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(57) **ABSTRACT**

The present invention relates to novel combinations of active agents and methods for treatment of retrovirus infections including HIV infections and AIDS. In particular, the present invention relates to novel use of administrations and combinations of a compound to stimulate the humoral immunity and/or peptides that elicit a cell-mediated immune response in a subject; and immunomodulatory compounds and/or a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor.

VACCINE

FIELD OF THE INVENTION

[0001] The present invention relates to novel combinations of active agents and methods for treatment of retrovirus infections including HIV infections and AIDS. In particular, the present invention relates to novel methods of treatments with combinations of a compound to stimulate the humoral immunity and/or peptides that elicit a cell-mediated immune response in a subject; and immunomodulatory compounds and/or reservoir purging agents, such as a histone deacetylase (HDAC) inhibitors.

BACKGROUND OF THE INVENTION

[0002] In recent years a large body of research evidence has accumulated supporting the concept that AIDS is an immunological disease induced by HIV-1 rather than simply being caused by loss of CD4+ T-lymphocytes as a result of chronic cytopathic viral infection. It is therefore important to develop interventions that also target the chronic immune stimulation induced by HIV-1 (Sommerfelt 2011, Recent Translational Research in HIV/AIDS Ed. Dr. Yi-Wei Tang. ISBN 979-953-307-189-2. pp 493-510).

[0003] The failure of antiretroviral therapy (ART) to eradicate HIV-1 infection lies in the observation that HIV-1 remains quiescent in latent reservoirs. Latently infected resting CD4+ cells (either naive or long lived memory cells) carry transcriptionally silent HIV-1 and represent the predominant reservoir of HIV-1 infection. Other cells may also act as reservoirs (Reviewed in Alexaki et al., 2008, Curr. HIV Res. 6:388-400) such as macrophages, dendritic cells and astrocytes (where HIV-1 infection occurs via a CD4-independent mechanism). It is these latent reservoirs that represent the major challenge to eradication of HIV-1 infection. Approaches towards eradication include attempts to purge reservoirs by selective activation of latently infected cells (such as memory cells) in the presence of ART such that released virus may not infect and replicate in neighbouring cells (Richman et al., 2009, Science 323:1304-1307). Agents include histone deacetylase inhibitors, cytokines, such as IL-2 and IL-7, as well as bryostatin, the protein kinase C activator (Kovochich et al., 2011, PLoS ONE 6 (4):e18270). Therapeutic vaccines have the advantage of being able to penetrate sanctuary sites less well accessed by ART such as lymphoid tissue (Pantaleo et al., 1991, Proc. Natl. Acad. Sci. USA 88:9838-42; Fox et al., 1991, J. Infect. Dis. 164:1051-57) and the central nervous system (Alexaki et al., 2008, Curr. HIV Res. 6:388-400), that represent regions for viral persistence. This relates to therapeutic interventions targeting both the virus itself as well as HIV-associated immune activation.

[0004] A number of studies have been conducted with the aim of providing compounds that can safely and effectively be used to treat diseases associated with abnormal production of TNF- α . See, e.g., Marriott, J. B., et al, Expert Opin. Biol. Ther. (4): 1-8 (2001); G. W. Muller, et al, Journal of Medicinal Chemistry, 39(17): 3238-3240 (1996); and G. W. Muller, et al, Bioorganic & Medicinal Chemistry Letters, 8: 2669-2674 (1998). Some studies have focused on a group of compounds selected for their capacity to potentially inhibit TNF- α production by LPS stimulated PBMC. L. G. Corral, et al, Ann. Rheum. Dis., 58 (suppl 1): 1107-1113 (1999). These compounds, often referred to as immunomodulatory compounds, show not only potent inhibition of TNF- α , but also marked

inhibition of LPS induced monocyte IL1B and IL12 production. LPS induced IL6 is also inhibited by immunomodulatory compounds, albeit partially. These compounds are potent stimulators of LPS induced IL10. Particular examples include, but are not limited to, the substituted 2-(2,6-dioxopiperidin-3-yl)phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles as described in U.S. Pat. No. 6,281,230 and U.S. Pat. No. 6,316,471. Monocyte/macrophage function is part of the Innate Immune System that serves as a first line of defense against an infection. By modulating the host's monocytes and macrophages, immunomodulatory compounds can change the dynamics of the response to a viral infection, such as influenza.

[0005] Histone deacetylases (HDAC) are a class of enzymes that remove acetyl groups from N-acetylated lysines amino acid on histone proteins. Currently 18 HDACs have been identified in mammals. They have been divided into four classes based on cellular localization, function, and sequence similarity. Class I includes HDACs 1, 2, 3, and 8 which are found primarily in the nucleus. Class II HDACs (HDACs 4, 5, 6, 7, 9, and 10) are found primarily in the cytoplasm but may be able to shuttle between the nucleus and the cytoplasm; class IIa comprises four HDACs (HDACs 4, 5, 7 and 9) while class IIb comprises two HDACs (HDACs 6 and 10) which are expressed only in the cytoplasm. HDAC11, which is ubiquitously expressed, shares sequence similarities with both class I and class II HDACs and represents Class IV. Class III (also called "sirtuin family") groups NAD⁺-dependent proteins which do not act primarily on histones.

[0006] Previous research shows that antibodies to the carboxyterminal C5 domain of HIV-1 gp120 has been associated with lower immune activation and slower disease progression (Loomis-Price et al. 1998 J. Inf Dis. 178:1306-1316; Warren R Q wt al. 1991 J. Clin Immunol 11: 13-21; Lifson et al. 1991 J. Inf. Dis 163:959-965). Indeed, disease progression was shown to accelerate if humoral (i.e. antibody) responses to this domain were lost (Wong et al. 1993 J. Inf. Dis 168: 1523-1527). Furthermore long term nonprogressors (LTNP) which represent 5% of HIV-infected individuals have sustained humoral responses to the carboxyterminal C5 domain of HIV-1 gp120 and can live in the absence of antiretroviral therapy for many years despite having some degree of viral load (Lieglert et al. 1998 J. Infect. Dis. 178: 669-79; Gougeon et al. 1996 J. Immunol 156:3509-20; Muro-Cacho et al. 1995 J. Immunol 154:5555-66; Easterbrook et al. 1999 J. Infect 28:71-73).

[0007] The C5 domain of gp120 is 13 amino acids long (amino acid residues 499-511 of gp120, and has the reference sequence B.FR 83.HXB2-TKAKRRVQREKR).

OBJECT OF THE INVENTION

[0008] It is an object of embodiments of the invention to provide effective combinations of therapeutic agents and methods which can be used in the prevention and/or treatment of retroviral infections, including HIV infection and AIDS.

[0009] It is an object of embodiments of the invention to enhance the effect of a therapeutic HIV vaccine by the use of immunomodulatory compounds and/or reservoir purging agents, such as histone deacetylase (HDAC) inhibitors in the prevention and/or treatment and/or eradication of HIV infection and AIDS.

SUMMARY OF THE INVENTION

[0010] It has been found by the present inventor(s) that it is advantageous to combine the effect of therapeutic vaccines

including compounds that stimulate a humoral and/or a cell-mediated immune response in a subject with immunomodulatory compounds and/or reservoir purging agents.

[0011] Thus in a first aspect the present invention relates to a method for treating a human retrovirus infection, such as human immunodeficiency virus I (HIV) infection in a human infected with such virus, the method comprising administering separately, sequentially in any order or simultaneously (1) a compound to stimulate the humoral immunity in a subject and/or one or more peptides that elicit a cell-mediated immune response in a subject; and (2) an immunomodulatory compound and/or a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor.

[0012] In a second aspect the present invention relates to a composition comprising

[0013] a) a compound to stimulate the humoral immunity in a subject and/or one or more peptides that elicit a cell-mediated immune response in a subject; and

[0014] b) an immunomodulatory compound and/or a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor.

[0015] In a further aspect the present invention relates to a method for reducing and/or delaying the risk of developing or the pathological effects of human retrovirus infection, such as immunodeficiency virus I (HIV) in a human infected with such virus, such as HIV, the method comprising administering sequentially in any order or simultaneously (1) a compound to stimulate the humoral immunity in a subject and/or one or more peptide that elicit a cell-mediated immune response in a subject; in combination with (2) an immunomodulatory compound and/or a reservoir purging agent, such as a Histone deacetylase (HDAC) inhibitor.

DETAILED DISCLOSURE OF THE INVENTION

Definitions

[0016] When terms such as “one”, “a” or “an” are used in this disclosure they mean “at least one”, or “one or more” unless otherwise indicated. Further, the term “comprising” is intended to mean “including” and thus allows for the presence of other constituents, features, conditions, or steps than those explicitly recited.

[0017] Human retroviruses that may be treated by the combinations according to the invention includes:

HTLV-I (originally called human T-cell leukaemia virus—now called human T-lymphotropic virus);

HTLV-II (human T-cell leukaemia virus also now called human T-lymphotropic virus);

HIV-1 (earlier also called LAV—lymphadenopathy virus, also called Human T-lymphotropic virus III i.e. HTLV-III); HIV-2 (has also been called HTLV-IV); as well as any subtype of any of the above viruses; and

[0018] Unless specified as otherwise, “HIV” generally denotes human immunodeficiency virus 1.

[0019] “HIV disease” is composed of several stages including the acute HIV infection which often manifests itself as a flu-like symptoms and the early and medium stage symptomatic disease, which has several non-characteristic symptoms such as skin rashes, fatigue, night sweats, slight weight loss, mouth ulcers, and fungal skin and nail infections. Most HIV infected will experience mild symptoms such as these before developing more serious illnesses. It is generally believed that it takes five to seven years for the first mild symptoms to appear. As HIV disease progresses, some individuals may

become quite ill even if they have not yet been diagnosed with AIDS (see below), the late stage of HIV disease. Typical problems include chronic oral or vaginal thrush (a fungal rash or spots), recurrent herpes blisters on the mouth (cold sores) or genitals, ongoing fevers, persistent diarrhea, and significant weight loss. “AIDS” is the late stage HIV disease and is a condition which progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumors.

[0020] When using the term “gp120” herein is meant the ≈ 120 kDa N-terminal glycoprotein enzymatic cleavage product of gp160, which in turn is the sole expression product of the HIV env gene. gp120 forms the “spikes” on infective HIV virions and is non-covalently bound to gp41.

[0021] “gp41” denotes the ≈ 41 kDa glycoprotein C-terminal enzymatic cleavage product of gp160. gp41 is located in the membrane of HIV infected cells or virions. gp41 has an N-terminal transmembrane domain which binds non-covalently to gp120. This transmembrane domain is termed “the transmembrane domain of gp41” or “tm-gp41” herein. The term includes within its scope naturally occurring mutated versions of the sequence as e.g. those set forth in Formula III.

[0022] “C5” or the “C5 domain” denotes the 13 C-terminal amino acid residues of gp120.

[0023] “C2” or the “C2 domain” denotes a conserved region in gp120. Regions in C2 form an antiparallel β -sheet with C5 in the inner proximal domain of gp120.

[0024] “Reducing and/or delaying pathological effect of HIV” is in the present context meant to denote that use of the methods of the invention provides for a statistically significant reduction and/or delay in morbidity seen in individual infected with HIV which are treated according to the present invention. That is, the time of onset of manifest disease symptoms characterizing AIDS is later compared to non-treated controls and/or the number of pathological manifestations is reduced compared to controls not receiving the treatment of the present invention.

[0025] The expression “association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120” means that C5 can interact non-covalently with both or one of the tm-gp41 and C2. The interaction with tm-gp41 is intermolecular, whereas the interaction with C2 is intramolecular.

[0026] An “agent capable of stabilising” association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120 is a composition of matter which prevents or statistically reduces release of C5 from its intermolecular binding to gp41 and/or from its intramolecular binding to C2. Generally, such an agent is any substance of matter capable of exerting this effect, but important examples are antibodies, antibody fragments, and antibody analogues. However, also other molecules having proper binding affinity for a complex between C5 on the one hand and tm-gp41 and/or C2 on the other, is an agent according the present invention—the precise molecular format is less important than the binding characteristics, and it is according to the invention also possible that such an agent may be a receptor or a receptor analogue, but also small molecule stabilisers are capable of functioning as an agent of the present invention.

[0027] The term “antibody” herein is used in the broadest sense and specifically includes full-length monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as

they exhibit the desired biological activity, i.e. to function as an agent described above. Various techniques relevant to the production of antibodies are provided in, e.g., Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988).

[0028] An “antibody fragment or antibody analogue” comprises a portion of a full-length antibody, preferably antigen-binding or variable regions thereof. Examples of antibody fragments/analogs include Fab, Fab', F(ab)₂, F(ab')₂, F(ab)₃, Fv (typically the VL and VH domains of a single arm of an antibody), single-chain Fv (scFv), dsFv, Fd fragments (typically the VH and CH1 domain), and dAb (typically a VH domain) fragments; VH, VL, VhH, and V-NAR domains; minibodies, diabodies, triabodies, tetrabodies, and kappa bodies (see, e.g., Ill et al., *Protein Eng* 1997; 10: 949-57); camel IgG; IgNAR; and multispecific antibody fragments formed from antibody fragments, and one or more isolated CDRs or a functional paratope, where isolated CDRs or antigen-binding residues or polypeptides can be associated or linked together so as to form a functional antibody fragment. Various types of antibody fragments have been described or reviewed in, e.g., Holliger and Hudson, *Nat Biotechnol* 2005; 23, 1126-1136; WO2005/040219, and published U.S. Patent Applications 20050238646 and 20020161201.

[0029] The term “antibody derivative”, as used herein, comprises a full-length antibody or a fragment of an antibody, preferably comprising at least antigen-binding or variable regions thereof, wherein one or more of the amino acids are chemically modified, e.g., by alkylation, PEGylation, acylation, ester formation or amide formation or the like, e.g., for linking the antibody to a second molecule. This includes, but is not limited to, PEGylated antibodies, cysteine-PEGylated antibodies, and variants thereof.

[0030] A “conjugate” as used herein comprises an agent to be used according to the invention such as an antibody derivative associated with or linked to a second agent, such as a cytotoxic agent, a detectable agent, etc. A conjugate may be constituted of covalently linked peptides (an example of a conjugate is a fusion peptide comprising two peptides linked via peptide bonds so that the conjugate in that case may be an expression product from a nucleic acid fragment), but a conjugate can also be a combination of peptides covalently linked via chemical conjugation (a traditional example is conjugation using glutaraldehyde). Another example of a more complex conjugation is the example where an agent or peptide multimer or other chemical substance of the present invention is linked to a carrier molecule, which in turn is coupled to other agents, peptide multimers or other chemical substances of the present invention (e.g. when such chemical substances are bound to a poly-lysine carrier (a lysine “tree”).

[0031] A “humanized” antibody is a human/non-human chimeric antibody that contains a minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit, or non-human primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In

general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR residues are those of a human immunoglobulin sequence. The humanized antibody can optionally also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, e.g., Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992), WO 92/02190, US Patent Application 20060073137, and U.S. Pat. Nos. 6,750,325, 6,632,927, 6,639,055, 6,548,640, 6,407,213, 6,180,370, 6,054,297, 5,929,212, 5,895,205, 5,886,152, 5,877,293, 5,869,619, 5,821,337, 5,821,123, 5,770,196, 5,777,085, 5,766,886, 5,714,350, 5,693,762, 5,693,761, 5,530,101, 5,585,089, and 5,225,539.

[0032] An antibody having a “biological characteristic” of a reference antibody, is one that possesses one or more of the biological characteristics of that antibody that distinguish it from other antibodies that bind to the same antigen.

[0033] The term “peptide” is in the present context intended to mean both short peptides of from 2 to 10 amino acid residues, oligopeptides of from 11 to 100 amino acid residues, and polypeptides of more than 100 amino acid residues. When referring to amino acids in peptides, it is intended that the amino acids are L-amino acids, unless other information is provided. Amino acids are referred to by their standard three letter or one letter designations unless otherwise stated. Some unusual amino acids referred to herein includes homoarginine usually abbreviated by Har, norleucine usually abbreviated as Nle or Nl, N-ε-methylated Lys usually abbreviated Lys(Me), Citrulline usually abbreviated Cit or with the single letter “B”, diaminopropionic acid usually abbreviated with Dpr and serinyl diaminopropionic acid usually abbreviated Dpr(Ser).

[0034] A “protein” is intended to denote a functional biomolecule comprising at least one peptide; when comprising at least two peptides, these may form complexes, be covalently linked, or may be non-covalently linked. The polypeptide(s) in a protein can be glycosylated and/or lipidated and/or comprise prosthetic groups.

[0035] A “peptide multimer” denotes a molecule which is constituted by at least two peptides in a non-natural configuration relative to each other. Examples are peptides from the same or from different proteins which are covalently linked via the side chains of at least one of their amino acids, or which are linked via their termini (e.g. via peptide bonds) but in a configuration which does not appear in nature. Typical examples of peptide multimers are detailed below.

[0036] A “variant” or “analogue” of a peptide refers to a peptide having an amino acid sequence that is substantially identical to a reference peptide, typically a native or “parent” polypeptide. The peptide variant may possess one or more amino acid substitutions, deletions, and/or insertions at certain positions within the native amino acid sequence.

[0037] “Conservative” amino acid substitutions are those in which an amino acid residue is replaced with an amino acid residue having a side chain with similar physicochemical properties. Families of amino acid residues having similar side chains are known in the art, and include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine,

threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). A particular form of conservative amino acid substitutions include those with amino acids, which are not among the normal 20 amino acids encoded by the genetic code. Since preferred embodiments of the present invention entail use of synthetic peptides, it is unproblematic to provide such “non-naturally occurring” amino acid residues in the peptides disclosed herein, and thereby it is possible to exchange the natural saturated carbon chains in the side chains of amino acid residues with shorter or longer saturated carbon chains—for instance, lysine may be substituted with an amino acid having an the side chain $-(CH_2)_nNH_3$, where n is different from 4, and arginine may be substituted with an amino acid having the side chain $-(CH_2)_nNHC(=NH_2)NH_2$, where n is different from 3, etc. Similarly, the acidic amino acids aspartic acid and glutamic acid may be substituted with amino acid residues having the side chains $-(CH_2)_nCOOH$, where $n \geq 2$.

[0038] A “retro form” of a peptide is a form of a peptide where the order of the amino acids in N- to C-terminal direction has been inverted. For instance, the retro form of ALDFR is the peptide RFDLA.

[0039] An “inverso” form is characterized by the fact that each amino acid in the inverso form is in the opposite stereochemical configurational compared to the corresponding amino acid in the peptide. So, if the peptide is composed of L-amino acids, the inverso form is composed of D-amino acids.

[0040] A “retro-inverso” form of a peptide is a form of a peptide which is both an inverso form and a retro form. The retro-inverso form of L-ala-L-Arg-L-Lys is D-Lys-D-Arg-D-ala.

[0041] The term “substantially identical” in the context of two amino acid sequences means that the sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 95, at least about 98, or at least about 99 percent sequence identity. In one embodiment, residue positions that are not identical differ by conservative amino acid substitutions. Sequence identity is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, the publicly available GCG software contains programs such as “Gap” and “BestFit” which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild-type protein and a mutein thereof. See, e.g., GCG Version 6.1. Polypeptide sequences can also be compared using FASTA or ClustalW, applying default or recommended parameters. A program in GCG Version 6.1, FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 1990; 183:63-98; Pearson, *Methods Mol. Biol.* 2000; 132: 185-219). Another preferred algorithm when comparing a sequence to a database containing a large number of sequences from various organisms, or when deducing the is

the computer program BLAST, especially blastp, using default parameters. See, e.g., Altschul et al., 3. *Mol. Biol.* 1990; 215:403-410; Altschul et al., *Nucleic Acids Res.* 1997; 25:3389-402 (1997); each herein incorporated by reference. “Corresponding” amino acid positions in two substantially identical amino acid sequences are those aligned by any of the protein analysis software mentioned herein, typically using default parameters.

[0042] The term “subsequence” in general means any consecutive stretch of at least 3 amino acids or, when relevant, of at least 3 nucleotides, derived directly from a naturally occurring amino acid sequence or nucleic acid sequence, respectively. However, when discussing peptide multimers of the present invention, the subsequence may be as short as 1 or 2 amino acids. This is because the inventive peptide multimers include amino acids from different peptide domains, where the amino acids together at least form a conformational epitope for an antibody. Hence, such a conformational epitope could be composed of 4 amino acids from C5, but only 1 or 2 from tm-gp41—the important point is here that this combined epitope from 2 domains is capable of being stabilised, i.e. that antibody binding to the same epitope in vivo will stabilise the configuration between C5 and tm-gp41 and/or C2.

[0043] A nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome-binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0044] An “isolated” molecule is a molecule that is the predominant species in the composition wherein it is found with respect to the class of molecules to which it belongs (i.e., it makes up at least about 50% of the type of molecule in the composition and typically will make up at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more of the species of molecule, e.g., peptide, in the composition). Commonly, a composition of an antibody molecule will exhibit 98%-99% homogeneity for antibody molecules in the context of all present peptide species in the composition or at least with respect to substantially active peptide species in the context of proposed use.

[0045] In the context of the present invention, “treatment” or “treating” refers to preventing, alleviating, managing, curing or reducing one or more symptoms or clinically relevant manifestations of a disease or disorder, unless contradicted by context. For example, “treatment” of a patient in whom no symptoms or clinically relevant manifestations of a disease or disorder have been identified is preventive or prophylactic therapy, whereas “treatment” of a patient in whom symptoms or clinically relevant manifestations of a disease or disorder have been identified generally does not constitute preventive or prophylactic therapy.

[0046] The term antigen denotes a substance of matter which is recognized by the immune system's specifically recognizing components (antibodies, T-cells).

[0047] The term "immunogen" is in the present context intended to denote a substance of matter, which is capable of inducing an adaptive immune response in an individual, where said adaptive immune response targets the immunogen. In relation to the present invention, an immunogen will induce antibodies that react with the immunogen. In other words, an immunogen is an antigen, which is capable of inducing immunity.

[0048] The terms "epitope", "antigenic determinant" and "antigenic site" are used interchangeably herein and denotes the region in an antigen or immunogen which is recognized by antibodies (in the case of antibody binding epitopes, also known as "B-cell epitopes") or by T-cell receptors when the epitope is complexed to an MHC molecule (in the case of T-cell receptor binding epitopes, i.e. "T-cell epitopes").

[0049] The term "immunogenically effective amount" has its usual meaning in the art, i.e. an amount of an immunogen, which is capable of inducing an immune response, which significantly engages pathogenic agents, which share immunological features with the immunogen.

[0050] The term "vaccine" is used for a composition comprising an immunogen and which is capable of inducing an immune response which is either capable of reducing the risk of developing a pathological condition or capable of inducing a therapeutically effective immune response which may aid in the cure of (or at least alleviate the symptoms of) a pathological condition.

[0051] The term "pharmaceutically acceptable" has its usual meaning in the art, i.e. it is used for a substance that can be accepted as part of a medicament for human use when treating the disease in question and thus the term effectively excludes the use of highly toxic substances that would worsen rather than improve the treated subject's condition.

[0052] A "T helper lymphocyte epitope" (a T_H epitope) is peptide, which binds an MHC Class II molecule and can be presented on the surface of an antigen presenting cell (APC) bound to the MHC Class II molecule. An "immunological carrier" is generally a substance of matter which includes one or many T_H epitopes, and which increase the immune response against an antigen to which it is coupled by ensuring that T-helper lymphocytes are activated and proliferate. Examples of known immunological carriers are the tetanus and diphtheria toxoids and keyhole limpet hemocyanin (KLH).

[0053] The term "adjuvant" has its usual meaning in the art of vaccine technology, i.e. a substance or a composition of matter which is 1) not in itself capable of mounting a specific immune response against the immunogen of the vaccine, but which is 2) nevertheless capable of enhancing the immune response against the immunogen. Or, in other words, vaccination with the adjuvant alone does not provide an immune response against the immunogen, vaccination with the immunogen may or may not give rise to an immune response against the immunogen, but the combined vaccination with immunogen and adjuvant induces an immune response against the immunogen which is stronger than that induced by the immunogen alone.

Peptides that Elicit Cell-Mediated Immunity (CMI)

[0054] Vaccination aims to stimulate the immune response to a specific pathogen in advance of infection. When an individual is exposed to that pathogen, a memory response is

triggered which prevents the establishment of infection. Vaccines therefore stimulate the adaptive immune response which unlike innate immunity, is long lived and has memory. There are two major arms to the adaptive immune system. Humoral immunity which involves the development of antibodies that can bind virus particles and certain antibodies that can neutralize infection. Cell mediated immunity that leads to the development of cytotoxic T-cells that kill infected cells exposing viral epitopes in the context of human leukocyte antigen (HLA) class I, in this way eliminating infected cells.

[0055] As used herein a peptide that elicits a cell-mediated immune response refers to any peptide that elicits an activation of antigen-specific cytotoxic T-lymphocytes. These peptides elicit a Cytotoxic T-lymphocyte immune (CTL) response that leads to the development of cytotoxic T-cells that kill infected cells exposing viral epitopes in the context of human leukocyte antigen (HLA) class I, in this way eliminating infected cells. Cell-mediated immunity (CMI) may also involve the activation of phagocytes, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. These peptides may be a helper T lymphocyte (HTL) inducing peptide comprising HTL epitopes. A "HTL inducing peptide" is a HLA Class II binding peptide that is capable of inducing a HTL response. Also the peptides may in other embodiments be CTL inducing peptides comprising CTL epitopes in addition to or as an alternative to being a HTL inducing peptide. A "CTL inducing peptide" is a HLA Class I binding peptide that is capable of inducing a CTL response.

[0056] A peptide that elicit a cell-mediated immune response as used according to the present invention includes but is not limited to any peptide described in any one of international patent applications WO0052040, WO 2012/092934 or WO 2012/072088, which patent applications are hereby incorporated by reference.

HIV-Specific Peptides

[0057] In some aspects, the methods and compositions according to the present invention utilise or comprise one or more peptides that elicits a cell-mediated immune response. In some embodiments, this peptide is at least one HIV-specific peptide.

[0058] One aspect of the peptides that elicits a cell-mediated immune response relates to HIV-specific peptides based on conserved regions of HIV gag p24, antigens in free or carrier-bound form comprising at least one of the said peptides.

[0059] The HIV-specific peptides to be used according to the invention may originate from conserved areas of the HIV-1 core protein p24, having the properties of maintaining the uniqueness (sensitivity and specificity) of the HIV-1-epitope. Further the new peptides to be used according to the invention possess no recognized cytotoxic T lymphocyte (CTL) antagonistic effect and shall have at least one potential CTL epitope.

[0060] In some embodiments, the HIV-specific peptides, to be used according to the present invention, and which have met the above criteria are selected from the following groups;

(SEQ ID NO: 47)

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Ala Xaa₈ Xaa₉ Gln Thr

Pro Trp Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈ Val Xaa₂₀;

wherein

Xaa in position 1 is Lys or Arg,
 Xaa in position 2 is Ala, Gly, Ser or Arg,
 Xaa in position 3 is Leu or Met,
 Xaa in position 4 is Gly or Arg,
 Xaa in position 5 is Pro, Thr, Val, Ser, Gln or Ala,
 Xaa in position 6 is Gly, Ala, Lys, Arg, Gln or Glu,
 Xaa in position 8 is Thr or Ser,
 Xaa in position 9 is Leu or Ile,
 Xaa in position 14 is Thr, Ser or Val,
 Xaa in position 15 is Ala or Ser,
 Xaa in position 16 is Cys or Ser,
 Xaa in position 17 is Gln or Leu,
 Xaa in position 18 is Gly, Glu or Arg, and
 Xaa in position 20 is Gly or Arg;

(SEQ ID NO: 50)

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Gly Leu Asn Pro Leu Val
 [Gly]_n Xaa₁₂ Xaa₁₃ Tyr Xaa₁₅ Pro Xaa₁₇ Xaa₁₈ Ile Leu
 Xaa₂₁ Xaa₂₂;

wherein

Xaa in position 1 is Arg, Lys, Asp or none,
 Xaa in position 2 is Trp, Gly, Lys or Arg,
 Xaa in position 3 is Ile, Leu, Val or Met,
 Xaa in position 4 is Ile, Val or Leu,
 Xaa in position 5 Leu, Met, Val or Pro,
 Xaa in position 12 is Arg or Lys,
 Xaa in position 13 is Met or Leu,
 Xaa in position 15 is Ser, Cys or Gln,
 Xaa in position 17 is Thr, Val, Ile, Ser or Ala,
 Xaa in position 18 is Ser, Gly or Thr,
 Xaa in position 21 is Asp, Glu, Cys or Gly,
 Xaa in position 22 is Gly or none, and
 n=0, 1, 2 or 3;

(SEQ ID NO: 55)

Xaa₁ Xaa₂ Xaa₃ Pro Ile Pro Xaa₇ Xaa₈ Xaa₉ Xaa₁₀
 Xaa₁₁ Xaa₁₂ [Gly]_n Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇
 Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄;

wherein

Xaa in position 1 is Asn, Ser, Gly, His, Ala, Pro, Arg or none,
 Xaa in position 2 is Asn, Ala or Lys,
 Xaa in position 3 is Pro, Gln, Gly, Ile or Leu,
 Xaa in position 7 is Val or Ala,
 Xaa in position 8 is Gly or Lys,
 Xaa in position 9 is Glu, Asp, Lys, Phe or Thr,
 Xaa in position 10 is Ile, Met, Val or Leu,
 Xaa in position 11 is Tyr, Leu or none,
 Xaa in position 12 is Ser or none,
 Xaa in position 13 is Arg or none,
 Xaa in position 14 is Asp, Arg, Trp, Ala or none,
 Xaa in position 15 is Ile or none,
 Xaa in position 16 is Tyr or none,
 Xaa in position 17 is Lys or Arg,
 Xaa in position 18 is Arg, Lys or Asp,
 Xaa in position 19 is Trp or Gly,
 Xaa in position 20 is Ile, Met, Val, Gln or Ala,
 Xaa in position 21 is Ile, Val or Ala,
 Xaa in position 22 is Leu, Met or Val,

Xaa in position 23 is Gly or Cys,
 Xaa in position 24 is Leu or none,
 n=1, 2 or 3, and

(SEQ ID NO: 61)

Xaa₁ Xaa₂ Ile Ile Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Leu
 Xaa₁₁ [Gly]_n [Arg]_m Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆
 Xaa₁₇ Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅;

wherein the Xaa in position 1 is Pro, Lys, Arg or none,

Xaa in position 2 is Glu, Arg, Phe or Lys,
 Xaa in position 5 is Pro or Thr,
 Xaa in position 6 is Met, Thr or Nleu,
 Xaa in position 7 is Phe or Leu,
 Xaa in position 8 is Ser, Thr, Ala or Met,
 Xaa in position 9 is Ala, Glu or Leu,
 Xaa in position 11 is Ser or none,
 Xaa in position 12 is Ala, Arg or none,
 Xaa in position 13 is Ile, Leu or none,
 Xaa in position 14 is Ser, Ala, Leu or none,
 Xaa in position 15 is Tyr, Glu or Asp,
 Xaa in position 16 is Gly or Asp,
 Xaa in position 17 is Ala or Leu,
 Xaa in position 18 is Thr, Ile, Val, Leu or Asn,
 Xaa in position 19 is Pro, Thr or Ser,
 Xaa in position 20 is Tyr, Phe, Nleu, His or Gln,
 Xaa in position 21 is Asp, Asn, Leu or Ala,
 Xaa in position 22 is Leu, Ile, Val or Asn,
 Xaa in position 23 is Asn, Tyr, Cys or Gly,
 Xaa in position 24 is Thr, Met, Ile, Ala, Val or none,
 Xaa in position 25 is Gly or none,
 n=1, 2 or 3 and m=0, 1, 2 or 3 independent of each other,
 wherein the terminal ends of each HIV specific peptide may
 be free carboxyl- or amino-groups, amides, acyls or acetyl
 or salts of any of the HIV specific peptides.

[0061] The HIV-specific peptide sequences have the potential to serve as a particularly good antigen wherein the antigen comprises at least one peptide selected from the group of sequences of SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:55 or SEQ ID NO:61. The antigenicity may be adapted through adjusting the ratio or concentration of different peptides or size of the peptides by for instance dimerisation or polymerisation and/or immobilisation to a solid phase. The antigen comprises two or more polypeptide sequences, to be used according to the invention, which are either linked by a bridge for instance a disulphide bridge between the Cys residues of the chains or bridges like C₁-C₈alkylene possibly intervened by one or more heteroatoms like O, S, or N or preferably they are unlinked. The chains may be immobilized to a solid phase in monomeric, dimeric or oligomeric forms. Further amino acids may be added to the ends in order to achieve an <<arm>> to facilitate immobilization.

[0062] All amino acids in the HIV-specific peptides to be used according to the invention can be in both D- or L-form, although the naturally occurring L-form is preferred.

[0063] The C- and N-terminal ends of the HIV-specific peptide sequences could deviate from the natural sequences by modification of the terminal NH₂-group and/or COOH-group, they may for instance be acylated, acetylated, amidated or modified to provide a binding site for a carrier or another molecule. When the C-terminal end of a peptide is an amide, suitable amides included those having the formula —C(O)—NR^xR^y, wherein R^x and R^y are independently

selected from hydrogen and C₁₋₆ alkyl, which alkyl group may be substituted with one of more fluoro atoms, for example —CH₃, —CH₂CH₃ and —CF₃, a particular amide group which may be mentioned is —C(O)NH₂. When the N-terminal end of the peptide is acetylated, suitable acetylated N-terminal ends include those of formula —NH—C(O)R^z, wherein R^z is hydrogen, C₁₋₆ alkyl, which alkyl group may be substituted with one of more fluoro atoms, for example —CH₃, —CH₂CH₃ and —CF₃, or phenyl.

[0064] The HIV-specific peptides to be used according to the invention consist of 6 to 50 amino acids, preferably between 10 and 30 amino acids. They are covering all natural variation of amino acids in the identified positions.

[0065] The polypeptide antigen to be used according to the invention is either in a free or in a carrier-bound form. The carrier or solid phase to which the peptide is optionally bound can be selected from a wide variety of known carriers. It should be selected with regard to the intended use of the immobilized polypeptide as a diagnostic antigen or as an immunizing component in a vaccine.

[0066] Examples of carriers that can be used for e.g. diagnostic purposes are magnetic beads or latex of co-polymers such as styrene-divinyl benzene, hydroxylated styrene-divinyl benzene, polystyrene, carboxylated polystyrene, beads of carbon black, non-activated or polystyrene or polyvinyl chloride activated glass, epoxy-activated porous magnetic glass, gelatine or polysaccharide particles or other protein particles, red blood cells, mono- or polyclonal antibodies or fab fragments of such antibodies.

[0067] In one embodiment of the vaccine for use according to the present invention comprises antigens containing the peptides of the SEQ ID NO:47, 50, 55 and 61, more preferably the peptides occur in the ratio 1:1:1:1 w/w.

[0068] In a further preferred embodiment the vaccine for use according to the invention comprises the following peptides:

(SEQ ID NO: 49)
RALGPAATLQTPWTASLGVG

RWLLGLNPLVGGGRLYSPTSILG (SEQ ID NO: 52)

RAIPIAGTLLSGGGRAYKRTAILG (SEQ ID NO: 57)
and

(SEQ ID NO: 64)
RFIIPNIFTALSGGRRALLYGATPYAIG
(NI in position 6 is Norleucine)

or salts thereof, particularly acetate salts.

[0069] In some embodiments the peptides for use according to the invention are modified at the C-terminus as follows:

(SEQ ID NO: 49)
RALGPAATLQTPWTASLGVG-NH₂

(SEQ ID NO: 52)
RWLLGLNPLVGGGRLYSPTSILG-NH₂

(SEQ ID NO: 57)
RAIPIAGTLLSGGGRAYKRTAILG-NH₂

-continued

and

(SEQ ID NO: 64)
RFIIPNIFTALSGGRRALLYGATPYAIG-NH₂
(NI in position 6 is Norleucine)

or salts thereof, particularly acetate salts.

[0070] One of the sequences contains a B-cell epitope and will activate the humoral immune system, whereas the other sequences contribute with CTL-epitopes and the amino acid changes implemented within the frame of the CTL-epitope are designed to achieve enhanced binding. Other amino acid changes have been conducted in order to facilitate the synthesis of the peptide and/or increase the solubility of the peptide.

Compound to Stimulate the Humoral Immunity in a Subject, Such as C5 Related Compounds:

[0071] The present invention also relates to the use of compounds that stimulate the humoral immunity in a subject. Humoral immunity involves the development of antibodies that can bind virus particles and certain antibodies that can neutralize infection.

[0072] A peptide that stimulate the humoral immunity in a subject as used according to the present invention includes but is not limited to any peptide described in any one of international patent applications WO2011/000962, WO00/52040, WO 2012/092934 or WO 2012/072088, which patent applications are hereby incorporated by reference.

[0073] In some embodiments these compounds are agents capable of stabilising the association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120. In other embodiments these compounds are immunogens, which induces antibodies that stabilise association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120.

[0074] One aspect of the invention relates to methods and compositions for reducing and/or delaying pathological effects of human retrovirus infection, such as immunodeficiency virus I (HIV) in a human infected with such virus, such as HIV, the method including administering an effective amount of an agent capable stabilising association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120.

[0075] Another aspect is much similar, but relates to compositions and methods of reducing the risk of developing acquired immunodeficiency syndrome (AIDS), the method including administering an effective amount of an agent capable of stabilising association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120.

[0076] These aspects primarily aim at treating retrovirus infections, such as HIV infected individuals with agents, which can mimic the antibodies which according to the present invention are characteristic for HIV infected long-term non-progressors—this is the most straightforward therapeutic utilisation of the findings underlying the present invention. Where the one aspect aims at reducing pathological effects of retrovirus infections, such as HIV or prolonging the time it takes to develop manifest AIDS, the other aspect aims at reducing the risk of developing AIDS altogether and may therefore be used in individuals which are currently treated prophylactically with antiretroviral therapy.

[0077] In one embodiment, the agent in these first aspects of the invention is a molecule comprising at least one amino acid sequence selected independently from an amino acid

sequence derived from the transmembrane domain of gp41 and an amino acid sequence derived from the C2 domain, wherein the at least one amino acid sequence binds the C5 domain and optionally comprises at least one D-amino acid; in certain embodiments all the amino acids in the amino acid sequence are D-amino acids. The molecule is preferably a peptide, and in certain embodiments this peptide consists of the at least one amino acid sequence. The amino acid sequences typically include at most 10 amino acid residues, such as at most 9, at most 8, at most 7, at most 6, and at most 5 amino acid residues. Preferred molecules are therefore peptides having 4, 5, 6, 7, 8, 9, or 10 amino acid residues. Specific embodiments of the at least one molecule are therefore the peptides having or comprising SEQ ID NO:34, 35, 36, 37, 39, 40, 42, 43 and 45, which may all be composed partly or entirely of D-amino acids. Also molecules comprising peptides having Formula III are interesting embodiments of the at least one molecule.

[0078] In one embodiment, the agent in these first aspects of the invention is selected from an antibody, an antibody fragment or an antibody analogue. The antibody may be a fully human antibody, a humanized antibody, or a chimeric antibody, or a derivative thereof. Typically, the antibody is an IgA, an IgD, an IgG, an IgE or an IgM—the antibody may be both monoclonal and polyclonal. The antibody fragment is typically selected from a Fab fragment, a Fab' fragment, a Fab'-SH fragment, a F(ab)₂ fragment, a F(ab')₂ fragment, an Fv fragment, a Heavy chain Ig (a llama or camel Ig), a V_{HH} fragment, a single domain Fv, and a single-chain antibody fragment, and the antibody analogue is typically selected from a scFv, a dsFv, a minibody, a diabody, a triabody, a kappa body, an IgNAR, a tandAb, a BiTE, and a multispecific antibody.

[0079] In one embodiment of these first aspects of the invention, the agent binds to and stabilises association between one or more amino acid residues in the amino acid stretch TZ¹AKRRVVZ²REKR, where Z¹ is K, R or E and where Z² is Q or E, and one or more amino acid residues in an amino acid stretch in the transmembrane domain of gp41 and/or in the constant C2 domain of gp120. This amino acid stretch from C5 is highly conserved across the multiple HIV clades known and effective interaction with this stretch by the agent is therefore believed to be highly advantageous.

[0080] A further aspect of the invention relates to a method for reducing the risk of or reducing and/or delaying pathological effects of human immunodeficiency virus I (HIV) in a human infected with HIV, the method including administering an effective amount of an immunogen, which induces antibodies that stabilise association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120, whereas other aspects relates to a prophylactic method using the same means. In other words, one aspect relates to therapeutic active immunotherapy, whereas another aspect relates to prophylactic immunotherapy of HIV disease, including AIDS. This also entails prophylaxis of HIV infection.

[0081] These particular aspects are based on the realisation that it is feasible to induce the same type of antibody repertoire in the average HIV infected individual as the one that is found in the HIV LTNP individuals. By carefully selecting peptide regions in both C5 and in tm-gp41 and/or C2 in order to prepare peptide multimers that mimic the antibody binding epitopes present in HIV composed of these regions, it becomes possible to prepare vaccines which will induce the

desired immunity—interestingly, this approach does not aim at vaccinating so as to obtain neutralizing antibodies in the classical sense.

[0082] In one embodiment the immunogen is selected from a peptide multimer detailed below when discussing these aspects of the invention, a composition detailed below, a nucleic acid fragment discussed in relation to other aspects, a virus or plasmid vector compositions discussed elsewhere.

[0083] In common for these aspects that they all include embodiments where the targeted association between the C5 domain and C2 and/or the transmembrane domain of gp41 involves at least one amino acid in the sequence TZ¹AKRRVVZ²REKR, where Z¹ is K, R or E and where Z² is Q or E and an amino acid and involves at least one amino acid in the transmembrane domain of gp41 or at least one amino acid in the constant C2 domain of gp120. As explained above, this particular sequence is extremely well-conserved across known HIV clades, and therefore it is the interaction between this sequence and tm-gp41 or C2 it is most feasible to target.

[0084] The immunogens to be used according to the invention may comprise (1) a peptide multimer, said multimer comprising

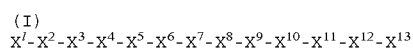
[0085] a first peptide comprising the amino acid sequence of the 13 amino acid residue amino acid sequence of the C5 domain of HIV gp120 including between 0 and 4 amino acid substitutions, a subsequence thereof, or an amino acid sequence comprising the inverso-, retro- or retro-inverso form of said amino acid sequence or subsequence, and

[0086] at least one second peptide comprising an amino acid stretch present in the transmembrane domain of gp41 or present in the constant C2 domain of gp120 or comprising an amino acid stretch present in any one of SEQ ID NOs. 6-13 or comprising an inverso-, retro- or retro-inverso form of an amino acid stretch present in the transmembrane domain of gp41 or present in the constant C2 domain of gp120,

wherein said peptide multimer is capable of inducing an antibody which can bind and stabilise the association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120, and wherein said peptide multimer lacks amino acids N-terminal of C5 in gp120.

[0087] In other words, this aspect relates to peptide multimers which have a resemblance in 3 dimensions with the epitopes which characterise the interacting areas in C5 on the one hand and tm-gp41 and/or C2 on the other. The peptide multimers to be used according to the invention are useful immunogens that can induce antibodies having the same characteristics as the antibodies found in HIV LTNP individuals, but the peptide multimers also are promising as diagnostic/prognostic tools. The inclusion of retro-, inverso-, and retro-inverso peptides i.a. enables production of proteolytically stable peptides as well as peptides are truly foreign compared to the HIV counterpart.

[0088] In one embodiment of the peptide multimer, said first peptide comprises the amino acid sequence having formula I:



[0089] wherein X^1 is Thr, X^2 is selected from Lys, Arg, Har and Glu, X^3 is selected from Ala and Val, X^4 is selected from Arg, Har, Lys and Cit (citrulline), X^5 is selected from Arg, Har, Lys and Cit, X^6 is selected from Arg, Har, Lys and Cit, X^7 is selected from Val, Leu, Ile and Nle (norleucin), X^8 is selected from Val, Leu Ile and Nle, X^9 is selected from Gln, Glu, Asn and Asp, X^{10} is selected from Arg, Har and Cit, X^{11} is selected from Glu and Asp, X^{12} is Lys, and X^{13} is selected from Arg, Har and Cit,

or comprises a subsequence the amino acid sequence of formula I, or comprising the inverso-, retro- or retro-inverso form of said amino acid sequence or subsequence. The first peptide may further comprise the dipeptide Ala-Pro linked to the N-terminus of the amino acid sequence having formula I and/or the first peptide may further comprise the dipeptide X^{14} - X^{15} linked to the C-terminus of the amino acid sequence having formula I, wherein X^{14} is selected from Ala and Val, and wherein X^{15} is selected from Val, Leu and Nle.

[0090] Particularly interesting peptides derived from C5 are set forth in the preamble to the Examples and constitute embodiments of a first peptide of the peptide multimers to be used according to the invention.

[0091] A number of naturally occurring mutants of gp41 and gp120 has been observed, so when stating that the second peptide comprises an amino acid stretch present in the trans-membrane domain of gp41 or present in the constant C2 domain of gp120, this is intended to denote that the amino acid stretch is present in any such naturally occurring form. So, the at least second peptide, when derived from gp41, is in certain embodiments one which includes the amino acid sequence having the formula:

(III)
 $Z^1-Z^2-Z^3-Z^4-Z^5-Z^6-Z^7-Z^8-Z^9-Z^{10}-Z^{11}-Z^{12}-Z^{13}-Z^{14}-Z^{15}-Z^{16}-Z^{17}$

[0092] wherein Z^1 is Asp, Z^2 is Arg, Z^3 is Pro, Z^4 is Glu or Gly, Z^5 is Gly or Arg, Z^6 is Ile, Z^7 is Glu, Z^8 is Glu, Z^9 is Glu, Z^{10} is Gly, Z^{11} is Gly, Z^{12} is Glu or is absent, Z^{13} is Arg or Gln, Z^{14} is Asp or Gly, Z^{15} is Arg or Lys, Z^{16} is Asp or Gly and Z^{17} is Arg,

or includes a subsequence of formula (III), such as a subsequence having at least 5 amino acid residues (such as at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, and at least 16 amino acid residues). Further, this embodiment of the second peptide may contain amino acid substitutions which result in a sequence identity of at least 80% with a corresponding amino acid sequence found in gp41.

[0093] Particularly interesting peptides derived from C2 and gp41, and gp120 are set forth in the preamble to the Examples and constitute embodiments of a second peptide of the peptide multimers to be used according to the invention.

[0094] In certain embodiments of the peptide multimer, the first peptide and the at least one second peptide are associated via a linker; the linker can be any peptide linker, such as a glycine, a lysine or an arginine linker, a polyhistidinyl tag, Protein G, and Protein A but it is also possible to use a bis-maleimide linker, a disulfide linker, or a polyethylene glycol (PEG) linker. In practice, any linker found useful in peptide chemistry is also useful as a linker according to the present invention. Thus, the invention contemplates the use of "simple" linear peptides which are conjugated or fused to each other, but also peptide multimers where the individual peptides derived from C5 and other regions of gp120 or gp41

are linked via non-peptide linkers e.g. complementary nucleic acids, nucleic acid derivatives or analogues e.g. PNA, LNA. Use of multiple linker types are also within the scope of the present invention, and it is e.g. also a part to be used according to the invention to utilise linear peptides which include intrachain disulphide linkers.

[0095] Particularly interesting peptide multimers to be used according to the invention are set forth in the preamble to the examples.

[0096] In certain embodiments, at least one of the first and at least one second peptides in the peptide multimer comprises an N- or C-terminal modification, such as an amidation, acylation, or acetylation. When the C-terminal end of a peptide is an amide, suitable amides included those having the formula $-C(O)-NR^xR^y$, wherein R^x and R^y are independently selected from hydrogen and C_{1-6} alkyl, which alkyl group may be substituted with one of more fluoro atoms, for example $-CH_3$, $-CH_2CH_3$ and $-CF_3$, a particular amide group which may be mentioned is $-C(O)NH_2$. When the N-terminal end of the peptide is acetylated, suitable acetylated N-terminal ends include those of formula $-NH-C(O)R^z$, wherein R^z is hydrogen, C_{1-6} alkyl, which alkyl group may be substituted with one of more fluoro atoms, for example $-CH_3$, $-CH_2CH_3$ and $-CF_3$, or phenyl.

[0097] Since the peptide multimers are contemplated as vaccine agents, they are in certain embodiments coupled to a carrier molecule, such as an immunogenic carrier. The peptides of the peptide multimers may thus be linked to other molecules either as recombinant fusions (e.g. via CLIP technology) or through chemical linkages in an oriented (e.g. using heterobifunctional cross-linkers) or nonoriented fashion. Linking to carrier molecules such as for example diphtheria toxin, latex beads (convenient in diagnostic and prognostic embodiments), and magnetic beads (also convenient in diagnostic and prognostic embodiments), polylysine constructs etc., are all possible carrier molecules to be used according to the invention.

[0098] The immunogenic carrier is conveniently selected from carrier proteins such as those conventionally used in the art (e.g. diphtheria or tetanus toxoid, KLH etc.), but it is also possible to use shorter peptides (T-helper epitopes) which can induce T-cell immunity in larger proportions of a population. Details about such T-helper epitopes can e.g. be found in WO 00/20027, which is hereby incorporated by reference herein—all immunologic carriers and "promiscuous" (i.e. universal) T-helper epitopes discussed therein are useful as immunogenic carriers in the present invention.

[0099] In certain embodiments, the carrier is a virus like particle, i.e. a particle sharing properties with virions without being infectious. Such virus-like particles may be provided chemically (e.g. Jennings and Bachmann Ann Rev Pharmacol. Toxicol. 2009. 49:303-26 Immunodrugs: Therapeutic VLP-based vaccines for chronic diseases) or using cloning techniques to generate fusion proteins (e.g. Peabody et al. J. Mol. Biol. 2008; 380: 252-63. Immunogenic display of diverse peptides on virus-like particles of RNA phage MS2). Another example is "Remune", an HIV vaccine originally made by Immune Response Corporation, which consists of formalin inactivated HIV that has been irradiated to destroy the viral genome. The company was started by Jonas Salk who used the same technique to generate the killed polio vaccine in widespread use today. However, on fixation of HIV, gp120 fell off leaving only gp41 on the virion surface. This opens for the possibility of directly admixing C5-derived

peptides disclosed herein with Remune particles, because it should still be possible to obtain the binding between C5 and gp41 on a Remune particle.

[0100] Embodiments of the aspect related to peptide multimers also include those wherein the first peptide is selected from the group consisting of SEQ ID NO:1, 2, 3, 4, and 5 or a fragment thereof, or the inverso-, retro- or retro-inverso form of a peptides selected from SEQ ID NO:1, 2, 3, 4, and 5 or a fragment thereof, and wherein the second peptide is selected from the group consisting of SEQ ID NO:6, 7, 8, 9, 10, 11, 12, 13, or 46 or a fragment thereof or the inverso-, retro- or retro-inverso form of a peptides selected from SEQ ID NO:6, 7, 8, 9, 10, 11, 12, 13, or 46 or a fragment thereof. As mentioned above, in such a case the fragment may be very short, as long as the peptide multimer provides for the ability to induce antibodies which will stabilise association between C5 and gp41 and/or C2. A number of interesting peptide multimers of the present invention are listed in the Preamble to the Examples.

[0101] In an embodiment, the peptide multimer to be used according to the invention comprises at most 70 amino acids, such as the most 69, at most 68, at most 67, at most 66, at most 65, at most 64, at most 63, at most 62, at most 61, at most 60, at most 59, at most 58, at most 57, at most 56, at most 55, at most 54, at most 53, at most 52, at most 51, at most 50, at most 49, at most 48, at most 47, at most 46, at most 45, at most 44, at most 43, at most 42, at most 41, at most 40, at most 39, at most 38, at most 37, at most 36, at most 35, at most 34, at most 33, at most 32, at most 31, at most 30, at most 29, at most 28, at most 27, at most 26, at most 25, at most 24, at most 23, at most 22, at most 21, at most 20, at most 19, at most 18, at most 17, at most 16, at most 15, at most 14, at most 13, at most 12, at most 11, at most 10, at most 9, at most 8, and at most 7 amino acids.

[0102] In an embodiment, the peptide multimer to be used according to the invention comprises at least 6 amino acid residues, such as at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, at least 50, at least 51, at least 52, at least 53, at least 54, at least 55, at least 56, at least 57, at least 58, at least 59, at least 60, at least 61, at least 62, at least 63, at least 64, at least 65, at least 66, at least 67, at least 68, and at least 69 amino acid residues.

[0103] In one embodiment, the peptide multimer to be used according to the invention consists of 6 amino acid residues or 7 amino acid residues or 8 amino acid residues or 9 amino acid residues or 10 amino acid residues or 11 amino acid residues or 12 amino acid residues or 13 amino acid residues or 14 amino acid residues or 15 amino acid residues or 16 amino acid residues or 17 amino acid residues or 18 amino acid residues or 19 amino acid residues or 20 amino acid residues or 21 amino acid residues or 22 amino acid residues or 23 amino acid residues or 24 amino acid residues or 25 amino acid residues or 26 amino acid residues or 27 amino acid residues or 28 amino acid residues or 29 amino acid residues or 30 amino acid residues or 31 amino acid residues or 32 amino acid residues or 33 amino acid residues or 34 amino acid residues or 35 amino acid residues or 36 amino

acid residues or 37 amino acid residues or 38 amino acid residues or 39 amino acid residues or 40 amino acid residues or 41 amino acid residues or 42 amino acid residues or 43 amino acid residues or 44 amino acid residues or 45 amino acid residues or 46 amino acid residues or 47 amino acid residues or 48 amino acid residues or 49 amino acid residues or 50 amino acid residues or 51 amino acid residues or 52 amino acid residues or 53 amino acid residues or 54 amino acid residues or 55 amino acid residues or 56 amino acid residues or 57 amino acid residues or 58 amino acid residues or 59 amino acid residues or 60 amino acid residues or 61 amino acid residues or 62 amino acid residues or 63 amino acid residues or 64 amino acid residues or 65 amino acid residues or 66 amino acid residues or 67 amino acid residues or 68 amino acid residues or 69 amino acid residues or 70 amino acid residues.

[0104] The peptides described above may be used according to the invention as an immunogenic composition (such as a vaccine composition) comprising a composition described herein optionally in combination with a pharmaceutically acceptable diluents, vehicle and/or one or more immunological adjuvant.

[0105] Preparation of immunogenic compositions includes the use of state-of-the-art constituents such as immunological adjuvants. Apart from these adjuvants, which are detailed below, immunogenic compositions are prepared as generally taught in the art:

[0106] Preparation of vaccines which contain peptide sequences as active ingredients is generally well understood in the art, as exemplified by U.S. Pat. Nos. 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and 4,578,770, all incorporated herein by reference. Typically, such vaccines are prepared as injectables either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active immunogenic ingredient is often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants which enhance the effectiveness of the vaccines; cf. the detailed discussion of adjuvants below.

[0107] The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously, intracutaneously, intradermally, subdermally or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral, buccal, sublingual, intraperitoneal, intravaginal, anal, epidural, spinal, and intracranial formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10-95% of active ingredient, preferably 25-70%.

[0108] The peptides and peptide multimers may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include acid addition salts (formed with the free amino groups of the peptide) 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 may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0109] The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to be treated, including, e.g., the capacity of the individual's immune system to mount an immune response, and the degree of immunity desired. Suitable dosage ranges are of the order of several hundred micrograms active ingredient per vaccination with a preferred range from about 0.1 µg to 2,000 µg (even though higher amounts in the 1-10 mg range are contemplated), such as in the range from about 0.5 µg to 1,000 µg, preferably in the range from 1 µg to 500 µg and especially in the range from about 10 µg to 100 µg. Suitable regimens for initial administration and booster shots are also variable but are typified by an initial administration followed by subsequent inoculations or other administrations.

[0110] Some of the peptides and peptide multimers are sufficiently immunogenic in a vaccine, but for some of the others the immune response will be enhanced if the vaccine further comprises an adjuvant substance. The immunogenic molecules described herein can be therefore be formulated with adjuvants:

[0111] The adjuvants—to be combined are known to induce humoral responses and include: i) Salt suspensions (e.g. varieties of salts containing aluminum ions or calcium ions), ii) Oil-in-water emulsions (e.g. varieties of squalene-based or squalene-based emulsions), iii) Water-in-oil emulsions (e.g. Montanide ISA51 or ISA720), iv) Neutral liposomes, v) Cationic liposomes, vi) Microspheres, vii) Immunostimulating complexes (e.g. ISCOMs or ISCOMATRIX), viii) Pattern-recognition receptor agonists (e.g. agonists for C-type lectin receptors (CLRs), NOD-like receptors (NLRs), RIG-like helicases (RLHs), Triggering receptor expressed on myeloid cells (TREM) and Toll-like receptors (TLRs)), ix) Saponins (i.e. Any saponin derived from *Quilaja saponaria* or *Platycodon grandiflorum*), x) Virosomes/Virus-like particles (e.g.), xi) Enterotoxins (i.e. Cholera toxin, CTA1-DD or *Escherichia coli* heat-labile enterotoxin), and combinations thereof.

[0112] Other suitable adjuvants include response-selective C5a agonists, such as EP54 and EP67 described in Hung C Y et al. An agonist of human complement fragment C5a enhances vaccine immunity against *Coccidioides* infection. Vaccine (2012) and Kollessery G et al. Tumor-specific peptide based vaccines containing the conformationally biased, response-selective C5a agonists EP54 and EP67 protect against aggressive large B cell lymphoma in a syngeneic murine model. Vaccine (2011) 29: 5904-10.

[0113] Various methods of achieving adjuvant effect for the vaccine are thus known. General principles and methods are detailed in "The Theory and Practical Application of Adjuvants", 1995, Duncan E. S. Stewart-Tull (ed.), John Wiley & Sons Ltd, ISBN 0-471-95170-6, and also in "Vaccines: New Generation Immunological Adjuvants", 1995, Gregoriadis G et al. (eds.), Plenum Press, New York, ISBN 0-306-45283-9, both of which are hereby incorporated by reference herein, but a number of later publications also deal with the technology of incorporating adjuvants: Roestenberg Metal., PLoS One. 2008; 3(12):e3960. Epub 2008 Dec. 18; Relyveld E and Chermann J C, Biomed Pharmacother. 1994; 48(2):79-83; Hsu F J et al., Blood. 1997 May 1; 89(9):3129-35; Galli G et al., Proc Natl Acad Sci USA. 2009 May 12; 106(19):7962-7. Epub 2009 Apr. 27; Bojang K A et al., Lancet. 2001 Dec. 8; 358(9297):1927-34; Odunsi K et al., Proc Natl Acad Sci USA. 2007 Jul. 31; 104(31):12837-42. Epub 2007 Jul. 25; Patel G B and Sprott G D; Crit Rev Biotechnol. 1999; 19(4): 317-57. Review; Agger E M et al., PLoS One. 2008 Sep. 8; 3(9):e3116; Kirby D J et al. J Drug Target. 2008 May; 16(4): 282-93; Florindo H F et al., Vaccine. 2008 Aug. 5; 26(33): 4168-77. Epub 2008 Jun. 17; Sun H X et al., Vaccine. 2009 May 28; Guy B, Nat Rev Microbiol. 2007 July; 5(7):505-17. Review.; Vandepapelière P et al., Vaccine. 2008 Mar. 4; 26(10):1375-86. Epub 2008 Jan. 14; Ghochikyan A et al. Vaccine. 2006 Mar. 20; 24(13):2275-82. Epub 2005 Dec. 5; Xie Y et al., Vaccine. 2008 Jun. 25; 26(27-28):3452-60. Epub 2008 May 1; Chung Y C et al., Vaccine. 2008 Mar. 28; 26(15): 1855-62. Epub 2008 Feb. 25; Maier M et al., Vaccine. 2005 Oct. 25; 23(44):5149-59; Sundling C et al., J Gen Virol. 2008 December; 89(Pt 12):2954-64.

[0114] When recombinantly producing the peptides to be used according to the invention by means of transformed cells, it is convenient, although far from essential, that the expression product is either exported out into the culture medium or carried on the surface of the transformed cell.

[0115] When an effective producer cell has been identified it is preferred, on the basis thereof, to establish a stable cell line which carries the vector to be used according to the invention and which expresses the nucleic acid fragment to be used according to the invention. Preferably, this stable cell line secretes or carries the peptide expression product, thereby facilitating purification thereof.

[0116] In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with the hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species (see, e.g., Bolivar et al., 1977). The pBR322 plasmid contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, promoters which can be used by the prokaryotic microorganism for expression.

[0117] Those promoters most commonly used in recombinant DNA construction include the β -lactamase (penicillinase) and lactose promoter systems (Chang et al., 1978; Itakura et al., 1977; Goeddel et al., 1979) and a tryptophan (trp) promoter system (Goeddel et al., 1979; EP-A-0 036

776). While these are the most commonly used, other microbial promoters have been discovered and utilized, and details concerning their nucleotide sequences have been published.

[0118] In addition to prokaryotes, eukaryotic microbes, such as yeast cultures may also be used, and also here the promoter should be capable of driving expression. *Saccharomyces cerevisiae*, or common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in *Saccharomyces*, the plasmid YRp7, for example, is commonly used (Stinchcomb et al., 1979; Kingsman et al., 1979; Tschemper et al., 1980).

[0119] Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzman et al., 1980) or other glycolytic enzymes (Hess et al., 1968; Holland et al., 1978), such as *enolase*, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also incorporated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination.

[0120] Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter region for alcohol dehydrogenase 2, isocytichrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Any plasmid vector containing a yeast-compatible promoter, origin of replication and termination sequences is suitable.

[0121] In addition to microorganisms, cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. Examples of such useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COS-7 293, *Spodoptera frugiperda* (SF) cells, *Drosophila melanogaster* cell lines (such as Schneider 2 (S₂)), and MDCK cell lines.

[0122] Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences.

[0123] For use in mammalian cells, the control functions on the expression vectors are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and most frequently Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., 1978). Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 bp sequence extending from the HindIII site toward the BglI site located in the viral origin of replication. Further, it is also possible, and often desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

[0124] An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g., other Polyoma viruses, Adeno, VSV, BPV) or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

[0125] As for routes of administration and administration schemes of polypeptide based vaccines which have been detailed above, these are also applicable for the nucleic acid vaccines of the invention and all discussions above pertaining to routes of administration and administration schemes for polypeptides apply mutatis mutandis to nucleic acids. To this should be added that nucleic acid vaccines can also be administered intravenously and intraarterially. Furthermore, it is well-known in the art that nucleic acid vaccines can be administered by use of a so-called gene gun and/or by use of electroporation, and hence also these and equivalent modes of administration are regarded as part of the present invention.

[0126] Under normal circumstances, the nucleic acid fragment is introduced in the form of a vector wherein expression is under control of a viral promoter. For more detailed discussions of vectors to be used according to the invention, cf. the discussion above. Also, detailed disclosures relating to the formulation and use of nucleic acid vaccines are available, cf. Donnelly J J et al, 1997, Annu. Rev. Immunol. 15: 617-648 and Donnelly J J et al., 1997, Life Sciences 60: 163-172. Both of these references are incorporated by reference herein.

[0127] An alternative of using peptide immunogens or nucleic acid immunogens is the use of live immunogen technology. This entails administering a non-pathogenic microorganism which has been transformed with a nucleic acid fragment or a vector of the present invention. The non-pathogenic microorganism can be any suitable attenuated bacterial strain (attenuated by means of passaging or by means of removal of pathogenic expression products by recombinant DNA technology), e.g. *Mycobacterium bovis* BCG., non-pathogenic *Streptococcus* spp., *E. coli*, *Salmonella* spp., *Vibrio cholerae*, *Shigella*, etc. Reviews dealing with preparation of state-of-the-art live vaccines can e.g. be found in Saliou P, 1995, Rev. Prat. 45: 1492-1496 and Walker P D, 1992, Vaccine 10: 977-990, both incorporated by reference herein. For details about the nucleic acid fragments and vectors used in such live vaccines, cf. the discussion below.

[0128] As an alternative to bacterial live immunogens, the nucleic acid fragment to be used according to the invention can be incorporated in a non-virulent viral vaccine vector such as a vaccinia strain or any other suitable poxvirus.

[0129] Normally, the non-pathogenic microorganism or virus is administered only once to a subject, but in certain cases it may be necessary to administer the microorganism/virus more than once in a lifetime in order to maintain protective immunity. It is even contemplated that immunization schemes as those detailed above for polypeptide vaccination will be useful when using live or virus vaccines.

[0130] Alternatively, live or virus immunization is combined with previous or subsequent polypeptide and/or nucleic acid immunization. For instance, it is possible to effect primary immunization with a live or virus vaccine followed by subsequent booster immunizations using the polypeptide or nucleic acid approach.

PREAMBLE TO EXAMPLES

-continued

Overview Sequences and Abbreviations

C5-Sequences:

[0131]

APTKAKRRVVQREKRAV (SEQ ID NO: 1)
 APTKAKRRVVEREKRAV (SEQ ID NO: 2)
 APTRAKRRVVQREKRAV (SEQ ID NO: 3)
 APTRAKRRVVEREKRAV (SEQ ID NO: 4)
 APTEAKRRVVEREKRAV (SEQ ID NO: 5)
 WWGC AKRRVCGGAKRRVVQREKRA (SEQ ID NO: 44)

(underlined amino acid residues in SEQ ID NO:44 are linked via a disulphide linker; the N-terminal W is preferably a D-amino acid and the C-terminal A may be amidated; the peptide is termed BI450-AdjBT_1, when having these two modifications).

C5-Complex Forming Sequences:

[0132] DRPEGIEEEGGGERDR (where amino acid 4 can be G and/or where amino acid 5 can be R and/or where amino acid 13 can be Q and/or where amino acid 14 can be G and/or where amino acid 15 can be K; SEQ ID NO:6);

[0133] DRPEGIENNGGERDR (SEQ ID NO:7 where amino acid 4 can be G and/or where amino acid 5 can be R and/or where amino acid 13 can be Q and/or where amino acid 14 can be G and/or where amino acid 15 can be K);

[0134] DRPEGIENNGGERDRDR (where amino acid 4 can be G and/or where amino acid 5 can be R and/or where amino acid 13 can be Q and/or where amino acid 14 can be G and/or where amino acid 15 can be K and/or where amino acid 16 can be G); SEQ ID NO:46).

VERYLKDQQLLG (SEQ ID NO: 8);
 VERYLKDEELLG (SEQ ID NO: 9);
 VERYLKDNLLG (SEQ ID NO: 10);
 QLLNGLSLAEEIIVI (SEQ ID NO: 11)
 QLLNGLSLAEEVVIV (SEQ ID NO: 12)
 QLLNGLSLAEEVVII (SEQ ID NO: 13)

GGAIVNGSLADDDIVI (SEQ ID NO: 37,
 also termed 204d herein)
 WWGCIEEEGCGGIEEEGGGERDR (SEQ ID NO: 45:

underlined amino acid residues are linked via a disulphide linker; the N-terminal W is preferably a D-amino acid and the C-terminal R may be amidated; the peptide is termed BI450-AdjBT_2, when having these two modifications).

Polypeptides I:

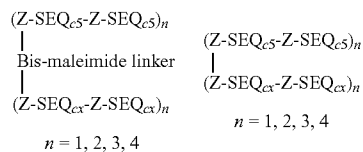
$$(Z\text{-SEQ}_{c5}\text{-Z-SEQ}_{c5})_n$$
[0135] $n=1, 2, 3, 4$

Polypeptides II:

$$(Z\text{-SEQ}_{cx}\text{-Z-SEQ}_{cx})_n$$
[0136] $n=1, 2, 3, 4$

Peptide Complexes:

[0137]



[0138] Examples of polypeptides I can be, but are not restricted to, the following sequences:

APTKAKRGGGAPTRAKRGGGAPTEAKR (SEQ ID NO: 14)
 RVVEREKGGGAKRRVVGGRVVQREK (SEQ ID NO: 15)
 GGAKRRVVGAKRRVVQREKRAV (SEQ ID NO: 16)
 CGGAKRRVVGAKRRVVQREKRAV (SEQ ID NO: 17)
 GGAKRRVVGAKRRVVGGQREKR (SEQ ID NO: 18)
 CGGAKRRVVGAKRRVVGGQREKR (SEQ ID NO: 19)
 GGAKRRVVGAKRRVV (SEQ ID NO: 20)
 GCGAKRRVVGAKRRVV (SEQ ID NO: 21)

[0139] Examples of polypeptides II can be, but are not restricted to, the following sequences:

(SEQ ID NO: 22)
GGGDQQLLGGAEEIIVGGIEEGGERDRDR

(SEQ ID NO: 23)
CGGGDQQLLGGAEEIIVGGIEEGGERDRDR

(SEQ ID NO: 24)
GGDQQLLGGAEEIIVGGGERDR

(SEQ ID NO: 25)
CGGGDQQLLGGAEEIIVGGIEEEGG

(SEQ ID NO: 26)
GGAEIEVVGGDQQLL

(SEQ ID NO: 27)
CGGAEEIEVVGGDQQLL

[0140] Examples of disulfide linked constructs can be, but are not restricted to, the following linked peptide sequences:

CGGAKRRVVGGAKRRVVQREKRAV (SEQ ID NO: 28)
|
CGGGDQQLLGGAEEIIVGGIEEGGERDRDR (SEQ ID NO: 29)
CGGAKRRVVGGAKRRVVGGQREKR (SEQ ID NO: 30)
|
CGGGDQQLLGGAEEIIVGGIEEEGG (SEQ ID NO: 31)
CGGAEEIEVVGGDQQLL (SEQ ID NO: 32)
|
GCGGAKRRVVGGAKRRVV (SEQ ID NO: 33)

[0141] The above disulfide linked constructs may e.g. be synthesised by titration of 2-pyridinesulfonyl (SPyr)-protected cysteine-containing peptides with thiol-unprotected peptides. This has proven to be a superior procedure to selectively generate disulfide-linked peptide heterodimers preventing the formation of homodimers (Schutz A et al., Tetrahedron, Volume 56, Issue 24, 9 Jun. 2000, Pages 3889-3891). Similar constructs where SEQ ID NO:28 is disulphide linked to SEQ ID NOs 31 or 33, or where SEQ ID NO:30 is disulphide linked to SEQ ID NOs: 29 or 33, or where SEQ ID NO:32 is disulphide linked to SEQ ID NOs: 29 or 31 are also within the scope of the present invention.

[0142] Examples of other linked constructs can be, but are not restricted to, the following linked peptide sequences, which have all been obtained from Bachem (UK) Ltd:

GAKRRVVGGCGGAKRRVVQREKRAGEREKRA (SEQ ID NO: 38)
|
GKGIEIEEGGRDRDRGGEQDRDR (SEQ ID NO: 39)

(the peptides are linked via the underlined Cys and Lys residues; the entire construct is termed BI400-B herein).

GAKRRVVGGCGGAKRRVVQREKRAGEREKRA (SEQ ID NO: 38)
|
GKGIEIEEGGERDRDRGGQDRDR (SEQ ID NO: 40)

(the peptides are linked via the underlined Cys and Lys residues; the entire construct is termed BI400-Bu1 herein).

GAKRRVVGGCGGAKRRVVQREKRAGEREKRA (SEQ ID NO: 41)
|
GKGIEIEEGGQDRDRGGGRDRDR (SEQ ID NO: 42)

(the peptides are linked via the underlined Cys and Lys residues; the entire construct is termed BI400-Bu2 herein).

GAKRRVVGGCGGAKRRVVQREKRAGEREKRA (SEQ ID NO: 41)
|
GKGIEIEEGGQDRDRGGGERDRD (SEQ ID NO: 43)

(the peptides are linked via the underlined Cys and Lys residues; the entire construct is termed BI400-Bu3 herein).

[0143] The Cys-Lys linker is typically established in the form of an amide bond between (2-oxo-ethyl) derivatized cysteine in one peptide and lysine in the other peptide.

[0144] Similar constructs where SEQ ID NO:38 is Cys-Lys linked to SEQ ID NOs: 42, 43, 68, or where SEQ ID NO:41 is Cys-Lys linked to SEQ ID NOs: 39 or 40 are also within the scope of the present invention.

Small Molecule Inhibitors:

[0145]

DQQLL (SEQ ID NO: 34)
AKRRVV (SEQ ID NO: 35)
AEEIEV (SEQ ID NO: 36)

[0146] SEQ ID NOs 34-36 are preferably composed partly or completely of D-amino acids.

[0147] One preferred immunogen which induces antibodies that stabilise association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120 is a compound of the following structure:

[0148] (H-Glycyl-L-alanyl-L-lysyl-L-arginyl-L-arginyl-L-valyl-L-valyl-glycyl-glycyl-L-cysteinyl(2-oxo-ethyl)-glycyl-glycyl-L-alanyl-L-lysyl-L-arginyl-L-arginyl-L-valyl-L-valyl-L-glutamyl-L-arginyl-L-glutamyl-L-lysyl-L-arginyl-L-alanyl-glycyl-L-glutamyl-L-arginyl-L-glutamyl-L-lysyl-L-arginyl-L-alanyl-NH₂) (H-Glycyl-L-lysyl-glycyl-glycyl-L-isoleucyl-L-glutamyl-L-glutamyl-L-glutamyl-glycyl-glycyl-L-arginyl-L-aspartyl-L-arginyl-L-aspartyl-L-arginyl-glycyl-glycyl-L-glutamyl-L-aspartyl-L-arginyl-L-aspartyl-L-arginyl-NH₂), acetate salt (amide bond between Cys(2-oxo-ethyl)¹⁰ (A-chain) and Lys² (B-chain))

[0149] This compound may also be referred to as:

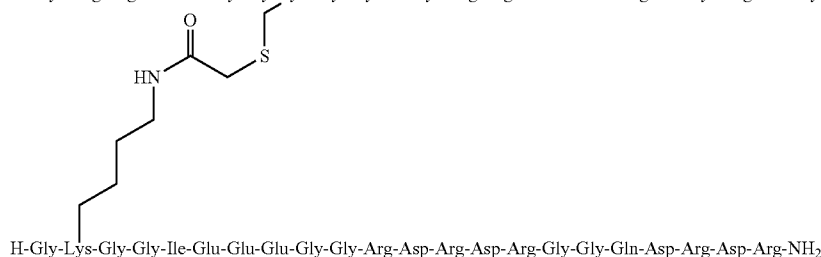
[0150] (H-Gly-Ala-Lys-Arg-Arg-Val-Val-Gly-Gly-Cys(2-oxo-ethyl)-Gly-Gly-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Gly-Glu-Arg-Glu-Lys-Arg-Ala-NH₂) (H-Gly-Lys-Gly-Gly-Ile-Glu-Glu-Glu-Gly-Gly-Arg-Asp-Arg-Asp-Arg-Gly-Gly-Gln-Asp-Arg-Asp-Arg-NH₂), acetate salt (amide bond between Cys(2-oxo-ethyl)¹⁰ (A-chain) and Lys² (B-chain))

[0151] This preferred C5 compound consists of two linear peptide amide chains with 31 amino acids (A-chain) and 22 amino acids (B-chain). Each chain has a free amino group at the N-terminus and an amide group at the C-terminus. The

chains are covalently linked via an amide bond between Cys (2-oxo-ethyl)¹⁰ of the A-chain and Lys² of the B-chain. All amino acid residues except the achiral Gly are in the L-configuration.

opiperidin-3-yl)-1-oxoisindoles including, but not limited to, those disclosed in U.S. Pat. No. 5,635,517, U.S. Pat. No. 6,281,230, U.S. Pat. No. 6,316,471, U.S. Pat. No. 6,403,613, U.S. Pat. No. 6,476,052 and U.S. Pat. No. 6,555,554; 1-oxo

H-Gly-Ala-Lys-Arg-Arg-Val-Val-Gly-Gly-Cys-Gly-Gly-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Gly-Glu-Arg-Glu-Lys-Arg-Ala-NH₂



[0152] The preferred C5 compound (also referred to in the examples as Vacc-C5) may be provided as an acetate salt. The counter ion acetate is bound in ionic form to basic groups of the peptide molecule.

Immunomodulatory Compounds

[0153] As used herein and unless otherwise indicated, the term “immunomodulatory compounds” encompasses certain small organic molecules that inhibit LPS induced monocyte TNF- α , IL-1B, IL-12, IL-6, MIP-1a, MCP-1, GM-CSF, G-CSF, and/or COX-2 production, COX-2 inhibitors, and Etoricoxib. Other immunomodulatory compounds includes Viriostatics VS411 (didanosine+hydroxyurea). Specific immunomodulatory compounds are discussed herein elsewhere.

[0154] In some embodiments, the immunomodulatory compounds used according to the present invention are co-stimulators of T cells and increase cell proliferation dramatically in a dose dependent manner. In one embodiment, immunomodulatory compounds provided herein may also have a greater co-stimulatory effect on the CD8+ T cell subset than on the CD4+ T cell subset. In one embodiment, the compounds have anti-inflammatory properties against myeloid cell responses, yet efficiently co-stimulate T cells to produce greater amounts of IL-2, IFN- γ , and to enhance T cell proliferation and CD8+ T cell cytotoxic activity. In one embodiment, without being limited by a particular theory, immunomodulatory compounds provided herein may be capable of acting both indirectly through cytokine activation and directly on Natural Killer (“NK”) cells and Natural Killer T (“NKT”) cells, and increase the NK cells’ ability to produce beneficial cytokines such as, but not limited to, IFN- γ , and to enhance NK and NKT cell cytotoxic activity.

[0155] In one embodiment, specific examples of immunomodulatory compounds include, but are not limited to, cyano and carboxy derivatives of substituted styrenes such as those disclosed in U.S. Pat. No. 5,929,117; 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl) isoindolines such as those described in U.S. Pat. No. 5,874,448 and U.S. Pat. No. 5,955,476; the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolines described in U.S. Pat. No. 5,798,368; 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines (e.g., 4-methyl derivatives of thalidomide), substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxo

and 1,3-dioxoisindolines substituted in the 4- or 5-position of the indoline ring (e.g., 4-(4-amino-1,3-dioxoisindoline-2-yl)-4-carbamoylbutanoic acid) described in U.S. Pat. No. 6,380,239; isoindoline-1-one and isoindoline-1,3-dione substituted in the 2-position with 2,6-dioxo-3-hydroxypiperidin-5-yl (e.g., 2-(2,6-dioxo-3-hydroxy-5-fluoropiperidin-5-yl)-4-aminoisoindolin-1-one) described in U.S. Pat. No. 6,458,810; a class of non-polypeptide cyclic amides disclosed in U.S. Pat. No. 5,698,579 and U.S. Pat. No. 5,877,200; and isoindole-imide compounds such as those described in U.S. patent publication no. 2003/0045552 published on Mar. 6, 2003, U.S. patent publication no. 2003/0096841 published on May 22, 2003, WO2007028047, WO2002094180 and WO 02/059106). The entireties of each of the patents and patent applications identified herein are incorporated herein by reference. In one embodiment, immunomodulatory compounds do not include thalidomide.

[0156] In one embodiment, the immunomodulatory compounds provided herein include, but are not limited to, 1-oxo and 1,3 dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines substituted with amino in the benzo ring as described in U.S. Pat. No. 5,635,517 which is incorporated herein by reference.

[0157] In one embodiment, other specific immunomodulatory compounds provided herein belong to a class of substituted 2-(2,6-dioxopiperidin-3-yl) phthalimides and substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindoles, such as those described in U.S. Pat. No. 6,281,230; U.S. Pat. No. 6,316,471; U.S. Pat. No. 6,335,349; U.S. Pat. No. 6,476,052, and WO 98/03502), each of which is incorporated herein by reference.

[0158] In one embodiment, other specific immunomodulatory compounds provided herein belong to a class of isoindole-imides disclosed in U.S. Patent Application Publication Nos. US 2003/0096841 and US 2003/0045552, and WO 02/059106), each of which are incorporated herein by reference.

[0159] In one embodiment, other specific immunomodulatory compounds provided herein belong to a class of isoindole-imides disclosed in U.S. Patent Application Publication Nos. US 2002/0045643, and WO 98/54170, and U.S. Pat. No. 6,395,754, each of which is incorporated herein by reference.

[0160] In one embodiment, other specific immunomodulatory compounds provided herein include, but are not limited to, 1-oxo-2-(2,6-dioxo-3-fluoropiperidin-3-yl) isoindolines and 1,3-dioxo-2-(2,6-dioxo-3-fluoropiperidine-3-yl) isoindolines

dolines such as those described in U.S. Pat. No. 5,874,448 and U.S. Pat. No. 5,955,476, each of which is incorporated herein by reference.

[0161] In one embodiment, other specific immunomodulatory compounds provided herein include, but are not limited to, the tetra substituted 2-(2,6-dioxopiperidin-3-yl)-1-oxoisindolines described in U.S. Pat. No. 5,798,368, which is incorporated herein by reference.

[0162] In one embodiment, other specific immunomodulatory compounds provided herein include, but are not limited to, 1-oxo and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl) isoindolines disclosed in U.S. Pat. No. 6,403,613, which is incorporated herein by reference.

[0163] In one embodiment, other specific immunomodulatory compounds provided herein include, but are not limited to, 1-oxo and 1,3-dioxoisindolines substituted in the 4- or 5-position of the indoline ring described in U.S. Pat. No. 6,380,239 and U.S. application Ser. No. 10/900,270, filed Jul. 28, 2004, which are incorporated herein by reference.

[0164] In one embodiment, other specific immunomodulatory compounds provided herein include, but are not limited to, isoindoline-1-one and isoindoline-1,3-dione substituted in the 2-position with 2,6-dioxo-3-hydroxypiperidin-5-yl described in U.S. Pat. No. 6,458,810, which is incorporated herein by reference.

Histone Deacetylase Inhibitors and Other Reservoir Purging Agents.

[0165] As used herein the term “histone deacetylase inhibitor”, “HDACi” refers to a compound that inhibits a Histone deacetylase.

[0166] Based on their chemical structure, HDACi have been subdivided into four different classes (for review see for instance Dokmanovic & Marks, *J. Cell. Biochem.*, 96, 293-304, 2005):

[0167] hydroxamic acids (Trichostatin A, Vorinostat=SAHA)

[0168] cyclic tetrapeptides (Depsipeptide)

[0169] short-chain aliphatic acids (Valproic Acid, phenyl butyrate)

[0170] benzamides (Entinostat).

[0171] These compounds are generally active (with depending on the compound, more or less specificity for a given HDAC) on histone deacetylases of Class I, II, and IV.

[0172] The inhibition of histone deacetylase by HDACi induces the accumulation of hyperacetylated nucleosome core histones. This chromatin remodeling results in modulation of gene expression, which can inhibit proliferation of cancer cells through biological processes such as cell cycle arrest, differentiation and/or apoptosis (for review see for instance Marks & Xu, *J. Cell. Biochem.*, 107, 600-608, 2009; Federico & Bagella, *J. Biomed. Biotechnol.*, 2011:475641).

[0173] Reservoir purging agents, such as histone deacetylase inhibitors, include but is not limited to e.g., Toll-like receptor-9 (TLR9) agonists, quercetin, trichostatin A, lipoic acid, an hydroxamic acid, valproic acid, sodium butyrate, vorinostat, romidepsin, TNF- α , PHA, Tat, interferon gamma (IFN- γ), CD154, IL-1 β , IL-2, IL-6, IL-7 or IL-7R agonist, IL-9 or IL-9R agonists, 5-hydroxynaphthalene-1,4-dione (5HN), siRNA or antagonists (anti-miRNA antisense) raised against miRNA controlling the expression of latent HIV, short hairpin RNAs (shRNAs) targeting transcription factor YY1 and/or cMyc, histone methyl transferase inhibitors as chaetocine, DNA methyltransferase inhibitors including nucleotide

analogue methylation inhibitors 5-azacytidine, 5-aza-2'-deoxycytidine, 5-fluoro-2'-deoxycytidine and zebularidine, non-nucleoside DNA methylation inhibitors procaine, procainamide, hydralazine and RG108.

[0174] Suitable histone deacetylase inhibitors (HDACi) in accordance with the invention include, but are not limited to M344 (4-(dimethylamino)-N-[7-(hydroxyamino)-7-oxoheptyl]benzamide), chidamide (CS055/HBI-800), 4SC-202, (4SC), Resminostat (4SC), hydroxamic acids such as vorinostat (SAHA), belinostat (PXD101), LAQ824, trichostatin A and panobinostat (LBH589); benzamides such as entinostat (MS-275), CI994, and mocetinostat (MGCD0103), cyclic tetrapeptides (such as trapoxin, such as trapoxin B), and the depsipeptides, such as romidepsin (ISTODAX), electrophilic ketones, and the aliphatic acid compounds such as phenylbutyrate, valproic acid, Oxamflatin, ITF2357 (generic givinostat), Apicidin, MC1293, CG05, and CG06.

[0175] Other suitable reservoir purging agents includes, but are not limited to the ones disclosed in any one of WO2013050422, WO2012051492 A3 and in Barton et al., *Clinical Pharmacology & Therapeutics* (2013); 93 1, 46-56I, including but not limited to a NF-kappa-B-inducer selected from the group comprising: PMA, prostratin, bryostatins and TNF-alpha, and/or b) a histone deacetylase inhibitor selected from the different families (hydroxamates, cyclic peptides, aliphatic acids, and benzamides) including: TSA, SAHA, MS-275, aminosuberoyl hydroxamic acids, M-Carboxycinnamic acid bishydroxamate, LAQ-824, LBH-589, belinostat (PXD-101), Panobinostat (LBH-589), a cinnamic hydroxamic acid analogue of M-carboxycinnamic acid bishydroxamate, IF2357, aryloxyalkanoic acid hydroxamides, depsipeptide, apicidin, cyclic hydroxamic acid-containing peptide group of molecules, FK-228, red FK, cyclic peptide mimic linked by an aliphatic chain to a hydroxamic acid, butyrate, phenylbutyrate, sodium butyrate, valproic acid, pivaloyloxymethyl butyrate, 5 NOX-275, and MGCD0103.

[0176] Any of the above virus reservoir purging agents may be used alone or in combination with any one other suitable virus reservoir purging agent, such as with another class of HIV inducers.

[0177] DNA methylation, probably together with repressive histone modifications, may also contribute to a “lock” in a silent state of the provirus and makes its return to an active state difficult. These observations suggest that HDAC or HMT or DNA methylation inhibitors together with efficient cART constitute good anti-latency drug candidates aimed at reducing/eliminating the pool of latent reservoirs to a level bearable by the host immune system.

[0178] Accordingly suitable immunomodulatory compounds or purging agents may be DNA methylation inhibitors selected from the two classes (non-nucleoside and nucleoside demethylating agents) including: 5-azacytidine (azacytidine), Sinefungin, 5-aza-2'-deoxycytidine (5-aza-CdR, decitabine), 1-3-Darabino-furanosyl-5-azacytosine (fazarabine) and dihydro-5-azacytidine (DHAC), 5-fluorodeoxycytidine (FdC), oligodeoxynucleotide duplexes containing 2-H pyrimidine, zebularine, antisense oligodeoxynucleotides (ODNs), MG98, (-)-epigallocatechin-3-gallate, hydralazine, procaine and procainamide.

[0179] Other suitable immunomodulatory compounds or purging agents to be used according to the present invention includes histone deacetylase inhibitor is selected from the different families of HDACi (hydroxamates, cyclic peptides, aliphatic acids, and benzamides) including TSA, SAHA,

MS-275, aminosuberoyl hydroxamic acids, M-Carboxycinnamic acid bishydroxamate, LAQ-824, LBH-589, belinostat (PXD-101), Panobinostat (LBH-589), a cinnamic hydroxamic acid analogue of M-carboxycinnamic acid bishydroxamate, IF2357, aryloxyalkanoic acid hydroxamides, depsipeptide, apicidin, cyclic hydroxamic acid-containing peptide group of molecules, FK-228, red FK, cyclic peptide mimic linked by an aliphatic chain to a hydroxamic acid, butyrate, phenylbutyrate, sodium butyrate, valproic acid, pivaloyloxymethyl butyrate, 5 NOX-275, and MGCD0103.

[0180] Other suitable immunomodulatory compounds or purging agents to be used according to the present invention includes histone methyltransferase inhibitors (chaetocin and BIX-01294); Inhibitors of Enhancers of Zeste 2 (EZH2)—such as 3-deazaneplanocin A (DZNep) used alone or in combination with other classes of immunomodulatory compounds or purging agents.

[0181] In the methods of the invention each of the components comprising (i) one or more peptides that elicit a cell-mediated immune response in a subject and/or (ii) a compound to stimulate the humoral immunity in a subject; and (iii) a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor, and/or (iv) an immunomodulatory compound, may be administered simultaneously, sequentially or separately in any order.

[0182] Thus the invention provides a pharmaceutical composition comprising at least one of components (i) and (ii) and at least one of components (iii) and (iv) optionally in combination with one or more pharmaceutically acceptable adjuvants, diluents or carriers.

[0183] Similarly, the invention also provides a combination product comprising at least one of components (i) and (ii) and at least one of components (iii) and (iv), wherein each component is optionally formulated in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier. In this aspect of the invention, the combination product may be either a single (combination) pharmaceutical formulation or a kit-of-parts. In a kit-of-parts some or all of the components may be formulated separately and may be each be provided in a form that is suitable for administration in conjunction with the other(s).

[0184] The component(s) may also be provided for use, e.g. with instructions for use, in combination with one or more further component(s) as defined above.

[0185] A therapeutic amount of HDACi is administered to a mammal in the present method, e.g. an amount sufficient to enhance the immunological effect of the viral vaccine. The HDACi may be administered using any suitable administrable form, including for example, oral, subcutaneous, intravenous, intraperitoneal, intranasal, enteral, topical, sublingual, intramuscular, intra-arterial, intramedullary, intrathecal, inhalation, ocular, transdermal, vaginal or rectal means.

[0186] Other suitable reservoir purging agents includes, but are not limited to compounds that activate transcription factors including NF-KappaB, Prostratin, auranofin, bryostatins, a nontumorigenic phorbol ester, DPP (12-deoxyphorbol-13-phenylacetate), PMA, and Phorbol 12-myristate 13-acetate (PMA); Compounds that activate HIV mRNA elongation including P-TEF-b kinase and hexamethylbisacetamide (HMBA); T-cell stimulating factors including anti-CD3/CD28-T-cell stimulating Ab's; Kinase inhibitors including Tyrphostin A, Tyrphostin B, and Tyrphostin C; TLR-7 agonists; PTEN (phosphatase and tensin homologue) gene

inhibitors including SF1670 (Echelon Bioscience), Disulfiram (DSF), an inhibitor of acetaldehyde dehydrogenase, Protein Tyrosine Phosphatase Inhibitors including bpV (HOPic), bpV(phen), and bpV(pic) (Calbiochem; EMD Millipore).

[0187] Activation and elimination of cells latently infected by HIV-1 by T-cells stimulated with HIV targeting peptides can be studied by a method described by Shan et al. (Immunity, 2012, Vol. 36, Issue 3, pp. 491-501). In this study the authors use methods to generate a model of for latent HIV-1 infection described by Yang et al. (3 Clin Invest. 2009, 119 (11):3473-3486). Other models for viral latency exists and might be used in a similar manner (Saleh et al. Blood 2007; 110:4161-4. Saleh et al. Retrovirology 2011; 8:80.). Furthermore, Shan et al. use the HDAC inhibitor SAHA to activate the latently infected cells, but in principle the method can be used, or adapted, to study other activators of latent HIV-1 in a similar manner. Briefly: Peripheral blood for the isolation of primary CD4+ and CD8+ T-cells was obtained from HIV-1-infected donors and healthy adult volunteers and used for Bcl-2 transduction and then HIV-1 reporter virus (GFP expressing) infection (Yang et al., 2009, J Clin Invest., 119 (11):3473-3486). CD8+ T-cells were isolated, optionally stimulated with HIV-1 specific peptides, and cocultured with in vitro infected autologous CD4+ T-cells. The fraction of residual GFP+CD4+ T-cells was measured by FACS. This procedure demonstrated CD8+ CTL mediated killing of the activated CD4+ T-cells.

Specific Embodiments of the Invention

[0188] As described above one aspect of the present invention relates to a composition comprising

[0189] a) a compound to stimulate the humoral immunity in a subject and/or one or more peptide that elicit a cell-mediated immune response in a subject; in combination with

[0190] b) an immunomodulatory compound and/or a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor.

[0191] In some embodiments, the composition according to the present invention comprises one or more peptides that elicit a cell-mediated immune response in a subject.

[0192] In some embodiments, the composition according to the present invention comprises a compound to stimulate the humoral immunity in a subject. In some embodiments, this compound stimulate the humoral immunity in a subject is an agent capable of stabilising the association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120. In some embodiments, this compound stimulate the humoral immunity in a subject is an immunogen, which induces antibodies that stabilise association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120. In some embodiments this compound stimulate the humoral immunity in a subject is a peptide described in any one of international patent applications WO2011/000962, WO0052040, WO 2012/092934 or WO 2012/072088, which references are hereby incorporated by reference.

[0193] In some embodiments, the composition according to the present invention comprises an immunomodulatory compound.

[0194] In some embodiments, the composition according to the present invention comprises a reservoir purging agent, such as a Histone deacetylase (HDAC) inhibitor.

[0195] In some embodiments, the immunomodulatory compounds as used according to the present invention is selected from anti-PD1 antibodies, such as MDX-1106 (Merck), THALOMID® (thalidomide), anti-PD1 antibodies, cyclophosphamide, Levamisole, lenalidomide, CC-4047 (pomalidomide), CC-11006 (Celgene), and CC-10015 (Celgene), and immunomodulatory compound described in any one of WO2007028047, WO2002059106, and WO2002094180.

[0196] In some embodiments, the immunomodulatory compound as used according to the present invention is selected from a 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoin-doline-1,3-dione and a 3-(4-amino-1-oxo-1,3-dihydro-isoin-dol-2-yl)-piperidine-2,6-dione.

[0197] In some embodiments, the immunomodulatory compound as used according to the present invention is enan-tiomERICALLY pure.

[0198] In some embodiments, the reservoir purging agent, such as a Histone deacetylase (HDAC) inhibitor as used according to the present invention is selected from M344 (4-(dimethylamino)-N-[7-(hydroxyamino)-7-oxoheptyl] benzamide, chidamide (CS055/HBI-800), 4SC-202, (4SC), Resminostat (4SC), hydroxamic acids such as vorinostat (SAHA), belinostat (PXD101), LAQ824, trichostatin A and panobinostat (LBH589); benzamides such as entinostat (MS-275), CI994, and mocetinostat (MGCD0103), cyclic tet-rapeptides (such as trapoxin, such as trapoxin B), and the depsipeptides, such as romidepsin (ISTODAX), electrophilic ketones, and the aliphatic acid compounds such as phenylbu-tyrate, valproic acid, Oxamflatin, ITF2357 (generic givi-nostat), Apicidin, MC1293, CG05, and CG06; compounds that activate transcription factors including NF-KappaB, Prostratin, auranofin, bryostatin, a nontumorigenic phorbol ester, DPP (12-deoxyphorbol-13-phenylacetate), PMA, and Phorbol 12-myristate 13-acetate (PMA); Compounds that activate HIV mRNA elongation including P-TEF-b kinase and hexamethylbisacetamide (HMB); IL-7; T-cell stimulat-ing factors including anti-CD3/CD28-T-cell stimulating Ab's; Kinase inhibitors including Tyrphostin A, Tyrphostin B, and Tyrphostin C; PTEN (phosphatase and tensin homo-logue) gene inhibitors including SF1670 (Echelon Bio-science), Disulfiram (DSF), an inhibitor of acetaldehyde dehydrogenase, Protein Tyrosine Phosphatase Inhibitors including bpV(HOPic), bpV(phen), and bpV(pic) (Calbio-chem; EMD Millipore), Toll-like receptors agonists includ-ing Toll-like receptor-9 (TLR9) and Toll-like receptor-7 (TLR9) agonists, quercetin, lipoic acid, sodium butyrate, TNF-alpha, PHA, Tat.

[0199] In some embodiments, the one or more peptide that elicit a Cell-mediated immune response as used according to the present invention is at least one HIV-specific peptide selected from the group of amino acid sequences:

[0200] Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Ala Xaa₈ Xaa₉ Gln Thr Pro Trp Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈ Val Xaa₂₀ (SEQ ID NO:47);

[0201] Wherein Xaa in position 1 of the peptide derivate is Lys or Arg,

Xaa in position 2 is Ala, Gly, Ser or Arg,

Xaa in position 3 is Leu or Met,

Xaa in position 4 is Gly or Arg,

Xaa in position 5 is Pro, Thr, Val, Ser, Gln or Ala,

Xaa in position 6 is Gly, Ala, Lys, Arg, Gln or Glu,

Xaa in position 8 is Thr or Ser,

Xaa in position 9 is Leu or Ile,

Xaa in position 14 is Thr, Ser or Val,

Xaa in position 15 is Ala or Ser,

Xaa in position 16 is Cys or Ser,

Xaa in position 17 is Gln or Leu

Xaa in position 18 is Gly, Glu or Arg, and

Xaa in position 20 is Gly or Arg;

(SEQ ID NO: 50)

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Gly Leu Asn Pro Leu Val

[Gly]_n Xaa₁₂ Xaa₁₃ Tyr Xaa₁₅ Pro Xaa₁₇ Xaa₁₈ Ile Leu

Xaa₂₁ Xaa₂₂

wherein Xaa in position 1 is Arg, Lys, Asp or none

Xaa in position 2 is Trp, Gly, Lys or Arg,

Xaa in position 3 is Ile, Leu, Val or Met

Xaa in position 4 is Ile, Val or Leu

Xaa in position 5 Leu, Met, Val or Pro

Xaa in position 12 is Arg, Lys

Xaa in position 13 is Met or Leu,

Xaa in position 15 is Ser, Cys or Gln,

Xaa in position 17 is Thr, Val, Ile, Ser or Ala,

Xaa in position 18 is Ser, Gly or Thr,

Xaa in position 21 is Asp, Glu, Cys or Gly,

Xaa in position 22 is Gly or none, and

n=0, 1, 2 or 3;

(SEQ ID NO: 55)

Xaa₁ Xaa₂ Xaa₃ Pro Ile Pro Xaa₇ Xaa₈ Xaa₉ Xaa₁₀

Xaa₁₁ Xaa₁₂ [Gly]_n Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇

Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄

wherein Xaa in position 1 is Asn, Ser, Gly, His, Ala, Pro, Arg or none

Xaa in position 2 is Asn, Ala or Lys

Xaa in position 3 is Pro, Gln, Gly, Ile or Leu

Xaa in position 7 is Val or Ala

Xaa in position 8 is Gly or Lys

Xaa in position 9 is Glu, Asp, Lys, Phe or Thr

Xaa in position 10 is Ile, Met, Val or Leu

Xaa in position 11 is Tyr, Leu or none

Xaa in position 12 is Ser or none

Xaa in position 13 is Arg or none

Xaa in position 14 is Asp, Arg, Trp, Ala or none

Xaa in position 15 is Ile or none

Xaa in position 16 is Tyr or none

Xaa in position 17 is Lys or Arg

Xaa in position 18 is Arg, Lys or Asp

Xaa in position 19 is Trp or Gly

Xaa in position 20 is Ile, Met, Val, Gln or Ala

Xaa in position 21 is Ile, Val or Ala

Xaa in position 22 is Leu, Met or Val

Xaa in position 23 is Gly or Cys

Xaa in position 24 is Leu or none,

n=1, 2 or 3, and

(SEQ ID NO: 61)
 Xaa₁ Xaa₂ Ile Ile Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Leu
 Xaa₁₁ [Gly]_n [Arg]_m Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆
 Xaa₁₇ Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅

wherein the Xaa in position 1 is Pro, Lys, Arg or none

Xaa in position 2 is Glu, Arg, Phe or Lys

Xaa in position 5 is Pro or Thr

Xaa in position 6 is Met, Thr or Nleu

Xaa in position 7 is Phe or Leu

Xaa in position 8 is Ser, Thr, Ala or Met

Xaa in position 9 is Ala, Glu or Leu

Xaa in position 11 is Ser or none

Xaa in position 12 is Ala, Arg or none

Xaa in position 13 is Ile, Leu or none

Xaa in position 14 is Ser, Ala, Leu or none

Xaa in position 15 is Tyr, Glu or Asp

Xaa in position 16 is Gly or Asp

Xaa in position 17 is Ala or Leu

Xaa in position 18 is Thr, Ile, Val, Leu or Asn,

Xaa in position 19 is Pro, Thr or Ser

Xaa in position 20 is Tyr, Phe, Nleu, His or Gln

Xaa in position 21 is Asp, Asn, Leu or Ala

Xaa in position 22 is Leu, Ile, Val or Asn

Xaa in position 23 is Asn, Tyr, Cys or Gly

Xaa in position 24 is Thr, Met, Ile, Ala, Val or none

Xaa in position 25 is Gly or none

n=1, 2 or 3 and m=0, 1, 2 or 3 independent of each other, the terminal ends of each HIV specific peptide may be free carboxyl- or amino groups, amides, acyls, acetyls or salts thereof. In some embodiments, two or more of the Cys residues of said HIV-specific peptide may form part of an intra-chain- or interchain disulphide binding, a —S—(CH₂)_p—S— or a —(CH₂)_p—bridge wherein p=1-8 optionally intervened by one or more heteroatoms such as O, N and S and/or the said peptide sequences are immobilized to a solid support. In some embodiments, the amino acid sequence of SEQ ID NO:47 is selected from the groups of SEQ ID NO:48 and SEQ ID NO:49. In some embodiments, the amino acid sequence of SEQ ID NO:50 is selected from the groups of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53 and SEQ ID NO:54. In some embodiments, the amino acid sequence of SEQ ID NO:55 is selected from the groups of SEQ ID NO:56 SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59 and SEQ ID NO:60. In some embodiments, the amino acid sequence of SEQ ID NO:61 is selected from the groups of SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65 and SEQ ID NO:66.

[0202] In some embodiments, the at least one HIV-specific peptide as used according to the present invention comprises at least two, three, or four peptides selected from each of the groups of SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:55 and SEQ ID NO:61.

[0203] In some embodiments, the at least one HIV-specific peptide as used according to the present invention consist of or comprises the peptides of the SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:57 and SEQ ID NO:64.

[0204] In some embodiments, the at least one agent capable stabilising association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120 as used according to the present invention is a molecule comprising at least one amino acid sequence selected independently from an amino acid sequence derived from the transmembrane domain of gp41

and an amino acid sequence derived from the C2 domain, wherein the at least one amino acid sequence binds the C5 domain, optionally comprising at least one D-amino acid. In some embodiments, this molecule is a peptide. In some embodiments, this peptide consists of at least one amino acid sequence. In some embodiments, the amino acid sequence derived from the transmembrane domain of gp41 has an amino acid sequence of at most 10 amino acid residues.

[0205] In some embodiments, the at least one agent capable stabilising association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120 as used according to the present invention is selected from an antibody, an antibody fragment or an antibody analogue. In some embodiments, this antibody which is a fully human antibody, a humanized antibody, or a chimeric antibody, or a derivative thereof. In some embodiments, the antibody is IgA, an IgD, an IgG, an IgE or an IgM. In some embodiments, the antibody fragment is selected from a Fab fragment, a Fab' fragment, a Fab'-SH fragment, a F(ab)₂ fragment, a F(ab')₂ fragment, an Fv fragment, a Heavy chain Ig (a llama or camel Ig), a V_HH fragment, a single domain FV, and a single-chain antibody fragment. In some embodiments, the antibody analogue is selected from a scFV, a dsFV, a minibody, a diabody, a triabody, a kappa body, an IgNAR, a tandAb, a BiTE, and a multispecific antibody.

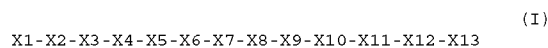
[0206] In some embodiments, the at least one agent capable of stabilising the association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120 binds to and stabilises association between one or more amino acid residues in the amino acid stretch TZ¹AKRRVVZ²REKR, where Z¹ is K, R or E and where Z² is Q or E, and one or more amino acid residues in an amino acid stretch in the transmembrane domain of gp41 and/or in the constant C2 domain of gp120.

[0207] In some embodiments, the immunogen referred to above to be used according to the present invention is a peptide multimer comprising

[0208] a first peptide comprising the amino acid sequence of the 13 amino acid residue amino acid sequence of the C5 domain of HIV gp120 including between 0 and 4 amino acid substitutions, a subsequence thereof, or an amino acid sequence comprising the inverso-, retro- or retro-inverso form of said amino acid sequence or subsequence, and

[0209] at least one second peptide comprising an amino acid stretch present in the transmembrane domain of gp41 or present in the constant C2 domain of gp120 or comprising an amino acid stretch present in any one of SEQ ID NOs. 6-13 or comprising a inverso-, retro- or retro-inverso form of an amino acid stretch present in the transmembrane domain of gp41 or present in the constant C2 domain of gp120, wherein said peptide multimer is capable of inducing an antibody which can bind and stabilise the association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120, and wherein said peptide multimer lacks amino acids N-terminal of C5 in gp120.

[0210] In some embodiments, the first peptide comprises the amino acid sequence having formula I:



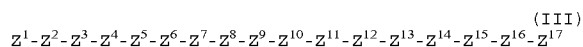
wherein X1 is Thr, X2 is selected from Lys, Arg, Har and Glu, X3 is selected from Ala and Val, X4 is selected from Arg, Har, Lys and Cit (citrulline), X5 is selected from Arg, Har, Lys and Cit, X6 is selected from Arg, Har, Lys and Cit, X7 is selected from Val, Leu, Ile and Nle (norleucin), X8 is selected from Val, Leu, Ile and Nle, X9 is selected from Gln, Glu, Asn and Asp, X10 is selected from Arg, Har and Cit, X11 is selected from Glu and Asp, X12 is Lys, and X13 is selected from Arg, Har and Cit,

or comprises a subsequence the amino acid sequence of formula I, or comprising the inverso-, retro- or retro-inverso form of said amino acid sequence or subsequence.

[0211] In some embodiments, the first peptide further comprises the dipeptide Ala-Pro linked to the N-terminus of the amino acid sequence having formula I.

[0212] In some embodiments, the first peptide further comprises the dipeptide X14-X15 linked to the C-terminus of the amino acid sequence having formula I, wherein X14 is selected from Ala and Val, and wherein X15 is selected from Val, Leu and Nle.

[0213] In some embodiments, the at least second peptide includes an amino acid sequence having the formula:



wherein Z^1 is Asp, Z^2 is Arg, Z^3 is Pro, Z^4 is Glu or Gly, Z^5 is Gly or Arg, Z^6 is Ile, Z^7 is Glu, Z^8 is Glu, Z^9 is Glu, Z^{10} is Gly, Z^{11} is Gly, Z^{12} is Glu or is absent, Z^{13} is Arg or Gln, Z^{14} is Asp or Gly, Z^{15} is Arg or Lys, Z^{16} is Asp or Gly and Z^{17} is Arg, or includes a subsequence of formula (III).

[0214] In some embodiments, the second peptide includes at least 5 consecutive amino acid residues from formula III.

[0215] In some embodiments, the first peptide and the at least one second peptide are associated via a linker. In some embodiments, the linker is selected from the group consisting of a bis-maleimide linker, a disulfide linker, a polyethylene glycol (PEG) linker, a glycine linker, a lysine linker, and an arginine linker.

[0216] In some embodiments, the at least one of the first and at least one second peptides comprises an N- or C-terminal modification, such as an amidation, acylation, or acetylation.

[0217] In some embodiments, the peptide multimer to be used according to the present invention is coupled to a carrier molecule, such as an immunogenic carrier. In some embodiments, the carrier is a virus like particle.

[0218] In some embodiments, the first peptide is selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 38, 41 and 44 or a fragment thereof, or the inverso-, retro- or retro-inverso form of a peptides selected from SEQ ID NO:1, 2, 3, 4, 5, 38, 41 and 44 or a fragment thereof, and wherein the second peptide is selected from the group consisting of SEQ ID NO:6, 7, 8, 9, 10, 11, 12, 13, 37, 39, 40, 42, 43, 45, 46 or a fragment thereof, or the inverso-, retro- or retro-inverso form of a peptides selected from SEQ ID NO:6, 7, 8, 9, 10, 11, 12, 13, 37, 39, 40, 42, 43, 45, 46 or a fragment thereof, and/or wherein the peptide multimer is selected from the peptides having SEQ ID NOs: 1-46.

[0219] In some embodiments, the peptide multimer to be used according to the present invention comprises at most 70 amino acids.

[0220] In some embodiments, the peptide multimer to be used according to the present invention comprises at least 6 amino acid residues.

[0221] In some embodiments, the peptide multimer to be used according to the present invention consist of a number of amino acid residues selected from the group consisting of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, and 70 amino acid residues.

[0222] In some embodiments, the peptide multimer to be used according to the present invention is selected from the group consisting of disulphide linked peptides between SEQ ID NO:28 and any one of SEQ ID NOs: 29, 31, and 33, between SEQ ID NO:30, and any one of SEQ ID NO:29, 31, and 33, or between SEQ ID NO:32 and any one of SEQ ID NO:29, 31, and 33; or selected from the group consisting of cysteine-lysine linked peptides between SEQ ID NO:38 and any one of SEQ ID NO:39, SEQ ID NO:40; SEQ ID NO:42, SEQ ID NO:43, and SEQ ID NO:68, or between SEQ ID NO:41 and any one of SEQ ID NO:39, SEQ ID NO:40; SEQ ID NO:42, and SEQ ID NO:43.

[0223] In some embodiments, the peptide multimer to be used according to the present invention is selected from the group consisting of:

CGGAKRRVVGGA	KRRVVGQREKRAV	(SEQ ID NO: 28)
CGGGDQQLLGG	AEIEIVGGIEEGGERDRDR,	(SEQ ID NO: 29)
CGGAKRRVVGGA	KRRVVGQREKR	(SEQ ID NO: 30)
CGGGDQQLLGG	AEIEIVGGIEEGG,	(SEQ ID NO: 31)
CGGAEEEVVGG	DQQLL	(SEQ ID NO: 32)
GCGGAKRRVVG	GAKRRVV,	(SEQ ID NO: 33)
GAKRRVVGCGG	AKRRVVQREKRAGEREKRA	(SEQ ID NO: 38)
GKGGIEEGGRDR	RGGEQDRDR,	(SEQ ID NO: 39)
GAKRRVVGCGG	AKRRVVQREKRAGEREKRA	(SEQ ID NO: 38)
GKGGIEEGGERDR	RGQDRDR,	(SEQ ID NO: 40)
GAKRRVVGCGG	AKRRVVEREKRAGQREKRA	(SEQ ID NO: 41)
GKGGIEEGGQDR	DRGGRDRDR,	(SEQ ID NO: 42)
GAKRRVVGCGG	AKRRVVEREKRAGQREKRA	(SEQ ID NO: 41)
GKGGIEEGGEQDR	DRGGERDRD and	(SEQ ID NO: 43)
GAKRRVVGCGG	AKRRVVQREKRAGEREKRA	(SEQ ID NO: 38)
GKGGIEEGGRDR	RGQDRDR.	(SEQ ID NO: 68)

[0224] In some embodiments, the peptide multimer to be used according to the present invention is selected (H-Gly-Ala-Lys-Arg-Arg-Val-Val-Gly-Gly-Cys(2-oxo-ethyl)-Gly-Gly-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Gly-Glu-Arg-Glu-Lys-Arg-Ala-NH2) (H-Gly-Lys-Gly-Gly-Ile-Glu-Glu-Glu-Gly-Gly-Arg-Asp-Arg-Asp-Gly-Gly-Gln-Asp-Arg-Asp-Arg-NH2), acetate salt (amide bond between Cys(2-oxo-ethyl)10 (A-chain) and Lys2 (B-chain)).

[0225] Another aspect of the present invention relates to a method for reducing and/or delaying the risk of developing or the pathological effects of human retrovirus infection, such as immunodeficiency virus I (HIV) in a human infected with such virus, such as HIV, the method comprising administering sequentially in any order or simultaneously (1) a compound to stimulate the humoral immunity in a subject and/or

one or more peptide that elicit a cell-mediated immune response in a subject; in combination with (2) an immunomodulatory compound and/or a reservoir purging agent, such as a Histone deacetylase (HDAC) inhibitor. In some embodiments, the human retrovirus infection is selected from HTLV-1 (human T-lymphotropic virus), HTLV-II (human T-lymphotropic virus), human immunodeficiency virus including HIV-1 and HIV-2, and any subtype thereof. In some embodiments, the immunomodulatory compound is as defined above. In some embodiments, the reservoir purging agent, such as a Histone deacetylase (HDAC) inhibitor is as defined above. In some embodiments, the one or more peptide that elicit a cell-mediated immune response is as defined above. In some embodiments, the compound to stimulate the humoral immunity in a subject is as defined above.

Example 1

[0226] Peptides to be used according to the present invention that elicit Cell-mediated immunity (CMI) or peptides that stimulate the humoral immunity in a subject were synthesized using conventional techniques for linear sequences as described in international patent applications WO0052040, WO 2012/092934 or WO 2012/072088.

[0227] Specifically compounds to stimulate the humoral immunity in a subject by inducing antibodies that stabilise association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120 were prepared as described in WO2011/000962.

[0228] Also compounds to stimulate the humoral immunity in a subject by stabilising the association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120 were prepared as described in WO2011/000962.

[0229] Immunomodulatory compound and a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor used according to the present invention are well known to the person skilled in the art, and are commercially available.

Synthesis of Peptides Using Conventional Techniques for Linear Sequences

[0230] Preparation of APTKAKRRVVQREKR

[0231] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of F-moc synthesis (Atherton et al. 1978 J. Chem. Soc. Chem Commun 539), which is below termed "the general description of synthesis."

[0232] Purity (HPLC): more than 90%.

[0233] Mass spectral analysis: Theoretical molecular weight: 1822.2

[0234] Experimental molecular weight: 1823.0 ES+

[0235] Preparation of APTKAKR

[0236] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0237] Purity (HPLC): more than 90%.

[0238] Mass spectral analysis: Theoretical molecular weight: 769.6

[0239] Experimental molecular weight: 760.7 ES+

[0240] Preparation of APTRAKR

[0241] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0242] Purity (HPLC): more than 90%.

[0243] Mass spectral analysis: Theoretical molecular weight: 797.6

[0244] Experimental molecular weight: 797.6 ES+

[0245] Preparation of APTEAKR

[0246] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0247] Purity (HPLC): more than 90%.

[0248] Mass spectral analysis: Theoretical molecular weight: 770.9

[0249] Experimental molecular weight: 770.9 ES+

[0250] Preparation of RVVEREK

[0251] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0252] Purity (HPLC): more than 90%.

[0253] Mass spectral analysis: Theoretical molecular weight: 914.1

[0254] Experimental molecular weight: 913.9 ES+

[0255] Preparation of RVVQREK

[0256] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0257] Purity (HPLC): more than 90%.

[0258] Mass spectral analysis: Theoretical molecular weight: 913.1

[0259] Experimental molecular weight: 913.0 ES+

[0260] Preparation of AKRRVV

[0261] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0262] Purity (HPLC): more than 90%.

[0263] Mass spectral analysis: Theoretical molecular weight: 726.9

[0264] Experimental molecular weight: 726.9 ES+

[0265] Preparation of DRPEGIEEGGERDR

[0266] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0267] Purity (HPLC): more than 90%.

[0268] Mass spectral analysis: Theoretical molecular weight: 1742.1

[0269] Experimental molecular weight: 1742.8

[0270] Preparation of VERYLKDQQLG

[0271] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0272] Purity (HPLC): more than 90%.

[0273] Mass spectral analysis: Theoretical molecular weight: 1460.7

[0274] Experimental molecular weight: 1460.1

[0275] Preparation of VERYLKDEELLG

[0276] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0277] Purity (HPLC): more than 90%.

[0278] Mass spectral analysis: Theoretical molecular weight: 1462.6

[0279] Experimental molecular weight: 1463.0

[0280] Preparation of VERYLKDNLLG

[0281] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

- [0282] Purity (HPLC): more than 90%.
- [0283] Mass spectral analysis: Theoretical molecular weight: 1432.6
- [0284] Preparation of QLLNGSLAEEIV
- [0285] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0286] Purity (HPLC): more than 90%.
- [0287] Mass spectral analysis: Theoretical molecular weight: 1639.9
- [0288] Preparation of QLLNGSLAEEVV
- [0289] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0290] Purity (HPLC): more than 90%.
- [0291] Mass spectral analysis: Theoretical molecular weight: 1625.9
- [0292] Preparation of QLLNSLAEEVV
- [0293] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0294] Purity (HPLC): more than 90%.
- [0295] Mass spectral analysis: Theoretical molecular weight: 1568.8
- [0296] Preparation of APTKAKRGGGAPTRAKRGG-GAPTEAKR
- [0297] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0298] Purity (HPLC): more than 90%.
- [0299] Mass spectral analysis: Theoretical molecular weight: 2647.0
- [0300] Experimental molecular weight: 2646.3 ES+
- [0301] Preparation of RVVEREKGGGAKRRV-VGGGRVVQREK
- [0302] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0303] Purity (HPLC): more than 90%.
- [0304] Mass spectral analysis: Theoretical molecular weight: 2862.3
- [0305] Experimental molecular weight: 2863.3 ES+
- [0306] Preparation of GGAKRRVVGAKRRV-VGQREKRAV
- [0307] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0308] Purity (HPLC): more than 90%.
- [0309] Mass spectral analysis: Theoretical molecular weight: 2590.1
- [0310] Preparation of CGGAKRRVVGAKRRV-VGQREKRAV
- [0311] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0312] Purity (HPLC): more than 90%.
- [0313] Mass spectral analysis: Theoretical molecular weight: 2693.2
- [0314] Preparation of GGAKRRVVGAKRRVVG-GQREKR
- [0315] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0316] Purity (HPLC): more than 90%.
- [0317] Mass spectral analysis: Theoretical molecular weight: 2476.9
- [0318] Preparation of CGGAKRRVVGAKRRVVG-GQREKR
- [0319] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0320] Purity (HPLC): more than 90%.
- [0321] Mass spectral analysis: Theoretical molecular weight: 2580.0
- [0322] Preparation of GGAKRRVVGAKRRVV
- [0323] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0324] Purity (HPLC): more than 90%.
- [0325] Mass spectral analysis: Theoretical molecular weight: 1665.0
- [0326] Preparation of GCGAKRRVVGAKRRVV
- [0327] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0328] Purity (HPLC): more than 90%.
- [0329] Mass spectral analysis: Theoretical molecular weight: 1768.1
- [0330] Preparation of GGGDQQLGGAEIVG-GIEEGGERDR
- [0331] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0332] Purity (HPLC): more than 90%.
- [0333] Mass spectral analysis: Theoretical molecular weight: 3127.2
- [0334] Preparation of CGGGDQQLGGAEIVG-GIEEGGERDR
- [0335] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0336] Purity (HPLC): more than 90%.
- [0337] Mass spectral analysis: Theoretical molecular weight: 3230.4
- [0338] Preparation of GGDQQLGGAEIVGG-ERDR
- [0339] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0340] Purity (HPLC): more than 90%.
- [0341] Mass spectral analysis: Theoretical molecular weight: 2242.4
- [0342] Preparation of CGGGDQQLGGAEIVG-GIEEGG
- [0343] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0344] Purity (HPLC): more than 90%.
- [0345] Mass spectral analysis: Theoretical molecular weight: 2402.5
- [0346] Preparation of GGAEIVVGGDQQL
- [0347] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.
- [0348] Purity (HPLC): more than 90%.
- [0349] Mass spectral analysis: Theoretical molecular weight: 1499.6

[0350] Preparation of CGGAEEVVGGDQQLL

[0351] The peptide was synthesized in amide form, from the corresponding starting point according to the general description of synthesis.

[0352] Purity (HPLC): more than 90%.

[0353] Mass spectral analysis: Theoretical molecular weight: 1602.7

Synthesis of Complexed C5 Related Peptides to be Used According to the Present Invention:

[0354] Preparation of

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CGGAKRRVVGGAKRRVVGGQREKRAV
|
CGGGDQQLLGGAAEEIIVGGIEEGGERDRD
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[0355] Purity (HPLC): more than 90%.

[0356] Mass spectral analysis: Theoretical molecular weight: 5750.4

[0357] Experimental molecular weight:

[0358] Preparation of

```
CGGAKRRVVGGAKRRVVGGQREKR
|
CGGGDQQLLGGAAEEIIVGGIEEGG
```

[0359] Purity (HPLC): more than 90%.

[0360] Mass spectral analysis: Theoretical molecular weight: 4965.6

[0361] Experimental molecular weight:

[0362] Preparation of

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CGGAEEVVGGDQQLL
|
CGGAKRRVVGGAKRRVV
```

[0363] Purity (HPLC): more than 90%.

[0364] Mass spectral analysis: Theoretical molecular weight: 3410.9

[0365] Experimental molecular weight:

[0366] In the following, a summary of the procedures for the synthesis and purification of C5-Peptide is given. Experience is still limited which may eventually lead to improvements in the manufacture and quality of this product.

[0367] The SPPS synthesis was started with 15 mmole (A-chain) and 30 mmole (B-chain) of the resin. After purification of a part of the crude C5-Peptide, 16.6 g of final product were obtained.

Stage 1: Solid Phase Peptide Synthesis

[0368] The amino acid sequences of the A-chain (H-Gly-Ala-Lys-Arg-Val-Val-Gly-Cys-Gly-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Gly-Glu-Arg-Glu-Lys-Arg-Ala-NH₂) and the B-chain (H-Gly-Lys-Gly-Gly-Ile-Glu-Glu-Glu-Gly-Gly-Arg-Asp-Arg-Asp-Arg-Gly-Gly-Gln-Asp-Arg-Asp-Arg-NH₂) are assembled by standard solid phase peptide synthesis (SPPS) using the Fmoc-strategy. (W. C. Chan and P. D. White; Solid Phase Peptide Synthesis—A Practical Approach, Oxford University Press Inc., New York, 2000, ISBN 978-0-19-963724-9)

[0369] The solid phase is transferred into an SPPS reactor and the synthesis cycle is started with the Fmoc-deprotection.

Following the deprotection step, the peptide chain is elongated by the coupling of the following N- α -protected AA derivative or the dipeptide according to the amino acid sequence in the presence of suitable activating agents. To avoid the formation of long peptide sequences as by-products, a systemic acetylation of unreacted peptide chains (capping procedure) can be performed after every coupling step.

[0370] For each single step, the solvents and/or reagents are added, and the reaction mixture is stirred and then filtered to remove solvents and/or reagents from the resin. Single steps of the SPPS cycle may be repeated in case the reaction is incomplete. The SPPS cycle is repeated until the solid phase carries the complete amino acid sequence of the A-chain or the B-chain.

[0371] For the A-chain, a final Fmoc-deprotection is performed and the SPPS is completed by drying the peptide resin under reduced pressure.

[0372] The B-chain is further modified with a bromoacetyl linker at Lys². This procedure consists of selectively cleaving the side-chain protecting group of Lys² and coupling bromoacetic acid to Lys² in the presence of a suitable activating agent. If the coupling reaction is incomplete, recoupling procedures can be performed. The SPPS is then completed by drying the peptide resin under reduced pressure.

Stage 2: Cleavage from the Resin Including Cleavage of the Acid Labile Protecting Groups

[0373] Cleavage of the peptides from the resin and concomitant cleavage of the acid labile protecting groups is accomplished by treatment with TFA in the presence of water. Scavengers are added as needed to trap reactive cations and to avoid alkylation of side-chain functions. After filtering off and washing the resin with TFA, the products are precipitated in IPE. They are filtered off, washed with IPE, and dried under reduced pressure.

Stage 3: Purification of the Intermediates by Preparative HPLC (TFA System)

[0374] The A-chain and the B-chain obtained in the previous stage are purified by preparative HPLC on reversed phase columns with ACN gradient elution (TFA system) and UV detection at $\lambda=220$ nm.

[0375] Portions of the peptides are dissolved in water or a mixture of water and acetic acid and loaded onto the column. Subsequently, the ACN gradient of the TFA system is started. The collected fractions are checked by analytical HPLC and pooled accordingly.

[0376] Side fractions can be repurified with the TFA system. Finally, the pooled fractions with adequate purity are lyophilized.

Stage 4: Coupling of A-Chain and B-Chain

[0377] The coupling of the two peptide chains is performed by the addition of a solution of the B-chain (1 equivalent) in aqueous TFA to a solution of the A-chain (1 equivalent) in TRIS buffer (adjusted to pH 8.5 by the addition of hydrochloric acid). Additional TRIS buffer is added to maintain a pH >8 in the reaction mixture. The reaction mixture is then stirred and the reaction progress is monitored by analytical HPLC. Upon completion, the pH of the reaction mixture is lowered to approx. pH 3 by the addition of TFA.

Stage 5: Purification by Preparative HPLC (TFA System)

[0378] The C5-peptide obtained in the previous stage is purified by preparative HPLC on reversed phase columns with ACN gradient elution (TFA system) and UV detection at $\lambda=220$ nm. Portions of the C5-peptide are directly loaded onto the column. Subsequently, the ACN gradient of the TFA system is started. The collected fractions are checked by analytical HPLC and pooled accordingly.

[0379] Side fractions can be repurified with the TFA system. Finally, the pooled fractions with adequate purity are lyophilized.

Stage 6: Ion Exchange, Microfiltration, and Lyophilization

[0380] The last stage of the manufacture of C5-Peptide is the ion exchange from the TFA salt, obtained in the previous stage, into the acetate salt.

[0381] The lyophilized material from the TFA purification is dissolved in 5% acetic acid and the solution loaded onto the ion exchange resin (acetate form). The elution is performed with 5% acetic acid and checked by TLC. The product solution is filtered through a 0.2 μ m membrane filter and lyophilized to yield the final product as a white to off-white material.

Preparation of HIV-Specific Peptides that Elicit a Cell-Mediated Immune Response to be Used in Compositions and Methods According to the Invention

[0382] The peptides to be used according to the present invention can be produced by any known method of producing a linear amino acid sequence, such as recombinant DNA techniques. A nucleic acid sequence which encodes a peptide to be used according to the invention or a multimer of the said peptides, is introduced into an expression vector. Suitable expression vectors are for instance plasmids, cosmids, viruses and YAC (yeast artificial chromosome) which comprise necessary control regions for replication and expression. The expression vector may be stimulated to expression in a host cell. Suitable host cells are for example bacteria, yeast cells and mammal cells. Such techniques are well known in the art and described for instance by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989. Other well-known techniques are degradation or synthesis by coupling of one amino acid residue to the next one in liquid phase or preferably on a solid phase (resin) for instance by the so-called Merrifield synthesis. See for instance Barany and Merrifield in the *Peptides, Analysis, Synthesis, Biology*, Vol. 2, E. Gross and Meinhofer, Ed. (Acad. Press, N.Y., 1980), Kneib-Coronier and Mullen *Int. J. Peptide Protein Res.*, 30, p. 705-739 (1987) and Fields and Noble *Int. J. Peptide Protein Res.*, 35, p. 161-214 (1990).

[0383] In case a linked or cyclic peptide is desired, the amino acid sequence is subjected to a chemical oxidation step in order to cyclize or link the two cysteine residues within one or between two peptide sequences, when the appropriate linear amino acid sequences are synthesized, see Akaji et al., *Tetrahedron Letter*, 33, 8, p. 1073-1076, 1992.

[0384] All peptide derivatives prepared in the Examples given below were synthesized on a Milligen 9050 Peptide Synthesizer using a standard program. The resin used was Tent Gel P RAM with a theoretical loading of 0.20 meq/g (RAPP POLYMERE GmbH, Tübingen). The final product of the synthesis was dried in vacuo overnight. The peptide was then cleaved from the resin by treatment with 90% trifluoro-

acetic acid in the presence of ethanedithiol (5%) and water (5%) as scavengers (1.5 hours at RT). Then the resin was filtered and washed on filter with additional trifluoroacetic acid (100%) (2x20 ml). The combined filtrates were evaporated in vacuo (water bath at RT) and the residue was triturated with ethyl ether (200 ml) and the precipitated product filtered off. The solid was promptly dissolved on filter with glacial acetic acid (100 ml) and added to 1.5 l of 20% acetic acid in methanol and treated with 0.1 M solution of iodine in methanol until a faint brown colour remained. Then Dowex 1x8 ion exchange in acetate form (15 g) (Bio-Rad, Richmond, Calif.) was added and the mixture filtered. The filtrate was evaporated and the residue freeze-dried from acetic acid. The product was then purified by reversed phase liquid chromatography on a column filled with Kromasil® 100-5 C8 (EKA Nobel, Surte, Sweden) in a suitable system containing acetonitrile in 0.1% trifluoroacetic acid water solution. The samples collected from the column were analyzed by analytical high performance liquid chromatography (HPLC) (Beckman System Gold, USA) equipped with a Kromasil® 100-5 C8 Column (EKA Nobel, Surte, Sweden). Fractions containing pure substance were pooled, the solvent was evaporated and the product freeze-dried from acetic acid. The final HPLC analysis was performed on final product, and the structure of the peptide was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0385] All amino acids used during the synthesis were L-amino acids and they were protected with a fluorenylmethoxy-carbonyl group at the α -amino function. The side chains were protected as follows:

Cys (Trt), Gln(Trt), Glu(OtBu), Thr(tBu).

[0386] The abbreviations, within the brackets are:

Trt=triphenylmethyl

t-Bu=tert. Butyl

OtBu=tert. Butylester

[0387] The amino acid derivatives was supplied by Bachem AG, Switzerland.

[0388] Preparation of K A L G P G A T L Q T P W T A C Q G V G-NH₂ (SEQ ID NO:48).

[0389] The peptide was synthesized in amide form, from corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0390] Purity (HPLC): 87%

[0391] Preparation of R A L G P A A T L Q T P W T A S L G V G (SEQ ID NO:49).

[0392] The peptide was synthesized in amide form, from corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0393] Purity (HPLC): more than 95%

[0394] Molecular weight (free base): 1966

[0395] Molecular formula: C₈₈H₁₄₄O₂₅N₂₆

[0396] Preparation of W I I P G L N P L V G G G K L Y S P T S I L C G-NH₂ (SEQ ID NO:51).

[0397] The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0398] Purity (HPLC): 95%

[0399] Mass spectral analysis: Theoretical molecular weight: 2454.9

[0400] Experimental molecular weight: 2454.8 ES+

[0401] Preparation of R W L L G L N P L V G G G R L Y S P T S I L G (SEQ ID NO:52).

[0402] The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0403] Purity (HPLC): more than 95%

[0404] Molecular weight (free base): 2552

[0405] Molecular formula: $C_{119}H_{195}O_{29}N_{33}$

[0406] Preparation of K I L L G L N P L V G G G R L Y S P T S I L G (SEQ ID NO:53), R L L L G L N P L V G G G R L Y S P T T I L G (SEQ ID NO:54) and N I P I P V G D I Y G G G D I Y K R W Q A L C L (SEQ ID NO:70). The peptides are synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity are determined by HPLC analysis and the structures are confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0407] Preparation of R N I P I P V G D I Y G G G D I Y K R W Q A L C L (SEQ ID NO:56).

[0408] The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0409] Purity (HPLC): 85%

[0410] Mass spectral analysis: Theoretical molecular weight: 2817.3

[0411] Experimental molecular weight: 2813.7 ES+

[0412] Preparation of R A I P I P A G T L L S G G G R A I Y K R W A I L G (SEQ ID NO:57).

[0413] The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0414] Purity (HPLC): more than 95%

[0415] Molecular weight (free base): 2707

[0416] Molecular formula: $C_{125}H_{208}O_{29}N_{38}$

[0417] Preparation of A L P I P A G F I Y G G G R I Y K R W Q A L G (SEQ ID NO:58), K I P I P V G F I G G W I Y K R W A I L G (SEQ ID NO:59) and K I P I P V G T L L S G G G R I Y K R W A I L G (SEQ ID NO:60). The peptides are synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity are determined by HPLC analysis and the structures are confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0418] Preparation of K F I I P N I F S A L G G A I S Y D L N T N I L N C I (SEQ ID NO:62).

[0419] The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. NI in the sequence is Norleucine. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0420] Purity (HPLC): more than 80%

[0421] Mass spectral analysis: Theoretical molecular weight: 2783.3

[0422] Experimental molecular weight: 2783.3 ES+

[0423] Preparation of K F I I P N I F S A L S G G G A I S Y D L N T F L N C I G (SEQ ID NO:63).

[0424] The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. NI in the sequence is Norleucine. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0425] Purity (HPLC): more than 80%

[0426] Mass spectral analysis: Theoretical molecular weight: 2932.4

[0427] Experimental molecular weight: 2931.8 ES+

[0428] Preparation of R F I I P N I F T A L S G G R R A L L Y G A T P Y A I G (SEQ ID NO:64).

[0429] The peptide was synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. NI in the sequence is Norleucine. The purity was determined by HPLC analysis and the structure was confirmed by amino acid analysis and mass spectrometry (LDI-MS).

[0430] Purity (HPLC): more than 95%

[0431] Molecular weight (free base): 2894

[0432] Molecular formula: $C_{137}H_{217}O_{32}N_{37}$

[0433] Preparation of K I I P N I F S A L G G G R L L Y G A T P Y A I G (SEQ ID NO:65), R I I P N I F T A L S G G R L L Y G A T P Y A I G (SEQ ID NO:66) and W I I P N I F S A L G G A I S Y D L N T N I L N C I (SEQ ID NO:71). The peptides are synthesized in amide form, from the corresponding starting materials according to the general description of synthesis. The purity are determined by HPLC analysis and the structures are confirmed by amino acid analysis and mass spectrometry (LDI-MS).

Dimerisation Via Disulphide Bridge.

[0434] The peptide sequences of examples above were linked via an oxidation step to form a dipeptide wherein the cysteine residues formed a disulphide bridge. The bridge was formed in either ways;

[0435] A) Oxidation with I_2 . Equal amounts of the peptides were dissolved in acetic acid/methanol (1:4) and 0.1 M I_2 in methanol was added yielding a mixture of the dimer.

or

[0436] B) Oxidation via [Cys(Spy)¹⁶]-SEQ ID NO:48. 2.3 mM of the peptide of SEQ ID NO:48 dissolved in 2 M AcOH (aq) and 2-propanol (1:1) was treated with 2,2 dithiodipyridin (3 eqv) to yield [Cys(Spy)¹⁶]-SEQ ID NO:48. Equal amounts of [Cys(Spy)¹⁶]-SEQ ID NO:48 and peptide of SEQ ID NO:51 were dissolved in 10 mM NH_4Oac (aq pH=6, 5) and methanol (5:2) to yield the dimer of SEQ ID NO:67.

[0437] The purity of the peptide was determined by HPLC analysis and the peptide structure was confirmed by amino acid analysis. The peptide content (aminoacid free base) was 80%.

[0438] Purity (HPLC): 92%.

Example 2

[0439] A vaccine comprising the peptides of the SEQ ID NO:49, 52, 57 and 64 was prepared. The freeze-dried peptides are dissolved in sterile water at a final concentration of

4 mg/ml. The final salt concentration was 0.9%. A preparation of a granulocyte-macrophage-colony stimulating factor (GM-CSF) was also prepared, according to the manufacturers directions for use, to a final concentration of 0.3 mg/ml. The two solutions are administered intracutaneously. A typical injection dose is 100 μ l.

Example 3

[0440] An antigen solution or suspension is mixed with equal parts of Freund's adjuvant of Behring, complete or incomplete, and is then finely emulsified by being drawn up into, and vigorously pressed out of, an injection syringe, or with a homogenator. The emulsion should remain stable for at least 30 minutes. The antigen-adjuvant emulsions is best injected subcutaneously as a depot.

Example 4

Toxicity Data

[0441] The dipeptides of the examples are diluted in 0.9% NaCl to a test solution concentration of 4 mg/ml. The peptide is administered by injection to NMFI female mice in a dose of 100 μ g per kg bodyweight. No toxicological effects are observed and the peptide is deemed not toxic.

[0442] Toxicity studies are performed in mice and rats on the peptide composition of the vaccine. The mouse is selected for the study to provide comparative data from a second commonly used rodent species. The test substance is a mixture of four peptides supplied as one vial containing lyophilised material for reconstitution with physiological saline, and dose levels are expressed in terms of total peptide load. The individual peptides is present in ratio 1:1:1:1 giving dose levels of each peptide of 0.0075 mg/kg body weight, 0.075 mg/kg body weight and 0.75 mg/kg body weight, which are up to 500 fold the intended human dose. The test animals are divided into four groups of ten animals each (five males and five females); a saline control group and groups for low, intermediate and high doses. The test composition is administered once, by intravenous infusion into a tail vein at a dose rate of 3 ml/minute. The animals are killed at day 15 and 16 by intraperitoneal injection of sodium pentobarbitone.

[0443] The results of these studies indicated that the dose levels administered to the mice and rats elicited no adverse reactions and that the no effect level is in excess of 3 mg/kg.

Example 5

Immunoassay for Detection of Antibodies Induced by HIV-1

[0444] The magnetic particle reagents are to be prepared according to the manufacturers recommended protocol. Dynal AS, is the manufacturer of the Dynabeads, which are employed. The magnetic particles coated with ligand are called Reagent 1. A peptide to be used according to the invention is covalently coupled to the pre-activated surface of the magnetic particles. It is also possible to physically absorb the peptide to the surface of the magnetic particles. The concentration of particles in Reagent 1 is within the range from 1 mg/ml to 15 mg/ml. The particle size varies between 0.2 μ m to 15 μ m. The concentration of peptides is within the range from 0.01 mg/mg particle to 1 mg/mg particle.

[0445] The anti human Ig Alkaline Phosphatase (AP) conjugated antibody reagent is prepared according to the recom-

mended protocol of Dako AS. This protocol is a standard procedure in this field. This reagent is called Reagent 2.

[0446] The substrate solution phenolphthalein-monophosphate is to be prepared according to the recommended protocol of Fluka AG. This protocol is a standard procedure in this field. The substrate solution is called Reagent 3.

[0447] The washing and incubation buffer which is used is standard 0.05M tris-base buffer with the following additional compounds; Tween 20 (0.01% to 0.1%), glycerol (0.1% to 10%) and sodium chloride (0.2% to 0.1%).

[0448] The assay procedure comprises an incubation step wherein 1 drop of Reagent 1 is mixed with 2 drops of washing buffer in each well. After mixing, 30 μ l of sample is added and the solution is incubated for 5 minutes. The magnetic particles can be trapped by a magnet and the liquid removed, before the magnet is separated. Then the wells are washed twice in 4 drops of washing solution, before incubation with Reagent 2. 1 drop of Reagent 2 is added with 2 drops of washing buffer and the solution is incubated for 5 minutes. The magnetic particles can be trapped by a magnet and the liquid removed, before the magnet is separated. Then the washing step is repeated before incubation with Reagent 3. 2 drops of Reagent 3 is added to each well and the solution is incubated for 3 minutes. The results can be read against a white background. Positive results are red (3+=strong red) whereas negative results are clearly light yellow/brown solutions as obtained in the negative control.

[0449] The immunoassay kit could be used in detection of antibodies, induced either by HIV virus or HIV-specific peptides or proteins, for instance the peptides of the present invention.

[0450] The above Examples are only meant as illustrating the invention. It must be understood that a person skilled in the art can modify the peptides, antigens and vaccines herein described without deviating from the concept and scope of this invention as set forth in the claims.

[0451] The polypeptides to be used according to the invention includes at least one peptide specifically disclosed in any of international patent applications WO2011/000962, WO0052040, WO 2012/092934 or WO 2012/072088 selected, including but not limited to any of SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:55 and SEQ ID NO:61 to as well as peptide multimers that are disulphide linked peptides between SEQ ID NO:28 and any one of SEQ ID NOs: 29, 31, and 33, between SEQ ID NO:30, and any one of SEQ ID NO:29, 31, and 33, or between SEQ ID NO:32 and any one of SEQ ID NO:29, 31, and 33; or cysteine-lysine linked peptides between SEQ ID NO:38 and any one of SEQ ID NO:39, SEQ ID NO:40; SEQ ID NO:42, SEQ ID NO:43, and SEQ ID NO:68, or between SEQ ID NO:41 and any one of SEQ ID NO:39, SEQ ID NO:40; SEQ ID NO:42, and SEQ ID NO:43, which form antigens and the active principle of a prophylactic or therapeutic vaccine provides for protection against the human immunodeficiency virus type 1 (HIV-1). The vaccine may include compounds having beneficial effects in protecting or stimulating the host's immune system (human being or vertebrate animal) for instance interleukins, interferons, granulocyte macrophage growth factors, haematopoietic growth factors or similar. Preferably the vaccine composition further contain an adjuvant or vehicle, more preferably the adjuvant or vehicle is Monophosphoryl Lipid A (MPL®) possibly with alum, Freund's adjuvant (complete or incomplete) or aluminum hydroxyd. The optimal amount of adjuvant/vehicle will depend on the type(s) which is chosen.

[0452] The peptide or vaccine formulation can be freeze-dried prior to storage. The vaccine may be stored preferably at low temperature, in ampoules containing one or more dosage units, ready for use. A typical dosage unit of the peptide to be used according to the invention is within the concentration range: 1 µg-1 mg per kg bodyweight, preferably within 2 µg-0.15 mg per kg body weight. Persons skilled in the art will appreciate that a suitable dose will depend on the body weight of the patient, the type of disease, severity of condition, administration route and several other factors. The vaccine might be administered up to twelve times and through injection, typically it will be administered about three times. In preparation of an injection solution the peptides are dissolved in sterile sodium chloride solution at a final concentration of 1 mg/ml per peptide and 0.9% sodium chloride. Typically an injection volume is 100 µl to 200 µl (2×100 µl). The peptide is preferably co-administered with a suitable adjuvant and/or a granulocyte-macrophage growth factor for instance Leucomax® (Shering Plough). Suitable administration may be intracutane, subcutane, intravenous, peroral, intramuscular, intranasal, mucosal or any other suitable route. Booster administrations may be required in order to maintain protection. For persons skilled in the art it will be understood that the vaccine compositions according to the invention are useful not only in prevention of infection, but also in treatment of infection.

Example 6

[0453] A clinical study testing the combination of the peptide composition of Example 8 and Vacc-C5 could be constructed based on pre-existing clinical data. The vaccination regime of Vacc-C5 could be 300 µg Vacc-C5 delivered intradermally 10 minutes following an intradermal injection of 0.33×10^6 IU Granulocyte-macrophage colony-stimulating factor (GM-CSF). The vaccination regime could be once weekly in weeks 1, 2, 4, 12, 13, 22 and 23. After period of immunological maturation, a vaccination schedule of the peptide composition could be 300 µg/peptide given intradermally 10 minutes following an intradermal injection of 0.33×10^6 IU GM-CSF. The peptide composition could be parentally administered at weeks 27, 28, 29, 30, 42, 44, with clinical and immunological parameters measured throughout the protocol, such as Delayed Hypersensitivity Reaction (DTH), serological parameters such as ELISA for Vacc-C5 antibody response and cellular parameters such as ELISPOT or proliferation for the peptide composition or corresponding native antigen. Alternatively, the peptide composition and Vacc-C5 (for instance 300 µg per peptide of the peptide composition and 300 µg Vacc-C5) could be combined in the same injection and be administered 10 minutes following intradermal injection of GM-CSF, once weekly at weeks 1, 2, 3, 4, 16 and 18, with clinical and immunological parameters measured throughout the protocol, such as DTH, serological parameters such as ELISA for Vacc-C5 antibody response and cellular parameters such as ELISPOT or proliferation for the peptide composition or corresponding native antigen. The adjuvant could also be ISA51, ISA720, Provac or other licenced or non-licenced adjuvant instead of GM-CSF, in both clinical studies.

Example 7

Test of Peptides Together with IMiDs for Increased Proliferation, Polyfunctionality, IL-2 Secretion and IFN-γ Production

[0454] Expansion of polyfunctional HIV-specific T-cells upon stimulation with Dendritic Cells, pre-incubated with

peptides to be used according to the invention, may be studied by methods described by Keersmaecker et al. (J. Virol., 2012 86:9351-9360) and referenced therein, HIV proteins Gag or Nef, they are incubated with peptides to be used according to the invention, before they are used to stimulate T-cells in a co-culture.

[0455] Keersmaecker et al. found that the presence of IMiDs (Lenalidomide (IMiD3; CC-5013) and pomalidomide (IMiD1; CC-4047) during in vitro T-cell stimulation with dendritic cells presenting Gag- or Nef-specific peptides, resulted in a number of improvements in the function of the T-cells. Among these were; polyfunctional HIV specific CD8+ T cells with enhanced lytic capacity, more Gag antigen epitopes recognized and at lower antigen peptide concentrations, reduced proliferation of CD4+ T cells with increased number of polyfunctional CD4+ T-cells, increased IL-2 production by CD8 T-cells, detectable IFN-γ production by CD8+ T-cells and CD4 T-cells after antigen stimulation.

[0456] "Expansion of Polyfunctional HIV-Specific T Cells upon Stimulation with mRNA Electroporated Dendritic Cells in the Presence of Immunomodulatory Drugs"

[0457] Brenda De Keersmaecker, Sabine D. Allard, Patrick Lacor, Rik Schots, Kris Thielemans, and Joeri L. Aerts

[0458] J. Virol. September 2012 86:9351-9360; published ahead of print 20 Jun. 2012, doi:10.1128/JVI.00472-12

Example 8

Suggested Clinical Study Protocol for the Test of Peptide Composition Comprising 4 Peptides in Combination with Lenalidomide and HDAC Inhibitor

[0459] Immunizations (four primary immunizations and two booster immunizations) at Weeks 1, 2, 3 and 4, and booster immunizations at Weeks 12 and 13 with either:

[0460] 1) Peptide composition with GM-CSF as adjuvant and Lenalidomide (CC-5013), or

[0461] 2) Peptide composition with GM-CSF as adjuvant and Placebo for Lenalidomide (CC-5013).

[0462] 3) Placebo

Suggested Doses:

[0463] Peptide composition: 0.6, 0.9, 1.2 and 1.5 mg (Equimolar amount of each peptide)

Lenalidomide: 5, 10, and 25 mg.

Vacc-C5: 100, 300 and 900 µg.

[0464] Subjects randomized to the Lenalidomide (CC-5013) arm will take a single oral dose of Lenalidomide (CC-5013) daily the two preceding days before immunization with the Peptide composition and on the day of each immunization.

[0465] The Peptide composition used according to this clinical trial setup consists of SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:57, and SEQ ID NO:64.

[0466] At week 20 subjects in all study arms will receive 20 mg panobinostat (LBH589) orally on days 1, 3, and 5 (i.e. 3 times a week) every other week for a period of 8 weeks (up to week 28) while maintaining background ART. This will be followed by a 24 week follow up period (up to week 52). Upon completion of the study, subjects may be invited to participate in an additional observational study in which ART

will be interrupted to evaluate the effect of study treatment on virological control. Enrolment into this part of the study will be optional and determined by the effect of study treatments on the latent HIV-1 reservoir. (Maximum duration of treatment interruption: 16 weeks).

In Summary:

[0467] Study arm 1: Peptide composition+IMiD+HDAC (panobinostat)

Study arm 2: Peptide composition+HDAC (panobinostat)

Study arm 3: HDAC (panobinostat)

[0468] Depletion of the viral reservoir as a result of the combination treatments according to the present invention may be quantified by for instance following the procedures set forth in Lehrman et al. (The Lancet (366), 2005, pp. 549-555) and references there in. In brief, this includes measuring in samples of patient blood obtained before, during and after treatment; p24 expression from stimulated latently infected cells, plasma HIV RNA concentration (viral load), and integrated HIV DNA by realtime PCR analysis.

Example 9

DC/T-Cell Proliferation Assay

[0469] Dendritic cells (DC) were generated from monocytes isolated from buffy coat preparations from healthy blood donors. Briefly, peripheral blood mononuclear cells were separated by a density gradient centrifugation and the monocytes were then negatively isolated using the Dynabeads Untouched Human Monocytes (Invitrogen, Carlsbad, Calif.) following the manufacturer's instructions. The monocytes were cultured with IL-4 (20 ng/ml; Immunotools, Friesoythe; Germany) and GM-CSF (100 ng/ml; Immunotools) in X-VIVO15 medium (Lonza, Basel, Switzerland) for 5-6 days to generate immature DC. Cytokines were replenished every 2-3 days. The maturation of the cells was performed for 24 hours with IFN- γ (1000 IU/ml), TNF- α (50 ng/ml), IL-1 β (25 ng/ml) IFN- α (3000 IU/ml). After maturation, the DC were pulsed for 2 hours at 37° C. with peptides at 10 μ g/ml, before extensive washing and co-culture with Peripheral blood mononuclear cells (PBMC) labelled with a fluorescent dye (VPD450, BD biosciences, San Jose, Calif.). Various ratios with DC:T cell were tested alongside with appropriate controls. IL-2 (50 U/ml) and IL-7 (50 ng/mL) (Both, Immunotools) and wells with or without IMiDs were added at the start of co-culture. At day 6-10, the level of T cell proliferation was analysed by flow cytometry. The supernatants from the co-culture wells were investigated with Luminex technology to establish any suppressor activity.

Example 10

Immunological Studies

Rabbit Immunizations

[0470] New Zealand White female rabbits were immunized intradermally at weeks 0, 2 & 6 with 1 ml of BI400-B vaccine consisting of 500 μ g BI400-B in 50% V/V Freund's adjuvant (i.e. Complete Freund's adjuvant used for priming, followed by boostings with Incomplete Freund's adjuvant). Animals optionally received a single dose of an immunomodulatory drug (IMiD), for instance daily the two preceding days before

immunization and on the day of each immunization. Individual blood serum was isolated for ELISA.

Direct ELISA for Human Sera

[0471] 50-100 μ l of a mixture of BI400-015 and -201 (pre-incubated in Coating buffer-0.05M Na₂CO₃ pH9.6; denoted CB-in cold at 16 μ g/ml for each peptide 1-3 days prior to coating) or just CB (background control) was used for coating wells in microtiter plates at 4° C. overnight. The microtiter plates were then washed 3 \times with washing buffer (PBS+1% v/v Triton-X100; denoted WB), followed by 2 h blocking at room temperature (RT) with 200 μ l/well of blocking buffer (PBS+1% w/v BSA). Plates were then washed 3 \times with WB, followed by 1 h incubation at 37° C. with 50-70 μ l/well of added human (serial dilutions ranging from 1:1-1:250 in dilution buffer (PBS+1% v/v Triton-X100+1% w/v BSA; denoted DB)). Plates were then washed 6 \times with WB, followed by 1 h incubation at RT with 70 μ l/well of Alkaline Phosphatase-conjugated Protein G (3 μ g/ml in DB; Calbiochem 539305). Plates were then washed 6 \times with WB, followed by 10-60 min incubation at room temperature with 100 μ l/well of 0.3% w/v of Phenolphthalein monophosphate (Sigma P-5758). Plates were finally quenched by adding 100 μ l/well of Quench solution (0.1M TRIS+0.1M EDTA+0.5M NaOH+0.01% w/v NaN₃; pH14), followed by ELISA reader (ASYS UVM 340) at 550 nm.

Competitive ELISA for Rabbit Sera after Immunization with BI400-B

[0472] 50-100 μ l of a mixture of BI400-015 and -201 (pre-incubated in Coating buffer-0.05M Na₂CO₃ pH9.6; denoted CB-in cold at 16 μ g/ml for each peptide 1-3 days prior to coating) or just CB (background control) was used for coating wells in microtiter plates at 4° C. overnight. Plates were then washed 3 \times with washing buffer (PBS+1% v/v Triton-X100; denoted WB), followed by 2 h blocking at room temperature (RT) with 200 μ l/well of blocking buffer (PBS+1% w/v BSA). Plates were then washed 3 \times with WB, followed by 1 h incubation at 37° C. with 60-100 μ l/well of added rabbit serum samples (diluted 1:10-1:250 final concentration) pre-incubated together (4° C. overnight) with serial dilutions (ranging from 10-1000 μ M final concentration) of 400-SEQ. B, BI400-015, BI400-201, BI400-204d, recombinant gp41 (Shin-Won Scientific, SWO 102 gp41), BI301-23 (irrelevant protein; control), no peptide (i.e. PBS; control), LTNP-sera pools (diluted 1:10 final concentration), or Blood donor sera-pools (diluted 1:10 final concentration; control). Plates were then washed 6 \times with WB, followed by 1 h incubation at RT with 70 μ l/well of Alkaline Phosphatase-conjugated Goat-anti-Rabbit-Ig (6 μ g/ml; Dako D0487). Plates were then washed 6 \times with WB, followed by 10-60 min incubation at RT with 100 μ l/well of 0.3% w/v of Phenolphthalein monophosphate (Sigma P-5758). Plates were finally quenched by adding 100 μ l/well of Quench solution (0.1M TRIS+0.1M EDTA+0.5M NaOH+0.01% w/v NaN₃; pH14), followed by ELISA reader (ASYS UVM 340) at 550 nm.

Example 11

[0473] The peptides according to the invention used in the following examples were synthesized by Schafer-N as c-terminal amides using the Fmoc-strategy of Sheppard, (1978) J. Chem. Soc., Chem. Commun., 539.

Cell Penetration Assay

Intracellular Staining for Biotinylated Peptides

[0474] 96-well U-bottom polystyrene plates (NUNC, cat no: 163320) were used for staining of human PBMCs. Briefly, 8 μ l of N- or C-terminally biotinylated peptides according to the invention (i.e. 5 mM, 2.5 mM & 1.25 mM tested for each peptide) were incubated at 37° C. for 2 h with 40 μ l of PBMC (12.5 \times 10⁶ cells/ml) from blood donors. Cells were then washed 3 \times with 150 μ l of Cellwash (BD, cat no: 349524), followed by resuspension of each cell pellet with 100 μ l of Trypsin-EDTA (Sigma, cat no: T4424), then incubated at 37° C. for 5 min. Trypsinated cells were then washed 3 \times with 150 μ l of Cellwash (BD, cat no: 349524), followed by resuspension with BD Cytofix/Cytoperm™ plus (BD, cat no: 554715), then incubated at 4° C. for 20 min according to manufacturer. Cells were then washed 2 \times with 150 μ l PermWash (BD, cat no: 554715). Cells were then stained with Streptavidin-APC (BD, cat no: 554067) & Anti-hCD11c (eBioscience, cat no: 12-0116) according to manufacturer at 4° C. for 30 min aiming to visualize biotinylated peptides & dendritic cells, respectively. Cells were then washed 3 \times with 150 μ l PermWash, followed by resuspension in staining buffer (BD, cat no: 554656) before flow cytometry. Dendritic cells were gated as CD11c+ events outside lymphocyte region (i.e. higher FSC & SSC signals than lymphocytes). 200000 total cells were acquired on a FACSCanto II flow cytometer with HTS loader, and histograms for both total cells & dendritic cells with respect to peptide-fluorescence (i.e. GeoMean) were prepared.

Extracellular Staining for Biotinylated Peptides

[0475] 96-well U-bottom polystyrene plates (NUNC, cat no: 163320) were used for staining of human PBMCs. Briefly, 8 μ l of N- or C-terminally biotinylated peptides according to table 1 or table 2 (i.e. 5 mM, 2.5 mM & 1.25 mM tested for each peptide; all peptides manufactured by solid phase synthesis by commercial suppliers) were incubated at 37° C. for 2 h with 40 μ l of PBMC (12.5 \times 10⁶ cells/ml) from blood donors. Cells were then washed 3 \times with 150 μ l of Cellwash (BD, cat no: 349524), then stained with Streptavidin-APC (BD, cat no: 554067) & Anti-hCD11c (eBioscience, cat no: 12-0116) according to manufacturer at 4° C. for 30 min aiming to visualize biotinylated peptides & dendritic cells, respectively. Cells were then washed 3 \times with 150 μ l of Cellwash (BD, cat no: 349524), followed by resuspension in staining buffer (BD, cat no: 554656) before flow cytometry. Dendritic cells were gated as CD11c+ events outside lymphocyte region (i.e. higher FSC & SSC signals than lymphocytes). 200000 total cells were acquired on a FACSCanto II flow cytometer with HTS loader, and histograms for both total cells & dendritic cells with respect to peptide-fluorescence (i.e. GeoMean) were prepared.

[0476] It was clearly seen that the CMI peptides according to the invention had improved ability to enter the cell compared to its native counterparts

[0477] The data are geomean-value of each tested peptide, as calculated by the FACS Diva software. The Geomean values by trypsinating/Cytofix/Cytoperm.

Example 12

[0478] Positive CTL response may alternatively be assayed by ELISPOT assay.

Human IFN-Gamma Cytotoxic T-Cell (CTL) Response by ELISPOT Assay

[0479] Briefly, at day 1, PBMC samples from HCV patients were incubated in flasks (430000 PBMCs/cm²) for 2 h at 37° C., 5% CO₂ in covering amount of culture media (RPMI 1640 Fisher Scientific; Cat No. PAAE15-039 supplemented with L-Glutamine, (MedProbe Cat. No. 13E17-605E, 10% Foetal Bovine serum (FBS), Fisher Scientific Cat. No. A15-101) and Penicillin/Streptomycin, (Fisher Scientific Cat. No. P11-010) in order to allow adherence of monocytes. Non-adherent cells were isolated, washed, and frozen in 10% V/V DMSO in FBS until further usage. Adherent cells were carefully washed with culture media, followed by incubation at 37° C. until day 3 in culture media containing 2 μ g/ml final concentration of hrGM-CSF (Xiamen amoytop biotech co, cat no: 3004.9090.90) & 1 μ g/ml hrIL-4 (Invitrogen, Cat no: PHC0043) and optionally an immunomodulating agent (IMiD), and this procedure was then repeated at day 6. At day 7, cultured dendritic cells (5000-10000 per well) were added to ELISPOT (Millipore multiscreen HTS) plates coated with 0.5 μ g/well anti-human γ Interferon together with thawed autologous non-adherent cells (200000 per well), antigen samples (1-8 μ g/ml final concentration for peptide antigens; 5 μ g/ml final concentration for Concanavalin A (Sigma, Cat no: C7275) or PHA (Sigma, Cat no: L2769)) & anti-Anergy antibodies (0.03-0.05 μ g/ml final concentration for both anti-PD-1 (eBioscience, cat no: 16-9989-82) & anti-PD-L1 (eBioscience, cat no: 16-5983-82)). Plates were incubated overnight and spots were developed according to manufacturer. Spots were read on ELISPOT reader (CTL-ImmunoSpot® S5 UV Analyzer).

Example 13

Influenza Specific M2e Response by ELISPOT Assay

[0480] At day one, PBMC samples from blood donors were thawed, washed with warm medium and incubated in flasks (250000 PBMCs/cm²) for 24 hours at 37° C., 5% CO₂ in covering amount of culture media (RPMI 1640 with ultra-glutamine, Lonza, BE12-702F701; 10% Foetal Bovine serum (FBS), Fisher Scientific Cat. No. A15-101; Penicillin/Strep-tomycin, Fisher Scientific Cat. No. P11-010) to allow the cells to recover after thawing. At day two, the cells were added to a Falcon Microtest Tissue Culture plate, 96 well flat bottom, at 500000 cells per well in a volume of 200 μ l total medium. Parallel wells were added the indicated stimuli in duplicate and optionally an immunomodulating agent (IMiD), or left with medium as a control for 6 days at 37° C., 5% CO₂. After the six days of incubation, 100 μ l of the cell suspension were transferred to an ELISPOT (Millipore multiscreen HTS) plate coated with 1 μ g/ml native influenza M2e protein. After a 24 hour incubation, the plate was washed four times with PBS+0.05% Tween20, and a fifth time with PBS, 200 μ l/well. A mouse Anti-human IgG or IgM biotin (Southern Biotech 9040-08 and 9020-08) was diluted in PBS with 0.5% FBS and incubated for 90 minutes at 37° C. The washing was repeated as described, before 80 μ l Streptavidin-Alkaline-Phosphatase (Sigma Aldrich, S289) was added each

well and incubated at 60 minutes in the dark, at room temperature. The wells were then washed 2 times with PBS+0.05% Tween20 and 4 times with PBS, 200 μ l/well, before the substrate, Vector Blue Alkaline Phosphatase Substrate kit III (Vector Blue, SK-5300) was added and let to develop for 7 minutes at room temperature. The reaction was stopped with running water, the plates let dry and the spot enumerated by an ELISPOT reader (CTL-ImmunoSpot® S5 UV Analyzer).

Influenza Specific M2e Response by ELISA

[0481] 100 μ l of antigen as indicated (pre-incubated in Coating buffer–0.05M Na₂CO₃ pH9.6; denoted CB-in cold at 8 μ g/ml 1-3 days) or just CB (background control) was used for coating wells in microtiter plates at 4° C. The microtiter plates are then washed 3 \times with washing buffer (PBS+1% v/v Triton-X100; denoted WB), followed by 2 h blocking at room temperature (RT) with 200 μ l/well of blocking buffer (PBS+1% w/v BSA). Plates are then washed 3 \times with WB, followed by 1 h incubation at 37° C. with 50-70 μ l/well of added human (or rabbit or sheep) sera (serial dilutions ranging from 1:5-1:

250 in dilution buffer (PBS+1% v/v Triton-X100+1% w/v BSA; denoted DB)). Plates are then washed 6 \times with WB, followed by 1 h incubation at RT with 70 μ l/well of Alkaline Phosphatase-conjugated Protein G (3 μ g/ml in DB; Calbiochem 539305) or goat anti-mouse IgG biotin (1 μ g/ml, Southern Biotech, 1030-08. In case of the goat anti-mouse IgG biotin, the plates were washed one extra step as described, before addition of 100 μ l Streptavidin-Alkaline-Phosphatase (1 μ g/ml, Sigma Aldrich, S289) and incubated 1 hour at RT. Plates are then washed 6 \times with WB, followed by 10-60 min incubation at room temperature with 100 μ l/well of 0.3% w/v of Phenolphthalein monophosphate (Sigma P-5758). Plates are finally quenched by adding 100 μ l/well of Quench solution (0.1M TRIS+0.1M EDTA+0.5M NaOH+0.01% w/v NaN₃; pH14), followed by a measurement with a ELISA reader (ASYS UVM 340) at 550 nm. The strength of the sera, i.e. the magnitude of the humoral immune response, is then reported as the dilution of sera that result in the described Optical Density (OD) value, or the OD value at the indicated dilution of sera.

SEQUENCE LISTING

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<213> ORGANISM: HIV

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Val

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<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: HIV

<400> SEQUENCE: 2

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Val

<210> SEQ ID NO 3
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: HIV

<400> SEQUENCE: 3

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Val

<210> SEQ ID NO 4
<211> LENGTH: 17
<212> TYPE: PRT
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<400> SEQUENCE: 4

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1	5	10	15
Val			
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Val

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<223> OTHER INFORMATION: Gly can be Arg
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<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Arg can be Gln
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<223> OTHER INFORMATION: Asp can be Gly
<220> FEATURE:
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<223> OTHER INFORMATION: Arg can be Lys

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<223> OTHER INFORMATION: Gly can be Arg
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<220> FEATURE:
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<223> OTHER INFORMATION: Asp can be Gly
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<400> SEQUENCE: 8

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1 5 10

<210> SEQ ID NO 9
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<212> TYPE: PRT
<213> ORGANISM: HIV

<400> SEQUENCE: 9

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1 5 10

<210> SEQ ID NO 10
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<212> TYPE: PRT
<213> ORGANISM: HIV

<400> SEQUENCE: 10

Val Glu Arg Tyr Leu Lys Asp Asn Asn Leu Leu Gly
1 5 10

<210> SEQ ID NO 11
<211> LENGTH: 15
<212> TYPE: PRT
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<400> SEQUENCE: 11

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1 5 10 15

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<211> LENGTH: 15
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<400> SEQUENCE: 12

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1 5 10 15

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<400> SEQUENCE: 13

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<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

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Gly Gly Gly Arg Val Val Gln Arg Glu Lys
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<210> SEQ ID NO 16

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<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

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Gly Gln Arg Glu Lys Arg Ala Val
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<210> SEQ ID NO 17

<211> LENGTH: 25

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

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Val Gly Gln Arg Glu Lys Arg Ala Val
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<211> LENGTH: 23

<212> TYPE: PRT

<213> ORGANISM: artificial sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

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Gly Gly Gln Arg Glu Lys Arg
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<210> SEQ ID NO 19

<211> LENGTH: 24

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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 19

Cys Gly Gly Ala Lys Arg Arg Val Val Gly Gly Ala Lys Arg Arg Val
1 5 10 15

Val Gly Gly Gln Arg Glu Lys Arg
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<210> SEQ ID NO 20
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

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1 5 10 15

<210> SEQ ID NO 21
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
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<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 21

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1 5 10 15

Val

<210> SEQ ID NO 22
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 22

Gly Gly Gly Asp Gln Gln Leu Leu Gly Gly Ala Glu Glu Glu Ile Val
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Gly Gly Ile Glu Glu Glu Gly Gly Glu Arg Asp Arg Asp Arg
20 25 30

<210> SEQ ID NO 23
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 23

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Val Gly Gly Ile Glu Glu Glu Gly Gly Glu Arg Asp Arg Asp Arg
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<210> SEQ ID NO 24
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<212> TYPE: PRT
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<220> FEATURE:
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<400> SEQUENCE: 24

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Gly Gly Glu Arg Asp Arg
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<210> SEQ ID NO 25
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 25

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1 5 10 15

Val Gly Gly Ile Glu Glu Glu Gly Gly
20 25

<210> SEQ ID NO 26
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 26

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1 5 10 15

<210> SEQ ID NO 27
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
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<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 27

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1 5 10 15

<210> SEQ ID NO 28
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 28

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Val Gly Gln Arg Glu Lys Arg Ala Val
20 25

<210> SEQ ID NO 29
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 29

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1 5 10 15

Val Gly Gly Ile Glu Glu Glu Gly Gly Glu Arg Asp Arg Asp Arg
20 25 30

<210> SEQ ID NO 30
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 30

Cys Gly Gly Ala Lys Arg Arg Val Val Gly Gly Ala Lys Arg Arg Val
1 5 10 15

Val Gly Gly Gln Arg Glu Lys Arg
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<210> SEQ ID NO 31
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<212> TYPE: PRT
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

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1 5 10 15

Val Gly Gly Ile Glu Glu Glu Gly Gly
20 25

<210> SEQ ID NO 32
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
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<400> SEQUENCE: 32

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1 5 10 15

<210> SEQ ID NO 33
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<212> TYPE: PRT
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<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 33

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Val Val

<210> SEQ ID NO 34
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<220> FEATURE:
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<400> SEQUENCE: 34

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<212> TYPE: PRT
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<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 35

Ala Lys Arg Arg Val Val
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<210> SEQ ID NO 36
<211> LENGTH: 6
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<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 36

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<211> LENGTH: 16
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 37

Gly Gly Ala Ile Val Asn Gly Ser Leu Ala Asp Asp Asp Ile Val Ile
1 5 10 15

<210> SEQ ID NO 38
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<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Cys may be linked to Lys in position 2 in SEQ
ID NO: 39 or 40 or 42 or 43

<400> SEQUENCE: 38

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1 5 10 15

Val Val Gln Arg Glu Lys Arg Ala Gly Glu Arg Glu Lys Arg Ala
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<210> SEQ ID NO 39
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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:

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<221> NAME/KEY: MUTAGEN
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Lys may be linked to Cys in position 10 in SEQ
ID NO: 38 or 41

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1 5 10 15

Gly Glu Gln Asp Arg Asp Arg
20

<210> SEQ ID NO 40
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<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
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<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Lys may be linked to Cys in position 10 in SEQ
ID NO: 38 or 41

<400> SEQUENCE: 40

Gly Lys Gly Gly Ile Glu Glu Glu Gly Gly Glu Arg Asp Arg Asp Arg
1 5 10 15

Gly Gly Gln Asp Arg Asp Arg
20

<210> SEQ ID NO 41
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<212> TYPE: PRT
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<223> OTHER INFORMATION: Cys may be linked to Lys in position 2 in SEQ
ID NO: 39 or 40 or 42 or 43

<400> SEQUENCE: 41

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1 5 10 15

Val Val Glu Arg Glu Lys Arg Ala Gly Gln Arg Glu Lys Arg Ala
20 25 30

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<223> OTHER INFORMATION: Lys may be linked to Cys in position 10 in SEQ
ID NO: 38 or 41

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Gly Arg Asp Arg Asp Arg
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<223> OTHER INFORMATION: Lys may be linked to Cys in position 10 in SEQ
ID NO: 38 or 41

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Gly Gly Glu Arg Asp Arg Asp
20

<210> SEQ ID NO 44
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<220> FEATURE:
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<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: Carboxyterminal may be amidated

<400> SEQUENCE: 44

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Val Val Gln Arg Glu Lys Arg Ala
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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Carboxyterminus may be amidated

<400> SEQUENCE: 45

Trp Trp Gly Cys Ile Glu Glu Glu Gly Cys Gly Gly Ile Glu Glu Glu
1 5 10 15

Gly Gly Glu Arg Asp Arg
20

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<210> SEQ ID NO 46
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Glu can be Gly
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Gly can be Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Arg can be Gln
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Asp can be Gly
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Arg can be Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Asp can be Gly

<400> SEQUENCE: 46

Asp Arg Pro Glu Gly Ile Glu Asn Asn Gly Gly Glu Arg Asp Arg Asp
1 5 10 15

Arg

<210> SEQ ID NO 47
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa in position 1 is Lys or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa in position 2 is Ala, Gly, Ser or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa in position 3 is Leu or Met
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa in position 4 is Gly or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa in position 5 is Pro, Thr, Val, Ser, Gln or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa in position 6 is Gly, Ala, Lys, Arg, Gln or Glu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa in position 8 is Thr or Ser
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa in position 9 is Leu or Ile
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa in position 14 is Thr, Ser or Val
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa in position 15 is Ala or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa in position 16 is Cys or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa in position 17 is Gln or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa in position 18 is Gly, Glu or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa in position 20 is Gly or Arg

<400> SEQUENCE: 47

Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Xaa Gln Thr Pro Trp Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Val Xaa
20

<210> SEQ ID NO 48
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 48

Lys Ala Leu Gly Pro Gly Ala Thr Leu Gln Thr Pro Trp Thr Ala Cys
1 5 10 15

Gln Gly Val Gly
20

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 49

Arg Ala Leu Gly Pro Ala Ala Thr Leu Gln Thr Pro Trp Thr Ala Ser
1 5 10 15

Leu Gly Val Gly
20

<210> SEQ ID NO 50
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa in position 1 is Arg, Lys, Asp or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa in position 2 is Trp, Gly, Lys or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa in position 3 is Ile, Leu, Val or Met
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (4)..(4)
<223> OTHER INFORMATION: Xaa in position 4 is Ile, Val or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa in position 5 Leu, Met, Val or Pro
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: Xaa in position 12 is 0, 1, 2 or 3 Gly residues
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa in position 13 is Arg or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa in position 14 is Met or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa in position 16 is Ser, Cys or Gln
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa in position 18 is Thr, Val, Ile, Ser or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa in position 19 is Ser, Gly or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa in position 22 is Asp, Glu, Cys or Gly
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: Xaa in position 22 is Gly or none

<400> SEQUENCE: 50

Xaa Xaa Xaa Xaa Xaa Gly Leu Asn Pro Leu Val Xaa Xaa Xaa Tyr Xaa
1 5 10 15
Pro Xaa Xaa Ile Leu Xaa Xaa
20

<210> SEQ ID NO 51
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 51

Trp Ile Ile Pro Gly Leu Asn Pro Leu Val Gly Gly Gly Lys Leu Tyr
1 5 10 15
Ser Pro Thr Ser Ile Leu Cys Gly
20

-continued

<210> SEQ ID NO 52
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 52

Arg Trp Leu Leu Leu Gly Leu Asn Pro Leu Val Gly Gly Gly Arg Leu
1 5 10 15

Tyr Ser Pro Thr Ser Ile Leu Gly
 20

<210> SEQ ID NO 53
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 53

Lys Ile Leu Leu Gly Leu Asn Pro Leu Val Gly Gly Gly Arg Leu Tyr
1 5 10 15

Ser Pro Thr Ser Ile Leu Gly
 20

<210> SEQ ID NO 54
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 54

Arg Leu Leu Leu Gly Leu Asn Pro Leu Val Gly Gly Gly Arg Leu Tyr
1 5 10 15

Ser Pro Thr Thr Ile Leu Gly
 20

<210> SEQ ID NO 55
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa in position 1 is Asn, Ser, Gly, His, Ala,
Pro, Arg or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa in position 2 is Asn, Ala or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (3)..(3)
<223> OTHER INFORMATION: Xaa in position 3 is Pro, Gln, Gly, Ile or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa in position 7 is Val or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)

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<223> OTHER INFORMATION: Xaa in position 8 is Gly or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa in position 9 is Glu, Asp, Lys, Phe or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Xaa in position 10 is Ile, Met, Val or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa in position 11 is Tyr, Leu or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: Xaa in position 12 is Ser or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa in position 13 is 1, 2 or 3 Gly residues
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa in position 14 is Arg or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa in position 15 is Asp, Arg, Trp, Ala or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa in position 16 is Ile or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa in position 17 is Tyr or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa in position 18 is Lys or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa in position 19 is Arg, Lys or Asp
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa in position 20 is Trp or Gly
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa in position 21 is Ile, Met, Val, Gln or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa in position 22 is Ile, Val or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: Xaa in position 23 is Leu, Met or Val
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: Xaa in position 24 is Gly or Cys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa in position 25 is Leu or none

<400> SEQUENCE: 55

Xaa Xaa Xaa Pro Ile Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25

<210> SEQ ID NO 56
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 56

Arg Asn Ile Pro Ile Pro Val Gly Asp Ile Tyr Gly Gly Gly Asp Ile
1 5 10 15

Tyr Lys Arg Trp Gln Ala Leu Cys Leu
20 25

<210> SEQ ID NO 57
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 57

Arg Ala Ile Pro Ile Pro Ala Gly Thr Leu Leu Ser Gly Gly Gly Arg
1 5 10 15

Ala Ile Tyr Lys Arg Trp Ala Ile Leu Gly
20 25

<210> SEQ ID NO 58
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 58

Ala Leu Pro Ile Pro Ala Gly Phe Ile Tyr Gly Gly Gly Arg Ile Tyr
1 5 10 15

Lys Arg Trp Gln Ala Leu Gly
20

<210> SEQ ID NO 59
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 59

Lys Ile Pro Ile Pro Val Gly Phe Ile Gly Gly Gly Trp Ile Tyr Lys
1 5 10 15

Arg Trp Ala Ile Leu Gly
20

<210> SEQ ID NO 60
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 60

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Lys Ile Pro Ile Pro Val Gly Thr Leu Leu Ser Gly Gly Gly Arg Ile
1 5 10 15

Tyr Lys Arg Trp Ala Ile Leu Gly
20

<210> SEQ ID NO 61
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: Xaa in position 1 is Pro, Lys, Arg or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (2)..(2)
<223> OTHER INFORMATION: Xaa in position 2 is Glu, Arg, Phe or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa in position 5 is Pro or Thr
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa in position 6 is Met, Thr or Nleu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (7)..(7)
<223> OTHER INFORMATION: Xaa in position 7 is Phe or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (8)..(8)
<223> OTHER INFORMATION: Xaa in position 8 is Ser, Thr, Ala or Met
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: Xaa in position 9 is Ala, Glu or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (11)..(11)
<223> OTHER INFORMATION: Xaa in position 11 is Ser or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (12)..(12)
<223> OTHER INFORMATION: Xaa in position 12 is 1, 2 or 3 Gly residues
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: Xaa in position 12 is 0, 1, 2 or 3 Arg residues
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (14)..(14)
<223> OTHER INFORMATION: Xaa in position 14 is Ala, Arg or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (15)..(15)
<223> OTHER INFORMATION: Xaa in position 15 is Ile, Leu or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa in position 16 is Ser, Ala, Leu or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (17)..(17)
<223> OTHER INFORMATION: Xaa in position 17 is Tyr, Glu or Asp
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa in position 18 is Gly or Asp
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE

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<222> LOCATION: (19)..(19)
<223> OTHER INFORMATION: Xaa in position 19 is Ala or Leu
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (20)..(20)
<223> OTHER INFORMATION: Xaa in position 20 is Thr, Ile, Val, Leu or Asn
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa in position 21 is Pro, Thr or Ser
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (22)..(22)
<223> OTHER INFORMATION: Xaa in position 22 is Tyr, Phe, Nleu, His or Gln
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: Xaa in position 23 is Asp, Asn, Leu or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: Xaa in position 24 is Leu, Ile, Val or Asn
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: Xaa in position 25 is Asn, Tyr, Cys or Gly
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (26)..(26)
<223> OTHER INFORMATION: Xaa in position 26 is Thr, Met, Ile, Ala, Val or none
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (27)..(27)
<223> OTHER INFORMATION: Xaa in position 27 is Gly or none

<400> SEQUENCE: 61

Xaa Xaa Ile Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25

<210> SEQ ID NO 62
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa in position 6 is Norleucine
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (21)..(21)
<223> OTHER INFORMATION: Xaa in position 21 is Norleucine

<400> SEQUENCE: 62

Lys Phe Ile Ile Pro Xaa Phe Ser Ala Leu Gly Gly Ala Ile Ser Tyr
1 5 10 15

Asp Leu Asn Thr Xaa Leu Asn Cys Ile
20 25

-continued

<210> SEQ ID NO 63
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (6)..(6)
<223> OTHER INFORMATION: Xaa in position 6 is Norleucine

<400> SEQUENCE: 63

Lys Phe Ile Ile Pro Xaa Phe Ser Ala Leu Ser Gly Gly Gly Ala Ile
1 5 10 15

Ser Tyr Asp Leu Asn Thr Phe Leu Asn Cys Ile Gly
 20 25

<210> SEQ ID NO 64
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 64

Arg Phe Ile Ile Pro Asn Leu Phe Thr Ala Leu Ser Gly Gly Arg Arg
1 5 10 15

Ala Leu Leu Tyr Gly Ala Thr Pro Tyr Ala Ile Gly
 20 25

<210> SEQ ID NO 65
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa in position 5 is Norleucine

<400> SEQUENCE: 65

Lys Ile Ile Pro Xaa Phe Ser Ala Leu Gly Gly Gly Arg Leu Leu Tyr
1 5 10 15

Gly Ala Thr Pro Tyr Ala Ile Gly
 20

<210> SEQ ID NO 66
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic peptide
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Xaa in position 5 is Norleucine

<400> SEQUENCE: 66

Arg Ile Ile Pro Xaa Phe Thr Ala Leu Ser Gly Gly Gly Arg Leu Leu
1 5 10 15

Tyr Gly Ala Thr Pro Tyr Ala Ile Gly
 20 25

-continued

<210> SEQ ID NO 67

<400> SEQUENCE: 67

000

<210> SEQ ID NO 68

<211> LENGTH: 22

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 68

Gly Lys Gly Gly Ile Glu Glu Gly Gly Arg Asp Arg Asp Arg Gly
1 5 10 15

Gly Gln Asp Arg Asp Arg
20

<210> SEQ ID NO 69

<400> SEQUENCE: 69

000

<210> SEQ ID NO 70

<211> LENGTH: 24

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<400> SEQUENCE: 70

Asn Ile Pro Ile Pro Val Gly Asp Ile Tyr Gly Gly Gly Asp Ile Tyr
1 5 10 15

Lys Arg Trp Gln Ala Leu Cys Leu
20

<210> SEQ ID NO 71

<211> LENGTH: 24

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic peptide

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (5)..(5)

<223> OTHER INFORMATION: Xaa in position 5 is Norleucine

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (20)..(20)

<223> OTHER INFORMATION: Xaa in position 20 is Norleucine

<400> SEQUENCE: 71

Trp Ile Ile Pro Xaa Phe Ser Ala Leu Gly Gly Ala Ile Ser Tyr Asp
1 5 10 15

Leu Asn Thr Xaa Leu Asn Cys Ile
20

1. A method for treating a human retrovirus infection, such as immunodeficiency virus I (HIV) in a human infected with such virus, such as HIV, the method comprising administering separately, sequentially in any order or simultaneously (1) one or more peptides that elicit a cell-mediated immune response in a subject and/or a compound to stimulate the humoral immunity in a subject; and (2) a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor, and/or an immunomodulatory compound.

2. The method according to claim 1, comprising the administering of one or more peptides that elicit a cell-mediated immune response in a subject.

3. The method according to claim 1 or 2, comprising the administering of a compound to stimulate the humoral immunity in a subject.

4. The method according to claim 3, wherein said compound to stimulate the humoral immunity in a subject is an immunogen, which induces antibodies that stabilise association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120.

5. The method according to any one of claims 1-4, comprising the administering of an immunomodulatory compound.

6. The method according to any one of claims 1-5, comprising the administering of a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor.

7. The method according to any one of claims 1-6, wherein said immunomodulatory compounds is selected from anti-PD1 antibodies, such as MDX-1106 (Merck), THALOMID® (thalidomide), anti-PD1 antibodies, cyclophosphamide, Levamisole, lenalidomide, CC-4047 (pomalidomide), CC-11006 (Celgene), and CC-10015 (Celgene), and immunomodulatory compound described in any one of WO2007028047, WO2002059106, and WO2002094180.

8. The method according to any one of claims 1-7, wherein said immunomodulatory compound is selected from 4-(amino)-2-(2,6-dioxo(3-piperidyl))-isoindoline-1,3-dione and 3-(4-amino-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione.

9. The method according to any one of claims 1-8, wherein said immunomodulatory compound is lenalidomide.

10. The method according to any one of claims 1-9, wherein said reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor is selected from M344 (4-(dimethylamino)-N-[7-(hydroxyamino)-7-oxoheptyl]benzamide), chidamide (CS055/HBI-800), 4SC-202, (4SC), Resminostat (4SC), hydroxamic acids such as vorinostat (SAHA), belinostat (PXD101), LAQ824, trichostatin A and panobinostat (LBH589); benzamides such as entinostat (MS-275), CI994, and mocetinostat (MGCD0103), cyclic tetrapeptides (such as trapoxin, such as trapoxin B), and the depsipeptides, such as romidepsin (ISTODAX), electrophilic ketones, and the aliphatic acid compounds such as phenylbutyrate, valproic acid, Oxamflatin, ITF2357 (generic givinostat), Apicidin, MC1293, CG05, and CG06; compounds that activate transcription factors including NF-KappaB, Prostratin, auranoquin, bryostatin, a nontumorigenic phorbol ester, DPP (12-deoxyphorbol-13-phenylacetate), PMA, and Phorbol 12-myristate 13-acetate (PMA); Compounds that activate HIV mRNA elongation including P-TEF-b kinase and hexamethylbisacetamide (HMBA); IL-7; T-cell stimulating factors including anti-CD3/CD28-T-cell stimulating Ab's; Kinase inhibitors including Tyrphostin A, Tyrphostin B, and Tyrphostin C; PTEN (phosphatase and tensin homo-

logue) gene inhibitors including SF1670 (Echelon Bioscience), Disulfiram (DSF), an inhibitor of acetaldehyde dehydrogenase, Protein Tyrosine Phosphatase Inhibitors including bpV(HOpic), bpV(phen), and bpV(pic) (Calbiochem; EMD Millipore), Toll-like receptors agonists including Toll-like receptor-9 (TLR9) and Toll-like receptor-7 (TLR9) agonists, quercetin, lipoic acid, sodium butyrate, TNF-alpha, PHA and Tat.

11. The method according to any one of claims 1-10, wherein said reservoir purging agent is romidepsin.

12. The method according to any one of claims 1-11, wherein said one or more peptide that elicits a cell-mediated immune response is at least one HIV-specific peptide selected from the group of amino acid sequences:

(SEQ ID NO: 47)

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Ala Xaa₈ Xaa₉ Gln Thr

Pro Trp Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈ Val Xaa₂₀;

wherein

Xaa in position 1 is Lys or Arg,
Xaa in position 2 is Ala, Gly, Ser or Arg,
Xaa in position 3 is Leu or Met,
Xaa in position 4 is Gly or Arg,
Xaa in position 5 is Pro, Thr, Val, Ser, Gln or Ala,
Xaa in position 6 is Gly, Ala, Lys, Arg, Gln or Glu,
Xaa in position 8 is Thr or Ser,
Xaa in position 9 is Leu or Ile,
Xaa in position 14 is Thr, Ser or Val,
Xaa in position 15 is Ala or Ser,
Xaa in position 16 is Cys or Ser,
Xaa in position 17 is Gln or Leu,
Xaa in position 18 is Gly, Glu or Arg, and
Xaa in position 20 is Gly or Arg;

(SEQ ID NO: 50)

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Gly Leu Asn Pro Leu Val

[Gly]_n Xaa₁₂ Xaa₁₃ Tyr Xaa₁₅ Pro Xaa₁₇ Xaa₁₈ Ile Leu

Xaa₂₁ Xaa₂₂;

wherein

Xaa in position 1 is Arg, Lys, Asp or none,
Xaa in position 2 is Trp, Gly, Lys or Arg,
Xaa in position 3 is Ile, Leu, Val or Met,
Xaa in position 4 is Ile, Val or Leu,
Xaa in position 5 Leu, Met, Val or Pro,
Xaa in position 12 is Arg or Lys,
Xaa in position 13 is Met or Leu,
Xaa in position 15 is Ser, Cys or Gln,
Xaa in position 17 is Thr, Val, Ile, Ser or Ala,
Xaa in position 18 is Ser, Gly or Thr,
Xaa in position 21 is Asp, Glu, Cys or Gly,
Xaa in position 22 is Gly or none, and
n=0, 1, 2 or 3;

(SEQ ID NO: 55)

Xaa₁ Xaa₂ Xaa₃ Pro Ile Pro Xaa₇ Xaa₈ Xaa₉ Xaa₁₀

Xaa₁₁ Xaa₁₂ [Gly]_n Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇

Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄;

wherein

Xaa in position 1 is Asn, Ser, Gly, His, Ala, Pro, Arg or none,
 Xaa in position 2 is Asn, Ala or Lys,
 Xaa in position 3 is Pro, Gln, Gly, Ile or Leu,
 Xaa in position 7 is Val or Ala,
 Xaa in position 8 is Gly or Lys,
 Xaa in position 9 is Glu, Asp, Lys, Phe or Thr,
 Xaa in position 10 is Ile, Met, Val or Leu,
 Xaa in position 11 is Tyr, Leu or none,
 Xaa in position 12 is Ser or none,
 Xaa in position 13 is Arg or none,
 Xaa in position 14 is Asp, Arg, Trp, Ala or none,
 Xaa in position 15 is Ile or none,
 Xaa in position 16 is Tyr or none,
 Xaa in position 17 is Lys or Arg,
 Xaa in position 18 is Arg, Lys or Asp,
 Xaa in position 19 is Trp or Gly,
 Xaa in position 20 is Ile, Met, Val, Gln or Ala,
 Xaa in position 21 is Ile, Val or Ala,
 Xaa in position 22 is Leu, Met or Val,
 Xaa in position 23 is Gly or Cys,
 Xaa in position 24 is Leu or none,
 n=1, 2 or 3, and

(SEQ ID NO: 61)

Xaa₁ Xaa₂ Ile Ile Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Leu
 Xaa₁₁ [Gly]_n [Arg]_m Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆
 Xaa₁₇ Xaa₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅;

wherein

Xaa in position 1 is Pro, Lys, Arg or none,
 Xaa in position 2 is Glu, Arg, Phe or Lys,
 Xaa in position 5 is Pro or Thr,
 Xaa in position 6 is Met, Thr or Nleu,
 Xaa in position 7 is Phe or Leu,
 Xaa in position 8 is Ser, Thr, Ala or Met,
 Xaa in position 9 is Ala, Glu or Leu,
 Xaa in position 11 is Ser or none,
 Xaa in position 12 is Ala, Arg or none,
 Xaa in position 13 is Ile, Leu or none,
 Xaa in position 14 is Ser, Ala, Leu or none,
 Xaa in position 15 is Tyr, Glu or Asp,
 Xaa in position 16 is Gly or Asp,
 Xaa in position 17 is Ala or Leu,
 Xaa in position 18 is Thr, Ile, Val, Leu or Asn,
 Xaa in position 19 is Pro, Thr or Ser,
 Xaa in position 20 is Tyr, Phe, Nleu, His or Gln,
 Xaa in position 21 is Asp, Asn, Leu or Ala,
 Xaa in position 22 is Leu, Ile, Val or Asn,
 Xaa in position 23 is Asn, Tyr, Cys or Gly,
 Xaa in position 24 is Thr, Met, Ile, Ala, Val or none,
 Xaa in position 25 is Gly or none,
 n=1, 2 or 3 and m=0, 1, 2 or 3 independent of each other,
 wherein the terminal ends of each HIV specific peptide may be free carboxyl- or amino-groups, amides, acyls or acetyls,
 or a salts of any one of the HIV specific peptides.

13. The method according to claim 12, wherein two or more of the Cys residues of said HIV-specific peptide may form part of an intrachain- or interchain disulphide binding, a —S—(CH₂)_p—S— or a —(CH₂)_p—bridge wherein p=1-8

optionally intervened by one or more heteroatoms such as O, N and S and/or the said peptide sequences are immobilized to a solid support.

14. The method according to claim 12 or 13, wherein the amino acid sequence of SEQ ID NO:47 is selected from the group of SEQ ID NO:48 and SEQ ID NO:49.

15. The method according to any one of claims 12-14, wherein the amino acid sequence of SEQ ID NO:50 is selected from the group of SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53 and SEQ ID NO:54.

16. The method according to any one of claims 12-15, wherein the amino acid sequence of SEQ ID NO:55 is selected from the group of SEQ ID NO:56 SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59 and SEQ ID NO:60.

17. The method according to any one of claims 12-16, wherein the amino acid sequence of SEQ ID NO:61 is selected from the group of SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65 and SEQ ID NO:66.

18. The method according to any one of claims 12-17, wherein at least one HIV-specific peptide comprises at least, two, three, or four peptides selected from each of the groups of SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:55 and SEQ ID NO:61.

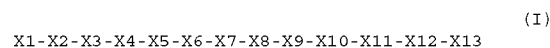
19. The method according to any one of claims 12-18, wherein at least one HIV-specific peptide consist of or comprises the peptides of the SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:57 and SEQ ID NO:64, or salts thereof.

20. The method according to any one of claims 4-19, wherein said immunogen is a peptide multimer comprising

a first peptide comprising the amino acid sequence of the 13 amino acid residue amino acid sequence of the C5 domain of HIV gp120 including between 0 and 4 amino acid substitutions, a subsequence thereof, or an amino acid sequence comprising the inverso-, retro- or retro-inverso form of said amino acid sequence or subsequence, and

at least one second peptide comprising an amino acid stretch present in the transmembrane domain of gp41 or present in the constant C2 domain of gp120 or comprising an amino acid stretch present in any one of SEQ ID NOs. 6-13 or comprising a inverso-, retro- or retro-inverso form of an amino acid stretch present in the transmembrane domain of gp41 or present in the constant C2 domain of gp120, wherein said peptide multimer is capable of inducing an antibody which can bind and stabilise the association of the C5 domain of HIV gp120 with the transmembrane domain of gp41 and/or with the constant C2 domain of gp120, and wherein said peptide multimer lacks amino acids N-terminal of C5 in gp120.

21. The method according to claim 20, wherein said first peptide comprises the amino acid sequence having formula I:



wherein X1 is Thr, X2 is selected from Lys, Arg, Har and Glu, X3 is selected from Ala and Val, X4 is selected from Arg, Har, Lys and Cit (citrulline), X5 is selected from Arg, Har, Lys and Cit, X6 is selected from Arg, Har, Lys and Cit, X7 is selected from Val, Leu, Ile and Nle (nor-leucin), X8 is selected from Val, Leu, Ile and Nle, X9 is selected from Gln, Glu, Asn and Asp, X10 is selected

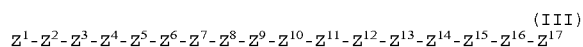
from Arg, Har and Cit, X11 is selected from Glu and Asp, X12 is Lys, and X13 is selected from Arg, Har and Cit,

or comprises a subsequence the amino acid sequence of formula I, or comprising the inverso-, retro- or retro-inverso form of said amino acid sequence or subsequence.

22. The method according to claim **20** or **21**, wherein the first peptide further comprises the dipeptide Ala-Pro linked to the N-terminus of the amino acid sequence having formula I.

23. The method according to any one of claims **20-22**, wherein the first peptide further comprises the dipeptide X14-X15 linked to the C-terminus of the amino acid sequence having formula I, wherein X14 is selected from Ala and Val, and wherein X15 is selected from Val, Leu and Nle.

24. The method according to any one of claims **20-23**, wherein the at least second peptide includes an amino acid sequence having the formula:



wherein Z^1 is Asp, Z^2 is Arg, Z^3 is Pro, Z^4 is Glu or Gly, Z^5 is Gly or Arg, Z^6 is Ile, Z^7 is Glu, Z^8 is Glu, Z^9 is Glu, Z^{10} is Gly, Z^{11} is Gly, Z^{12} is Glu or is absent, Z^{13} is Arg or Gln, Z^{14} is Asp or Gly, Z^{15} is Arg or Lys, Z^{16} is Asp or Gly and Z^{17} is Arg,

or includes a subsequence of formula (III).

25. The method according to any one of claims **20-24**, wherein the second peptide includes at least 5 consecutive amino acid residues from formula III.

26. The method according to any one of claims **20-25**, wherein the first peptide and the at least one second peptide are associated via a linker.

27. The method according to any one of claims **20-26**, wherein the linker is selected from the group consisting of a bis-maleimide linker, a disulfide linker, a polyethylene glycol (PEG) linker, a glycine linker, a lysine linker, and an arginine linker.

28. The method according to any one of claims **20-27**, where at least one of the first and at least one second peptides comprises an N- or C-terminal modification, such as an amidation, acylation, or acetylation.

29. The method according to any one of claims **20-28**, wherein said peptide multimer is coupled to a carrier molecule, such as an immunogenic carrier.

30. The method according to any one of claims **20-29**, wherein the carrier is a virus like particle.

31. The method according to any one of claims **20-30**, wherein the first peptide is selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 38, 41 and 44 or a fragment thereof, or the inverso-, retro- or retro-inverso form of a peptides selected from SEQ ID NO:1, 2, 3, 4, 5, 38, 41 and 44 or a fragment thereof, and wherein the second peptide is selected from the group consisting of SEQ ID NO:6, 7, 8, 9, 10, 11, 12, 13, 37, 39, 40, 42, 43, 45, 46 or a fragment thereof, or the inverso-, retro- or retro-inverso form of a peptides selected from SEQ ID NO:6, 7, 8, 9, 10, 11, 12, 13, 37, 39, 40, 42, 43, 45, 46 or a fragment thereof, and/or wherein the peptide multimer is selected from the peptides having SEQ ID NOs: 1-46.

32. The method according to any one of claims **20-31**, wherein said peptide multimer comprises at most 70 amino acids.

33. The method according to any one of claims **20-32**, wherein said peptide multimer comprises at least 6 amino acid residues.

34. The method according to any one of claims **20-33**, wherein said peptide multimer consist of a number of amino acid residues selected from the group consisting of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, and 70 amino acid residues.

35. The method according to any one of claims **30-34**, wherein said peptide multimer is selected from the group consisting of disulphide linked peptides between SEQ ID NO:28 and any one of SEQ ID NOs: 29, 31, and 33, between SEQ ID NO:30, and any one of SEQ ID NO:29, 31, and 33, or between SEQ ID NO:32 and any one of SEQ ID NO:29, 31, and 33; or selected from the group consisting of cysteine-lysine linked peptides between SEQ ID NO:38 and any one of SEQ ID NO:39, SEQ ID NO:40; SEQ ID NO:42, SEQ ID NO:43, and SEQ ID NO:68, or between SEQ ID NO:41 and any one of SEQ ID NO:39, SEQ ID NO:40; SEQ ID NO:42, and SEQ ID NO:43.

36. The method according to any one of claims **20-35**, wherein said peptide multimer is selected from the group consisting of:

```
CGGAKRRVVGAKRRVVGQREKRAV (SEQ ID NO: 28)
|
CGGGDQQLLGGAAEEIVGGIEEGGERDRD (SEQ ID NO: 29),
|
CGGAKRRVVGAKRRVVGQREKR (SEQ ID NO: 30)
|
CGGGDQQLLGGAAEEIVGGIEEGG (SEQ ID NO: 31),
|
CGGAEEIVVGDDQQLL (SEQ ID NO: 32)
|
GCGAKRRVVGAKRRVV (SEQ ID NO: 33),
|
GAKRRVVGCGGAKRRVVQREKRAGEREKRA (SEQ ID NO: 38)
|
GKGGIEEGGERDRDRGGEQDRDR (SEQ ID NO: 39),
|
GAKRRVVGCGGAKRRVVQREKRAGEREKRA (SEQ ID NO: 38)
|
GKGGIEEGGERDRDRGGQDRDR (SEQ ID NO: 40),
|
GAKRRVVGCGGAKRRVVVEREKRAGQREKRA (SEQ ID NO: 41)
|
GKGGIEEGGQDRDRGGRDRDR (SEQ ID NO: 42),
|
GAKRRVVGCGGAKRRVVVEREKRAGQREKRA (SEQ ID NO: 41)
|
GKGGIEEGGQDRDRGGERDRD (SEQ ID NO: 43) and
|
GAKRRVVGCGGAKRRVVQREKRAGEREKRA (SEQ ID NO: 38)
|
GKGGIEEGGERDRDRGGQDRDR (SEQ ID NO: 68)
```

or a salt of any one thereof.

37. The method according to any one of claims **20-36**, wherein said peptide multimer is (H-Gly-Ala-Lys-Arg-Arg-Val-Val-Gly-Gly-Cys(2-oxo-ethyl)-Gly-Gly-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-Ala-Gly-Glu-Arg-Glu-Lys-Arg-Ala-NH₂) (H-Gly-Lys-Gly-Gly-Ile-Glu-Glu-Glu-Gly-Gly-Arg-Asp-Arg-Asp-Arg-Gly-Gly-Gln-Asp-Arg-Asp-Arg-NH₂), acetate salt (amide bond between Cys(2-oxo-ethyl)10 (A-chain) and Lys2 (B-chain)).

38. A composition comprising

- one or more peptides that elicit a cell-mediated immune response in a subject and/or a compound to stimulate the humoral immunity in a subject; and
- a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor, and/or an immunomodulatory compound.

39. A pharmaceutical composition comprising at least one of (i) one or more peptides that elicit a cell-mediated immune response in a subject and (ii) a compound to stimulate the humoral immunity in a subject; and at least one of (iii) a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor, and (iv) an immunomodulatory compound; optionally in combination with one or more pharmaceutically acceptable adjuvants, diluents or carriers.

40. A kit-of-parts comprising at least one of components (i) and (ii) and at least one of components (iii) and (iv), wherein at least two of the components are formulated separately from one another, and each component is optionally formulated in admixture with a pharmaceutically-acceptable adjuvant, diluent or carrier.

41. The composition or kit-of-parts according to any one of claims **38** to **40**, which composition or kit-of-parts comprises one or more peptides that elicit a cell-mediated immune response in a subject and/or a compound to stimulate the humoral immunity in a subject; and a reservoir purging agent, such as a histone deacetylase (HDAC) inhibitor, and/or an immunomodulatory compound, as defined in any one of claims **2-37**.

42. The method according to any one of claims **1-37**, wherein said human retrovirus infection is selected from HTLV-1 (human T-lymphotropic virus), HTLV-II (human T-lymphotropic virus), human immunodeficiency virus including HIV-1 and HIV-2, and any subtype thereof.

43. The method according to claim **42**, wherein said human retrovirus infection is human immunodeficiency virus-1 infection.

* * * * *