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(54) Title: TRANS-ACTIVATOR OF TRANSCRIPTION PROTEIN

(57) Abstract: The present invention deals with a polypeptide consisting of the amino acid sequence $PX_1X_2EPWX_3H-PGSQPX_4TX_5CGGX_6EX_7DX_8EX_9X_{10}X_{11}X_{12}VGGFX_{13}X_{14}KX_{15}LGI SYGRKKRX_{16}CC$ (SEQ ID NO. 1) in which X_n represents any amino acid residue except cysteine, and in which the cysteines at position 17 and 49 or at position 17 and 50 are linked by a disulfide bond, the numbering corresponding to that of SEQ ID No.1.



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Trans-activator of transcription protein

The present invention relates to a novel polypeptide suitable for inducing an immune response against the Trans-activator of transcription (Tat) protein of human immunodeficiency virus (HIV).

5 BACKGROUND OF THE INVENTION

The HIV protein Tat plays an important role in viral pathogenesis. Tat is secreted by HIV infected cells and, having the capacity to cross cell membranes, it is taken up by infected and uninfected cells. In infected cells, its main function is to activate viral transcription. In infected and uninfected cells, Tat induces a variety of other deleterious effects, such as apoptosis contributing to the collapse of the cellular immune response against HIV infected cells (Campbell & Loret, 2009 Retrovirology 6, 50-63). Therefore, a vaccine eliciting neutralizing antibodies against Tat may be helpful in the prophylaxis or therapy of HIV infection. However, neutralization of extracellular Tat by natural antibodies of HIV infected patients is rarely observed in human cohorts. Tat epitopes appear to be highly conformational, and the development of a vaccine able to generate neutralizing antibodies against Tat will have to take into account Tat folding (Campbell & Loret, 2009, Retrovirology 6, 50-63). Furthermore, Tat recognition appears to be repressed due to sequence homologies with human proteins such as protamine (Campbell & Loret, 2009 Retrovirology 6, 50-63).

A virus strain called HIV-1 Oyi, was cloned from one patient in a cohort of 25 seropositive patients in Gabon (Huet et al., 1989). Importantly, 23 patients in the cohort retro-seroconverted against gp120 during a follow up of two years but maintained a CTL activity against HIV infected cells, and ten years later all patients were still alive with no trace of HIV infection (Campbell & Loret, 2009, Retrovirology 6: 50-63). HIV-1 Oyi has genes similar to regular HIV-1 strains except for the *tat* gene, which had mutations never found in other Tat variants (Gregoire & Loret, 1996, J. Biol. Chem., 22641-46). Immunization with Tat Oyi raised antibodies in rabbits that were able to recognize different Tat variants even with mutations of up to 38%, which is not possible with other Tat variants (Opi et al., 2002). Tat Oyi appears to induce a humoral immune response against a three-dimensional epitope that is conserved in Tat variants, this humoral response enabling neutralization of extracellular Tat. Different vaccines against Tat are being developed, aiming to restore the cellular immune response against HIV infected cells.

Seven rhesus macaques were immunized with synthetic Tat Oyi mixed with classical adjuvants, and a heterologous challenge with the European SHIV BX08 was carried out on the vaccinated macaques and an unvaccinated control group of macaques. Tat Oyi vaccinated macaques had a lower viremia associated with a increase in the CD8 T cells compared with control macaques. Moreover, SHIV infected cells were no longer detectable at 8 weeks post-challenge in the Tat Oyi vaccinated macaques (Watkins et al., Retrovirology 2006, 3-8). At that time point, the unvaccinated macaques had lower viremia than during the peak of the infection, but had not returned to undetectable

levels of SHIV infected cells. This result is promising but the use of a synthetic protein of 101 residues for mass vaccination is not currently technically feasible. Furthermore, Tat Oyi needs to be folded to be efficient as a vaccine and the presentation of Tat Oyi on a carrier such as a virosome might disrupt the Tat folding. Therefore there is a need to obtain a mimotope of Tat Oyi that could be a shorter peptide and could be presented by a virosome without loss of immune activity.

SUMMARY OF THE INVENTION

The present invention relates to a novel polypeptide suitable for inducing an immune response against the Trans-activator of transcription (Tat) protein of human immunodeficiency virus (HIV), said polypeptide forming a three dimensional epitope (mimotope) inducing an immune response against a highly conserved region in Tat variants, a conjugate comprising said mimotope and a carrier, preferentially a virosome, a pharmaceutical preparation including said mimotope linked to said virosome, constituting a therapeutic and/or a preventive vaccine against HIV infection.

According to a first aspect the invention provides a polypeptide consisting of the following amino acid sequence
PX₁X₂EPWX₃HPGSQPX₄TX₅CGGX₆EX₇DX₈EX₉X₁₀X₁₁X₁₂VGGFX₁₃X₁₄KX₁₅LGI
SYGRKKRX₁₆CC as described in SEQ ID No. 1 in which X_n represents any amino acid residue except cysteine, and in which the cysteines at position 17 and 49 or at position 17 and 50 are linked by a disulfide bond, the numbering corresponding to that of SEQ ID No.1

Said polypeptide of the invention can be elongated at its N-terminus and/or C-terminus by one to ten amino acids said amino acid being any amino acid residue except cysteine.

In a preferred embodiment the polypeptide of the invention is elongated at its
 5 N-terminus by to ten amino acids, more preferably by five amino acids such as
 MX₁₇X₁₈VDPX₁X₂EPWX₃HPGSQPX₄TX₅CGGX₆EX₇DX₈EX₉X₁₀X₁₁X₁₂VGGFX₁₃
 X₁₄KX₁₅LGISYGRKKRX₁₆CC as represented by SEQ ID No. 2.

The cysteines at position 22 and 54 or at position 22 and 55 of the polypeptide
 represented by SEQ ID No.2, are linked by a disulfide bond, the numbering
 10 corresponding to that of SEQ ID No.2.

According to a still preferred embodiment, the amino acids designated as X_n
 within the sequence the polypeptide of the invention can be further defined as:

- X₁ is an amino acid residue selected from the group consisting of R or N,
- X₂ is an amino acid residue selected from the group consisting of L or I
- 15 - X₃ is an amino acid residue selected from the group consisting of K or N
- X₄ is an amino acid residue selected from the group consisting of K, T or R
- X₅ is an amino acid residue selected from the group consisting of A or P
- X₆ is an amino acid residue selected from the group consisting of D or A
- X₇ is an amino acid residue selected from the group consisting of P or R
- 20 - X₈ is an amino acid residue selected from the group consisting of T or A,
- X₉ is an amino acid residue selected from the group consisting of T or A
- X₁₀ is an amino acid residue selected from the group consisting of E or K
- X₁₁ is an amino acid residue selected from the group consisting of R, D or
 S,

- X₁₂ is an amino acid residue selected from the group consisting of E or A
 - X₁₃ is an amino acid residue selected from the group consisting of T, Q, I or L
 - X₁₄ is an amino acid residue selected from the group consisting of K, S, T or N,
 - X₁₅ is an amino acid residue selected from the group consisting of G or A,
 - X₁₆ is an amino acid residue selected from the group consisting of R or K
 - X₁₇ is an amino acid residue selected from the group consisting of E or D
 - X₁₈ is an amino acid residue selected from the group consisting of P or L
- 10 Such as defined in SEQ ID No. 4 or SEQ ID No. 5.

In the most preferred embodiment of the invention, the polypeptide consists of the amino acid sequence as described in SEQ ID No. 3.

The polypeptides of the invention as defined above form a tridimensional structure.

- 15 Such polypeptide tridimensional structures are recognized by an antibody raised against the Tat protein under non reducing conditions.

The polypeptides of the present invention can be synthetically produced by chemical synthesis methods which are well known in the art, either as an isolated peptide or as a part of another peptide or polypeptide. As such a polypeptide of the invention can be elongated by further amino acids.

20

Alternatively, the peptide mimotope can be produced in a microorganism or cell comprising a vector expressing a polynucleotide encoding the polypeptide, which is then isolated and if desired, further purified. If an elongated

polypeptide is to be produced, the nucleic acid sequence should encode the longer polypeptide. Said polypeptides provide further aspects of the invention. These polypeptides can be produced in microorganisms such as bacteria, yeast or fungi, in eukaryotic cells such as a mammalian or an insect cell, or in a recombinant virus vector such as adenovirus, poxvirus, herpes virus, Semliki Forest virus, baculovirus, bacteriophages, Sindbis virus or Sendai virus. Suitable bacteria for producing the peptide mimotope include E.coli, B.subtilis or any other bacterium that is capable of expressing polypeptides. Suitable yeast types for expressing the polypeptide of the invention include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Candida, Pichia pastoris or any other yeast capable of expressing polypeptides. Corresponding methods are well known in the art (Sambrook and Russell. Molecular Cloning: A laboratory Manual, 2001, ISBN 978-087969577)

Methods for isolating and purifying recombinantly produced polypeptides are well known in the art and include gel filtration, affinity chromatography, ion exchange chromatography etc.

MIMOTOPE

In a further aspect, the invention deals with an antigenic Tat mimotope consisting of the polypeptide or a tridimensional structure of the invention as described above.

Within the meaning of the present invention the term "mimotope" refers to a molecule which has a conformation that has a topology equivalent to the epitope of which it is a mimic. The mimotope will elicit an immunological

response in a host that recognizes the antigen of which it is a mimic. The mimotope may also act as a competitor for the epitope of which it is a mimic in *in vitro* inhibition assays (e.g. ELISA inhibition assays) which involve the epitope and an antibody binding to said epitope.

- 5 The antigenic Tat mimotope of the invention is recognized by an antibody raised against the Tat protein under non reducing conditions.

CONJUGATE

- The present invention also deals with a conjugate comprising the, a tridimensional structure, an antigenic Tat mimotope or a polypeptide as defined
10 above and a pharmaceutically acceptable carrier.

In a preferred aspect, the pharmaceutically acceptable carrier of the conjugate is a virosome.

Within the meaning of the invention, an effective agent shall mean a polypeptide, a tridimensional structure, a mimotope or a conjugate.

15 VIROSOME-LIKE VESICLE

A virosome-like vesicle suitable for the instant invention comprises at least virosomal lipids and preferably exhibits fusion membrane properties.

According to an embodiment, a virosome-like vesicle of the invention may comprise an unilamellar lipid bilayer.

- 20 According to an embodiment, a virosome-like vesicle of the invention may be a bi- or a multilamellar vesicle.

According to an embodiment, a virosome-like vesicle may have a diameter generally in the range of 50 to 600 nm, and in particular a diameter from 100 nm to 300 nm, and in particular from 200 nm to 400 nm.

Virosome-like vesicles of the invention may be spherical unilamellar vesicles with a mean diameter with approximately 150 nm. Virosome-like vesicles
5 comprise, incorporated into the lipid bilayer, membrane fusion proteins or fragments thereof.

The expression "fusion proteins or fragments thereof" is intended to refer to proteins or fragments thereof capable of inducing and/or promoting a fusion
10 reaction between a virosome-like vesicle membrane and a biological membrane of the target cell.

For example, fusion proteins may be influenza membrane glycoproteins such as hemagglutinin (HA).

According to an embodiment, at least two different fusion proteins or fragments
15 thereof may be used, that may display distinct fusion characteristic. According to another embodiment, distinct fusion characteristics may be, for example, different sensitivity to temperature, to ion concentration, to acidity, to cell type and to tissue type specificity.

According to an embodiment, a virosome-like vesicle may contain fusion
20 proteins that mediate fusion at two distinct temperatures. According to another embodiment, hemagglutinin (HA) from different virus strains may be used to construct a virosome-like vesicle.

As an example, HA molecules from both X-31 and PR8/34 virions may be capable of catalyzing two distinct fusion reactions at distinct temperatures.

Fusion proteins with different fusion characteristics may be derived from different influenza strains or fusion proteins may be derived from other viruses,
5 such as the vesicular stomatitis virus (VSV) E1 protein, the Semliki Forest virus (SFV) envelope protein complex, or the Sendai virus F protein.

An antigen coupled to the membrane of a virosome-like vesicle may be degraded within the endosome and may be presented to the immune system by MHC class II receptors. An antigen contained within the lumen of a virosome
10 can be delivered to the cytosol of an antigen-presenting cell by membrane fusion and degraded in the cytosol, after which it may be presented MNC-1. Cross-presentation of antigens delivered by virosomes may also occur.

Therefore, a virosome-like vesicle may be able to induce a humoral and/or a cellular immune response.

15 In particular, a virosome-like vesicle might induce the production of IgA antibodies, such as secretory IgA, as well as IgG or IgM. Protocols of preparation are well-known by the skilled person in the art. Suitable protocols for the preparation of virosomes are described, for example, in WO 2004/045582 or EP 0 538 437, EP 1 633 395, EP 1 594 466, which are
20 incorporated herein by reference.

According to an embodiment, a virosome-like vesicle according to the invention may be obtained either from a virosome vesicle as such, or from a vesicle resulting from the fusion of a virosome vesicle with a liposome vesicle.

Preparation of virosome vesicles may be made by any known method of the skilled person in the art such as described by Stegmann et al., EMBO J. 6,
5 1987, no. 9, 2651-9, or de Jonge et al., Biochim. Biophys. Acta, 1758, 2006, 527-539, incorporated herein by reference. Virosome vesicles, for example, may be reconstituted from original viral membrane lipids and spike glycoproteins after solubilization of, for example, intact influenza virus with
10 octaethyleneglycol mono-N-dodecyl ether (OEG), sedimentation of the nucleocapsid (the viral glycoproteins and lipids will remain in the supernatant), and removal of the detergent from the supernatant with a hydrophobic resin (Bio-Beads SM2) (Stegmann T, et al., EMBO J. 6, 1987 2651-9).

Virosomes may also be reconstituted from original viral membranes by
15 solubilizing viral membranes with a short-chain phospholipid, sedimentation of the nucleocapsid (only the viral membrane glycoproteins and lipids will remain in the supernatant), and removal of the short-chain lipid in the supernatant by dialysis.

After solubilization of the virus with a detergent or short-chain phospholipid, and
20 the removal of the nucleocapsid as described above, antigens or adjuvants, solubilized in detergent or short-chain phospholipid, may be added to the supernatant prior to the removal of the detergent or short-chain lipid, leading to incorporation of the antigen or adjuvant in the virosome so formed. Likewise,

lipids solubilized in detergent or short-chain phospholipid, may be added to the supernatant for inclusion in the virosomal membrane. Preparation of virosome vesicles containing fusion proteins from different viruses, may be performed by mixing supernatants containing solubilized viral membranes as described
 5 above, or by adding purified fusion proteins to such supernatant, before said removal of detergent or short-chain lipid.

According to one embodiment, a virosome-like vesicle according to the invention may be obtained from a fusion of a virosome vesicle with a liposome vesicle.

10 Therefore, according to one embodiment, a virosome-like vesicle of the invention may comprise virosomal and liposomal lipids. According to one embodiment, a virosome-like vesicle of the invention may comprise a lipid bilayer comprising lipids chosen from cationic lipids, synthetic lipids, glycolipids, phospholipids, glycerophospholipids, glycosphingolipids like galactosylceramid,
 15 sphingolipids, cholesterol and derivatives thereof.

Phospholipids may comprise in particular phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatide acid, cardiolipin and phosphatidylinositol with varying fatty acyl compositions.

20 Cationic lipids may be chosen from DOTMA (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride), DOTAP (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride, DODAC (N,N-dioleoyl-N,N-

dimethylammonium chloride), DDAB (didodecyldimethylammonium bromide) and stearylamine or other aliphatic amines and the like.

The lipids used in the invention may be formulated as small unilamellar liposomes in a mixture with DOPE (dioleoylphosphatidyl ethanolamine) that is
5 widely used as helper lipid to facilitate disruption of the endosomal membrane.

According to another embodiment, co-emulsifying agent may be also used in order to improve the rigidity and/or the sealing of the vesicles. As an example of co-emulsifying agent, mention may be made of cholesterol and derivatives, as for example cholesterol ester charged or neutral as cholesterol sulphate;
10 derivatives with a sterol backbone, for example derived from plants, such as phytosterol (sitosterol, stigmasterol); ceramides; and mixtures thereof.

Virosomes or their contents may be subject to hydrolysis and physical degradation upon storage. According to one embodiment, virosomes may be preserved for long-term storage by freeze-drying, and reconstituted with an
15 aqueous solution before use. Lyoprotectants such as inulin may be added prior to lyophilization to help preserve virosome integrity during lyophilization and upon reconstitution (Wilschut, J. et al., J. Liposome Res. 17, 2007, 173-182). Preferably, spray freeze-drying is employed (Amorij, J.P. et al. Vaccine 17, 2007, 8707-17).

20 A virosome-like vesicle of the invention may further comprise a targeting moiety that target said vesicle to a specific cell or tissue.

According to one embodiment, a virosome-like vesicle of the invention may further comprise a targeting moiety that target said vesicle to a specific cell or tissue.

A suitable targeting moiety may be chosen from a cell-surface receptor, a chemokine, a cytokine, a growth-factor, an antibody or an antibody fragment, a peptide sequence with specificity or specific charge complementary to an adhesion molecule such as an integrin. A targeting moiety may be incorporated into, or attached to the lipid bilayer of said vesicle, by any known techniques of the skilled person in the art.

10

According to one embodiment, the mimotope located to the external surface of virosome-like vesicle of the invention may be:

- covalently linked with a lipid of said virosome-like vesicle, or
- intercalated into a lipid bilayer of said virosome-like vesicle by a peptide transmembrane domain.

15

According to one embodiment, the mimotope may be contained within the virosome.

Modifications of the mimotope of the invention and methods for cross-linking said modified mimotope to the external surface of a virosome-like vesicle may be as those described in WO 2004/078099.

20

According to one embodiment, the mimotope may be covalently linked to the external surface of a virosome-like vesicle by crosslinking with a lipid or a phospholipid. According to another embodiment, the mimotope may be covalently linked to the external surface of a virosome-like vesicle by cross-
5 linking with a carbohydrate. According to an embodiment, a covalently linked mimotope may comprise at least one C-terminally positioned cross-linking residue.

For example, cross-linking residue may be chosen from cysteine (Cys) or lysine (Lys). According to another embodiment a covalently linked mimotope may
10 further comprise at least one spacer residue between said C-terminally positioned cross-linking residues and a corresponding C-terminal mimotope extremity.

A suitable spacer residue may be chosen, for example, from Gly (glycine), Ala (alanine), Ser (serine), Asp (aspartate), Lys (lysine), Gln (glutamine), His
15 (histidine), Ile (isoleucine) and Leu (leucine) residues. From 2 to 12, in particular from 3 to 10, and more particularly from 4 to 8, spacer residues may be linked to form spacer sequences. Suitable spacer sequences may be chosen, for example, from Gly-Gly or Lys- Gly.

Crosslinking of the mimotope to the surface of a virosome-like vesicle may be,
20 for example, performed by the use of amphiphilic PEG derivatives, a phosphatidylethanolamine (PE), a phosphatidylcholine (PC), a phosphatidylserine, a cholesterol, or a mixture thereof, readily incorporated into lipids bilayer. Cross-linking of the mimotope to a lipid of a virosome-like vesicle

of the invention may be performed by any method known to those skilled in the art.

The cross-linking may be operated in a lipid solution and the lipid-peptide conjugate may be subsequently incorporated into a virosome-like vesicle.

- 5 According to an embodiment of the invention, the mimotope may be linked to a lipid of a vesicle of the invention, for example, by a bifunctional succinate linker, in particular a [gamma]-maleinidobutyric acid N-hydroxysuccinimide ester or a N-[gamma]- maleimidobutyryloxy-succinimide-ester.

- 10 Mimotopes, lipid linked mimotopes, phospholipids and adjuvants may be added to the supernatant formed after solubilization of a virus with a detergent or short-chain phospholipid, and the removal of the nucleocapsid as described above. Virosomes may be then formed, as previously described, by detergent removal for example using Bio-Beads SM-2 (Biorad), Amberlyte XM, or short-chain phospholipid may be removed by dialysis.

15

The present invention also provides antigenic or immunogenic composition comprising a polypeptide, a tridimensional structure or an antigenic Tat mimotope or a conjugate according to the invention.

- 20 Said composition can comprises at least another distinct polypeptide, said distinct polypeptide being in the form of a conjugate and more preferably conjugated with a virosome.

The composition of the invention can further comprise an adjuvant.

ADJUVANTS

According to an embodiment, the immunostimulatory effect of effective agent of the invention may be further increased by mixing or associating those effective agents with at least one adjuvant.

- 5 Said adjuvant may be encapsulated inside and/or incorporated in the lipid bilayer of, and/or freely combined with a vesicle, if said effective agent is a virosome-like vesicle according to the invention.

According to one embodiment, a virosome-like vesicle may additionally
10 comprise at least one adjuvant enhancing and/or mediating an immune response chosen from an innate immune response and/or an adaptative immune response.

Usable adjuvants may enhance the immunological response by activating antigen presenting cells (APC), macrophages and/or stimulating specific sets of
15 lymphocytes.

An adjuvant that may convene to the instant invention may be any ligand suitable for the activation of a pathogen recognition receptor (PRR) expressed in and on dendritic cells (DCs), T-cells, B-cells or other antigen presenting cells.
20

Ligands activating the nucleotide-binding oligomerization domain (NOD) receptor pathway may be suited for the purpose of the invention. Adjuvants suitable for these ligands may be muramyl dipeptide derivatives. Ligands activating the Toll-like receptors (TLRs) may also convene for the purpose of

the invention. Those receptors are member of the PRR family and are widely expressed on a variety of innate immune cells, including DCs, macrophages, mast cells and neutrophils.

5 As example of ligands activating TLR, mention may be made, for TLR4 of monophosphoryl lipid A, 3-O-deacytlylated monophosphoryl lipid A, LPS from *E. coli*, taxol, RSV fusion protein, and host heat shock proteins 60 and 70, for TLR2 of lipopeptides such as *N*-palmitoyl-S-2,3(bispalmitoyloxy)-propyl-cysteinyl-seryl-(lysyl)₃-lysine, peptidoglycan of *Staphylococcus aureus*,
10 lipoproteins from *M. tuberculosis*, *Sacharomyces cerevisiae* zymosan, and highly purified *P. gingivalis* LPS, for TLR3 of dsRNA, for TLR5 of flagellin, for TLR7 synthetic compounds such as imidazoquinolines or for TLR9 of certain types of CpG- rich DNA. Other useful adjuvants for the invention may be T helper epitopes.

15

A T helper epitope is a peptide usually derived from exogenous proteins that have undergone proteolytic degradation and processing within the endocytic pathway of antigen presenting cells (APCs). In those cells the Major Histocompatibility Complex of class II (MHC II) associates with those peptides
20 in endosomes. This complex transported to the surface of the APCs may interact with a specific T cell receptor of T lymphocytes CD4 leading to their activation. According to the helper epitope, the T cell response may be of Th1 and/or Th2 type, as known in the art.

As an example of a Th-oriented response epitope one may mention pan DR helper T cell epitope (PADRE). This epitope is engineered to bind most common HLA-DR molecules with high affinity and to act as a powerful immunogen. The PADRE HTL epitope has been shown to augment the
5 potency of vaccines designed to stimulate a cellular immune response (Alexander J. et al., Immunol Res. 18, 1998, 79-92).

According to an embodiment, an adjuvant that may be used with the effective agents of the present invention may be chosen from aluminum salts, aluminum
10 phosphate gels, mycobacteria such as BCG, M. Vaccae, or corynebacterium parvum, peptides, keyhole limpet hemocyanin, , interleukin-2 (IL-2), IL-12, GM-CSF, ligands from the chemokine family, such as RANTES (Regulated upon Activation Normal T cell Expressed and Secreted), a lipoprotein of Gram bacteria, a yeast cell wall component, a double- stranded RNA, a
15 lipopolysaccharide of Gram-negativebacteria, flagellin, a U-rich single-stranded viral RNA, a CpG containing DNA, a Suppressor of Cytokine Signalling small interfering RNA (SOCS siRNA), mellitin derived peptides, a pan DR epitope (PADRE) and mixtures thereof.

ANTIGENIC OR IMMUNOGENIC COMPOSITIONS

20 According to an embodiment, is related to an antigenic or immunogenic composition comprising at least an effective agent chosen among the polypeptide, the tridimensional structure, the antigenic Tat mimotope or a conjugate of the invention.

In a further embodiment, said antigenic or immunogenic composition may comprise at least another distinct antigen, more preferably in the form of a conjugate and even more preferably with a virosome.

Said distinct antigen may be a gp41-derived antigen corresponding to a peptide
5 of amino acid sequence represented in all or part by a sequence set forth as
SEQ ID NO 4 of WO 2007/099446 or SEQ ID No. 5, or an analogue thereof.

The above described compositions may further comprise an adjuvant.

The antigenic or immunogenic compositions according to the invention may be
10 used for the preparation in immunotherapy, in particular prophylactic
immunotherapy, to induce an immune response directed against the Tat protein
of a human immunodeficiency virus and for the preparation of a vaccine.

Such antigenic or immunogenic pharmaceutical composition may comprise a
15 solution of effective agent suspended in a pharmaceutical acceptable carrier,
for example an aqueous carrier, forming a pharmaceutical composition. A
variety of aqueous carriers may be used e.g., water, buffered water, saline, 0.
3% glycine, hyaluronic acid and the like. A pharmaceutical composition may be
sterilized by conventional, well-known sterilization techniques, or may be sterile
20 filtered. Lyoprotectants suitable for lyophilization of virosomes, such as inulin,
are known in the art (Wilschut, J. et al., J. Liposome Res. 17, 2007;173-82),
and may be added.

The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. A pharmaceutical composition may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, lyoprotectants and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, sucrose, inulin, trehalose, dextran, calcium chloride, sorbitan monolaurate, triethanolamine oleate, among many others, acceptable concentrations of salt, antioxidants, preservatives, compatible carriers, adjuvants as previously described and optionally other therapeutic agents.

The effective agents of the invention, and in particular the virosome-like vesicles and the pharmaceutical compositions comprising thereof may be used for the preparation of a vaccine.

As such, a further aspect of the invention relates to an anti-HIV vaccine comprising an effective agent such as a polypeptide, a tridimensional structure an antigenic mimotope or a conjugate according to the invention.

Said effective agent of the invention may be used for the manufacture of a medicament intended to induce an adaptative immune response and/or an innate immune response directed against a Tat protein of a human immunodeficiency virus.

The invention also deals with a prophylactic method comprising administering to a patient at least an effective agent or a composition according to the invention, said effective agent of composition being administered in an effective amount.

- 5 In a further aspect, of said method, the effective agent of the invention is administered in combination with an additional distinct antigen.

According to another embodiment, the virosome-like vesicles that may be used in accordance with the instant method may be virosome-like vesicles comprising a Tat mimotope located to the external surface and virosome-like
10 vesicles comprising another distinct antigen located to the external surface of distinct virosomes as a combined preparation for simultaneous, separate or sequential use in immunotherapy.

- In a preferred aspect of the above method said effective agent or composition
15 is administered systematically by injection or topically by a mucosal route, or a combination thereof.

Injection routes may be, for example, intraperitoneal, intradermal, subcutaneous intravascular or intramuscular route.

20

Any mucosal route may be used, such as genito-urinary route as for example vaginal route, gastro-intestinal route, anorectal route, respiratory route, upper mucosal tissue, mouth-nasal route and mixtures thereof.

Any immunization protocols standard in the art may be used.

An effective amount is that amount of effective agent or composition that alone, or together with further doses may evoke the desired response. An effective amount may depend upon a variety of factors, such as the route for administration, whether the administration is in single or multiple doses, and individual patient parameters including age, physical condition, size, weight, and the stage of the disease. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

10

In one embodiment, the effective agent or the pharmaceutical composition of the invention may be provided as oral dosage forms, such as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions.

15

According to another of its aspects, the instant invention also relates to a kit for inducing an immune response against a Tat protein of a human immunodeficiency virus comprising: at least a first effective agent according to the instant invention such as a polypeptide, a tridimensional structure, an antigenic Tat mimotope, a conjugate and more preferably a virosome-like vesicle as described above, and at least a second gp41 -derived antigen more preferably in the form of a conjugate such as a virosome-like vesicle of the invention said first and second antigens being different from one each other.

20

The various aspects of the instant invention will be further illustrated by the following examples, which should not in any case be constructed as limiting the scope of the instant invention.

5

Figure Legends

Figure 1 Structure and sequence of Tat Oyi, and the Tat mimotope. Panel a: structure of Tat Oyi (left) and the mimotope (right). Panel b: sequence of Tat

10 Oyi. Tat is usually divided into six regions (Campbell & Loret, 2009, Retrovirology 6: 50-63). The N-terminal Tat Oyi region I (residues 1-22), region II (cysteine-rich region or residues 23-37), region III (residues 38-48), region IV (basic region or residues 49 to 62), region V (residues 63 to 72), region VI (residues 73-101). The mimotope comprises the N-terminal region, residues 1-

15 20 (1), followed by the inverted sequence of the C-terminal region III, and t. Glycines serve as a linker between the three Tat regions are purple. The position of the crucial glutamine 100 residue in Tat Oyi is indicated by an arrow. Two of the three cysteines create a disulfide bridge that is essential to have a 3D epitope in the mimotope equivalent to the 3D epitope in Tat Oyi that induce

20 cross clade immunity and neutralising antibodies. If this disulfide bridge is broken, the mimotope is no longer recognised by Tat antibodies. The disulfide bridge in the mimotope of the invention is indicated by a line linking cysteine 22

with either the one but C-terminal or the C-terminal cysteine (panels c and d, SEQ ID No. 3).

Figure 2 IgG (A) and IgM (B) in serum and vaginal secretion of rats vaccinated with the virosome-coupled Tat mimotope 2 weeks after the last vaccination. Pooled serum samples from the groups vaccinated with 5 or 25 µg of mimotope per injection were analysed by ELISA. Closed triangles: serum samples 5 µg group, open triangles vaginal wash 5 µg, crosses: serum samples, 25 µg, open squares, vaginal washes.

Figure 3 Sequences of the six Tat variants synthesized, selected as representative examples of the five main HIV-1 subtypes, compared to Tat Oyi. Ug11RP HIV-1 strain was identified in Uganda by the Medical Research Council Program on AIDS and corresponds to subtype A. HXB2 HIV-1 strain was isolated in France and corresponds to subtype B. 96Bw is a C subtype identified in Botswana. *Eli* is a subtype D identified in the Democratic Republic of Congo. CM240 is a subtype AE identified in Thailand.

Figure 4 Recognition of Tat variants by serums of rats the virosome-coupled Tat mimotope, compared recognition of the mimotope itself and to Tat Oyi. IgG ELISA with decreasing dilutions of serum was performed as in example 4. The Tat proteins of Figure 3 were used: Clade A (Ug11RP), B (HXB2), C (96Bw), D (Eli), E (CM240), and compared to free mimotope (MIMO) and Tat Oyi. Pooled serum samples were from the group vaccinated 25 µg of

mimotope coupled to virosome per injection, collected two weeks after the last injection.

Figure 5 Reduction of the disulfide bridge in the mimotope affects
5 **recognition by anti-Tat antibodies.**

ELISA plates were coated with reduced (black), or oxidized (white) forms of the mimotope and an ELISA was carried out using two different monoclonal antibodies (Mab) specific for Tat and a polyclonal rabbit serum specific for Tat
10 Oyi.

Example 1: Mimotope construction

Molecular modelling was carried out with the Insight II 2002 package including Biopolymer, Discover and Homology (Accelrys, San Diego, CA). Tat Oyi
15 structure was obtained from molecular modelling using the Tat Eli NMR structure as a model (Watkins JD et al., Retrovirology 5, 2008:83). Molecular modelling was carried out with the Consistent Valence Force Field, using Steepest Descent and Gradient Conjugate algorithms for energy minimization and dynamic steps.

20 Figure 1 shows the three-dimensional (3D) structure of Tat Oyi. Three regions of Tat in particular were considered important for mimotope construction. Figure 1 shows that these three regions are located at the N and C terminus and in the middle of the Tat Oyi sequence. A suitable structure for the mimotope was determined from molecular modelling of these three Tat Oyi

- regions in positions similar to their location in Tat Oyi. Glu 100 appears to be very important in the 3D epitope, as this mutation is observed only in Tat Oyi (Campbell GR and Loret E.P. Retrovirology, 6, 2009, 50-63). To be able to obtain the structure of the mimotope as shown in the figure, the order of these
- 5 three regions was changed with respect to the Tat Oyi sequence. The sequence at the C terminus was inverted and placed in the middle of the mimotope sequence. A disulfide bridge was necessary to block the sequence of the mimotope (56 residues) into the conformation of the 3D epitope resembling that of Tat Oyi.
- 10 The mimotope was synthesized by fast Fmoc solid phase synthesis and then cleaved with trifluoroacetic acid (TFA). The peptide was purified by high pressure liquid chromatography (HPLC) on a reverse phase C8 column. Purification was carried out under reducing conditions (0.1 % TFA). After purification, oxidation was carried out in 100 mM phosphate buffer at pH 8.2 for
- 15 6 hours. Precipitation was observed if oxidation was carried out for 24 hours. After 6 hours a shift of 1 minute was observed in analytical HPLC (data not shown). Acetylation of cysteine was carried out with iodoacetamide, and mass spectroscopy revealed that there was a disulfide bridge since only one acetylated cysteine per three cysteines was identified. The only possible
- 20 disulfide bridge is between Cys 22 and one of the two cysteines located at the C terminus (Figure 1, panels c and d, SEQ ID No. 3).

Example 2: Recognition of the mimotope by sera from HIV infected people.

From a cohort of 100 seropositive patients never vaccinated with Tat, ten sera capable of recognizing Tat Oyi significantly at serum dilutions of 1/20, 1/100, 1/500, and 1/2500 were selected by ELISA. ELISA plates were coated with 200 ng of the Tat mimotope per well, in 100 mM phosphate buffer pH 6. Five of the
5 ten sera were able to recognise significantly the mimotope protein under the same conditions at these same dilutions.

Example 3: Immunization of rats with virosome-coupled mimotope.

Virosome-like vesicles were prepared essentially as as described in WO
10 2007/099387. Briefly, influenza A/Singapore/6/86 virus was dissolved in 100 mM of octaethyleneglycolmonodecylether (OEG) in phosphate buffered saline (PBS), and the viral nucleocapsid was removed by ultracentrifugation. The solubilized membranes, containing 4 mg of hemagglutinin, were mixed with 32 mg egg phosphatidylcholine (PC) and 8 mg of phosphatidylethanolamine (PE)
15 dissolved in 2 ml of PBS containing 100 mM OEG.

The Tat mimotope (SEQ ID No. 3) was conjugated through a succinate linker at the N-terminus to a regioisomer of phosphatidylethanolamine (PE) as follows. Phosphatidylethanolamine (PE) was dissolved in methanol and 0.1% (v/v)
20 triethylamine was added. The solution was then mixed with the heterobifunctional cross- linker N-[gamma]-maleimidobutyryloxy-succinimide-ester (GMBS), (Pierce Chemical Company, Rockford, IL) (ratio PE: GMBS = 5:1), dissolved in dimethylsulfoxide (DMSO) (20 [mu]l). After an incubation for 30 minutes at room temperature, the solvents were evaporated for 1 h under

vacuum in a speedvac centrifuge. The GMBS-PE was then dissolved in 1 ml of PBS containing 100 mM octaethyleneglycol (OEG) (Fluke Chemicals, Switzerland), and the Tat mimotope (SEQ ID No. 3) was added (ratio PE-GMBS:mimotope = 5:1). In this step, the phosphatidylethanolamine-GMBS
5 reacts with a free C-terminal cysteine of the Tat mimotope. After incubation time of 30 minutes, free cysteine was added, in order to inactivate free GMBS (ratio cysteine:GMBS = 10:1).

The lipid-coupled mimotope was then mixed with the solubilized viral
10 membrane/lipid mixture at a ratio of 1 mg of hemagglutinin per mg of mimotope conjugate. The mixture was sonicated for 1 minute, centrifuged at 100.000 g for one hour and then the supernatant was sterilized by filtration, and virosomes were formed from them by detergent absorption on Bio-Beads SM-2 (Bio-Rad).

15 Two groups of five Wistar rats were vaccinated subcutaneously with A/Singapore/6/86 influenza vaccine (1 µg/rat). 3 weeks later one group was vaccinated intramuscularly (i.m.) with 5 µg per rat of mimotope conjugated to virosomes, the other with 25 µg. The i.m. immunizations with the vaccine, at the same dose, were repeated one month and two months later. Vaginal
20 washes were collected seven days after the last vaccination and blood was collected at various time points, including two weeks after the last vaccination. All of the rats survived the vaccination, and there were no obvious deleterious side effects, such as weight loss.

Example 4: Recognition of the mimotope by sera and vaginal secretions of rats immunized with virosome-coupled mimotope.

The serums and vaginal samples of Example 3 were analyzed by ELISA
5 (Figure 2). The ELISA plates were coated with the mimotope protein at a concentration of 200 nM, in 100 mM phosphate buffer pH 6. Under these conditions, Tat proteins adopts an active tertiary structure (Opi et al., Vaccine 22, 2004, 3105-3119). It was found that the virosome-coupled mimotope induces IgG and IgM antibodies in serum capable of recognizing the mimotope.

10

Example 5: Production of synthetic Tat proteins, and recognition by rat sera after immunization of rats with virosome-coupled mimotope.

Tat proteins representing different clades of HIV-1 (Figure 3) were assembled
15 according to the method of Barany and Merrifield (1980) on Hydroxymethylphenoxy preloaded resin (0.5-0.65 mmol; Perkin Elmer, Applied Biosystem Inc., Forster City, CA) on an automated synthesizer (ABI 433A, Perkin Elmer, Applied Biosystem Inc.) and purified as described elsewhere (Péloponèse et al, 1999). The purified Tat proteins were fully monomeric and
20 had full biological activity, as assessed by a transactivation assay. The trans-activation activities of the synthetic Tat proteins was analyzed by monitoring the production of β -galactosidase after activation of lacZ expression in HeLa-P4 cells. These cells contain the *lacZ* gene under the control of an integrated HIV-1 LTR. Briefly, 2×10^5 cells per well were incubated in 24-well flat-bottomed

plates (Falcon) at 37°C, 5% CO₂, in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 100 µg/ml neomycin (all Invitrogen). After 24 h, cells were washed with phosphate-buffered saline. Tat protein was mixed with DMEM supplemented with 0.01% (w/v) protamine (Sigma) and 0.1% (w/v) bovine serum albumin (BSA; Sigma) and immediately added to the cells. After 16 hours at 37°C, 5% CO₂, cells were washed with phosphate-buffered saline, lysed and the β-galactosidase content was measured with a commercially available antigen capture enzyme-linked immunosorbent assay (β-galactosidase ELISA, Roche Diagnostics). The concentrations of Tat used was 0,5 µM. It was found that all the produced Tat proteins had trans-activation activity. The serum anti-Tat antibodies were detected by ELISA using Maxisorp U96 immunoplates (Nunc). At the coating of the ELISA plates, the concentration of antigen was 200 nM diluted in 100 mM phosphate buffer pH 6. Under these conditions, the Tat proteins adopt an active tertiary structure (Opi et al., Vaccine 22, 2004, 3105-3119).

It was found (Figure 4) that the rat serums were capable of broadly recognizing the Tat proteins representing five different clades of HIV-1, indicating that the virosome-coupled mimotope could provide a vaccine that induces broadly neutralizing high-titer anti Tat antibodies.

Example 6: Importance of the disulfide bridge in the mimotope for recognition by anti-Tat antibodies.

Reduced and oxidized forms of the mimotope were coated onto ELISA plates at 33 ng/well and each one of three different antibodies capable of recognizing Tat was diluted 1/100. Under these conditions, monoclonal antibody (Mab) 8D6 (rat IgM) , Mab 27A8 (rat IgG) and a rabbit polyclonal antibody against Tat Oyi
5 recognized 7, 9 and 3 times more the oxidized mimotope form (white) than the reduced reduced (black), respectively. These results indicate that the oxidized mimotope has a conformational structure similar to the 3D epitope of Tat Oyi and that the mimotope disulfide bridge is crucial to maintain this conformation.

Claims

1. Polypeptide

5 - consisting of the amino acid sequence :

PX₁X₂EPWX₃HPGSQPX₄TX₅CGGX₆EX₇DX₈EX₉X₁₀X₁₁X₁₂VGGFX₁₃X₁₄KX₁₅LGISYGRKKRX₁₆CC (SEQ ID No. 1) in which X_n represents any amino acid residue except cysteine, and in which

- the cysteines at position 17 and 49 or at position 17 and 50 are

10 linked by a disulfide bond, the numbering corresponding to that of SEQ ID No.1.
2. A polypeptide according to claim 1 elongated at its N-terminus and/or C-terminus by an additional one to ten amino acids said amino acids

15 being any amino acid residue except cysteine.
3. A polypeptide according to claim 2 elongated at its N-terminus by an additional one to ten amino acids, more preferably by five amino acids such as represented by

20 MX₁₇X₁₈VDPX₁X₂EPWX₃HPGSQPX₄TX₅CGGX₆EX₇DX₈EX₉X₁₀X₁₁X₁₂VGGFX₁₃X₁₄KX₁₅LGISYGRKKRX₁₆CC (SEQ ID No. 2).
4. A polypeptide according to claim 3 elongated at its N-terminus by five amino acids as represented by SEQ ID No. 2, the cysteines at

position 22 and 54 or at position 22 and 55 being linked by a disulfide bond, the numbering corresponding to that of SEQ ID No.2.

5. Polypeptide according to claim 1 to 4 in which :

- 5 - X_1 is an amino acid residue selected from the group consisting of R or N,
- X_2 is an amino acid residue selected from the group consisting of L or I
- X_3 is an amino acid residue selected from the group consisting of K or N
- X_4 is an amino acid residue selected from the group consisting of K, T or R
- X_5 is an amino acid residue selected from the group consisting of A or P
- 10 - X_6 is an amino acid residue selected from the group consisting of D or A
- X_7 is an amino acid residue selected from the group consisting of P or R
- X_8 is an amino acid residue selected from the group consisting of T or A,
- X_9 is an amino acid residue selected from the group consisting of T or A
- X_{10} is an amino acid residue selected from the group consisting of E or K
- 15 - X_{11} is an amino acid residue selected from the group consisting of R, D or
 S,
- X_{12} is an amino acid residue selected from the group consisting of E or A
- X_{13} is an amino acid residue selected from the group consisting of T, Q, I
 or L
- 20 - X_{14} is an amino acid residue selected from the group consisting of K, S, T
 or N,
- X_{15} is an amino acid residue selected from the group consisting of G or A,
- X_{16} is an amino acid residue selected from the group consisting of R or K
- X_{17} is an amino acid residue selected from the group consisting of E or D

- X_{18} is an amino acid residue selected from the group consisting of P or L
As represented by SEQ ID No. 4 or SEQ ID No.5.

6. Polypeptide according to claim 4 or 5 consisting of the amino acid sequence as described in SEQ ID No. 3.

5

7. A tridimensional structure formed by the polypeptide according to anyone of claims 1 to 6.

8. An antigenic Tat mimotope consisting of a peptide according to
10 anyone of claims 1 to 6 or a tridimensional structure according to claim 7.

9. A polypeptide according to anyone of claims 1 to 6, a tridimensional structure according to claim 7, or an antigenic Tat mimotope according to claim 8, which are recognized by an antibody raised against
15 the Tat protein under non-reducing conditions.

10. Polynucleotide encoding for the polypeptide according to anyone of claims 1 to 6.

20 11. A conjugate comprising the polypeptide according to anyone of claims 1 to 6, a tridimensional structure according to claim 7 or an antigenic Tat mimotope according to claim 8, a polypeptide according to claim 8 and a pharmaceutically acceptable carrier.

12. The conjugate according to claim 11 in which the pharmaceutically acceptable carrier is a virosome.
13. An antigenic or immunogenic composition comprising a polypeptide according to anyone of claims 1 to 6, a tridimensional structure according to claim 7, or an antigenic Tat mimotope according to claim 8, or a conjugate according to claim 11 or 12.
14. The antigenic or immunogenic composition of claim 13 further comprising at least another distinct polypeptide.
15. The antigenic or immunogenic composition of claim 14 wherein said at least distinct polypeptide is in the form of a conjugate, preferably conjugated with a virosome.
16. The antigenic according to anyone of claims 13 to 16 further comprising an adjuvant.
17. The composition according to the preceding claims for its use in immunotherapy, in particular prophylactic immunotherapy.
18. The composition according to claim 17 for the preparation of a vaccine.

19. An anti-HIV vaccine comprising a polypeptide according to anyone of claims 1 to 6, a tridimensional structure according to claim 7, an antigenic Tat mimotope according to claim 8 or a conjugate according to claim 11 or 12.
- 5
20. Use of a polypeptide according to anyone of claims 1 to 6, a tridimensional structure according to claim 7 or an antigenic Tat mimotope according to claim 8, a conjugate according to anyone of claims 11 or 12, or a composition according to any one of claims 13 to 16, for the manufacture of a medicament intended to induce an adaptative immune response and/or an innate immune response directed against a Tat protein of a human immunodeficiency virus.
- 10
21. A prophylactic vaccination method comprising administering to a patient at least a peptide according to anyone of claims 1 to 6, a tridimensional structure according to claim 7, or an antigenic Tat mimotope according to claim 8, or a conjugate according to claim 11 or 12, or a composition according to anyone of claims 13 to 16.
- 15
22. The method according to claim 21, wherein said polypeptide, conjugate or composition is administered systematically by injection and/or topically by the mucosal route.
- 20

23. The method according to claims 22, wherein said mucosal route is chosen from genito-urinary tract, gastro-intestinal tract, anorectal route, respiratory tract, upper mucosal tissue, mouth-nasal route and mixtures thereof.
- 5 24. The method according to anyone of claims 21 to 23, wherein said polypeptide, conjugate or composition is administered in combination with an additional distinct antigen.
- 10 25. A kit for inducing an immune response against a Tat protein of human immunodeficiency virus comprising at least a conjugate according to anyone of claims 11 or 12.
- 15 26. A kit for inducing an immune response according to claim 25 further comprising a second conjugate comprising a polypeptide distinct from the polypeptide according to anyone of claims 1 to 6.

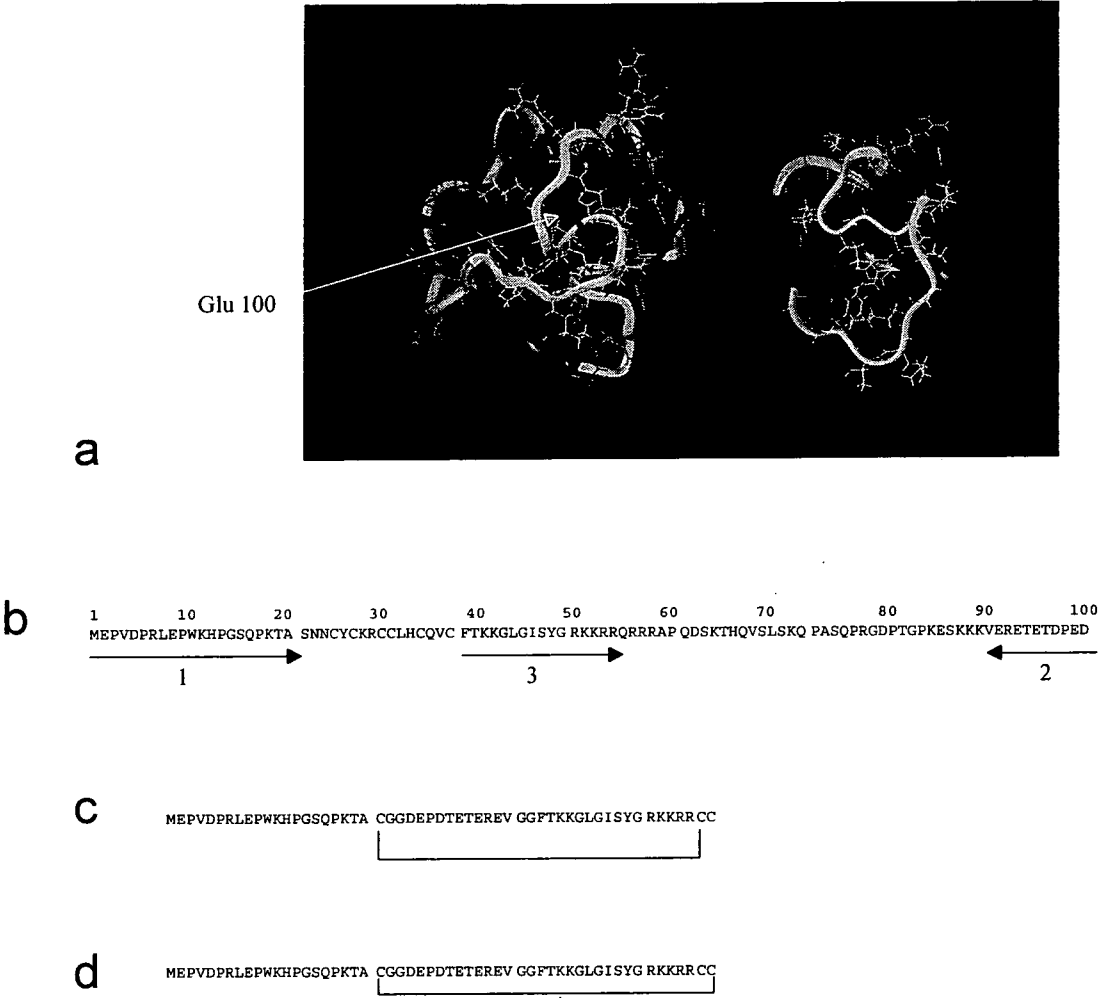
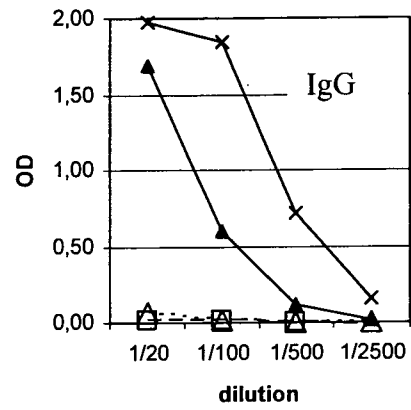
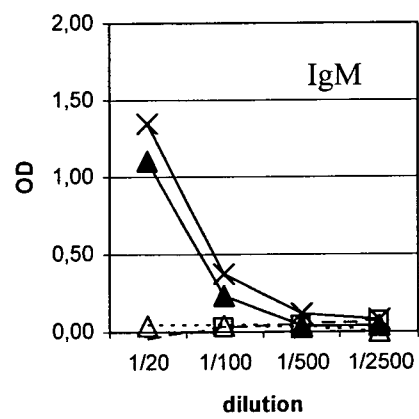


Figure 1

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A



B

Figure 2

1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100
Tat_HV10yi MEVDPRLPEPKHPGSPKTAASNNCYCKRCCLHCOVCFKKGLGISYGRKKRRQRRAPQDSKTHQVSLSKQPASQPRGD-PTGPKESKKKVERETETDPED 101
Tat_HV1UG11RP D...NI...N...T.PC.R...V.Y.L...QS.....K...GPT.SN.Q.NPIP...IPTQ.I-S...E.S...DK...RR. 101
Tat_HV1HXB2 CT...K.F.....IT.A.....H.N.Q...A.....T.....-.....F. 100
Tat_HV196BW N.....R..CTK...Y.Y.L...QT.....ST.PS.ES.NLI.E.LPRTQ.N-...SE.....SK..A..FA101
Tat_HV1E1i D...N...N...R.PC.K.H.K.Y.P...LN.....GP.GGQA...PIP...S.....Q.....S.A.... 99
Tat_HV1CM240 L...N...N...T..CSK...K.W...L..L.....H..GT..S..D..NPIP...LPIT.RN-.D.....E.ASKA...OC. 101

Figure 3

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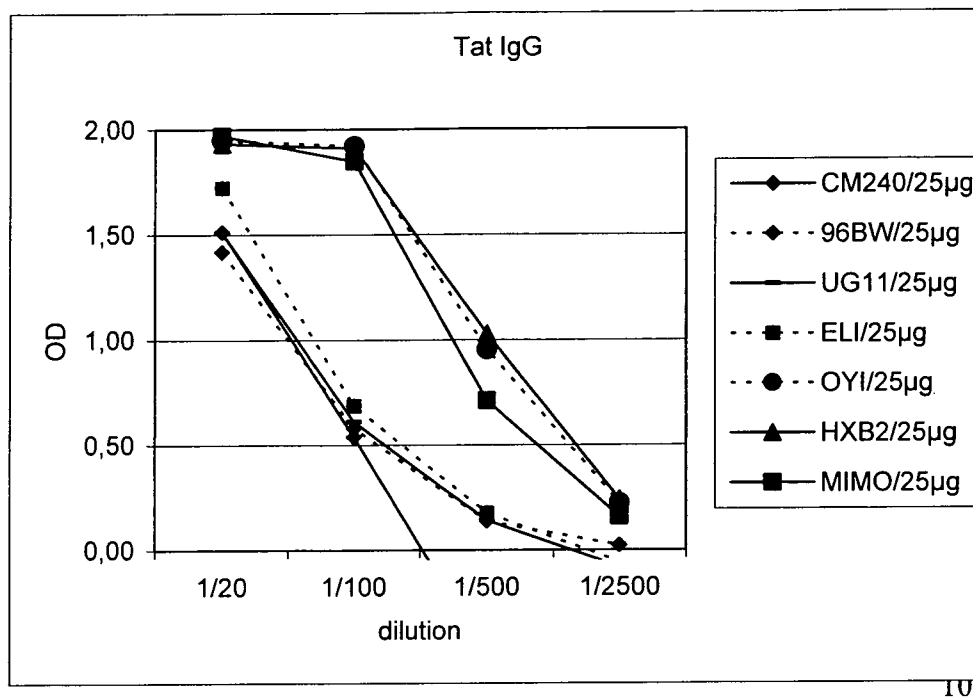


Figure 4

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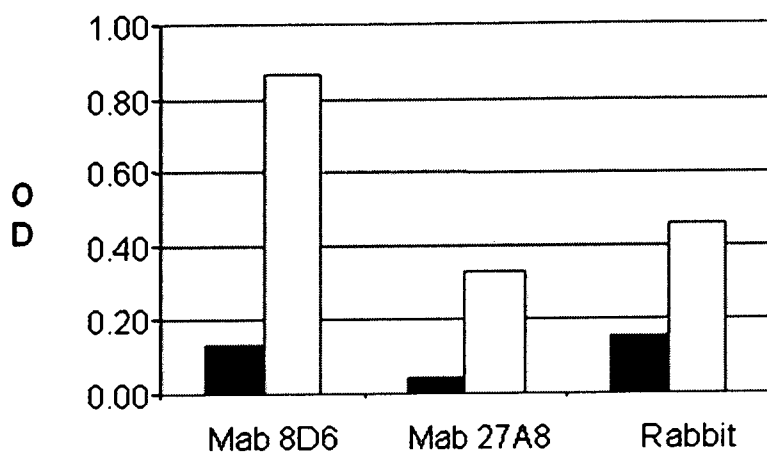


Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/001263

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/16 A61K39/21 C12N15/113
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/130256 A2 (UNIV AIX MARSEILLE II [FR]; CENTRE NAT RECH SCIENT [FR]; LORET ERWANN) 29 October 2009 (2009-10-29) pages 7,10-12; claims 1-25; sequences 1,2 -----	1-26
A	WO 00/61067 A2 (CENTRE NAT RECH SCIENT [FR]; LORET ERWANN [FR]) 19 October 2000 (2000-10-19) claims 1-18; figure 1A -----	1-26
A	WO 2007/099387 A1 (MYMETICS CORP [US]; INST NAT SANTE RECH MED [FR]; PEVION BIOTECH LTD []) 7 September 2007 (2007-09-07) page 2; claim 1 ----- -/-	1-26

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

8 October 2010

Date of mailing of the international search report

26/10/2010

Name and mailing address of the ISA/

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Schmidt, Harald

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2010/001263

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2010/001263

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