PEPTIDE INHIBITORS OF LFA-1/ICAM-1 INTERACTION

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ABSTRACT
Cyclic peptides inhibit LFA-1 interaction with ICAM-1 and are useful in treatment of hematopoietic neoplasms and in adjunct therapy in prevention of retinoic acid syndrome and diseases involving emigration of leukocytes into organ tissue.

9 Claims, 2 Drawing Sheets
A library of phage, each displaying a different peptide sequence is applied to surface coated with ICAM-1.

Unbound phage is washed away.

Specifically-bound phage is eluted with 84H10 mAb.

After 3-4 rounds, individual clones are isolated and sequenced.

Eluted pool of phage is amplified, and the process is repeated for a total of 3-4 rounds.

**FIG. 2**

![Diagram](image)

**FIG. 3**

![Peptide Sequence](image)
PEPTIDE INHIBITORS OF LFA-1/ICAM-1 INTERACTION


BACKGROUND

1. Field of the Invention

The present invention relates to the field of biologically active peptide containing formulations for use in the prevention and treatment of hematopoietic neoplastic diseases, particularly leukemia.

2. Description of Related Art

LFA-1 (lymphocyte function associated antigen-1) is an integrin β2 heterodimer (Carlos and Harlan, 1994; Springer, 1994; Larson and Springer, 1990; McEver, 1990; Picker and Butcher, 1992). Although three other integrins restrict in expression to leukocytes share the same β subunit and have homologous α subunits (Mac-1, p150,95, and alpha d), only LFA-1 is expressed on normal and leukemia T cells (Larson and Springer, 1990). LFA-1 binds ICAM-1 (intracellular adhesion molecule), and although LFA-1 is constitutively expressed on all leukocytes, LFA-1 binding to ICAM-1 requires cellular activation. Activation, in part, results in conformational changes in LFA-1 that affect its avidity for ICAM-1. In contrast, ICAM-1 is constitutively avid and expressed on a wide array of cell types including leukocytes, endothelium, stromal cells, and fibroblasts. In a model developed by the present inventor, a stromal cell derived soluble factor cooperates with LFA-1 on the surface of T lineage acute lymphoblastic leukemia (T-ALL) cells (Winter et al., 1998). The LFA-1 on T-ALL cells results in bone marrow (BM) stromal cell binding via ICAM-1 that leads to enhanced leukemia cell survival. Furthermore, aberrant LFA-1/ICAM-1 dependent interaction between circulating leukemia cells and endothelial cells lining blood vessels promotes extravasation of leukemia cells into tissue as seen in the life-threatening therapeutic complication of acute leukemia, retinoic acid syndrome (Brown et al., 1999).

Hence, the development of effective in vivo inhibitors of LFA-1/ICAM-1 interaction would be useful in the therapy of acute leukemia and prevention of therapeutic complications.

The present inventor has shown, for example, that inhibition of LFA-1/ICAM-1 dependent stromal cell binding with mAbs decreases survival of T-ALL cell lines and T-ALL cells isolated from patients. In one study, a representative sample from a patient with T-ALL showed that survival of T-ALL cells is augmented by BM stromal cells and that survival is inhibited by mAbs directed against LFA-1 (mAb 1SI22.5 μg/ml) or its ligand ICAM-1 (mAb 84H10, 10 μg/ml). This observation has been replicated for T-ALL cell lines Jurkat and Sup T1 as well as a subset of patients with T-ALL. However, even though in vivo use of mAbs against LFA-1 or ICAM-1 blocks LFA-1 function in a number of disease models, unfortunately anaphylactic reactions and secondary physiologic effects have hampered this approach (McMuray, 1996; DeMeester et al., 1996; Jackson et al., 1997; Cuthbertson et al., 1997; Gundel et al., 1992; Haming et al., 1993; Nakano et al., 1995).

Another means to interfere with protein—protein interactions is through the use of small peptide inhibitors. In fact, small peptide inhibitors to adhesion molecules structurally related to LFA-1 have recently been approved for clinical use in coagulopathies (Ohman et al., 1995; Adgey et al., 1998; Lefevre and Topol, 1995). Short linear peptides (<30 amino acids) have also been described that prevent or interfere with integrin dependent firm adhesion using sequences derived from integrin or their ligands. In particular, these peptides have been derived from a number of integrin receptors: the β2 and β3 subunits of integrins, and the α6 subunit of ICAM-1, and VCAM-1 (Murayama et al., 1996; Jacobsson and Frykberg, 1996; Zhang and Plow, 1996; Budnik et al., 1996; Vanderslice et al, 1997; Suehiro et al., 1996; Endemann et al., 1996). However, the clinical applicability of these linear peptides is limited. The half maximal inhibitory concentration (IC50) at which aggregation is inhibited 50% for most of these peptides is 10-4 M with purified receptor-ligand pairs (univalent interactions) and they are ineffective at inhibiting multivalent interactions, during cell—cell adhesion. In addition, linear peptides have short serum half-lives because of proteolysis. Therefore, prohibitively high concentrations of peptide would have to be administered in a clinical setting and a biologic effect would not necessarily occur.

Longer peptides, ranging in length from 25–200 residues, have also been reported to block β1, β2, and β3 integrin dependent adhesion (Zhang and Plow, 1996; Budnik et al., 1996; Vanderslice et al, 1997; Suehiro et al., 1996; Endemann et al., 1996). In general, these peptide inhibitors may have higher affinities or slower off-rates than short peptides and, therefore, are better inhibitors. However, they are still susceptible to proteolysis.

Therefore, a need exists to develop novel and specific classes of pharmaceutical agents to inhibit the binding of LFA-1 and ICAM-1 and to be useful in the treatment of hematopoietic neoplastic diseases as well as other diseases that involve emigration of leukocytes from blood into tissue, such as myocardial infarction, radiation injury, asthma, rheumatoid arthritis, and lymphoma metastasis.

SUMMARY

The present invention addresses the problems in the art by providing compositions that include cyclic peptide inhibitors of binding interactions between the integrin, lymphocyte function associated antigen-1 (LFA-1) expressed on leukocytes, including leukemic T-cells, and intracellular adhesion molecule 1 (ICAM-1), expressed on a variety of cell types. As stated above, this binding of activated LFA-1 is implicated in a variety of diseases and inhibition of this binding interaction with a cyclic peptide inhibitor will have implications in the treatment or management of those diseases.

As a part of the present invention, phage display has been used to identify peptide sequences that bind ICAM-1 and block LFA-1/ICAM interaction. Phage that specifically bound ICAM-1 were identified by repeated selection from a cysteine-constrained heptapeptide phage display library. The peptide sequences expressed on ICAM-1 binding phage were determined by nucleotide sequencing. A consensus sequence, CLLRMRSC (SEQ ID NO:1) was derived from the analysis of the most frequently occurring sequences. Analysis of less frequently recurring amino acids of ICAM-1 binding-phage identified variants of SEQ ID NO: 1 wherein the second amino acid is methionine (SEQ ID NO: 17), or in which the 5th amino acid is proline (SEQ ID NO: 5), or in which the 6th amino acid is asparagine (SEQ ID NO: 9), or in which the 7th amino acid is leucine (SEQ ID NO: 3), or in which the 8th amino acid is arginine (SEQ ID NO: 2), or any combination of these substitutions.

Another aspect of the present disclosure is the application of an alanine scanning technique to the consensus sequence, SEQ ID NO: 1. An alanine was substituted at each position
in the heptapeptide LLRMRSL of the consensus sequence SEQ ID NO: 1, and each peptide was examined in for its ability to inhibit LFA-1/ICAM-1 mediated cell aggregation. Alanine substitution of the isoleucine at position 8 of SEQ ID NO: 1, i.e., CLLRMRSC (SEQ ID NO: 40), resulted in a more potent antagonist. Loss of inhibitory function resulted from alanine substitution of the leucines in positions 2 (SEQ ID NO: 34) and 3 (SEQ ID NO: 35), methionine in position 5 (SEQ ID NO: 37), and arginine in position 6 (SEQ ID NO: 38) of SEQ ID NO: 1, indicating that these amino acids are important to the antagonistic activity of the peptide. Substitution of the serine in position 7 (SEQ ID NO: 39) had no significant effect on the inhibitory activity of the peptide. Substitution of the arginine in position 4 (i.e., SEQ ID NO: 36) of SEQ ID NO: 1 was not soluble in aqueous solution at 1 mM and was not tested.

A further aspect of the present invention is the use of conservative amino acid substitutions of the key amino acid residues identified by the alanine screen. Sequences that exhibit greater inhibition of LFA-1/ICAM-1 mediated cell inhibition as compared to SEQ ID NO: 1, include CLLRMRSAC (SEQ ID NO: 43), CLLRMRSC (SEQ ID NO: 44), CLLRMKRSAC (SEQ ID NO: 45), CLLRMLRSAC (SEQ ID NO: 46), and CLLRMRSV (SEQ ID NO: 48).

The present invention may be described, therefore, in certain aspects as a composition comprising a cyclic peptide inhibitor of LFA-1/ICAM-1 interaction, wherein the composition has the amino acid sequence, CLLRMRSC (SEQ ID NO: 1) or CLLRMRSC (SEQ ID NO: 40), or a conservative variant thereof. Conservative variants are described elsewhere herein, and include the exchange of an amino acid for another of like charge, size, or hydrophobicity, and include, but are not limited to, CLRMRSC (SEQ ID NO: 43), CLLRMRSAC (SEQ ID NO: 44), CLLRMKRSAC (SEQ ID NO: 45), CLLRMLRSAC (SEQ ID NO: 46), and CLLRMRSV (SEQ ID NO: 48). The present disclosure would also include variants of SEQ ID NO: 1 in which the 3rd and 4th amino acids remain the same and other amino acids of the sequence are substituted and tested empirically for their ability to inhibit the LFA-1/ICAM-1 interaction. The amino acid sequence is numbered in the conventional sense, in that the first amino acid on the N-terminus is cysteine, followed by 7 amino acids and then a carboxy terminal cysteine. In the practice of the invention, the two terminal cysteines may form a disulfide bonded cystine residue resulting in a cyclic peptide as is well known in the art. Alternatively, the terminal cysteines may be linked by an amide peptide linkage, either directly or separated by one or more amino acids, leaving two free sulfhydryl groups.

Based on the empirical data obtained by the inventor and disclosed herein, a cyclic peptide of the invention may have the sequence of SEQ ID NO: 1, or it may be a derivative of that sequence in which the second amino acid is methionine (SEQ ID NO: 17), or in which the 5th amino acid is proline (SEQ ID NO: 5), or in which the 6th amino acid is asparagine (SEQ ID NO: 9), or in which the 7th amino acid is leucine (SEQ ID NO: 3), or in which the 8th amino acid is arginine (SEQ ID NO: 2), or any combination of these substitutions, or even conservative variants of any of these substitutions.

Another aspect of the present invention includes cyclic peptides inhibitors of LFA-1/ICAM-1 interaction comprising the heptapeptide sequences which include LLRMRSL (SEQ ID NO: 49), LLRMRSA (SEQ ID NO: 50), LLRMRSA (SEQ ID NO: 51), LLRMRSA (SEQ ID NO: 52), LLRMRSA (SEQ ID NO: 53), LLRMRSA (SEQ ID NO: 54) or LLRMRSV (SEQ ID NO: 55). Peptides that comprise the heptapeptide sequences of the present invention may be a peptide of 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15 amino acids in length, wherein additional amino acids may be any L-series or any D-series amino acid.

In preferred embodiments of the invention, the peptides or peptide mimetics of the invention exhibit an inhibition constant (IC₅₀) for the binding interaction of LFA-1/ICAM-1 of from about 10 μM to about 800 μM for cell aggregation or from about 10 to about 250 nM for non-augment ICAM-1 binding. The term "IC₅₀" is well known in the art, and means the half maximal inhibitory concentration, or concentration at which aggregation is inhibited by 50%.

Any of the compositions described herein may be formulated for pharmaceutical or therapeutic administration either to a mammal, or more preferably to a human. As such, the compositions may be contained in a pharmaceutically acceptable carrier. The preferred mode of administration of a peptide active agent is by injection, either intravenous, intra-arterial, intramuscular or subcutaneous. Other routes of administration may also be possible and would be included within the scope of the present disclosure.

The compositions may be administered parenterally or intraperitoneally. Solutions of the active compounds as free base or pharmaceutically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxpropyldiolus. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be suitable fluid. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.
As used herein, "pharmacologically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

A peptide can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035–1038 and 1570–1580). Some variation in dosages will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

It is an aspect of the present disclosure that the disclosed compositions may be used in adjunct therapy in standard treatments for diseases such as hematopoietic neoplasms. The present invention may be described therefore, in certain embodiments as a method of treating retinoic acid syndrome in a subject receiving all-trans retinoic acid, comprising administering to the subject an effective amount of a composition comprising a cyclic peptide, wherein the peptide comprises a heptapeptide sequence including, but not limited to, LLLMRSA (SEQ ID NO: 49), LLLMRSA (SEQ ID NO: 50), LVMRSA (SEQ ID NO: 51), ILLMRSA (SEQ ID NO: 52), LLLMRSA (SEQ ID NO: 53), ILLMRSA (SEQ ID NO: 54) or LLRMRSV (SEQ NO: 55). In some embodiments the cyclic peptide has the amino acid sequence CLLLMRSC (SEQ ID NO: 41) or CLLLMRSC (SEQ ID NO: 42), or a conservative variant thereof, such as CLLLMRSC (SEQ ID NO: 43), CLLLMRSC (SEQ ID NO: 44), CLLLMRSC (SEQ ID NO: 45), CLLLMRSC (SEQ ID NO: 46), or CLLLMRSC (SEQ ID NO: 48).

An embodiment of the invention may also be described as a method for inhibiting growth of leukemia cells comprising preventing an LFA-1/ICAM-1 interaction between the leukemia cells and support cells such as bone marrow stromal cells, wherein the method comprises contacting the leukemia cells with a cyclic peptide inhibitor of the LFA-1/ICAM-1 interaction, and further wherein the amino acid sequence of the cyclic peptide inhibitor is not a fragment of the amino acid sequence of LFA-1 or ICAM-1. In the practice of this method, the leukemia cells are preferably in a leukemia patient and contacting comprises administering the cyclic peptide to the patient.

An embodiment of the invention is also a therapeutic package for dispensing to, or for use in dispensing to, a mammal or human being treated for a hematopoietic neoplastic disease, myocardial infarction, radiation injury, asthma, rheumatoid arthritis, or lymphoma metastasis, wherein the package contains in a unit dose, an amount of a cyclic peptide comprising a heptapeptide sequence including, but not limited to, LLLMRSA (SEQ ID NO: 49), LLLMRSA (SEQ ID NO: 50), LLMRMRSA (SEQ ID NO: 51), LLMRMRSA (SEQ ID NO: 52), LRMRKSA (SEQ ID NO: 53), ILRMRSA (SEQ ID NO: 54) or LLRMRSV (SEQ ID NO: 55), effective to inhibit an LFA-1/ICAM-1 interaction in a subject when administered periodically. In some embodiments the cyclic peptide has the amino acid sequence CLLLMRSC (SEQ ID NO: 41) or LLRMRSC (SEQ ID NO: 40), or a conservative variant thereof, such as CLLLMRSC (SEQ ID NO: 43), CLLLMRSC (SEQ ID NO: 44), CLLLMRSC (SEQ ID NO: 45), CLLLMRSC (SEQ ID NO: 46), or LLRMRSC (SEQ ID NO: 48).

In certain embodiments a unit dose is from about 10 to about 500 μg/Kg, or is from about 50 to about 250 μg/Kg, or from about 120 to about 150 μg/Kg. The unit dose may be an initial bolus dose which may be followed by a continuous infusion of about 5 to about 250 μg/Kg/min, or from about 40 to about 60 μg/Kg/min, or may be about 50 μg/Kg/min. Alternatively, unit doses may be repeated daily, and administered multiple times per day. Dosage regimens are not limited to those exemplified, and the invention encompasses any dosage regimen that delivers an therapeutically effective dose. An example of clinical administration of a peptidomimetic inhibiting an integrin functions is documented by Ohman et al. (1995).

An embodiment of the present disclosure is a method of inhibiting emigration of leukocytes from blood into tissue in a subject comprising administering to the subject an amount of a cyclic peptide comprising a heptapeptide sequence including, but not limited to, LLMRSA (SEQ ID NO: 49), LLLMRSA (SEQ ID NO: 50), LLMRSA (SEQ ID NO: 51), LLRMRSA (SEQ ID NO: 52), LLMRSA (SEQ ID NO: 53), LLMRSA (SEQ ID NO: 54) or LLRMRSV (SEQ ID NO: 55), effective to inhibit an LFA-1/ICAM-1 interaction in the subject. In some embodiments the cyclic peptide has the amino acid sequence, CLLLMRSC (SEQ ID NO: 41) or CLLLMRSC (SEQ ID NO: 40), or a conservative variant thereof, such as CLLLMRSC (SEQ ID NO: 43), LLRMRSC (SEQ ID NO: 44), CLLLMRSC (SEQ ID NO: 45), CLLMRSC (SEQ ID NO: 46), or CLLMRSC (SEQ ID NO: 48).

In preferred embodiments the subject is susceptible to the development of or is suffering from a hematopoietic neoplastic disease, myocardial infarction, radiation injury, asthma, rheumatoid arthritis, or lymphoma metastasis. A further embodiment of the invention is a method comprising a competitive binding assay for screening the ability of candidate compounds to bind to ICAM-1. The method comprises assessing the displacement of candidate compounds by cyclic peptide inhibitors of LFA-1/ICAM-1 binding interaction. The cyclic peptide may comprise a heptapeptide sequence including, but not limited to, LLLMRSA (SEQ ID NO: 49), LLLMRSA (SEQ ID NO: 50), LLLMRSA (SEQ ID NO: 51), LLMRSA (SEQ ID NO: 52), LLMRSA (SEQ ID NO: 53), LLLMRSA (SEQ ID NO: 54) or LLRMRSV (SEQ ID NO: 55). In some embodiments the cyclic peptide has the amino acid sequence, CLLLMRSC.
will die in ex vivo culture, and BM stromal cells have been shown to support ex vivo survival of T-ALL cells. Furthermore, in co-culture experiments mAbs to LFA-1 and ICAM-1 dramatically decrease T-ALL cell survival by inhibiting adherence to bone marrow stromal cells. This latter finding indicates that survival of T-ALL cells in the marrow microenvironment is dependent on proper LFA-1/ICAM-1 binding. Since failure to eradicate leukemia cells in the marrow is a major reason for treatment failure, adjunct therapy that included inhibition of LFA-1/ICAM-1 ligation between leukemia cells and T-ALL cells may cause T-ALL cell death and improved patient outcome. This may have applicability to all T-cell leukemias.

The present inventor has also established a model and dissected the mechanism of a life-threatening therapeutic complication of acute promyelocytic leukemia (APL), retinoic acid syndrome. All-trans retinoic acid (ATRA) promotes a complete remission in up to 80% of patients with APL. However, retinoic acid syndrome occurs in up to 30% of patients and is caused by APL cells infiltrating organs after retinoic acid therapy. Using an apparatus known as a parallel plate flow chamber, the interactions that occur in blood vessels between circulating leukemia cells and endothelial cells have been recapitated under physiologic flow conditions (Brown et al., 1999; Larson et al., 1997). Upregulation of LFA-1 activity on APL cells after retinoic acid treatment results in APL cell binding to and transmigration through endothelium, analogous to the organ infiltration seen in APL patients being treated with retinoic acid. mAbs against LFA-1 and ICAM-1 completely inhibit the binding to and transmigration through endothelium. Hence, inhibition of LFA-1/ICAM interaction in patients with APL being treated with retinoic acid may protect against the development of retinoic acid syndrome. LFA-1/ICAM inhibition is also applicable to the use of alternatives to retinoic acid, such as 9-cis-retinoic acid or various synthetic retinoids, in APL treatment, to the extent that they cause retinoic acid syndrome.

Inhibition of the LFA-1 and ICAM-1 binding has potential therapeutic benefits relating to blocking allotraft rejection allografts, including cardiac, renal and thyroid allografts (Isobe et al., 1992; Steptoe et al., 1994; Cosimi et al., 1990; Nakamura et al., 1993; Talento et al., 1993), bone marrow transplants (Tibbetts et al., 1999; Cavazzana-Calvo et al., 1995) T-cell mediated sensitization reactions (Ma et al., 1994; Cumberbatch et al., 1992), diabetes (Hasegawa et al., 1994) and rheumatoid arthritis (Davis et al., 1995; Kavanaugh et al., 1994). Expression of ICAM-1 by keratinocytes is also implicated in the etiology of psoriasis, and inhibition of LFA-1/ICAM-1 binding presents a possible point of therapeutic intervention (Servitje et al., 1996). Thus the peptide compositions of the present invention may be used in treatment of the above conditions and more generally in any condition T-cell mediated condition wherein T-cells are activated via interaction of LFA-1 and ICAM-1.

The present disclosure provides novel peptide containing compositions that inhibit the LFA-1/ICAM-1 interaction. Preferred are compositions including nine amino acid peptides in which the terminal amino acids are cysteines, thus allowing the peptide to exist in a heterodetic cyclic form by disulfide bond formation or in a homodetic form by amide peptide bond formation between the terminal amino acids. Cyclizing small peptides through disulfide or amide bonds between the N- and C-terminus cysteines may circumvent problems of affinity and half-life. Disulfide bonds connecting the amino and carboxy terminus decrease proteolysis and also increase the rigidity of the structure, which may
yield higher affinity compounds. Peptides cyclized by disulfide bonds have free amino- and carboxy-termini which still may be susceptible to proteolytic degradation, while peptides cyclized by formation of an amide bond between the N-terminal amine and C-terminal carboxyl, no longer contain free amino or carboxy termini. Cyclic peptides may have longer half-lives in serum (Picken and Butcher, 1992; Huang et al., 1997). Moreover, the side-effects from peptide therapy are minimal, since ana phylaxis and other responses against the small peptide occur only rarely (Ohman et al., 1995; Adgey, 1998). Finally cyclic peptides have been shown to be effective inhibitors in vivo of integrins involved in human and animal disease (Jackson et al., 1997; Cuthbertson et al., 1997; Lefevre and Topol, 1995; Goligorsky et al., 1998; Ojima et al., 1995; Noiri et al., 1994). Thus the peptides of the present invention, including CLRMRSIC (SEQ ID NO: 1), CLRMRSAC (SEQ ID NO: 40), CLIIRMRSAC (SEQ ID NO: 43), CLIIRMRSAC (SEQ ID NO: 44), CLIIRMRSAC (SEQ ID NO: 45), CLIIRMRSAC (SEQ ID NO: 46), and CLIIRMRSVC (SEQ ID NO: 48), can be linked either by a C-N linkage or a disulfide linkage.

The present invention is not limited by the method of cyclization of peptides, but encompasses peptides whose cyclic structure may be achieved by any suitable method of synthesis. Thus, heterodetic linkages may include, but are not limited to formation via disulfide, alkylene or sulfide bridges. Methods of synthesis of cyclic homodetic peptides and cyclic heterodetic peptides, including disulfide, sulfide and alkylene bridges, are disclosed in U.S. Pat. No. 5,643,872, herein incorporated in entirety by reference. Other examples of cyclization methods are disclosed in U.S. Pat. No. 6,008,058, herein incorporated in entirety by reference. Cyclic peptides can also be prepared by incorporation of a type II β-turn dipeptide (Doyle et al., 1996). In certain aspects, embodiments of the present invention include cyclic peptides comprising the heptapeptide represented by residues 2 through 8 of the exemplified cysteine-containing nonapeptide. Thus, embodiments of the present invention include cyclic peptides comprising the heptapeptide sequences LLRMRSL (SEQ ID NO: 49), LLRMRSA (SEQ ID NO: 50), LLRMRSA (SEQ ID NO: 51), LLRMRSA (SEQ ID NO: 52), LLRMRSA (SEQ ID NO: 53), LLRMRSA (SEQ ID NO: 54), and LLRMRSA (SEQ ID NO: 55). Peptides that comprise the heptapeptide sequences of the present invention may be a peptide of 7, 8, or 9, 10, 11, or 12, 13, or 14, or 15 amino acids in length, wherein additional amino acids may be any L-series or any D-series amino acid.

Phage Display and Consensus Sequence

As a part of the present disclosure, phage display has been used to identify peptide sequences that bind ICAM-1 and block LFA-1/ICAM interaction. Briefly a library of cysteine-constrained heptapeptides was purchased from New England Labs (Cambridge, Mass.) and screened for its ability to bind the LFA-1 ligand, ICAM-1. Human ICAM-1 has been previously isolated in functional form (Larson et al., 1990), and a variation of this technique was used to obtain purified recombinant soluble ICAM-1 for use in phage display. Each phage in the library has the potential to display a unique cyclic heptapeptide fused to its gene III coat on its surface. The linkage of the displayed random peptide with a phage surface protein forms the basis of the technique. The library consists of approximately 2.8×10^13 random heptapeptide sequences expressed on phage, compared to 20^7 (20 possible amino acids in 7 different positions) or 1.28×10^6 possible heptapeptide sequences. The phage were then screened for their ability to bind purified ICAM-1 by interaction with the displayed heptapeptide sequences. The phage were then screened for their ability to bind purified ICAM-1 by phanning (FIG. 2). Bound phage is eluted using the anti-ICAM-1 mAb 84h410. This mAb binds to amino acid residues on ICAM-1 that are similar to those to which LFA-1 binds (Staunton et al., 1990). Elution with R6.5 allowed for isolation of phage expressing cyclic peptides that bind a region on ICAM-1 that is shared with LFA-1 binding. In addition, phage were eluted with mAb for 1 hour so that the peptides with highest affinity and slower off-rates (i.e., peptides most likely to be potent in vivo inhibitors) would be included. Thus, peptide sequences that block ICAM-1/LFA-1 interactions are identified.

Adherent phage were selected and amplified in ER2537 bacteria through four rounds of phanning. The sequences of 12-18 phage in each round were determined. A working consensus peptide was determined after nucleotide sequencing of 18 phage in the fourth and final round (Table 1). Amino acids that were recurring but occurred at lower frequency and did not fit into the consensus sequence are also shown below. The recurring amino acids form the basis of derivative structures. The ability of each phage isolated after four rounds of phanning to specifically bind ICAM-1 was also determined in an ELISA assay with serial dilutions of phage.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td><strong>PEPTIDE SEQUENCES EXPRESSED ON PHAGE THAT BIND ICAM-1</strong></td>
</tr>
<tr>
<td>Amino acid position</td>
</tr>
<tr>
<td>Consensus</td>
</tr>
<tr>
<td>Recurring amino acids</td>
</tr>
</tbody>
</table>

* N-terminus cysteine residues are disulfide linked to C-terminus cysteines.

The following techniques have been developed or modified for use in the present examples described below. Rapid Aggregation Assay for Screening Peptide Effectiveness

LFA-1 dependent cell aggregation has been previously studied using an aggregation assay it with a variety of leukocyte subclasses and cell lines (Larson et al., 1990; Wang et al., 1988; Larson et al., 1997). JY cells were obtained from American Type Tissucue Collection and were maintained in RPMI 1640 supplemented with 10% FBS at 37° C. in 5% CO₂. Aggregation of cells was measured in a homotypic aggregation assay using ICAM-1 as a stimulus. JY cells were washed twice with serum free medium and resuspended at a concentration of 4×10^5 is cells/ml. Cells were preincubated with the desired concentration of peptide for 15 min at room temperature. In a final volume of 100 µl, 50 µl of cells and peptide were seeded in 96 well flat bottom microtiter plates. Cells were allowed to aggregate at 37° C. in humidified air with 5% CO₂. Cells were visualized and counted by inverted phase contrast microscopy at the time indicated. Within each well of aggregates as well as the total number of free (single) cells were counted. percent aggregation was determined by the following equation:

\[
\text{Percent aggregation}=100\times (1-\text{number of free cells/total number of cells})
\]

Assay for Measuring LFA-1 Dependent Ex Vivo Survival of Leukemic Cells

A co-culture assay has been developed by the present inventor that quantifies ex vivo survival of T-ALL cells.
which prevent firm attachment to and transmigration through activated endothelium of APL cells in a parallel plate flow chamber (Larson et al., 1997; Brown et al., 1999). Flowing ATRA-treated APL cell lines over activated endothelial cell monolayers in a parallel flow chamber determines the effectiveness of peptides to inhibit LFA-1-dependent firm adherence and subsequent transmigration under physiologic flow conditions. ICAM-1 expressed on activated endothelial monolayers are incubated with cyclic peptides over a range of concentrations (10^{-7} to 10^{-6} M) and the IC_{50} is determined.

For investigating neutrophil binding, neutrophils are isolated from heparin anticoagulated venous blood of healthy adult donors by centrifugation on Ficoll-Hypaque density gradients as described by Simon et al. (1992). Isolated neutrophils are suspended at a concentration of 10^{6}/ml in Hanks’ Balanced Salt Solution supplemented with 10 mmol/L HEPES, pH 7.4 and 0.2% human serum albumin (Armour Pharmaceutical, Karakakee, III.) and used within 2 hours of preparation. Neutrophils are kept on ice and resuspended in RPMI pre-warmed to 37°C immediately before use.

Monoclonal Antibodies

In order to have adequate monoclonal antibodies at a reasonable cost, hybridomas were grown by the present inventor and mAbs were purified for blocking studies. The following monoclonal antibodies have been isolated from hybridoma supernatants: mAbs against LFA-1 (TS2/4 and TSI22) and ICAM-1 (RR1/1, R6.6 and 8HI10) (Larson et al., 1997).

Screening Assay

The ability of candidate compounds to bind to ICAM-1 is assessed by a competitive binding assay. Candidate compounds may be peptide or non-peptide compounds. Binding to ICAM-1 is quantified by the ability to displace a peptide of the present invention, including the peptides CLLRMRSAC (SEQ ID NO: 1), CLLRMRSAC (SEQ ID NO: 40), CLLRMRSAC (SEQ ID NO: 43), CLLRMRSAC (SEQ ID NO: 44), CLLRMRSAC (SEQ ID NO: 45), CLLRMRSAC (SEQ ID NO: 46), and CLLRMRSAC (SEQ ID NO: 48), from ICAM-1. The displaced peptide can be assayed by a number of techniques. For example, radiolabeled peptide can be synthesized using commercial available radiolabeled amino acids precursors. Peptides radiolabeled with H\textsuperscript{3}, C\textsuperscript{14} or S\textsuperscript{35} can be quantified by routine liquid scintillation techniques. Alternatively, a fluorescent labeled peptide can be synthesized. For example, lysine can be inserted in a non-critical position and labeled with fluorescein isothiocyanate ("FITC"). In addition to FITC, the peptide may be labeled with any suitable fluorophore. A carboxy fluorescein derivative of SEQ ID NO: 1 has been prepared. The S(6)-carboxy fluorescein derivative of the disulfide constrained cyclic form of SEQ ID NO: 40, as shown by FIG. 3, has been synthesized by Biopeptide (San Diego, Calif.). Alternatively, peptides cyclized with an amide peptide linkage have free sulfhydryl groups available for linkage to fluorescent compounds such as thiocyanates. Separation of bound from unbound peptide and quantitation of displaced peptide can be performed by routine techniques known to one of skill in the art. This embodiment of the invention is not limited by the method used to quantify the displaced peptide, and any suitable analytical technique may be used and be within the scope of the invention.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques
discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

Consensus Sequence Peptide Blocks LFA-1/ICAM-1 Dependent Cell Aggregation

The consensus peptide (SEQ ID NO: 1) and the randomized sequence CLMRMRMLRC (SEQ ID NO: 33), cyclized in the disulfide-linked form, were synthesized by Bioprotein (San Diego, Calif.) and tested in a screening assay for the ability to inhibit aggregation of the cell line JY, which aggregates in an LFA-1/ICAM-1 dependent manner. Aggregation measurements are relatively rapid and quantifiable. Furthermore, small volumes are used to minimize peptide consumption. Finally, cell aggregation in vitro depends on a multivalent LFA-1/ICAM-1 interaction. A peptide that is identified as being able to block cell aggregation in vitro is, therefore, likely to have affinity and off-rate characteristics that would make it an effective in vivo inhibitor, since cell-cell interaction in vivo also involves multivalent LFA-1/ICAM-1 interaction. Using this assay, the consensus peptide sequence has an IC50 of 500 μM (FIG. 1).

EXAMPLE 2

Derivative Peptide Antagonists

The consensus peptide sequence (SEQ ID NO: 1) identified by phage display and described in Example 1 has an IC50 of 800 μM for cell aggregation and 250 nM for monovalent ICAM binding. Monovalent ICAM-1 binding is measured by standard ELISA techniques, wherein, for example, the inhibition of 84H110 binding to ICAM-1 is assayed. For in vivo use, preferred peptides would have IC50’s of about 10 μM to 800 μM for cell aggregation and from about 10 to 250 nM for monovalent ICAM binding. In order to obtain more effective inhibitory peptides, standard techniques of designing derivative structures may be used to produce candidates to be tested for enhanced inhibitory capacity. This strategy has been shown to be effective in developing in vivo peptide inhibitors to other adhesion molecules (Ohman et al., 1995; Adgey, 1998).

For example, heptapeptide sequences are proposed on the basis of the observed consensus sequence of different phage sequences that were discovered in the phage display studies described above. Two of the amino acid positions in the consensus sequence show greater than 80% identity among phage and therefore are fixed (positions 3 and 4 of Table 1). The other positions are altered using 1 of 2 possible amino acids as shown in Table 2. The amino acids employed in the altered positions are identical to the recurring amino acids identified by phage display, but are represented at too low a frequency to be incorporated in the consensus sequence peptide. Using these combinations, 32 cysteine restrained heptapeptides are available as candidate inhibitors.

Peptides are initially tested for their ability to block LFA-1/ICAM dependent aggregation of the cell line JY (Wang et al., 1988; Larsen et al., 1997). Peptides are preferably tested over a range of concentrations (104 to 108 μM, for example) and the concentration leading to half maximal inhibition of aggregation (IC50) is determined for all the peptide sequences.

### TABLE 2

Peptide Antagonists Identified by Phage Display

<table>
<thead>
<tr>
<th>No.</th>
<th>Sequence</th>
<th>No.</th>
<th>Sequence</th>
</tr>
</thead>
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<tr>
<td>SEQ ID NO: 1</td>
<td>CLMRMRSLC</td>
<td>SEQ ID NO: 17</td>
<td>CMLRMRSLC</td>
</tr>
<tr>
<td>SEQ ID NO: 2</td>
<td>CLMRMSRC</td>
<td>SEQ ID NO: 18</td>
<td>CMLRMRSLC</td>
</tr>
<tr>
<td>SEQ ID NO: 3</td>
<td>CLMRMLRC</td>
<td>SEQ ID NO: 19</td>
<td>CMLRMRMLC</td>
</tr>
<tr>
<td>SEQ ID NO: 4</td>
<td>CLMRMLRC</td>
<td>SEQ ID NO: 20</td>
<td>CMLRMRMLC</td>
</tr>
<tr>
<td>SEQ ID NO: 5</td>
<td>CLMRPSRC</td>
<td>SEQ ID NO: 21</td>
<td>CMLRPSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 6</td>
<td>CLMRPSRC</td>
<td>SEQ ID NO: 22</td>
<td>CMLRPSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 7</td>
<td>CLMRPSRC</td>
<td>SEQ ID NO: 23</td>
<td>CMLRPSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 8</td>
<td>CLMRPSRC</td>
<td>SEQ ID NO: 24</td>
<td>CMLRPSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 9</td>
<td>CLMRMSRC</td>
<td>SEQ ID NO: 25</td>
<td>CMLRMSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 10</td>
<td>CLMRMSRC</td>
<td>SEQ ID NO: 26</td>
<td>CMLRMSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 11</td>
<td>CLMRMLRC</td>
<td>SEQ ID NO: 27</td>
<td>CMLRMLRC</td>
</tr>
<tr>
<td>SEQ ID NO: 12</td>
<td>CLMRMLRC</td>
<td>SEQ ID NO: 28</td>
<td>CMLRMLRC</td>
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<tr>
<td>SEQ ID NO: 13</td>
<td>CLMRPSRC</td>
<td>SEQ ID NO: 29</td>
<td>CMLRPSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 14</td>
<td>CLMRMLRC</td>
<td>SEQ ID NO: 30</td>
<td>CMLRPSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 15</td>
<td>CLMRMLRC</td>
<td>SEQ ID NO: 31</td>
<td>CMLRPSRC</td>
</tr>
<tr>
<td>SEQ ID NO: 16</td>
<td>CLMRMLRC</td>
<td>SEQ ID NO: 32</td>
<td>CMLRPSRC</td>
</tr>
</tbody>
</table>

Alanine Screen of Consensus Sequence

An alternative derivative peptide strategy was undertaken in which the consensus sequence peptide was subjected to an alanine-screening procedure as described by Cunningham and Wells, 1989. Peptides cyclized in the disulfide-linked form were synthesized by Bioprotein (San Diego, Calif.) wherein an alanine was substituted at each position in the heptapeptide LLRMRSL of the consensus sequence SEQ ID NO: 1, and each peptide was examined in parallel for its ability to inhibit LFA-1/ICAM-1 mediated JY cell aggregation. The results are shown in Table 2. Six of the seven peptides were soluble in aqueous solution. SEQ ID NO: 376, representing a substitution of the arginine in position 4 of SEQ ID NO: 1, was not soluble at 1 nm and could not be tested. Loss of inhibitory function resulted from alanine substitution of the leucines in positions 2 (SEQ ID NO: 34) and 3 (SEQ ID NO: 35), methionine in position 5 (SEQ ID NO: 36), and arginine in position 6 (SEQ ID NO: 38) of SEQ ID NO: 1, indicating that these amino acids are important to the antagonistic activity of the peptide. Substitution of the serine in position 7 (SEQ ID NO: 39) had no significant effect on the inhibitory activity of the peptide. Alanine substitution of the isoleucine at position 8 of SEQ ID NO: 1, i.e., CLMRMRSLC (SEQ ID NO: 40), resulted in a more potent antagonist. The randomized sequence CLLRSMRRC (SEQ ID NO: 56) was used as a control. This randomized sequence has a greater aqueous solubility than the alternative randomized sequence CLMRMLRSLC (SEQ ID NO: 33). Conservative substitutions in other peptide antagonists been shown to result in more potent inhibitors (Jennings and White, 1998).

### TABLE 3

Inhibition of JY cell aggregation with alanine-substituted derivatives

<table>
<thead>
<tr>
<th>SEQ ID NO</th>
<th>Sequence</th>
<th>Percent JY Aggregation</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Peptide</td>
<td>76(12)</td>
<td>0(16)</td>
<td></td>
</tr>
<tr>
<td>SEQ ID NO: 56*</td>
<td>CLLRSMRMC</td>
<td>74(14)</td>
<td>4(18)</td>
</tr>
<tr>
<td>SEQ ID NO: 1</td>
<td>CLMRMRSLC</td>
<td>41(0)</td>
<td>46(1)</td>
</tr>
<tr>
<td>SEQ ID NO: 34</td>
<td>CMLRMRSLC</td>
<td>71(15)</td>
<td>7(19)</td>
</tr>
<tr>
<td>SEQ ID NO: 35</td>
<td>CMLRMSRC</td>
<td>69(17)</td>
<td>9(22)</td>
</tr>
<tr>
<td>SEQ ID NO: 36</td>
<td>CMLRMRSLC</td>
<td>Not Soluble</td>
<td>Not Soluble</td>
</tr>
<tr>
<td>SEQ ID NO: 37</td>
<td>CMLRMRSLC</td>
<td>72(11)</td>
<td>5(14)</td>
</tr>
</tbody>
</table>
TABLE 3-continued

Inhibition of JY cell aggregation with alanine-substituted derivations

<table>
<thead>
<tr>
<th>SEQ ID NO:</th>
<th>Sequence</th>
<th>Percent JY Aggregation</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>CLLRMASCIC</td>
<td>68(9)</td>
<td>11(22)</td>
</tr>
<tr>
<td>39</td>
<td>CLLRMRACIC</td>
<td>56(16)</td>
<td>27(21)</td>
</tr>
<tr>
<td>40</td>
<td>CllRMsSac</td>
<td>29(13)</td>
<td>61(17)</td>
</tr>
</tbody>
</table>

Percent Inhibition = (1 - (Observed/Max)) x 100

(All in parentheses)

* = Randomized cyclic peptide

Conservative Substitutions of Amino Acids

The important amino acids of SEQ ID NO: 40, as identified by the alanine substitutions, were substituted with amino acids of like charge, e.g., as described by Dayoff et al., Atlas of Protein Sequence and Structure; vol 5, Suppl. 3, pp345-362 (M. O. Dayoff, ed., Nat'l BioMed Research Fdn., Washington, D.C. 1979). Peptides cyclized in the disulfide-linked form were synthesized by Biopetide (San Diego, Calif.) and were examined for their ability to inhibit LFA-1/ICAM-1 mediated JY cell aggregation. The results are summarized in Table 4. Five peptide sequences exhibited greater inhibition of JY cell inhibition as compared to SEQ ID NO: 1, i.e., CllRMRSAC (SEQ ID NO: 43), CllRMRSAC (SEQ ID NO: 44), CllKMRSac (SEQ ID NO: 45), CllKMRSac (SEQ ID NO: 46), and CllRMRSVC (SEQ ID NO: 48). This type of derivative strategy has previously been shown to be successful at identifying higher affinity peptides (Ohman et al., 1995; Adgey, 1998). In addition to binding to ICAM, conservative variants can also be screened for other desirable properties such as a longer serum-half life or desirable other pharmacokinetic properties.

TABLE 4

Inhibition of JY cell aggregation with homologous amino acid substitutions

<table>
<thead>
<tr>
<th>SEQ ID NO:</th>
<th>Sequence</th>
<th>Percent JY Aggregation</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>43</td>
<td>No Peptide</td>
<td>55(3)</td>
<td>0(5)</td>
</tr>
<tr>
<td>44</td>
<td>CllRMRSAC</td>
<td>57(4)</td>
<td>3,6(7)</td>
</tr>
<tr>
<td>45</td>
<td>CllKMRSac</td>
<td>53(1)</td>
<td>0(1)</td>
</tr>
<tr>
<td>46</td>
<td>CllKMRSac</td>
<td>24(3)</td>
<td>56(6)</td>
</tr>
<tr>
<td>47</td>
<td>CllRMRSAC</td>
<td>5(5)</td>
<td>92(9)</td>
</tr>
<tr>
<td>48</td>
<td>CllKMRSac</td>
<td>6(2)</td>
<td>90(4)</td>
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<tr>
<td>49</td>
<td>CllRMRSAC</td>
<td>61(7)</td>
<td>12(13)</td>
</tr>
<tr>
<td>50</td>
<td>CllKMRSac</td>
<td>4(1)</td>
<td>92(3)</td>
</tr>
</tbody>
</table>

Percent Inhibition = (1 - (Observed/Max)) x 100

(All in parentheses)

* = Randomized cyclic peptide

EXAMPLE 3

Investigation Of Peptide Inhibition Of LFA-1/ICAM-1 Interaction

The cyclic peptide inhibitors having the amino acid sequences, CLLMRSCIC (SEQ ID NO: 1) and CllRMKSAC (SEQ ID NO: 46) have been shown to block neutrophil-binding to endothelium under physiologic flow conditions in a parallel plate flow chamber. The data is shown in Table 5. In similar fashion to the inhibition of JY cell aggregation, SEQ ID NO: 46 exhibited greater inhibition of attachment of neutrophils to activated endothelium.

TABLE 5

Inhibition of Firm Attachment of Neutrophils to Simulated Endothelial Cells in a Parallel Plate Flow Chamber

<table>
<thead>
<tr>
<th>SEQ ID NO:</th>
<th>Sequence</th>
<th>Cells/mm²</th>
<th>Percent Inhibition</th>
</tr>
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<tbody>
<tr>
<td>43</td>
<td>No Peptide</td>
<td>82(19)</td>
<td>0(12)</td>
</tr>
<tr>
<td>44</td>
<td>CllRMRSAC</td>
<td>67(152)</td>
<td>25(19)</td>
</tr>
<tr>
<td>45</td>
<td>CllKMRSac</td>
<td>43(120)</td>
<td>47(15)</td>
</tr>
<tr>
<td>46</td>
<td>CllRMKSAC</td>
<td>28(3)</td>
<td>66(0)</td>
</tr>
</tbody>
</table>

Percent Inhibition = (1 - (Observed/Max)) x 100

(All in parentheses)

* = Randomized cyclic peptide

The peptides of the present invention bind to ICAM-1 and block cell—cell adhesion that is dependent on LFA-1 binding to its ligand, ICAM-1. In contrast to other peptide inhibitors, these compounds have no structural similarity to fragments or portions of LFA-1 or ICAM-1. These compounds are, therefore, effective agents in the treatment of myocardial infarction, rheumatoid arthritis, asthma, and leukemia/lymphoma, and are contemplated to be especially effective in adjunct therapy of acute T-ALL and retinoic acid syndrome in APL.

Regarding the clinical utility of the disclosed compositions, preliminary data indicate that approximately 80% of cases of T-ALL may utilize LFA-1 dependent adhesion for survival. Therefore, the peptide inhibitor may be useful in 80% of cases of T-ALL when incorporated as adjunct therapy. Side effects related to use of a small peptide are contemplated to be minimal, since peptides mimicking inhibiting other integrin functions, and now in clinical trial do not have significant side-effects (Ohman et al., 1995; Adgey, 1998). However, LFA-1 may be critical to an aspect of hematopoiesis that is as yet undefined.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are chemically or physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.


Cosimi et al., In vivo effects of monoclonal antibody to ICAM-1 (CD54) in nonhuman primates with renal allografts,” J Immunol, 144:4604, 1990.


Jacobsson and Frykberg, “Phage display shot-gun cloning of ligand-binding domains of prokaryotic receptors approaches 100% correct clones,” Biotechniques 20:1070, 1996.


Liesveld et al., “Adhesive interactions of normal and leukemic human CD34+ myeloid progenitors: Role of mar-


Winter et al., “Combinatorial effects of LFA-1 dependent stromal cell adherence and soluble factor(s) on T-ALL cell survival (abstract),” *Blood*, 89 (suppl 1):81a, 1998.

Cys Leu Leu Arg Met Arg Ser Arg Cys
1 5

Cys Leu Leu Arg Met Arg Leu Ile Cys
1 5

Cys Leu Leu Arg Met Arg Ser Ile Cys
1 5

Cys Leu Leu Arg Met Arg Leu Arg Cys
1 5

Cys Leu Leu Arg Pro Arg Ser Ile Cys
1 5

Cys Leu Leu Arg Pro Arg Ser Arg Cys
1 5
Cys Leu Leu Arg Pro Arg Leu Ile Cys
1  5

Cys Leu Leu Arg Pro Arg Leu Arg Cys
1  5

Cys Leu Leu Arg Met Asn Ser Ile Cys
1  5

Cys Leu Leu Arg Met Asn Ser Arg Cys
1  5

Cys Leu Leu Arg Met Asn Met Leu Ile Cys
1  5

Cys Leu Leu Arg Met Asn Leu Arg Cys
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Cys Leu Leu Ala Met Arg Ser Ile Cys

<210> SEQ ID NO 37
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic

<400> SEQUENCE: 37
Cys Leu Leu Arg Ala Arg Ser Ile Cys
Cys Leu Leu Arg Met Ala Ser Ile Cys
1 5

Cys Leu Leu Arg Met Arg Ala Ile Cys
1 5

Cys Leu Leu Arg Met Arg Ser Ala Cys
1 5

Cys Leu Val Arg Met Arg Ser Ala Cys
1 5

Cys Val Leu Arg Met Arg Ser Ala Cys
1 5

Cys Ile Leu Arg Met Arg Ser Ala Cys
1 5
Cys Leu Ile Arg Met Arg Ser Ala Cys
1 5

Cys Leu Leu Lys Met Arg Ser Ala Cys
1 5

Cys Leu Leu Arg Met Lys Ser Ala Cys
1 5

Cys Leu Leu Arg Met Arg Ser Leu Cys
1 5

Cys Leu Leu Arg Met Arg Ser Leu Cys
1 5

Cys Leu Leu Arg Met Arg Ser Val Cys
1 5

Leu Leu Arg Met Arg Ser Ile
1 5

<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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Cys Leu Ile Arg Met Arg Ser Ala Cys
1 5

<210> SEQ ID NO 45
<211> LENGTH: 9
<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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Cys Leu Leu Lys Met Arg Ser Ala Cys
1 5

<210> SEQ ID NO 46
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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Cys Leu Leu Arg Met Lys Ser Ala Cys
1 5

<210> SEQ ID NO 47
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
<400> SEQUENCE: 47

Cys Leu Leu Arg Met Arg Ser Leu Cys
1 5

<210> SEQ ID NO 48
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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Cys Leu Leu Arg Met Arg Ser Val Cys
1 5

<210> SEQ ID NO 49
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
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Leu Leu Arg Met Arg Ser Ile
1 5

<210> SEQ ID NO 50
Leu Leu Arg Met Arg Ser Ala
15

Leu Ile Arg Met Arg Ser Ala
15

Leu Leu Lys Met Arg Ser Ala
15

Leu Leu Arg Met Lys Ser Ala
15

Ile Leu Arg Met Arg Ser Ala
15

Leu Leu Arg Met Arg Ser Val
15
What is claimed is:

1. A composition comprising a cyclic peptide inhibitor of LFA-1/ICAM-1 interaction, wherein the amino acid sequence of said cyclic peptide inhibitor consists of an amino acid sequence selected from the group consisting of SEQ ID NO: 40, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46 and SEQ ID NO: 48.

2. The composition of claim 1, wherein said composition is contained in a pharmaceutically acceptable carrier.

3. The composition of claim 1, wherein said composition exhibits an IC_{50} of cell aggregation mediated by LFA-1/ICAM-1 binding of from about 10 \text{ nM} to about 850 \text{ nM}.

4. The composition of claim 1, wherein said composition exhibits an IC_{50} of monovalent ICAM-1 binding of from about 10 \text{ nM} to about 250 \text{ nM}.

5. A therapeutic package, wherein said package contains in a unit dose, an amount of a cyclic peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 40, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46 and SEQ ID NO: 48.

6. The package of claim 5, wherein said package is formulated for intravenous administration.

7. The package of claim 5, wherein said package includes said unit dose in a dry powder form.

8. The package of claim 5, wherein said unit dose will deliver a dose from about 10 \text{ \mu g/Kg} to about 500 \text{ \mu g/Kg}.

9. The package of claim 5, wherein said unit dose will deliver a dose from about 50 \text{ \mu g/Kg} to about 250 \text{ \mu g/Kg}.

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