and gels, in which peptide 4-29 is present in a physiologically compatible solution. The solution may also contain minor amounts of emulsifiers or dispersing agents, buffering agents, preservatives, wetting agents and gelling agents as are known in the art.

Peptide 4-29 in nasal spray form may be applied by an individual prior to, or immediately after being exposed to other individuals that have a cold. In this way, the exposure of an individual to rhinovirus by an infected individual can be minimized or eliminated since the peptide 4-29 would prevent or greatly reduce the amount of virus that binds to nasal endothelial.

The present invention has been described with reference to specific embodiments. However, this application is intended to cover those changes and substitutions which may be made by those skilled in the art without departing from the spirit and the scope of the appended claims.

WE CLAIM:

1. A leukocyte ß subunit, CD18, integrin peptide that interferes with the adhesion of leukocytes to biological material.

2. A peptide as described in claim 1, wherein said peptide is selected from the group consisting of:

a) NH_2-Try-Pro-Ile-Asp-Leu-Tyr-Tyr-Leu-Met-Asp-Leu-Ser-Tyr-Ser-MET-Leu-Asp-Asp-Leu-Arg-Asn-Val-Lys-Lys-Leu-Gly-COOH;

b) NH_2-Phe-Asp-Tyr-Pro-Ser-Val-Gly-Gln-Leu-Ala-His-Lys-Leu-Ala-Glu-Asn-Asn-Ile-Gln-Pro-Ile-Phe-Ala-Val-Thr-COOH;

c) NH_2-Ile-Pro-Lys-Ser-Ala-Val-Gly-Glu-Leu-Ser-Glu-Asp-Ser-Ser-Asn-Val-Val-His-Leu-Ile-Lys-Asn-Ala-Tyr-Asn-Lys-Leu-Ser-Ser-COOH;

d) NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH;

e) NH_2-Val-Gly-Lys-Gln-Leu-Ile-Ser-Gly-Asn-Leu-Asp-Ala-Pro-Glu-Gly-Gly-Leu-Asp-Ala-Met-Met-Gln-Val-Ala-COOH; and

f) NH_2-Arg-Ile-Gly-Phe-Gly-Ser-Phe-Val-Asp-Lys-Thr-Val-Leu-Pro-Phe-Val-Asn-Thr-His-Pro-Asp-Lys-Leu-Arg-Asn-COOH.

3. A peptide as described in claim 2, wherein said peptide comprises: NH_2-Try-Pro-Ile-Asp-Leu-Tyr-Tyr-Leu-Met-Asp-Leu-Ser-Tyr-Ser-Met-Leu-Asp-Asp-Leu-Arg-Asn-Val-Lys-Lys-Leu-Gly-COOH.

4. A peptide as described in claim 2, wherein said peptide comprises: NH_2-Phe-Asp-Tyr-Pro-Ser-Val-Gly-Gln-Leu-Ala-His-Lys-Leu-Ala-Glu-Asn-Asn-Ile-Gln-Pro-Ile-Phe-Ala-Val-Thr-COOH.

5. A peptide as described in claim 2, wherein said peptide comprises: NH_2-Ile-Pro-Lys-Ser-Ala-Val-Gly-Glu-Leu-Ser-Glu-Asp-Ser-Ser-Asn-Val-Val-His-Leu-Ile-Lys-Asn-Ala-Tyr-Asn-Lys-Leu-Ser-Ser-COOH.

6. A peptide as described in claim 2, wherein said peptide comprises: NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH.

7. A peptide as described in claim 2, wherein said peptide comprises: NH_2-Val-Gly-Lys-Gln-Leu-Ile-Ser-Gly-Asn-Leu-Asp-Ala-Pro-Glu-Gly-Gly-Leu-Asp-Ala-Met-Met-Gln-Val-Ala-COOH.

8. A peptide as described in claim 2, wherein said peptide comprises: NH_2-Arg-Ile-Gly-Phe-Gly-Ser-Phe-Val-Asp-Lys-Thr-Val-Leu-Pro-Phe-Val-Asn-Thr-His-Pro-Asp-Lys-Leu-Arg-Asn-COOH.

9. A peptide as described in claim 1, wherein said biological material is characterized by having ICAM.

10. A peptide as described in claim 9, wherein said biological material is endothelium.

11. A leukocyte ß subunit, CD18, integrin peptide that comprises the properties of interfering with or preventing binding of leukocytes to biological material and interfering with or preventing chemotaxis of leukocytes to said biological material .

12. A method for treating disease, comprising administering to a living subject an effective amount of one or more leukocyte ß subunit, CD18, integrin peptide(s) that prevents or interferes with adhesion of leukocytes to biological material.

13. A method for treating disease as described in claim 12, wherein said disease comprises an inflammatory response.

14. A method for treating disease as described in claim 13, wherein said leukocyte ß subunit, CD18, integrin peptide is selected from the group consisting of:

a) NH_2-Try-Pro-Ile-Asp-Leu-Tyr-Tyr-Leu-Met-Asp-Leu-Ser-Tyr-Ser-Met-Leu-Asp-Asp-Leu-Arg-Asn-Val-Lys-Lys-Leu-Gly-COOH;

b) NH_2-Phe-Asp-Tyr-Pro-Ser-Val-Gly-Gln-Leu-Ala-His-Lys-Leu-Ala-Glu-Asn-Asn-Ile-Gln-Pro-Ile-Phe-Ala-Val-Thr-COOH;

c) NH_2-Ile-Pro-Lys-Ser-Ala-Val-Gly-Glu-Leu-Ser-Glu-Asp-Ser-Ser-Asn-Val-Val-His-Leu-Ile-Lys-Asn-Ala-Tyr-Asn-Lys-Leu-Ser-Ser-COOH;

d) NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH;

e) NH_2-Val-Gly-Lys-Gln-Leu-Ile-Ser-Gly-Asn-Leu-Asp-Ala-Pro-Glu-Gly-Gly-Leu-Asp-Ala-Met-Met-Gln-Val-Ala-COOH; and

f) NH_2-Arg-Ile-Gly-Phe-Gly-Ser-Phe-Val-Asp-Lys-Thr-Val-Leu-Pro-Phe-Val-Asn-Thr-His-Pro-Asp-Lys-Leu-Arg-Asn-COOH.

15. A method for treating disease as described in claim 14, wherein said leukocyte ß subunit, CD18, integrin peptide comprises 4-29: NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH.

16. A method for treating disease as described in claim 12, wherein said disease is caused by rhinovirus.

17. Antibody to leukocyte ß subunit, CD18, integrin chemotactic peptide(s).

18. A method for treating disease, comprising administering to a living subject an effective amount of one or more antibodies that bind to a peptide selected from the group consisting of:

a) NH_2-Try-Pro-Ile-Asp-Leu-Tyr-Tyr-Leu-Met-Asp-Leu-Ser-Tyr-Ser-Met-Leu-Asp-Asp-Leu-Arg-Asn-Val-Lys-Lys-Leu-Gly-COOH;

b) NH_2-Phe-Asp-Tyr-Pro-Ser-Val-Gly-Gln-Leu-Ala-His-Lys-Leu-Ala-Glu-Asn-Asn-Ile-Gln-Pro-Ile-Phe-Ala-Val-Thr-COOH;

c) NH_2-Ile-Pro-Lys-Ser-Ala-Val-Gly-Glu-Leu-Ser-Glu-Asp-Ser-Ser-Asn-Val-Val-His-Leu-Ile-Lys-Asn-Ala-Tyr-Asn-Lys-Leu-Ser-Ser-COOH;

d) NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH;

e) NH_2-Val-Gly-Lys-Gln-Leu-Ile-Ser-Gly-Asn-Leu-Asp-Ala-Pro-Glu-Gly-Gly-Leu-Asp-Ala-Met-Met-Gln-Val-Ala-COOH; and

f) NH_2-Arg-Ile-Gly-Phe-Gly-Ser-Phe-Val-Asp-Lys-Thr-Val-Leu-Pro-Phe-Val-Asn-Thr-His-Pro-Asp-Lys-Leu-Arg-Asn-COOH.

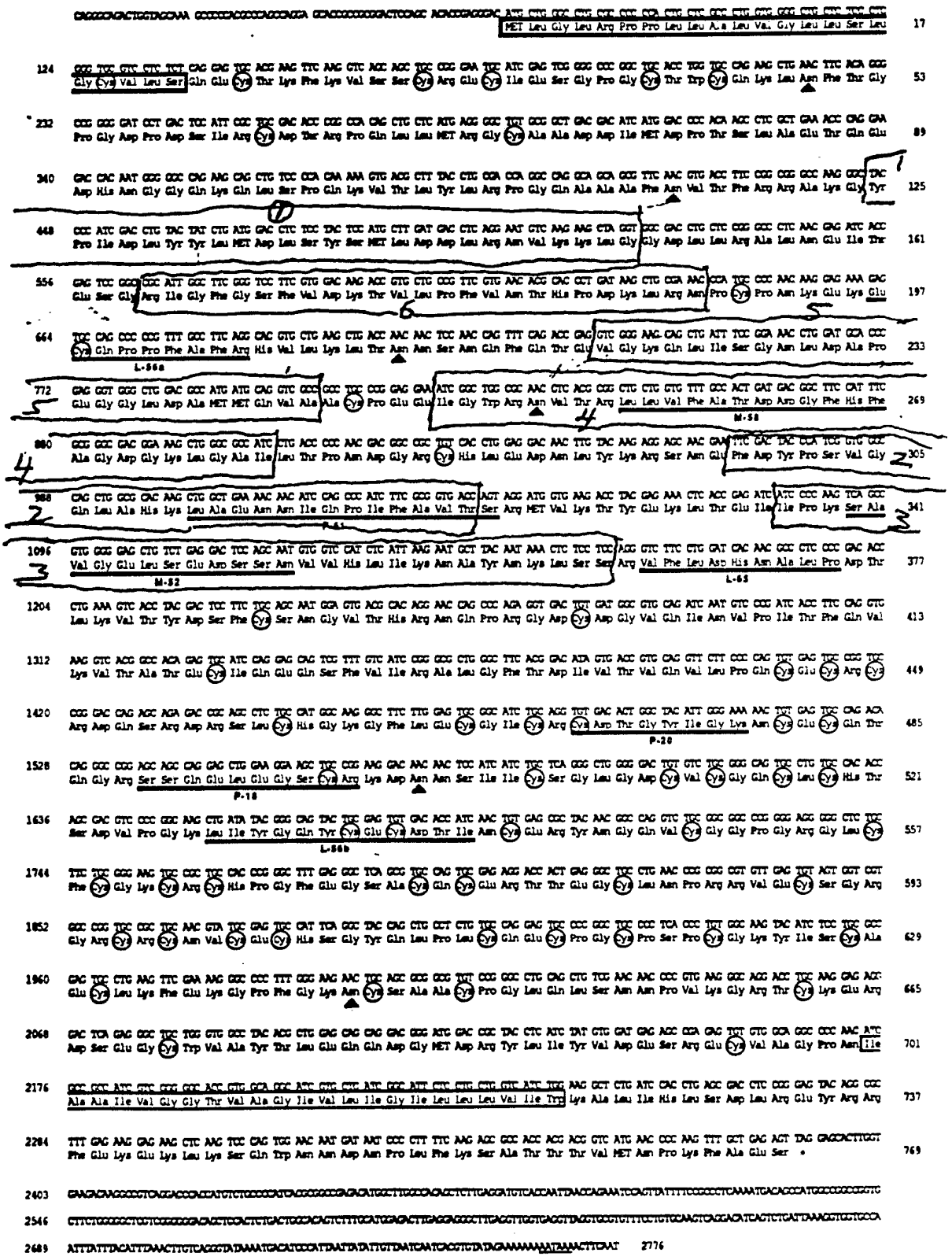
19. A method for treating disease, comprising administering to a living subject an effective amount of an antibody that binds to 4-29: NH_2-Ile-Gly-Trp-Arg-Asn-Val-Thr-Arg-Leu-Leu-Val-Phe-Ala-Thr-Asp-Asp-Gly-Phe-His-Phe-Ala-Gly-Asp-Gly-Lys-Leu-Gly-Ala-Ile-COOH.

20. A method for treating disease as described in claim 18, wherein said disease is caused by rhinovirus.

21. A method for treating disease as described in claim 19, wherein said disease is caused by rhinovirus.

22. A peptide that interferes with the adhesion of leukocytes to biological material, said peptide selected from the group consisting of WT, JUN-k, or 2X.

Figure 1



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/06053

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 K 7/00 C 12 P 21/08 A 61 K 37/02
A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 K C 12 P A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	CELL, vol. 48, no. 4, 27 February 1987, Cell Press, (Cambridge, MA, US), T.K. KISHIMOTO et al.: "Cloning of the beta subunit of the leukocyte adhesion proteins: Homology to an extracellular matrix receptor defines a novel supergene family", pages 681-690, see figures 4,6 (cited in the application) ---	1-22
Y	WO,A,9003983 (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 19 April 1990, see page 11, lines 13-14; claims ---	1-22
Y	WO,A,8806592 (DANA FARBER CANCER INSTITUTE) 7 September 1988, see the whole document --- -/-	1-22

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13-12-1991	13. 01. 92
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: M. PEIS M. Peis

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	EMBO JOURNAL, vol. 9, no. 5, May 1990, Oxford University Press, (Oxford, GB), H. RAMASWAMY et al.: "Cloning, primary structure and properties of a novel human integrin beta subunit", pages 1561-1568, see the abstract; figure 5; page 1561, right-hand column, lines 39-48 ---	1-22
Y	NATURE, vol. 344, 1 March 1990, (London, GB), S.D. MARLIN et al.: "A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection", pages 70-72, see the whole document (cited in the application) ---	18-22
A	IMMUNOGENETICS, vol. 31, no. 3, March 1990, Springer-Verlag, D.L. ZEGER et al.: "Mouse macrophage beta subunit (CD11b) cDNA for the CR3 complement receptor/Mac-1 antigen", pages 191-197, see the abstract -----	1-22

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12-16 and 18-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged facts of the compound/composition.

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.