(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 September 2005 (09.09.2005)

PCT

(10) International Publication Number
WO 2005/082003 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/US2005/005878

(22) International Filing Date: 22 February 2005 (22.02.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/547,169	24 February 2004 (24.02.2004)	US
60/637,362	17 December 2004 (17.12.2004)	US

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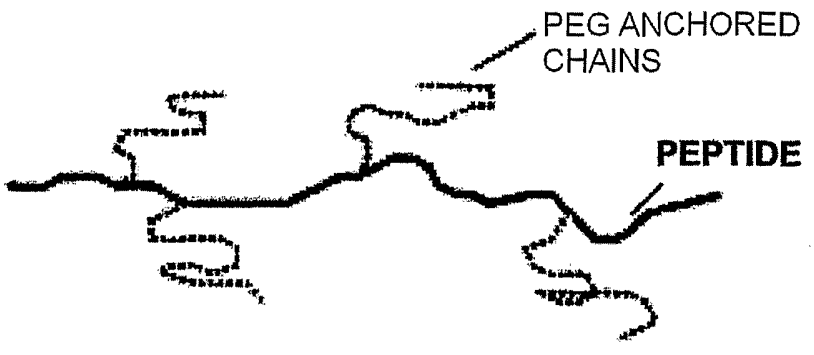
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PEPTIDES AND USES THEREOF



(57) Abstract: Peptides that bind to at least one molecule to which a binding domain of LSIGN binds, antibodies to such peptides, and antibody/peptide constructs containing such peptides are administered as therapeutics to modulate immune response in a subject and/or block entry or transmission of pathogen.

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PEPTIDES AND USES THEREOF

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial Nos. 60/637,362 and 60/547,169 which were filed on December 17, 2004 and February 24, 2004, respectively, the contents of which are incorporated herein by this reference.

BACKGROUND

Technical Field

The present disclosure relates to compositions and methods for modulating the immune response and/or blocking the entry or transmission of pathogens in an animal such as a human or another mammal.

Background of Related Art

Liver/lymph node-specific intercellular adhesion molecule-3 grabbing integrin ("L-SIGN", also sometimes referred to as "DC-SIGN-R," "DC-SIGN2" or "CD209L") is highly expressed on liver and lymph node endothelial cells. L-SIGN belongs to the family of pathogen internalization receptors that internalize receptor bound protein and facilitate antigen presentation.

Similar to dendritic cell-specific ICAM-3 grabbing non-integrin, or DC-SIGN, L-SIGN interacts with ICAM-3 on T cells in a Ca^{2+} dependent manner. Both DC-SIGN and L-SIGN have been shown to be capturing receptors for a variety of pathogens such as HIV, Hepatitis C virus, Ebola, Mycobacterium tuberculosis, Schistosoma mansonii and others.

Compositions and methods for modulating the activity of T cells would be desirable. Furthermore, compositions and methods for blocking viral entry and/or preventing viral transmission are desirable.

SUMMARY

The present disclosure is directed to isolated peptides that bind to at least one molecule to which a binding domain of L-SIGN and/or DC-SIGN binds. In one embodiment, an isolated peptide is described that has the amino acid sequence:

R Y W N S G E P N N S G N E D C A E F S G S G W N D N R C D V D N (SEQ ID NO: 1).

Peptides that are substantially related to the peptide set forth in SEQ ID NO: 1 and variants of such peptides are also described. Therapeutic compositions that contain one or more of the foregoing peptides and a pharmaceutically acceptable carrier are also described herein.

The present disclosure also provides antibodies to peptides that bind to at least one molecule to which a binding domain of L-SIGN and/or DC-SIGN binds. Antibody/peptide constructs wherein one or more peptides in accordance with this disclosure are grafted onto an antibody are also described.

Methods for modulating a T cell response, inhibiting pathogen entry into antigen presenting cells and/or preventing pathogen transmissions are described herein. These methods involve administration of one or more peptides, antibodies, or antibody/peptide constructs described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a schematic diagram of pegylated peptides in accordance with one aspect of the present disclosure.

Figure 1B is a schematicdiagram of pegylated peptides in conjunction with PLGA particles in accordance with another aspect of the present disclosure.

Figure 2 is a schematic representation of a multimeric protein in accordance with one embodiment of the present disclosure.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present disclosure is directed to peptides that bind to at least one molecule to which a binding domain of L-SIGN and/or DC-SIGN binds. As those skilled in the art will appreciate, L-SIGN and DC-SIGN bind to both natural ligands and pathogens, such as, for example, envelope proteins of viruses.

In one embodiment, the peptide includes 33 amino acid residues of L-SIGN, and has the amino acid sequence:

R Y W N S G E P N N S G N E D C A E F S G S G W N D N R C D V D N (SEQ ID NO: 1).

This sequence includes eleven residues that make direct contact with a ligand, e.g., high mannose sugar. These eleven residues are underlined in SEQ ID NO: 1. The cysteines (double underline in SEQ ID NO: 1) form a disulfide bond. The peptide of SEQ ID NO: 1 also contains many residues (including a C-terminal motif), which are uniquely present in L-SIGN but not the closely related DC-SIGN protein.

Peptides that are substantially related to the peptide set forth in SEQ ID NO: 1, these containing one or more amino acid variations as compared to SEQ ID NO: 1, are also encompassed by the present disclosure. An "amino acid variation" denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of amino acid variations are known to those skilled in the art and include substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, or glutamic acid for aspartic acid, and the like. Other illustrative examples of amino acid variations include the following changes: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamate to aspartate; glycine to proline; phenylalanine to tyrosine, leucine or methionine; serine to threonine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine. The term "amino acid variation" also includes the use of a substituted amino acid in place of an unsubstituted original amino acid,

provided that binding to at least one molecule to which the peptide of SEQ ID NO: 1 binds is preserved. Where the peptide is substantially related to the peptide set forth in SEQ ID NO: 1 but for a plurality of amino acid variations, such peptide preferably has greater than 50% identity with the peptide of SEQ ID NO: 1, more preferably greater than 75% identity, and most preferably greater than 90% identity with the peptide of SEQ ID NO: 1.

In other embodiments, variants of the peptide of SEQ ID NO: 1 are contemplated. The variants bind to at least one molecule to which the peptide of SEQ ID NO: 1 binds. The degree of binding to the molecule can be greater or lesser than the binding exhibited by the peptide of SEQ ID NO: 1, depending on the specific variant and the molecule(s) to which the variant binds. For instance, where the variant binds selectively to one molecule to which the peptide of SEQ ID NO: 1 binds, but not to all such molecules, a lesser degree of binding can be tolerated in exchange for a desired selectivity. In other examples, the variant has a higher binding affinity for a molecule to which the peptide of SEQ ID NO: 1 binds.

In one embodiment, the variant includes the eleven residues of the peptide of SEQ ID NO: 1 that make direct contact with a ligand. Such variants have at least the following amino acid sequence:

G E X N X S X X E X X X X X S G S X X N D N (SEQ ID NO: 2)

wherein X can be any amino acid so long as the peptide is capable of binding under physiological conditions to at least one ligand selected from the group consisting of L-SIGN ligands and DC-SIGN ligands, and wherein the peptide is not L-SIGN.

"Binds under physiological conditions" means forming a covalent or non-covalent association with an affinity of at least 10^6 M^{-1}, most preferably at least 10^9 M^{-1}, either in the body, or under conditions which approximate physiological conditions with respect to ionic strength, e.g., 140 mM NaCl, 5 mM $MgCl_2$. It will be recognized that the concentration of various salts depends on the organ,

organism, cell, or cellular compartment used as a reference. The salt concentration and ionic strength in an aqueous solution that characterize fluids found in human metabolism (commonly referred to as physiological buffer or physiological saline) typically are represented by an intracellular pH of 7.1 and salt concentrations (in mM) of Na^+ : 3-15; K^+ : 140; Mg^{+2} : 6.3; Ca^{+2} : 10^{-4} ; Cl^- : 3-15, and an extracellular pH of 7.4 and salt concentrations (in mM) of Na^+ : 145; K^+ : 3; Mg^{+2} : 1-2; Ca^{+2} : 1-2; and Cl^- : 110.

In other embodiments, variants include at least the amino acid sequence:

R Y W N S XX P X N X G N X D C A E F XXX G W XXX R C D V D N

(SEQ ID NO: 3)

wherein X can be any amino acid so long as the peptide is capable of binding under physiological conditions to at least one ligand selected from the group consisting of L-SIGN.

In other embodiments, it is also contemplated to make variants that can bind to DC-SIGN ligand specifically, L-SIGN ligand specifically, or both DC-SIGN and L-SIGN ligands. This can be accomplished by randomizing the amino acids in the residues outlined in SEQ ID NO: 1, followed by selection for binders to the appropriate ligand or ligands. This would be particularly advantageous for interfering with infectious diseases and inflammatory conditions.

It is also contemplated by this disclosure that the peptides in accordance with this disclosure (e.g., the peptide of SEQ ID NO: 1, substantially related peptides or variants) can be modified in order to enhance stability of the peptide and/or extend the useful half-life in vivo of the peptide. By "increase in serum half-life" is meant the positive change in circulating half-life of a modified peptide relative to its non-modified form. Serum half-life is measured by taking blood samples at various time points after administration of the peptide, and determining the concentration of that molecule in each sample. Correlation of the serum concentration with time allows calculation of the serum half-life. The increase is desirably at least about two-fold, but a smaller increase may be useful, for example where it enables a satisfactory dosing regimen or avoids a

toxic effect. Preferably the increase is at least about three-fold, more preferably at least about five-fold, even more preferably at least about ten-fold, and most preferably at least about fifteen-fold.

Any technique within the purview of those skilled in the art to improve the structural stability and serum half-life of peptides can be used. For example, the amino terminus and the carboxy terminus of the peptide can be blocked by acetylation and amidation, respectively. These modifications can enhance the serum half-life of the peptides by preventing enhanced proteolysis as occurs with peptides containing unblocked termini. Alternatively, or in addition to terminal blocking, the peptides can be constructed with D-amino acids. As D-amino acids do not make up the natural proteins, this modification can decrease the proteolytic degradation of the peptides. As those skilled in the art will appreciate, D-and L- amino acids are optical isomers and have opposite chirality. Thus, it is also contemplated that variants can be prepared wherein the D-amino acid peptides are sequenced in the reverse order compared to SEQ ID NO: 1 to preserve the overall structure of the peptides. In addition, it is contemplated that a disulfide bond-pair can be introduced between cysteines (see double underline in SEQ ID NO: 1) to further stabilize the structure of the peptides in accordance with this disclosure.

Methods to increase the half-life of peptides, antibodies or other biological molecules include glycosylation, pegylation, or fusion proteins such as immunoadhesions or albumin fusion proteins. Additionally, fusing the desired peptide to a second peptide sequence that in turn associates with an abundant serum protein can increase the in-vivo half-life. In one example, one would fuse the first peptide to a second peptide sequence that encodes an albumin binding sequence. The two peptide sequences could be fused directly, or alternatively, an intervening flexible linker could be placed in between. An example of one albumin binding sequence is the following:

RLIEDICLPRWGCLWEDD (SEQ ID NO: 18)

Other albumin binding sequences could be identified by those skilled in the art, through methods such as peptide phage display. Ideally, though not

necessary, the albumin binding sequence binds to albumin from multiple species. When the first peptide is fused to the albumin binding peptide and is administered in vivo, the albumin binding sequence quickly associates with albumin abundantly present in the serum. In humans, administration of the dual peptide would associate with human serum albumin, a.0 versatile transporter protein and the most important carrier for acidic drugs in human plasma. As human serum albumin has been shown to bind a large number of different compounds in a reversible manner, the peptide/protein interaction should modify peptide availability and elimination from the body.

An illustrative list of possible peptide modifications are shown in Table 1 using the unmodified sequence of the L-SIGN peptide as an example. The binding affinity and serum stability of these peptides can be determined both in tissue culture and in animal models as described in Srinivasan, et al., J. Immunol., volume 169, page 2180 (2002); and Srinivasan, et al., J. Immunol., volume 167, page 578 (2001).

Table 1

Exemplary Modifications To Enhance
The Stability Of The Peptide Of SEQ ID NO: 1

Nature of L- SIGN Peptide	**Sequence**
Native with L amino acids	NH_2-SEQ ID NO: 1-COOH
End group blocked and L-amino acids	CH_3CO- SEQ ID NO: 1- $CONH_2$
End group blocked and D-amino acids	CH_3CO-D[SEQ ID NO: 1]-$CONH_2$
End group blocked and D-amino acids in reverse order	CH_3CO-D[ReverseSEQ ID NO: 1*]-$CONH_2$
End group blocked and L-amino acids in reverse order	CH_3CO- ReverseSEQ ID NO: 1*-$CONH_2$

*ReverseSEQ ID NO:1 is NDVDCRNDNWGSGSFEACDENGSNNPEGSNWYR (SEQ ID NO: 6)

All peptides listed in Table 1 and variants thereof can be made with or without a disulfide bond between the double underlined cysteines shown in SEQ ID NO: 1. In addition, all peptides listed in Table 1 and variants thereof can be made with or without their amino terminus and/or carboxy terminus blocked.

It is also contemplated that the peptides of the present disclosure can be pegylated to enhance their serum half-life. Pegylation of proteins has been found to considerably enhance the serum half life of peptides and proteins. (See, Grace, et al., J. Interferon Cytokine Res., volume 21, page 1103 (2001); Yamamoto, et al., Nat. Biotechnol., volume 21, page 546 (2003).) By "pegylated" is meant the covalent attachment of at least one molecule of polyethylene glycol (PEG) to a peptide. Techniques for pegylation of peptides are within the purview of those skilled in the art. A schematic diagram of pegylated peptides in accordance with this embodiment is shown in Figure 1. The average molecular weight of the reactant PEG is preferably between about 5,000 and about 50,000 daltons, more preferably between about 10,000 and about 40,000 daltons, and most preferably between about 15,000 and about 30,000 daltons. Particularly preferred are PEGs having nominal average sizes of about 20,000 and about 25,000 daltons. The method of attachment is not critical, but preferably does not alter, or only minimally alters, the activity of the peptide. Preferably the increase in half-life is greater than any decrease in biological activity. A preferred method of attachment is via N-terminal linkage to a peptide of the present disclosure.

Once pegylated, the peptide can be linked to the surface of a microsphere as shown schematically in Figure 2. By attaching several pegylated peptides to the same microsphere, a multivalent high affinity product is provided. Techniques for preparing such microspheres are disclosed, for example, by Coombes, et al., Biomaterials, volume 18, page 1153 (1997). Alternatively, the pegylated peptide can be linked to the surface of a liposome using, for example, the methods disclosed by Park, et al., Clin. Cancer Res., volume 8, page 1172 (2002) or Mamot, et al., Cancer Res., volume 63, page 3154 (2003).

It is further contemplated to increase the serum half life of the peptide by making peptide/Fc fusion proteins using G2/G4 Fc (see published PCT patent

application number WO 05/007809A2) or any other suitable human antibody constant region.

Techniques for the production of peptides in accordance with this disclosure are within the purview of those skilled in the art. For example, commercial services are available that will synthetically produce any desired peptide if provided with the desired amino acid sequence. Alternatively, nucleic acid encoding the peptide can be incorporated into a suitable expression vector. The vector can then be transfected into an appropriate host cell and the desired peptide expressed and recovered. Suitable techniques for peptide expression are within the purview of those skilled in the art. Expression of the peptide provides the advantage of allowing randomization of the peptide at desired locations in the peptide sequence, for example, through the use of degenerate codons in the nucleic acid sequence.

In other embodiments, antibodies to the peptides of the present disclosure are provided. Such antibodies can be produced by methods within the purview of those skilled in the art. The antibody can be a natural antibody (isolated using well known techniques) or an antibody that is synthetically prepared by recombinant methods within the purview of those skilled in the art. As used herein, "antibody" refers to an entire immunoglobulin molecule or molecules that contain immunologically active portions of whole immunoglobulin molecules and includes but is not limited to Fab, F(ab')2, scFv, Fv, heavy chain variable regions and light chain variable regions. The terms immunoglobulin and antibody are used interchangeably herein. Antibodies can be polyclonal, monoclonal, chimeric or "humanized" antibodies. See, e.g., U.S. Patent Nos.5,225,539, 5,585,089 and 5,693,761 and WO 90/07861 and WO 98/49306, which describe methods for producing humanized immunoglobulins. The antibody can be, for example, a fully human antibody, a non-human antibody, a humanized antibody, a chimeric antibody or any of the foregoing types of antibodies that have been manipulated in any way (e.g., site-specific modifications or de-immunization), as well as fragments thereof.

For instance, antibodies can be obtained by immunizing a suitable host such as a goat, rabbit, sheep, rat, pig, mouse or human with a peptide of the present disclosure or an immunogenic portion, fragment or fusion thereof, optionally with the use of an immunogenic carrier (such as bovine serum albumin or keyhole limpet hemocyanin) and/or an adjuvant such as Freund's, saponin, ISCOM's, aluminum hydroxide or a similar mineral gel, or keyhole limpet hemocyanin or a similar surface active substance. After an immunoresponse against the peptide has been raised (usually within 1-7 days), the antibodies can be isolated from blood or serum taken from the immunized animal in a manner known per se, which optionally may involve a step of screening for an antibody with desired properties (i.e. specificity) using known immunoassay techniques.

Monoclonal antibodies may be produced using continuous cell lines in culture, including hybridoma and similar techniques within the purview of those skilled in the art. In a further aspect, the disclosure provides a cell line such as a hybridoma that produces antibodies, preferably monoclonal antibodies, against the peptides of the present disclosure. In yet another embodiment, the disclosure relates to a method for producing an antibody to the peptides of the present disclosure by cultivating a cell or a cell line that produces said antibody and harvesting/isolating the antibody from the cell culture.

The antibody can be advantageously selected from a library of antibodies using techniques within the purview of those skilled in the art, such as, for example, immune repertoire cloning, phage display and panning.

Once selected, nucleic acid encoding the antibody can be amplified using techniques within the purview of those skilled in the art such as, for example, conventional PCR (see, Rader and Barbas, Phage Display, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000) or the amplification technique described in International Application WO 03/025202 A2, the disclosures of which are incorporated herein by reference.

In yet another aspect, peptides of the present disclosure can be linked to an antibody to prepare an antibody/peptide construct having increased stability and an extended serum half-life. Antibodies suitable for use in formation of the

antibody/peptide construct include any antibody or antibody fragment. In some embodiments a suitable antibody for preparation of an antibody/peptide construct is an anti-tetanus toxoid antibody. Techniques for the preparation of such antibody/peptide constructs are within the purview of one skilled in the art. See, e.g., U.S. Patent No. 6,403,769 which describe methods for producing antibody/peptide fusions, the disclosure of which is incorporated herein by this reference. In particularly useful embodiments, a peptide in accordance with this disclosure is incorporated into or replaces at least a portion of a CDR of an antibody. Such "rationally designed antibodies" can be produced using the techniques described in WO 02/46238 A2, the disclosure of which is incorporated herein by this reference. In certain embodiments, the peptide is incorporated into a domain-exchanged antibody such as, for example, the antibody disclosed in Calarese, et al., Science, volume 300, pages 2065-2071 (2003).

In one aspect, the peptides, antibodies or antibody/peptide constructs of the present disclosure may be used for modulating one or more interactions between a cell of a sinusoid endothelial layer, in particular a liver sinusoid endothelial cell (LSEC), and a cell expressing ICAM-2 and/or ICAM-3, in particular a T cell. More particularly, the present peptides, antibodies or antibody/peptide constructs are used for modulating the adhesion between a cell of a sinusoid endothelial layer and a cell expressing ICAM-2 and/or ICAM-3, in particular a T cell. In particularly useful embodiments, the present peptides, antibodies or antibody/peptide constructs modulate adhesion between a C-type lectin on the surface of a LSEC and an ICAM receptor (e.g., an ICAM-2 or ICAM-3 receptor) on the surface of a T cell. By competing with ICAM-binding to L-SIGN on tolerance inducing cells, induction of immune tolerance could be prevented with the peptide or peptide/antibody construct.

Peptides, antibodies or peptide/antibody constructs selected to bind to both L-SIGN and DC-SIGN ligands, in particular ICAM-2 and/or ICAM-3, will be useful in an inflammatory or immune stimulatory environment. Competition with ICAM-binding to DC-SIGN on dendritic cells could prevent immune stimulation.

In yet another aspect, the peptides, antibodies or antibody/peptide constructs of the present disclosure can be utilized to block entry of pathogen (e.g., viruses) into antigen presenting cells such as liver sinusoidal cells and their infection into other cells. In some embodiments, the antibodies of the present disclosure can bind to L-SIGN on an antigen presenting cell, thereby blocking the ability of the cell to bind to a pathogen. In addition, the peptidesor antibody/peptide constructs of the present disclosure can provide competitive binding sites for pathogen, and thus can be utilized as therapeutics to treat or prevent an infection by providing a competitive binding site to L-SIGN. In such a case, pathogen particles which may bind to L-SIGN on antigen presenting cells such as liver sinusoidal endothelial cells instead bind to the peptides, peptide Fc fusion proteins or antibody/peptide constructs of the present disclosure, thereby limiting the binding and entry of pathogen into the antigen presenting cells and their infection of other cells. Based on the high homology between L-SIGN and DC-SIGN, it is expected that the peptide or peptide antibody construct could also prevent binding to DC-SIGN, thereby preventing the pathogen entry into both DC-SIGN and L-SIGN expressing cells.

Thus the peptides, antibodies or antibody/peptide constructs of the present disclosure may be utilized to treat a subject animal such as a human having an existing viral infection. In the alternative, the peptides, antibodies or antibody/peptide constructs of the present disclosure may be included in vaccines to prevent infection of a subject animal such as a human by a virus.

Administration of the peptide or variants thereof can be used to block pathogen infection by binding to the pathogen and preventing its interaction with the cell. Also, administration of the peptide can induce antibodies to the pathogen binding region of L-SIGN and DC-SIGN. Thereby, blockage of the pathogen and blockage of the entry receptor on the cell can be accomplished simultaneously.

The present peptides, antibodies or antibody/peptide constructs can be administered in accordance with known methods, *e.g.,* injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, subcutaneous,

intraarterial, intrathecal, inhalation or intralesional routes, topically or by sustained release systems as noted below. The peptide is preferably administered continuously by infusion or by bolus injection. One may administer the peptide in a local or systemic manner.

The peptide, antibody or antibody/peptide construct may be prepared in a mixture with a pharmaceutically acceptable carrier. Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. This therapeutic composition can be administered intravenously, parenterally or subcutaneously as desired. When administered systemically, the therapeutic composition should be sterile, pyrogen-free and in a parenterally acceptable solution having due regard for pH, isotonicity, and stability. These conditions are within the purview of those skilled in the art.

Briefly, dosage formulations of the present peptides, antibodies or antibody/peptide constructs are prepared for storage or administration by mixing the compound having the desired degree of purity with physiologically acceptable carriers, excipients, or stabilizers. Such materials are non-toxic to the recipients at the dosages and concentrations employed, and may include buffers such as TRIS HCl, phosphate, citrate, acetate and other organic acid salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) peptides such as polyarginine, proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidinone; amino acids such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium and/or nonionic surfactants such as TWEEN, PLURONICS or polyethylene glycol.

When used for *in vivo* administration, the peptide, antibody or antibody/peptide construct formulation should be sterile and can be formulated according to conventional pharmaceutical practice. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization

and reconstitution. The antibody ordinarily will be stored in lyophilized form or in solution. Other vehicles such as naturally occurring vegetable oil like sesame, peanut, or cottonseed oil or a synthetic fatty vehicle like ethyl oleate or the like may be desired. Buffers, preservatives, antioxidants and the like can be incorporated according to accepted pharmaceutical practice.

Pharmaceutical compositions suitable for use include compositions wherein one or more peptides, antibodies or antibody/peptide constructs are contained in an amount effective to achieve their intended purpose. More specifically, a therapeutically effective amount means an amount of peptide or antibody effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. Therapeutically effective dosages may be determined by using *in vitro* and *in vivo* methods.

A typical daily dosage might range from about 1μg/kg to up to 1000mg/kg or more, depending on the factors mentioned above. Typically, the clinician will administer the peptide, antibody or antibody/peptide construct until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

To prolong the release of the drug in the blood and decrease the number of doses of the peptide injected to the patient, pegylated peptides can be encapsulated in biodegradable microspheres as described in Kim, et al., Biomaterials, volume 23, page 2311 (2002). Biodegradable microspheres made of poly (D, L-lactide-co-glycolide), also referred to as PLGA, can be used. PLGA is a polymer approved by the FDA as safe for human use. PLGA based microspheres have been extensively used to prolong the release of therapeutic peptides and proteins in the human body. (See, Frangione-Beebe, et al., J. Microencapsul., volume 18, page 663 (2001); Putney, Curr. Opin. Chem. Biol., volume 2, page 548 (1998); and Putney, et al., Nat. Biotechnol., volume 16, page 153 (1998).)

Kits according to the present disclosure include frozen or lyophilized peptides, antibodies or antibody/peptide constructs to be reconstituted, respectively, by thawing (optionally followed by further dilution) or by suspension in a (preferably buffered) liquid vehicle. The kits may also include buffer and/or excipient solutions (in liquid or frozen form), or buffer and/or excipient powder preparations to be reconstituted with water, for the purpose of mixing with the peptides, antibodies or antibody/peptide constructs to produce a formulation suitable for administration. Thus, preferably the kits containing the peptides, antibodies or antibody/peptide constructs are frozen, lyophilized, pre-diluted, or pre-mixed at such a concentration that the addition of a predetermined amount of heat, water, or solution provided in the kit will result in a formulation of sufficient concentration and pH as to be effective for in vivo or in vitro use as immunological tolerance-inducing agents. Preferably, such a kit will also comprise instructions for reconstituting and using the peptides, antibodies or antibody/peptide constructs as an immunological tolerance-inducing agent. The kit may also comprise two or more component parts for the reconstituted active composition. For example, a first component can include the peptides, antibodies or antibody/peptide constructs and the second component can include a bifunctional chelate or a therapeutic agent such as a radionuclide, which when mixed with the peptides, antibodies or antibody/peptide constructs forms a conjugated system therewith. The above-noted buffers, excipients, and other component parts can be sold separately or together with the kit.

Practice of the present methods, including additional preferred aspects and embodiments thereof, will be more fully understood from the following examples, which are presented for illustration only and should not be construed as limiting in any way.

EXAMPLE 1

Selection of peptide binding to either L-SIGN ligands, DC-SIGN ligands or both

Peptides are selected based on their capability to block binding of L-SIGN specific or DC-SIGN-specific antibodies or antibodies cross-reactive with both DC-SIGN and L-SIGN to L-SIGN- or DC-SIGN-expressing cells. Antibodies are incubated with a peptide of SEQ ID NO: 1 or a variant thereof. Subsequently, the antibody peptide mixture is added to K562 cells expressing DC-SIGN or L-SIGN. Binding of antibody to the cells is assessed by FACS analysis after adding a fluorochrome-conjugated anti-mouse Fab antibody. Loss of binding of only L-SIGN antibodies to L-SIGN expressing cells indicates selection of a peptide specific for L-SIGN ligands, loss of binding of only DC-SIGN antibodies to DC-SIGN expressing cells indicates selection of a DC-SIGN-specific ligand, and loss of binding to both cell types indicates a peptide interacting with cross-reactive ligands.

Furthermore, peptides can be selected based on their ability to block binding of both ICAM-2 and ICAM-3 to DC-SIGN-expressing cells, or their ability to block pathogen binding to both DC-SIGN and L-SIGN as outlined in example 2.

EXAMPLE 2

Therapeutic Evaluation - Immunomodulatory effects

An immunomodulatory effect of the peptide having SEQ ID NO: 1 or variants thereof is tested in vitro in an allogeneic T cell response. Resting or activated (cultured in the presence of 400 U IL-2/ml 0.2 mg/ml PHA for 2 days) responder T cells (100 x 10^3) are added to dendritic cells (1.5 x 10^3) in the presence of the peptide in a 96-well plate. The cells are cultured for 3 days and then pulsed with 0.5 μCi ^{3}H-thymidine/well. Cells are harvested the next day and incorporated radioactivity is determined using a Topcount (Packard Instruments, Meriden, CT).

EXAMPLE 3

Therapeutic Evaluation – Blocking Viral Entry

The capacity of the peptide of or modifications thereof to block viral entry into cells is tested using L-SIGN or DC-SIGN transfected K-562 cells. Serum from virus+ or virus- donors is incubated with the peptide for 30 minutes before adding to the transfected cells. After 1 hour incubation at 37° C, cells are washed 5 times and viral RNA is extracted from the cells using Qiagen's viral RNA mini spin kit (Qiagen Inc., Valencia, CA). Viral RNA is then amplified by RT-PCR as described by Gardner, et al., Proc. Natl. Acad. Sci. USA, volume 100, page 4498 (2003), and a southern blot is performed. The peptide or peptide/antibody construct blocking binding to DC-SIGN and L-SIGN most efficiently is selected.

EXAMPLE 4

Therapeutic Evaluation-Blocking Virus Binding

The capacity of the peptide of SEQ ID NO: 1 or variants thereof to block binding of the virus to cells is accomplished by a fluorescent bead adhesion assay as previously described by Geijtenbeek et al., Blood, Volume 94, pages 754-764 (1999). Briefly, fifty thousand K562/L-SIGN or DC-SIGN transfected cells are incubated with peptide of SEQ ID NO: 1 or variants thereof (0 to 100 µg/mL) for 10 minutes at RT in a 96-well V-shaped-bottom plate. Fluorescent beads (20 beads/cell) coated with viral envelope proteins e.g., HCV E1/E2 or HIVgp120 are added and the suspension is incubated for an additional 30 minutes at 37°C. After washing, the cells are resuspended in Tris-sodium-BSA buffer. The extent of blocking by antibodies of virus coated beads to K562/SIGN cells is measured using a FACScalibur. The percentage of cells bound to the virus beads (negative control) in the absence of peptides is set at 100 and the decrease in binding in the presence of SIGN peptides is expressed as % blocking. The peptide or peptide/antibody construct blocking binding to DC-SIGN and L-SIGN most efficiently is selected.

EXAMPLE 5

Therapeutic Evaluation – Preventing Virus Transmission

To test whether peptide of SEQ ID NO: 1 or variants thereof can prevent transfer of the virus from receptor positive endothelial cells to either human T-cells or liver cells, K562/L-SIGN or DC-SIGN cells or freshly isolated human liver sinusoidal endothelial cells (L-SIGN+) or dendritic cells (DC-SIGN+) are incubated with peptide of SEQ ID NO: 1 or variants thereof (0 to 100 µg/mL) for 30 minutes before adding luciferase or green fluorescent protein reporter viruses expressing envelope proteins of interest e.g., HCV-E2 as previously described by Cormier et al., Proc Natl Acad Sci U S A, volume 101, pages 14067- 72 (2004), HIVgp120, as previously described by Pohlmann et al., J Virol, volume 75, pages 4664-4672 (2001), Ebola as previously described by Alvarez et al., J Virol, volume 76, pages 6841-6844 (2002) or sindbis as previously described by Klimstra et al., J Virol, volume 77, pages 12022-12032 (2003). After washing with culture medium, the cells are co-cultured with T (C8166) cells or human liver (Huh-7) cells. Reporter virus transmission is assessed either by measuring luciferase activity (relative light units) in target cell lysates or by flow cytometric analysis of GFP positive target cells in combination with suitable surface marker double staining on target cells (e.g., CD3 on T-cells).

EXAMPLE 6

Blocking Infection by Mycobacterium tuberculosis

Mannosylated lipoarabinomannan (ManLAM), a carbohydrate rich structure present on the surface of *M. tuberculosis* has been reported to interact with both DC-SIGN (Geijtenbeek et al., J Exp Med, volume 197, pages 7-17 (2003) and L-SIGN (Koppel et al., Immunobiology, volume 209, pages 117-127 (2004). High antibody titers against ManLAM are observed in people with active tuberculosis and have been shown to reduce bacterial loads in passive protection experiments (Hamasur et al., Clin Exp Immunol, volume 138, pages 30-38 (2004). Based on these observations, it is possible to inhibit mycobacterial binding and infection using either SIGN antibodies or SIGN peptide mimics

capable of binding to ManLAM with high affinities. To test this possibility, available strains of the bacterium e.g., *M, bovis and M.tuberculosis* are labelled with fluorescein isothiocyanate (FITC) as detailed in Geijtenbeek et al., J Exp Med, volume 197, pages 7-17 (2003) and incubated K562/L-SIGN or DC-SIGN cells with FITC conjugated bacteria at a ratio 1 to 20 in presence of peptide of SEQ ID NO: 1 or variants thereof (0 to 100 µg/mL). The extent of blocking (reduction in fluorescence) by peptide inhibitor is determined by flow cytometry analysis.

EXAMPLE 7

Improvement of affinity

Each of the amino acid residues in the peptide of SEQ ID NO: 1 or variants thereof is serially mutated with alanine to determine the amino acids that result in loss of binding to ICAM-3 and HCV, HIV or other pathogen's envelope glycoproteins. Loss of binding is evaluated in either whole cell or ELISA-based assays as described in Pal, et al., J. Mol. Biol., volume 332, page 195 (2003); Cunningham, et al., Science, volume 244, page 1081 (1989); and Jin, et al., Protein Sci., volume 3, page 2351 (1994).

After identifying residues critical for binding to L-SIGN ligands or, for pathogen targeting, also DC-SIGN ligands, each of the critical residues is substituted with nineteen other amino acids to identify peptide variants that have enhanced binding to ICAM-3 and/or HCV envelope proteins. Each of these peptides is tested for high affinity binding to ICAM-3 and HCV or other pathogen's envelope proteins using techniques within the purview of those skilled in the art. Additionally, peptides that have selective binding to HCV or other pathogen's envelope protein but not T-cell ligands, e.g., ICAM-3 and/or ICAM-2, are identified from this pool of combinatorial peptides. The peptides that show reduced binding to ICAM-3 but retain binding to HCV envelope protein are useful to develop therapeutics that selectively interfere with the entry of the virus into cells but do not inhibit L-SIGN-T cell interactions. On the other hand, peptides that show increased binding to ICAM-3 but reduced binding to an envelope protein can be useful in down-modulating immune response.

All the productive amino acid substitutions can be combined into one peptide and, in parallel, a set of peptides containing the productive amino acids in various combinations can be made by combinatorial peptide synthesis methods as detailed in Balse-Srinivasan, et al. J Med Chem, volume 46, page 3728 (2003); Nitz, et al. Chembiochem, volume 4, page 272 (2003); and Katchalski-Katzir, et al. Biophys Chem, volume 100, page 293 (2003).

EXAMPLE 8

Testing Whether Treatment Of Transplanted HCV Infected Liver With Peptide Can Prevent Replication Of The Virus And Spread Of The Disease To Recipient T Cells

If virus transmission is prevented, the peptide can be used in a transplant setting in which donors potentially have HCV infections. To test this, mildly HCV-infected human donor liver is transplanted into immunodeficient mice such as NOD/SCID alongside with injection of primary blood lymphocytes from a healthy, HLA matched human donor. Mice are treated with peptide over a period of one to 6 months. One to six months after transplantation, the mice are sacrificed, and the extent of HCV infection in the liver is assessed. Also, T cells are examined for infection with virus by PCR.

EXAMPLE 9

Grafting of Peptide Into Antibody Scaffold

The peptide of SEQ ID NO: 1 or variants thereof are grafted into the CDR3 region of an anti-tetanus toxoid antibody scaffold or a domain-exchanged antibody scaffold. With either scaffold, two grafting approaches are taken. The flanking sides can be modified by adding two glycines. This is to reduce the structural constraints on the grafted peptide so that it can more easily adopt the needed conformation. In another approach, two amino acid positions on each side of the peptide graft are randomized in order that the best presentation of the peptide can be achieved. These two approaches are taken in order to determine whether the peptide alone is sufficient or if specific residues are required for

proper presentation of the peptide on the antibody scaffold, thereby conferring its activity to the antibody.

Overlap PCR is performed to insert peptide sequence into heavy chain CDR3 of Tetanus toxoid antibody (TTH3) and also incorporate 2 randomized amino acids in the framework 1 region of the heavy chain. It consists of 2 overlap PCR reactions. First overlap PCR was performed with primers L-SIGN TT3R (SEQ ID NO: 7 – see below) and L-SIGN TT3F (SEQ ID NO: 8 – see below) that incorporate peptide sequence into TTH3 paired with primers (LeadVH (SEQ ID NO: 9 – see below) and SeqG3-R (SEQ ID NO: 10 – see below)) that hybridize to the vector sequence. Insertion of L-SIGN peptide into TTH3 included 2 additional primers Int-F (SEQ ID NO: 11 – see below) and Int-R (SEQ ID NO: 12 – see below) that do not contain a hybridizing portion to the TT antibody gene but were used to make hybriding portion between the two amplified products. The template for this PCR was pRL4-TT (pRL4 vector that has TT antibody gene). The amplified products were purified and used as templates for the overlap PCR with primers H2H3SSTOXX-F (SEQ ID NO: 13 – see below) and N-dP (SEQ ID NO: 14 – see below). H2H3SSTOXX-F primer hybridizes to TT heavy chain framework region 1 immediately after the 2 randomized amino acids and N-dP hybridizes to hemagglutinin sequence in the vector. The amplified product was purified and used for the second overlap PCR. Separately, TT heavy chain framework region 1 was amplified with primers LeadVH (SEQ ID NO: 9 – see below) and H2H3SSTOXX-R (SEQ ID NO: 15 – see below) using pRL4-TT as a template. H2H3SSTOXX-R hybridizes to framework region 1 of TT heavy chain including degeneracy to randomized two amino acids.

This product is also purified and used as a template for the second overlap PCR. The second overlap PCR is amplified using primers LeadVH and N-dP. The amplified product is gel purified and digested with Xho I/Spe I and cloned into pRL4-TT and heavy chain of TT is replaced by each peptide inserted heavy chain sequence in its CDR3.

L-SIGN TT3R

5'GCGCAATCTTCGTTGCCTGAGTTATTCGGTTCGCCGCTGTTCCAATAGCG MNNMNNTCTCGCACAATAATATATGGC 3' (SEQ ID NO: 7)

L-SIGN TT3F

5'GGAATTTAGCGGCAGTGGTTGGAATGATAATCGCTGTGACGTGGATAACN NKNNKTGGGGCCAAGGGACCACGGTC 3' (SEQ ID NO: 8)

LeadVH

5' GCT GCC CAA CCA GCC ATG GCC 3' (SEQ ID NO. 9)

SeqG3-R

5'ATC AAA ATC ACC GGA ACC AGA GC 3' (SEQ ID NO. 10)

INT-F

5'AACCGAATAACTCAGGCAACGAAGATTGCGCGGAATTTAGCGGCAGTGGT TGGAATGATAATCG3' (SEQ ID NO: 11)

INT-R

5'CGATTATCATTCCAACCACTGCCGCTAAATTCCGCGCAATCTTCGTTGCCT GAGTTATTCGGTT3' (SEQ ID NO: 12)

H2H3SSTOXX-F

5' TAT GCC ATC AGC TGG GTG CGA CAG 3' (SEQ ID NO: 13)

N-dP

5' AGC GTA GTC CGG AAC GTC GTA CGG 3' (SEQ. ID. NO: 14)

H2H3SSTOXX-R

5' TCG CAC CCA GCT GAT GGC ATA MNN MNN GAA GGT GCC TCC AGA AGC CCT 3' (SEQ ID NO: 15)

Nucleotide Sequence Encoding Peptide Of SEQ ID NO: 1

5' CGCTATTGGAACAGCGGCGAACCGAATAACTCAGGCAACGAAGATTGCG CGGAATTTAGCGGCAGTGGTTGGAATGATAATCGCTGTGACGTGGATAAC3' (SEQ ID NO: 16)

Complement of Nucleotide Sequence of SEQ ID NO: 5

5' GTTATCCACGTCACAGCGATTATCATTCCAACCACTGCCGCTAAATTCCG CGCAATCTTCGTTGCCTGAGTTATTCGGTTCGCCGCTGTTCCAATAGCG3' (SEQ ID NO: 17)

EXAMPLE 10

Use Of Peptide For Immunization To Produce Antibodies Against The L-SIGN Ligand Binding Domain

The peptide of SEQ ID NO: 1 or variants thereof are administered with an adjuvant such as CpG or Titermax or dendritic cells or other stimulatory peptides as e.g. tetanus toxoid to laboratory animals such as mice or rabbits. Immunizations are repeated two to three times 1-6 weeks apart. Serum is taken and tested for an antibody response against the peptide by ELISA. If the anti-peptide titers are satisfactory after the third immunization, spleen and bone marrow are collected and a phage library constructed from RNA of these organs. Alternatively, spleen cells are used to produce hybridomas. Resulting antibodies are used therapeutically as described above.

EXAMPLE 11

Use Of The Peptide As A Screening Tool For Antibodies Against The L-SIGN Ligand Binding Domain

The peptide of SEQ ID NO: 1 or variants thereof are used to screen antibodies produced by immunizing with L-SIGN expressing cells or recombinant L-SIGN protein for antibodies specifically recognizing the ligand binding domain. This can be accomplished by either simply coating the peptide on ELISA plates and determining which antibody binds to the peptide, or in competition studies. Competition is performed by coating plates with L-SIGN protein and then adding both antibody and peptide followed by detection of bound antibody. A similar assay is performed by FACS using L-SIGN or DC-SIGN expressing cells and incubating them with antibody in the presence of peptide. In both experimental

approaches, antibodies recognizing the ligand binding domain show reduced or no binding in the presence of peptide.

Furthermore, this peptide is used to screen for antibodies cross-reactive with human and mouse L-SIGN or DC-SIGN proteins binding to the ligand recognition domain. In that case, antibodies produced by immunizing lab animals with the mouse homologue of L-SIGN (mSIGNR1) or mSIGNR1 expressing cells are tested for their capacity to bind to peptide coated on ELISA plates or their performance in the competition assays described above. Similarly, antibodies cross-reactive with human L-SIGN and DC-SIGN are identified by screening antibodies derived from lab animals immunized with DC-SIGN or DC-SIGN expressing cells. Alternatively, antibodies from a peptide immunized lab animal are screened on human L-SIGN, DC-SIGN or their mouse homologues to identify antibodies to either of these proteins.

EXAMPLE 12

L-SIGN active site peptide-antibody fusion proteins to enhance serum stability

The first encounter of a number of viruses (e.g., HIV and Ebola) with the host is through binding to attachment receptors such as L-SIGN and DC-SIGN (Altmeyer, Curr Pharm Des 10, 3701-3712, 2004.). Therefore peptides designed based on the active site of these receptors e.g., SEQ ID NO: 1 may be used therapeutically for blocking the binding and infection by these pathogens. L-SIGN active site peptide (SEQ ID NO: 1) is produced as antibody-fusion conjugates to enhance the serum half-life and biological activity of the drug. The L-SIGN peptide fusions are made in at least two different formats. The first format is produced as a carboxy-terminus fusion of the Fc portion of immunoglobulin G_2G_4 (to prevent Fc receptor uptake) using a flexible linker made of amino acids glycine and serine $(G_4S)_2$. The recombinant fusion proteins are produced in bacteria or mammalian cells naturally as a dimer as shown below:

CH1–Hinge-CH2-CH3 - $(G_4S)_2$ linker – L-SIGN peptide of SEQ ID NO: 1

or as a monomer by mutating selected cysteine residues to alanine in the Fc portion as illustrated below:

CH1–Hinge-CH2-CH3 - $(G_4S)_2$ linker – L-SIGN peptide of SEQ ID NO: 1

In an alternative embodiment, an amino-terminus fusion can be prepared having the following structure:

L-SIGN peptide of SEQ ID NO: 1 - $(G_4S)_2$ linker – CH1–Hinge-CH2-CH3

Another useful format is a multimer, wherein the active site L-SIGN peptide (SEQ ID NO: 1) is grown as branches on a tree trunk using the side chain free amino groups of lysine on synthetic linker. The multimeric protein is produced synthetically as described earlier (Sundaram, et al., J Biol Chem 279, 24141-24151, 2004), the structure of which is schematically shown in Figure 2.

It will be understood that various modifications may be made to the embodiments disclosed herein. For example, as those skilled in the art will appreciate, the specific sequences described herein can be altered slightly without necessarily adversely affecting the functionality of the peptides or antibody/peptide constructs. For instance, substitutions of single or multiple amino acids in the peptide sequence can frequently be made without destroying the functionality of the peptide, antibody or antibody/peptide construct. Thus, it should be understood that peptides having a degree of identity greater than about 70% to the specific peptides described herein are within the scope of this disclosure. In particularly useful embodiments, peptides having a identity greater than about 80% to the specific peptides described herein are contemplated. In other useful embodiments, peptides having a identity greater than about 90% to the specific peptides described herein are contemplated. Therefore, the above

description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of this disclosure.

The following references are incorporated herein in their entirety by this reference:

- Altmeyer, R. 2004. Virus attachment and entry offer numerous targets for antiviral therapy. Curr Pharm Des 10, 3701-3712.
- Alvarez, C. P., Lasala, F., Carrillo, J., Muniz, O., Corbi, A. L., and Delgado, R. 2002. C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. J Virol 76, 6841-6844.
- Appelmelk, B. J., I. van Die, S. J. van Vliet, C. M. Vandenbroucke-Grauls, T. B. Geijtenbeek, and Y. van Kooyk. 2003. Cutting edge: carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. *J Immunol 170:1635.*
- Balse-Srinivasan, P., P. Grieco, M. Cai, D. Trivedi, and V. J. Hruby. 2003. Structure-activity relationships of novel cyclic alpha-MSH/beta-MSH hybrid analogues that lead to potent and selective ligands for the human MC3R and human MC5R. J Med Chem 46:3728.
- Bashirova A.A., Geijtenbeek T.B., van Duijnhoven G.C. et al. 2001. A dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. J Exp Med 193: 671-678.
- Coombes, A. G., S. Tasker, M. Lindblad, J. Holmgren, K. Hoste, V. Toncheva, E. Schacht, M. C. Davies, L. Illum, and S. S. Davis. 1997. Biodegradable polymeric microparticles for drug delivery and vaccine formulation: the surface attachment of hydrophilic species using the concept of poly(ethylene glycol) anchoring segments. Biomaterials 18:1153.
- Cormier, E. G., Durso, R. J., Tsamis, F., Boussemart, L., Manix, C., Olson, W. C., Gardner, J. P., and Dragic, T. 2004. L-SIGN (CD209L) and DC-

SIGN (CD209) mediate transinfection of liver cells by hepatitis C virus. Proc Natl Acad Sci U S A 101(39):14067.

- Cunningham, B. C., and J. A. Wells. 1989. High-resolution epitope mapping of hGH-receptor interactions by alanine-scanning mutagenesis. Science 244:1081.
- Feinberg, H., D. A. Mitchell, K. Drickamer, and W. I. Weis. 2001. Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. Science 294:2163.
- Frangione-Beebe, M., R. T. Rose, P. T. Kaumaya, and S. P. Schwendeman. 2001. Microencapsulation of a synthetic peptide epitope for HTLV-1 in biodegradable poly(D,L-lactide-co-glycolide) microspheres using a novel encapsulation technique. J Microencapsul 18:663.
- Gardner, J. P., R. J. Durso, R. R. Arrigale, G. P. Donovan, P. J. Maddon, T. Dragic, and W. C. Olson. 2003. L-SIGN (CD 209L) is a liver-specific capture receptor for hepatitis C virus. *Proc Natl Acad Sci U S A 100:4498.*
- [1]Geijtenbeek, T. B., Van Vliet, S. J., Koppel, E. A., Sanchez-Hernandez, M., Vandenbroucke-Grauls, C. M., Appelmelk, B., and Van Kooyk, Y. 2003. Mycobacteria target DC-SIGN to suppress dendritic cell function. J Exp Med 197, 7-17.
- [2]Geijtenbeek T.B., Groot P.C., Nolte M.A., van Vliet S.J., Gangaram-Panday S.T., van Duijnhoven G.C., Kraal G., van Oosterhout A.J., van Kooyk Y. 2002. Marginal zone macrophages express a murine homologue of DC-SIGN that captures blood-borne antigens in vivo. Blood 100: 2908-2916.
- [3]Geijtenbeek, T. B., S. J. van Vliet, G. C. van Duijnhoven, C. G. Figdor, and Y. van Kooyk. 2001. DC-SIGN, a dentritic cell-specific HIV-1 receptor present in placenta that infects T cells in trans-a review. Placenta 22 Suppl A:S19.
- [4]Geijtenbeek T.B., Torensma R., van Vliet S.J., van Duijnhoven G.C., Adema G.J., van Kooyk Y., Figdor C.G. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary

immune responses. Cell 100: 575-585.

- [5]Geijtenbeek, T. B., van Kooyk, Y., van Vliet, S. J., Renes, M. H., Raymakers, R. A., and Figdor, C. G. 1999. High frequency of adhesion defects in B-lineage acute lymphoblastic leukemia. Blood 94, 754-764.
- Grace, M., S. Youngster, G. Gitlin, W. Sydor, L. Xie, L. Westreich, S. Jacobs, D. Brassard, J. Bausch, and R. Bordens. 2001. Structural and biologic characterization of pegylated recombinant IFN-alpha2b. J Interferon Cytokine Res 21:1103.
- Hamasur, B., Haile, M., Pawlowski, A., Schroder, U., Kallenius, G., and Svenson, S. B. 2004. A mycobacterial lipoarabinomannan specific monoclonal antibody and its F(ab') fragment prolong survival of mice infected with Mycobacterium tuberculosis. Clin Exp Immunol 138, 30-38.
- Jin, L., and J. A. Wells. 1994. Dissecting the energetics of an antibody-antigen interface by alanine shaving and molecular grafting. Protein Sci 3:2351.
- Katchalski-Katzir, E., R. Kasher, M. Balass, T. Scherf, M. Harel, M. Fridkin, J. L. Sussman, and S. Fuchs. 2003. Design and synthesis of peptides that bind alpha-bungarotoxin with high affinity and mimic the three-dimensional structure of the binding-site of acetylcholine receptor. Biophys Chem 100:293.
- Kim, T. H., H. Lee, and T. G. Park. 2002. Pegylated recombinant human epidermal growth factor (rhEGF) for sustained release from biodegradable PLGA microspheres. Biomaterials 23:2311.
- Klimstra, W. B., E. M. Nangle, M. S. Smith, A. D. Yurochko, and K. D. Ryman. 2003. DC-SIGN and L-SIGN can act as attachment receptors for alphaviruses and distinguish between mosquito cell- and mammalian cell-derived viruses. J Virol 77:12022.
- Knolle PA, Germann T, Treichel U, Uhrig A, Schmitt E, Hegenbarth S, Lohse AW, Gerken G (1999) Endotoxin down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells. J Immunol 162: 1401 -1407.

- Koppel, E. A., Ludwig, I. S., Hernandez, M. S., Lowary, T. L., Gadikota, R. R., Tuzikov, A. B., Vandenbroucke-Grauls, C. M., van Kooyk, Y., Appelmelk, B. J., and Geijtenbeek, T. B. 2004. Identification of the mycobacterial carbohydrate structure that binds the C-type lectins DC-SIGN, L-SIGN and SIGNR1. Immunobiology 209, 117-127.
- Limmer A, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA (2000) Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med 6: 1348-1354.
- Mamot, C., D. C. Drummond, U. Greiser, K. Hong, D. B. Kirpotin, J. D. Marks, and J. W. Park. 2003. Epidermal growth factor receptor (EGFR)-targeted immunoliposomes mediate specific and efficient drug delivery to EGFR- and EGFRvIII-overexpressing tumor cells. Cancer Res 63:3154.
- Nitz, M., K. J. Franz, R. L. Maglathlin, and B. Imperiali. 2003. A powerful combinatorial screen to identify high-affinity terbium(III)-binding peptides. Chembiochem 4:272.
- Pal, G., A. A. Kossiakoff, and S. S. Sidhu. 2003. The functional binding epitope of a high affinity variant of human growth hormone mapped by shotgun alanine-scanning mutagenesis: insights into the mechanisms responsible for improved affinity. J Mol Biol 332:195.
- Park, J. W., K. Hong, D. B. Kirpotin, G. Colbern, R. Shalaby, J. Baselga, Y. Shao, U. B. Nielsen, J. D. Marks, D. Moore, D. Papahadjopoulos, and C. C. Benz. 2002. Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery. Clin Cancer Res 8:1172.
- Pohlmann S, Soilleux EJ, Baribaud F et al. 2001. DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. Proc Natl Acad Sci USA 98: 2670-2675.
- Pohlmann, S., Baribaud, F., Lee, B., Leslie, G. J., Sanchez, M. D., Hiebenthal-Millow, K., Munch, J., Kirchhoff, F., and Doms, R. W. 2001.

DC-SIGN interactions with human immunodeficiency virus type 1 and 2 and simian immunodeficiency virus. J Virol 75, 4664-4672.

- [1]Putney, S. D. 1998. Encapsulation of proteins for improved delivery. Curr Opin Chem Biol 2:548.
- [2]Putney, S. D., and P. A. Burke. 1998. Improving protein therapeutics with sustained-release formulations. Nat Biotechnol 16:153.
- Soilleux EJ, Barten R, Trowsdale J (2000) Cutting Edge: DC-SIGN; a related gene, DC-SIGNR; and CD23 form a cluster on 19p13. J Immunol 165: 2937-2942.
- [1]Srinivasan, M., I. E. Gienapp, S. S. Stuckman, C. J. Rogers, S. D. Jewell, P. T. Kaumaya, and C. C. Whitacre. 2002. Suppression of experimental autoimmune encephalomyelitis using peptide mimics of CD28. J Immunol 169:2180.
- [2]Srinivasan, M., R. M. Wardrop, I. E. Gienapp, S. S. Stuckman, C. C. Whitacre, and P. T. Kaumaya. 2001. A retro-inverso peptide mimic of CD28 encompassing the MYPPPY motif adopts a polyproline type II helix and inhibits encephalitogenic T cells in vitro. J Immunol 167:578.
- Sundaram, R., Lynch, M. P., Rawale, S. V., Sun, Y., Kazanji, M., and Kaumaya, P. T. 2004. De novo design of peptide immunogens that mimic the coiled coil region of human T-cell leukemia virus type-1 glycoprotein 21 transmembrane subunit for induction of native protein reactive neutralizing antibodies. J Biol Chem 279, 24141-24151.
- Yamamoto, Y., Y. Tsutsumi, Y. Yoshioka, T. Nishibata, K. Kobayashi, T. Okamoto, Y. Mukai, T. Shimizu, S. Nakagawa, S. Nagata, and T. Mayumi. 2003. Site-specific PEGylation of a lysine-deficient TNF-alpha with full bioactivity. Nat Biotechnol 21:546.

WHAT IS CLAIMED IS:

1. An isolated peptide comprising SEQ ID NO: 1 wherein the peptide is not L-SIGN.

2. A peptide as in claim 1 wherein the amino acids of said peptide are L-isomers.

3. A peptide as in claim 1 wherein the amino acids of said peptide are D-isomers.

4. Nucleic acid encoding the peptide of claim 1.

5. Nucleic acid encoding the peptide of claim 1 wherein said peptide is comprised of L-isomers.

6. A composition comprising the peptide of claim 1 and a pharmaceutically acceptable carrier.

7. A peptide as in claim 1 wherein the peptide is bound to an Fc fragment of an antibody.

8. A peptide as in claim 1 wherein the peptide is pegylated.

9. A peptide as in claim 1 wherein the amino terminus is blocked by acetylation.

10. A peptide as in claim 1 wherein the carboxy terminus is blocked by amidation.

11. A peptide comprising the amino acid sequence:

G E X N X S X X E X X X X X S G S X X N D N (SEQ ID NO: 2)

wherein X can be any amino acid so long as said peptide is capable of binding under physiological conditions to at least one ligand selected from the group consisting of L-SIGN ligands and DC-SIGN ligands, wherein said peptide is not L-SIGN.

12. A peptide as in claim 11 wherein the amino acids of said peptide are L-isomers.

13. A peptide as in claim 11 wherein the amino acids of said peptide are D-isomers.

14. A peptide of claim 11 wherein said peptide is capable of binding to ICAM-2, ICAM-3 or a pathogen under physiological conditions.

15. A peptide of claim 11 wherein a sequence of 22 consecutive amino acids of said peptide is at least 50% identical to amino acids 6-27 of SEQ ID NO: 1.

16. A peptide of claim 11 wherein a sequence of 22 consecutive amino acids of said peptide is at least 70% identical to amino acids 6-27 of SEQ ID NO: 1.

17. A peptide of claim 11 wherein a sequence of 22 consecutive amino acids of said peptide is at least 90% identical to amino acids 6-27 of SEQ ID NO: 1.

18. Nucleic acid encoding the peptide of claim 11.

19. Nucleic acid encoding the peptide of claim 11 wherein said peptide is comprised of L-isomers.

20. A composition comprising the peptide of claim 11 and a pharmaceutically acceptable carrier.

21. A peptide as in claim 11 wherein the peptide is pegylated.

22. A peptide as in claim 11 wherein said peptide is bound to an Fc fragment of an antibody.

23. A peptide as in claim 11 wherein the amino terminus is blocked by acetylation.

24. A peptide as in claim 11 wherein the carboxy terminus is blocked by amidation.

25. A peptide comprising the amino acid sequence:
RYWNSXXPXNXGNXDCAEFXXXGWXXXRCDVDN (SEQ ID NO: 3)
wherein X is any amino acid so long as said peptide is capable of binding under physiological conditions to at least one ligand to which the peptide of SEQ ID NO: 1 can bind under physiological conditions, wherein said peptide is not L-SIGN.

26. A peptide as in claim 25 wherein the amino acids of said peptide are L-isomers.

27. A peptide as in claim 25 wherein the amino acids of said peptide are D-isomers.

28. A peptide of claim 25 wherein said peptide is capable of binding to ICAM-2, ICAM-3 or a pathogen under physiological conditions.

29. A peptide of claim 25 wherein a sequence of 33 consecutive amino acids of said peptide is at least 50% identical to the sequence of SEQ ID NO: 1.

30. A peptide of claim 25 wherein a sequence of 33 consecutive amino acids of said peptide is at least 70% identical to the sequence of SEQ ID NO: 1.

31. A peptide of claim 25 wherein a sequence of 33 consecutive amino acids of said peptide is at least 90% identical to the sequence of SEQ ID NO: 1.

32. Nucleic acid encoding the peptide of claim 25.

33. Nucleic acid encoding the peptide of claim 25 wherein said peptide is comprised of L-isomers.

34. A peptide as in claim 25 wherein said peptide is bound to an Fc fragment of an antibody.

35. A composition comprising the peptide of claim 25 and a pharmaceutically acceptable carrier.

36. A peptide as in claim 25 wherein the peptide is pegylated.

37. A peptide as in claim 25 wherein the amino terminus is blocked by acetylation.

38. A peptide as in claim 25 wherein the carboxy terminus is blocked by amidation.

39. A method for prevention of the induction of immune tolerance by blocking access of ICAM-expressing T cells to LSEC's or other tolerance inducing cells expressing L-SIGN by administering an effctive amount of a peptide in accordance with one of claims 1, 11 or 25 to a subject.

40. A method for downregulating the immune response or preventing immune activation by blocking ICAM-dendritic cell interaction by administering an effective amount of a peptide in accordance with one of claims 1, 11 or 25 to a subject.

41. A method of inhibiting pathogen infection in a subject comprising administering an effective amount of a peptide in accordance with one of claims 1, 11 or 25 to a subject.

42. A method of inhibiting viral transmission in a subject comprising administering an effective amount of a peptide in accordance with one of claims 1, 11 or 25 to said subject.

43. An antibody that binds a peptide in accordance with one of claims 1, 11 or 25, wherein said antibody does not bind L-SIGN.

44. A nucleic acid encoding an antibody of claim 42.

45. A method of inhibiting pathogen infection in a subject comprising administering to said subject an effective amount of an antibody of claim 42.

46. A method of inhibiting viral transmission in a subject comprising administering to said subject an effective amount of an antibody of claim 42.

47. An antibody made by an antibody producing cell in response to a peptide of one of claims 1, 11 or 24.

48. A method of inhibiting pathogen infection in a subject comprising administering to said subject an effective amount of an antibody of claim 46.

49. A method of inhibiting viral transmission in a subject comprising administering to said subject an effective amount of an antibody of claim 46.

50. A nucleic acid encoding an antibody of claim 46.

51. An antibody/peptide construct comprising at least a portion of the constant region of an antibody joined to a peptide in accordance with one of claims 1, 11 or 24.

52. An antibody/peptide construct in accordance with claim 50 wherein a linker is positioned between the peptide and the antibody.

53. An antibody/peptide construct in accordance with claim 50 wherein the at least a portion of the constant region of an antibody is an Fc fragment.

54. An antibody/peptide construct in accordance with claim 50 wherein the at least a portion of the constant region of an antibody is a whole antibody.

55. An antibody having at least a portion of one CDR replaced with a peptide in accordance with one of claims 1, 11 or 24.

56. A peptide comprising SEQ ID NO: 6 wherein the amino acids are D amino acids.

57. A multimer comprising one or more peptides in accordance with any of claims 1, 11 or 24.

58. A multimer as in claim 56 comprising multiple copies of the same peptide.

59. A multimer as in claim 56 comprising at least two different peptides.

60. A multimer as in claim 56 wherein the peptides are joined to a synthetic peptide backbone.

61. A method of screening antibodies comprising:

coating a plate with a peptide in accordance with claim 1; and

contacting the coated plate under physiological conditions with a composition containing a plurality of antibodies produced by a subject in response to L-SIGN immunization; and

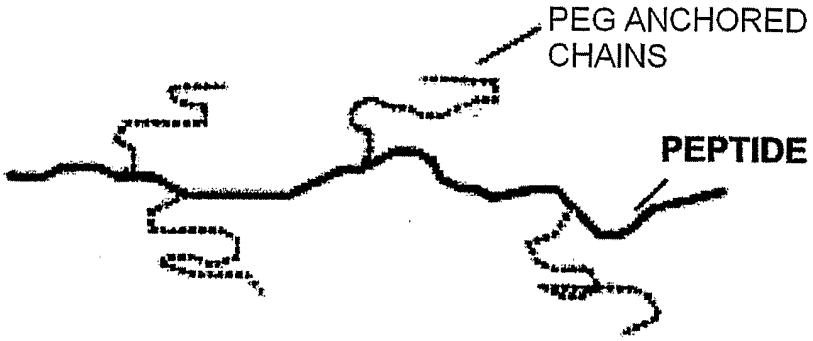
determining which antibodies bind to the coated plate.

62 A fusion protein comprising a peptide in accordance with any of claims 1, 11 or 25 fused to albumin.

62. A fusion protein as in claim 61 further comprising an albumin fusion sequence.

63. A fusion protein as in claim 61 wherein the albumin fusion sequence comprises SEQ ID NO: 18.

FIGURE 1A



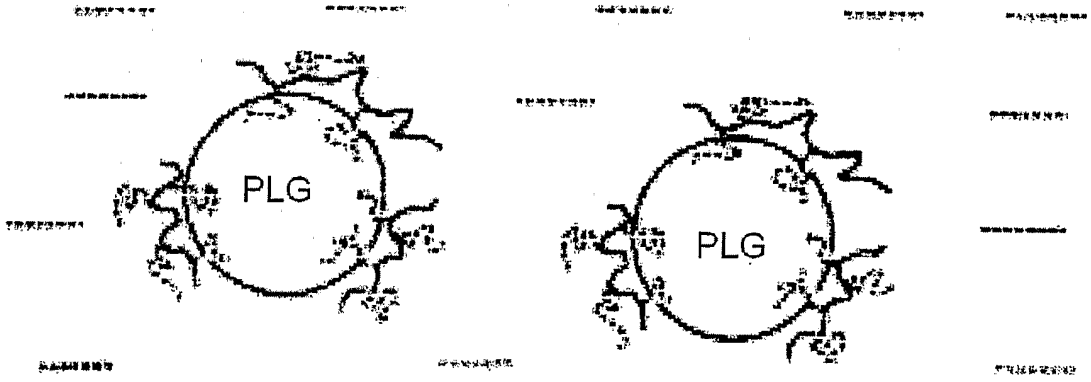
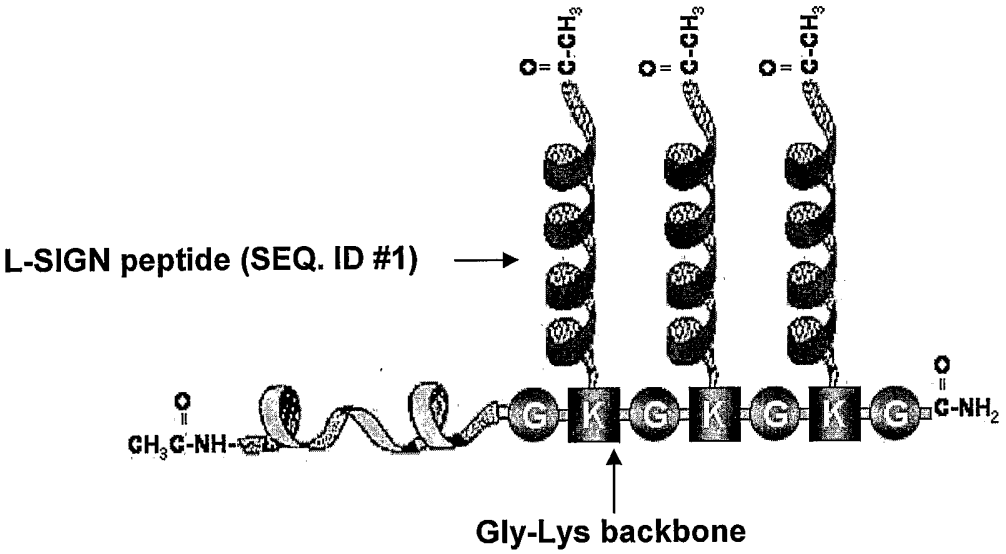


FIGURE 1B

FIGURE 2


L-SIGN peptide (SEQ. ID #1)
O= C-CH3
O= C-CH3
O= C-CH3
CH3C-NH-
G K G K G K G
C-NH2
Gly-Lys backbone