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- (71) **Applicant:** RUTGERS, THE STATE UNIVERSITY OF
NEW JERSEY [US/US]; Old Queen's Somerset Street,
New Brunswick, NJ 08909 (US).
- (72) **Inventor:** PINTER, Abraham; 1250 E. 22nd Street,
Brooklyn, NY 11210 (US).
- (74) **Agents:** NORTON, Gerard, P. et al.; Fox Rothschild Llp,
997 Lenox Drive, Building 3, Lawrenceville, NJ 08648-
2311 (US).
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(54) **Title:** HIV-1 ANTIGENS WITH DISCRETE CONFORMATIONAL FORMS OF THE V1/V2 DOMAIN AND METHODS OF USE THEREOF

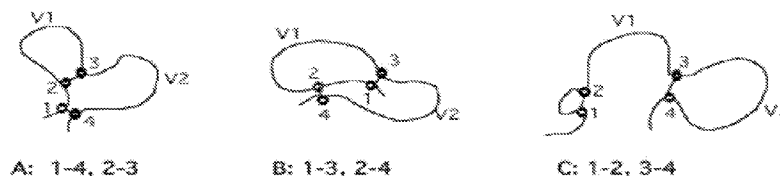


FIGURE 6

(57) **Abstract:** The present invention relates to a novel composition of HIV-1 Env proteins that contain structurally and immunologically distinct V1/V2 domains. Methods of isolating such proteins, and methods of using such proteins as immunogens, therapeutic agents, vaccines, and test compounds for use in identifying a HIV antiviral are also provided.



HIV-1 ANTIGENS WITH DISCRETE CONFORMATIONAL FORMS OF THE V1/V2 DOMAIN AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

5 The present application claims the benefit of U.S. Provisional Patent Application Serial No. 61/697,979, filed September 7, 2012. The contents of the foregoing application is incorporated by reference in its entirety.

GOVERNMENT INTERESTS

10 This invention was made with government support under grant numbers R01 AI-46383, R01 AI102718-01 and P01 AI-0888610 from the National Institutes of Health. The United States government has certain rights to this invention.

FIELD OF THE INVENTION

 The present invention relates to a novel composition of recombinant HIV-1 Env proteins that contain structurally and immunologically distinct V1/V2 domains.

BACKGROUND OF THE INVENTION

15 Recent studies have shown a role for the V1/V2 domain of the HIV-1 ENV protein as a target in HIV-1 vaccines. A series of potent and broadly neutralizing monoclonal antibodies (MAbs) targeting a class of quaternary epitopes that were dependent on several positions in the V2 region were isolated in one study (Walker et al., 2009, Science 326:285-289; Moore et al., 20 2011, Journal of Virology 85:3128-3141). These antibodies react preferentially with native trimeric Env complexes. Further studies have shown that a small conserved sequence in the V2 domain interacted with $\alpha 4\beta 7$ integrin, the mucosal homing receptor for activated T cells, and that this interaction strongly enhanced infection of those cells (Nawaz et al., 2011, PLoS Pathog 7:e1001301; Cicala et al., 2009, Proc Natl Acad Sci USA 106:20877-20882). A third finding 25 came from the analysis of correlates of protection in the recently concluded RV144 vaccine trial conducted in Thailand, the first HIV vaccine trial that resulted in some protection against infection (Rerks-Ngarm et al., 2009, N Engl J Med 361:2209-2220). Protection in this trial was shown to correlate with an increased titer of antibodies that bound to a V1/V2 fusion protein, but not to any other factor analyzed, including avidity and titers of Env-specific IgG and IgA, viral

neutralizing activity or levels of Env-specific CD4+ T cells (Haynes et al., 2012, The New England Journal of Medicine 366:1275-1286).

SUMMARY OF THE INVENTION

5 The present invention relates in part to isolated and purified polypeptides comprising the V1/V2 domain of a HIV envelope (Env) protein having a structural configuration of a "C" form, as at least shown in Figure 6 and Figure 7. As used herein, "HIV" is meant to represent either HIV-1 or HIV-2, and any and all subtypes thereof. Upon review of the specification, it will be understood that any such polypeptide is encompassed within the scope of invention which
10 possesses the ability present and maintain the conformational integrity of the "C" form of the V1/V2 domain. It is disclosed herein that this "C" form presents one of several structural conformations based on an alternative form of disulfide bonding within this V1/V2 domain, which dramatically impacts the immunoreactivity of these proteins. To this end, an embodiment of the present invention further relates to a polypeptide which presents and maintains the
15 conformational, antigenic, and immunoreactive integrity of the alternative "C" form of the V1/V2 domain, including but not limited to wherein the polypeptide is native gp120, or wherein the polypeptide native is gp140, or wherein the polypeptide is a recombinant form of gp120, or wherein the polypeptide is a recombinant form of gp140. Thus, another embodiment of the present invention further relates to such a polypeptide maintaining the structural integrity as
20 discussed herein where the recombinant gp120-based form comprises a portion of the gp120 coding region, again, such that the antigenicity immunoreactivity of the V1/V2 structural configuration of the "C" form is maintained. A specific, but non-limiting embodiment of the present invention relates to such a presented polypeptide wherein the amino-terminal sequence represents a portion of MuLV gp70, including but not limited to a polypeptide presenting the "C"
25 form which has the amino acid sequence as set forth in Figure 14 (SEQ ID NO: 2). An additional embodiment of the present invention relates to nucleic acid molecules encoding a polypeptide with at least 90% amino acid sequence identity to a polypeptide which presents and maintains the conformational, antigenic, and immunoreactive integrity of the alternative "C" form of the V1/V2 domain, as well as related recombinant DNA molecules housing such a
30 nucleic acid molecule, and any cell line transfected or transformed with any such recombinant DNA molecule.

The present invention also relates to isolated and purified polypeptides comprising the V1/V2 domain of a HIV envelope (Env) protein having a structural configuration of yet another form, identified herein as a "B" form, as at least shown in Figure 6 and Figure 7, or in the alternative, presented as both a "B" form and an "A" form ("B"/"A" form"). Again, upon review of the specification, it will be understood that any such polypeptide is encompassed within the scope of invention which possesses the ability to present and maintain the conformational integrity of the "B" form of the V1/V2 domain, or in the alternative a "B"/"A" combined form. Either form/forms as disclosed herein will again present one of several structural conformations based on an alternative form of disulfide bonding within this V1/V2 domain, dramatically impacting the immunoreactivity of these proteins. As disclosed immediately above in regard to the "C" forms, the present invention further relates to a polypeptide which presents and maintains the conformational, antigenic, and immunoreactive integrity of the alternative "B" form (or in the alternative a "B"/"A" combined form) of the V1/V2 domain, including but not limited to wherein the polypeptide is native gp120, native gp140, recombinant gp120, recombinant form gp140, a construction based upon a portion of the gp120 or gp 140 coding region, again, such that the antigenicity and immunoreactivity of the V1/V2 structural configuration of the "B" form (or in the alternative a "B"/"A" combined form) are maintained. An additional, but non-limiting embodiment of the present invention relates to such a presented polypeptide wherein the amino-terminal sequence represents a portion of MuLV gp70, including but not limited to a polypeptide presenting the "B" form (or a "B"/"A" combined form) which has the amino acid sequence as set forth in Figure 14 (SEQ ID NO:2). An additional embodiment of the present invention relates to nucleic acid molecules encoding a polypeptide with at least 90% amino acid sequence identity to a polypeptide which presents and maintains the conformational, antigenic, and immunoreactive integrity of the alternative "B" form of the V1/V2 domain, or in the alternative a combined "B"/"A" form) as well as related recombinant DNA molecules housing such a nucleic acid molecule, and any cell line transfected or transformed with any such recombinant DNA molecule. It will be evident upon review of this specification that combinations of an "A" form, a "B" form and/or a "C" form are also contemplated herein as relating to the present invention.

The present invention also relates to utilizing the polypeptides of the present invention, including but not limited to polypeptides which maintain the conformational integrity disclosed

herein as a "C" form, a "B" form and/or a combined "B/A" form in methods of treating a HIV positive subject which involves administering to such a subject a pharmaceutical composition comprising any such polypeptide disclosed herein, or combination thereof, with such a pharmaceutical composition optionally containing a pharmaceutically active carrier. It will be the goal of any such treatment to result in a reduction in the HIV viral load of a subject.

The present invention further relates to utilizing the polypeptides of the present invention, including but not limited to polypeptides which maintain the conformational integrity disclosed herein as a "C" form, a "B" form and/or a combined "B/A" form in methods of vaccinating a human subject against infection or progression of HIV which comprises administration of a pharmaceutical composition comprising any, or combination thereof, of such polypeptides disclosed herein, with the pharmaceutical composition optionally containing a pharmaceutically active carrier, wherein administration of the pharmaceutical composition results in generation of an immune response against HIV infection.

To this end, the present invention relates to any such methodology utilized to induce an enhanced immunological response against a HIV antigen in a mammalian host, wherein the mammalian host is inoculated with a polypeptide disclosed herein as a "C" form, a "B" form and/or a combined "B/A" form and, optionally, a pharmaceutically acceptable carrier. Thus, the present invention also relates to any such pharmaceutical composition which comprises an effective amount of such a polypeptide and, optionally, a pharmaceutically acceptable carrier.

Another embodiment relates to methods of identifying an HIV antiviral compound, which comprises the steps of: (a) combining component (i) -- a test compound, component (ii) -- a "C" form-specific antibody, or a "B" form-specific antibody and/or an antibody which specifically recognizes a combination of the "B" and "A" form, and component (iii) -- a polypeptide of "C" form, a "B" form and/or a combined "B/A" form; (b) measuring the effect the test compound has on the affinity of component (ii) for component (iii) of step (a); and, (c) comparing the effect the test compound has on the affinity of component (ii) for component (iii) versus the affinity of component (ii) for component (iii) in the absence of the test compound. Such methods may utilize test compounds selected from the group consisting of a peptide, a protein, a non-proteinaceous organic or inorganic molecule, DNA and RNA.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Evidence of structural/immunological heterogeneity of the V1/V2 domain was seen for several rgp120s upon sequential radioimmunoprecipitations. (a) Analyses by sequential immunoprecipitations revealed that a substantial fraction (~25%) of three rgp120s tested were non-reactive with 697D, directed against a conformational V2 epitope. (b) C108g is a mAb isolated from an HIV-infected chimp that recognizes a glycan-dependent V1/V2 epitope overlapping with the PG9/PG16 site and possesses potent neutralizing activity against a limited set of viruses. C108g also recognized only a fraction of BaL rgp120 containing the correctly-folded V2 domain.

Figure 2. To facilitate the structural analysis of the native V1/V2 domain, this region of a primary clade B Env (CaseA2) sequence was expressed as a fusion protein, joined to an N-terminal fragment of the MuLV Env protein, gp70. This fusion protein system allowed the expression of the V1/V2 domain as a native glycosylated and disulfide-bonded protein. Antibody reactivity with this V1/V2 fusion protein was the only variable shown to have a significant inverse correlation with risk of infection in the Thailand RV144 vaccine.

Figure 3. Separation of the V1/V2 domain from the gp70 domain by cleavage at a proteolytic site engineered between the two domains shows that these regions form distinct and independent folded domains.

Figure 4. The deglycosylated gp70-V1/V2_{CaseA2} protein is resolved as a doublet by SDS-PAGE under non-reducing conditions, indicating distinct conformational forms.

Figure 5. The two V1/V2 conformational forms possess distinct immunoreactivities with V1/V2-specific mAbs. Mabs isolated from infected humans and mice immunized with rgp120 all specifically recognize the V1/V2 lower band form. Mabs from rats immunized with V1/V2 fusion protein recognize upper band or both bands.

Figure 6. Three V1/V2 conformers can exist, which differ in their disulfide bond patterns. Structure A was determined for the H XB2 strain of HIV gp120 by Leonard et al., 1990, J. Biol. Chem. 265:10373-10382.

Figure 7. Homogeneous conformational forms of the V1/V2 fusion protein were isolated by immunoaffinity chromatography and analyzed by mass spectrometry. The two forms were fractionated by immunoaffinity on columns containing lower band-specific mab 238. The flow-through contained upper band forms, while the column eluate contained the lower band form.

The upper band form contained peptides at mass of 1573 and 2634 predicted for the C form, while the lower band form contained the complex peptide with mass of 4206 characteristic of forms A and B.

Figure 8. Analysis of alternate V1/V2 conformeric forms of the CaseA2 and BaL V1/V2 fusion proteins. For the CaseA2 protein, rat Mab K10A11 (directed against a site in the gp70 carrier domain) pulls down both forms, mouse Mab SC-258 recognizes only lower form and human Mab 8.22.2 (from immunized Xenomouse, which contained only human Ig genes) recognized predominantly upper band form. The two forms were also seen for BaL V1/V2 fusion protein, although they were not resolved as well. Again, K10A11 recognized both bands, rat Mab 10/76b, directed against a linear V2 epitope, recognized both upper and lower band equally well, while chimp Mab C108g recognizes only the lower band form. The C108g epitope was not well-expressed in this protein, perhaps because of its glycan-dependence.

Figure 9. Analysis of reactivity of human vaccinee sera with conformers of the gp70-V1/V2_{CaseA2} protein.

Figure 10. Titrations of HIV-1-positive human immune sera show different patterns against specific V1/V2 conformers.

Figure 11. Alignment of V1/V2 region of sequences analyzed in Fig. 12. Indicated are plasmid numbers and corresponding names of Envs these sequences were obtained from. Sequences are divided to indicate different regions of the V1/V2 domain; the conserved flanking regions, the V1 hypervariable region, the semi-conserved V2 region, the V2 hypervariable region and the V2 flank. The sequences are arranged by decreasing size of the V1 hypervariable region, and this order is reflected in the gel shown in Fig. 12.

Figure 12. Analysis of conformational heterogeneity in V1/V2 domain demonstrated by SDS-PAGE analysis of deglycosylated gp70-fusion proteins (+PngaseF, Non-Reduced). The plasmid number and sequence name are indicated above each sample. Samples are arranged according to the list in Fig. 11, according to size of the V1 hypervariable region. The CaseA2 sequence (p3020) is repeated in each gel to serve as a standard. Protein markers are also included as size standards. All samples were deglycosylated by treatment with PNGaseF before analysis. The data presented in this Fig. 12 represents gels run in unreduced form, while the bottom gels.

Figure 13. Analysis of conformational heterogeneity in V1/V2 domain demonstrated by SDS-PAGE analysis of deglycosylated gp70-fusion proteins (+PngaseF, Reduced). See also, Fig. 12 legend. The data presented in this Fig. 13 represents the same samples analyzed as per Fig. 12, but after reduction of disulfide bonds by treatment with DTT.

5 **Figure 14.** The CaseA2 gp70-V1/V2 sequence.

Figure 15. The sequence of P565 plasmid.

Figure 16. The gp70-V1/V2 sequence of P565.

DETAILED DESCRIPTION OF INVENTION

10 The present invention is based on the discovery that the V1/V2 domain of HIV Env-based antigens possesses conformational heterogeneity resulting from alternative disulfide bonding, which dramatically impacts the immunoreactivity of these proteins. In accordance with the present invention, the conformationally heterogeneous antigens can be resolved to provide HIV Env antigens with homogeneous conformational forms of the V1/V2 domain.

15 HIV envelope (Env) glycoproteins, including for example gp120 and p140, are known in the art and contain five constant and five variable domains. The V1/V2 domain comprises about 90 amino acids, including four cysteine residues designated herein as C1, C2, C3, and C4, with C1 being the most N-terminal and C4 being the most C-terminal. (Figs. 2 6, and 7). The HIV Env antigens with homogeneous conformational forms of the V1/V2 domain provided herein are designated Form A, Form B, and Form C. In Form A, there is a disulfide bond between C1 and C4, and a disulfide bond between C2 and C3. In Form B, there is a disulfide bond between C1 and C3, and a disulfide bond between C2 and C4. In Form C, there is a disulfide bond between C1 and C2, and a disulfide bond between C3 and C4.

25 Forms A, B and C are present in native and recombinant HIV Env glycoproteins, and can be isolated from one another in accordance with the present invention. In particular, the present invention provides a method for isolating HIV Env antigens with Form A, or Form B, or Form C of the V1/V2 domain from each other to provide HIV Env antigens with a homogeneous conformational form of the V1/V2 domain. In particular, the different forms in a native or recombinant composition of HIV Env proteins can be separated from one another by immunoprecipitation with antibodies that are specific for the different conformations. For
30 example, human Mab 8.22.2 (He et al., 2002, J. Immunol. 169:595-605) is specific for Form C.

One of skill in the art can identify antibodies that are specific for the different conformations, and can perform immunoprecipitation, including serial immunoprecipitation, by methods known in the art to separate the different conformations. These forms can be fractionated by immunoaffinity methods using monoclonal antibodies that are specific for one of the distinct
5 forms. The homogeneous forms may be obtained either in the depleted fraction or by elution of bound molecules.

Accordingly, in another embodiment, the present invention provides a composition of structurally and immunologically distinct HIV-1 antigens in which V1/V2 domains possess alternate disulfide bond patterns, i.e. Form A, Form B, or Form C, or combinations thereof, as
10 disclosed herein. The antigens include at least the V1/V2 domain, and optionally other domains of the Env protein or other fusion partners. These antigens may consist of fusion glycoproteins that express the isolated V1/V2 domains as well as native gp120 and gp140 proteins that have been fractionated so that they contain homogeneously folded V1/V2 domains.

The antigens of the present invention are useful as antigens in immunoassays to
15 characterize the specificity of antibodies directed against the V1/V2 domain in infected or immunized people. Accordingly, the invention further provides compositions comprising the antigens of the invention for use in such assays.

The antigens of the present invention are also useful as protein therapeutic agents for inhibiting functions mediated by the V1/V2 domain, and as immunogens in HIV vaccines.
20 Accordingly, the present invention further provides pharmaceutical compositions comprising the antigens of the invention.

Formulations of the compositions useful in certain embodiments such as polypeptides, polynucleotides, or antibodies may be prepared for storage by mixing the selected composition having the desired degree of purity with optional physiologically pharmaceutically-acceptable
25 carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 18th edition, A. R. Gennaro, ed., Mack Publishing Company (1990)) in the form of a lyophilized cake or an aqueous solution. Acceptable carriers, excipients or stabilizers are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular
30 weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic

polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween,
5 Pluronic or polyethylene glycol (PEG).

Compositions to be used for *in vivo* administration should be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The composition for parenteral administration ordinarily will be stored in lyophilized form or in solution.

10 Therapeutic compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. The route of administration of the composition is in accord with known methods, e.g. oral, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, or by sustained release systems or
15 implantation device. Where desired, the compositions may be administered continuously by infusion, bolus injection or by implantation device.

An effective amount of the compositions to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it may be necessary for the therapist to titer the dosage and modify the
20 route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage may range from about 1 µg/kg to up to 100 mg/kg or more, depending on the factors mentioned above. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays designed to evaluate blood glucose levels or other particular conditions of
25 interest in a particular subject.

Pharmaceutical compositions may be produced by admixing a pharmaceutically effective amount of protein with one or more suitable carriers or adjuvants such as water, mineral oil, polyethylene glycol, starch, talcum, lactose, thickeners, stabilizers, suspending agents, etc. Such compositions may be in the form of solutions, suspensions, tablets, capsules, creams, salves,
30 ointments, or other conventional forms.

In certain embodiments, compounds are formulated with pharmaceutically acceptable diluents, adjuvants, excipients, or carriers. The phrase "pharmaceutically or pharmacologically acceptable" refers to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human, e.g., orally, topically, 5 transdermally, parenterally, by inhalation spray, vaginally, rectally, or by intracranial injection. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intracisternal injection, or infusion techniques. Administration by intravenous, intradermal, intramuscular, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary injection and/or surgical implantation at a particular site is contemplated as well.) Generally, this will also 10 entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals. The term "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, liposomes, capsids, nanocapsules, microcapsules and the like. The use of such media and agents for pharmaceutically active substances is well known 15 in the art.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The carrier can be a solvent or dispersion medium containing, for example, 20 water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many 25 cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

In certain embodiments, the present invention provides a method of treating a subject comprising administration of a composition. As used herein, the term "subject" is used to mean 30 an animal, preferably a mammal, including a human. The terms "patient" and "subject" may be used interchangeably.

The therapeutic compositions may be administered by any route that delivers an effective dosage to the desired site of action, with acceptable (preferably minimal) side-effects. Numerous routes of administration of agents are known, for example, oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary
5 injections, as well as intrathecal, intraperitoneal, intranasal, cutaneous or intradermal injections; inhalation, and topical application.

Therapeutic dosing is achieved by monitoring therapeutic benefit and monitoring to avoid side-effects. Preferred dosage provides a maximum localized therapeutic benefit with minimum local or systemic side-effects. Suitable human dosage ranges for the polynucleotides or
10 polypeptides can be extrapolated from these dosages or from similar studies in appropriate animal models. Dosages can then be adjusted as necessary by the clinician to provide maximal therapeutic benefit for human subjects.

When a therapeutically effective amount of a composition of the present invention is administered by e.g., intradermal, cutaneous or subcutaneous injection, the composition is
15 preferably in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein or polynucleotide solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred composition should contain, in addition to protein or other active ingredient of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose
20 Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. The agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer.
25 For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The compositions of the invention may be in the form of a complex of the protein(s) or other active ingredient of present invention along with protein or peptide antigens.

The composition may further contain other agents which either enhance the activity of
30 the protein or other active ingredient or complement its activity or use in treatment. Such

additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein or other active ingredient, or to minimize side effects.

Techniques for formulation and administration of the therapeutic compositions of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

The antigens of the present invention are useful for identifying, for example by screening, and for generating antibodies or antigen-binding fragments thereof that are specific for antigens with homogeneous conformational forms of the V1/V2 domain.

Antibodies may take the form of any type of relevant antibody fragment, antibody binding portion, specific binding member, a non-protein synthetic mimic, or any other relevant terminology known in the art which refers to an entity which at least substantially retains the binding specificity/neutralization activity. Thus, the term "antibody" as used in any context within this specification is meant to include, but not be limited to, any specific binding member, immunoglobulin class and/or isotype (e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA, IgD, IgE and IgM); and biologically relevant fragment or specific binding member thereof, including but not limited to Fab, F(ab')₂, Fv, and scFv (single chain or related entity). Therefore, it is well known in the art, and is included as review only, that an "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. A heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (CH1, CH2 and CH3). A light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The variable regions of both the heavy and light chains comprise framework regions (FWR) and complementarity determining regions (CDR). The four FWR regions are relatively conserved while CDR regions (CDR1, CDR2 and CDR3) represent hypervariable regions and are arranged from NH₂ terminus to the COOH terminus as follows: FWR1, CDR1, FWR2, CDR2, FWR3, CDR3, FWR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an

antigen while, depending of the isotype, the constant region(s) may mediate the binding of the immunoglobulin to host tissues or factors. That said, also included in the working definition of "antibody" are chimeric antibodies, humanized antibodies, a recombinant antibody, as human antibodies generated from a transgenic non-human animal, as well as antibodies selected from
5 libraries using enrichment technologies available to the artisan. Antibody fragments are obtained using techniques readily known and available to those of ordinary skill in the art, as reviewed below. Therefore, an "antibody" is any such entity or specific binding member, which specifically binds the conformational epitope of the of the V1/V2 domain, as described herein. Therefore, the term "antibody" describes an immunoglobulin, whether natural or partly or wholly
10 synthetically produced; any polypeptide or protein having a binding domain which is, or is substantially homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd and diabodies, as discussed without limitation,
15 infra. It is known in the art that it is possible to manipulate monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may evolve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus
20 framework regions, of a different immunoglobulin. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced. Antibodies can be modified in a number of ways, and the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody
25 fragments, derivatives, functional equivalents and homologues of "antibody" including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Such an entity may be a binding fragment encompassed within the term "antigen-binding portion" or "specific binding member" of an antibody including but not limited to (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH domains; (ii)
30 a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH domains; (iv) a Fv

fragment consisting of the VL and VH domains of a single arm of an antibody (v) a dAb fragment, which comprises a VH domain; (vi) an isolated complementarity determining region (CDR); (vii) a 'scAb', an antibody fragment containing VH and VL as well as either CL or CH; and (viii) artificial antibodies based upon protein scaffolds, including but not limited to
5 fibronectin type III polypeptide antibodies (e.g., see U.S. Pat. No. 6,703,199, issued to Koide on Mar. 9, 2004 and PCT International Application Publication No. WO 02/32925). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules
10 (known as single chain Fv (scFv)). In one embodiment, the invention provides a method for generating HIV-1 Env proteins with structurally homogeneous V1/V2 domains, and the use of such uniform structures as antigens, immunogens and therapeutic reagents.

Polyclonal or monoclonal antibodies for use in accordance with the present invention may be raised by known techniques. Monospecific murine (mouse) antibodies showing
15 specificity to a conformational epitope of a target of choice may be purified from mammalian antisera containing antibodies reactive against this region, or may be prepared as monoclonal antibodies using the technique of Kohler and Milstein (1975, Nature 256: 495-497). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics, such as the mouse monoclonal
20 antibodies exemplified herein with the 13C3 series of monoclonal antibodies. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. The splenic antibody producing cells and myeloma cells are fused, selected, and screened for antibody production. Hybridoma cells from antibody positive wells are cloned by a technique
25 such as the soft agar technique of MacPherson (1973, Soft Agar Techniques, in Tissue Culture Methods and Applications, Kruse and Paterson, Eds, Academic Press). Monoclonal antibodies are produced in vivo by injecting respective hybridoma cells into pristine primed mice, collecting ascite fluid after an interval of time, and prepared by techniques well known in the art.

Beyond species specific monoclonal antibodies described above, the antibodies of the
30 present invention may also be in the form of a "chimeric antibody", a monoclonal antibody constructed from the variable regions derived from say, the murine source, and constant regions

derived from the intended host source (e.g., human; for a review, see Morrison and Oi, 1989, *Advances in Immunology*, 44: 65-92). For example, the variable light and heavy DNA sequences from the rodent (e.g., mouse) antibody may be cloned into a mammalian expression vector. These light and heavy "chimeric" expression vectors are cotransfected into a recipient cell line and selected and expanded by known techniques. This cell line may then be subjected to known cell culture techniques, resulting in production of both the light chain and heavy chain of a chimeric antibody. Such chimeric antibodies have historically been shown to have the antigen-binding capacity of the original rodent monoclonal while significantly reducing immunogenicity problems upon host administration.

A logical improvement to the chimeric antibody is the "humanized antibody," which arguably reduces the chance of the patient mounting an immune response against a therapeutic antibody when compared to use of a chimeric or full murine monoclonal antibody. The strategy of "humanizing" a murine Mab is based on replacing amino acid residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grafting of entire complementarity determining regions (Jones et al., 1986, *Nature* 321: 522-526). This technology is again now well known in the art and is represented by numerous strategies to improve on this technology; namely by implementing strategies including, but not limited to, "reshaping" (see Verhoeyen, et al., 1988, *Science* 239: 1534-1536), "hyperchimerization" (see Queen, et al., 1991, *Proc. Natl. Acad. Sci.* 88:2869-2873) or "veneering" (Mark, et al., 1994, *Derivation of Therapeutically Active Humanized and Veneered anti-CD18 Antibodies* Metcalf and Dalton, eds. *Cellular Adhesion Molecular Definition to Therapeutic Potential*. New York: Plenum Press, 291-312). These strategies all involve to some degree sequence comparison between rodent and human sequences to determine whether specific amino acid substitutions from a rodent to human consensus is appropriate. Whatever the variations, the central theme involved in generating a humanized antibody relies on CDR grafting, where these three antigen binding sites from both the light and heavy chain are effectively removed from the rodent expressing antibody clone and subcloned (or "grafted") into an expression vector coding for the framework region of the human antibody. For example, utilizing the above techniques a humanized antibody may be expressed wherein the CDR1, CDR2, and CDR3 regions of the variable light chain are prepared, and the CDR1, CDR2, and CDR3 regions of the variable heavy chain are prepared. Therefore, a "humanized antibody" is effectively an antibody constructed

with only murine CDRs (minus any additional improvements generated by incorporating one or more of the above mentioned strategies), with the remainder of the variable region and all of the constant region being derived from a human source.

5 The invention also encompasses nucleic acid molecules encoding the proteins of the invention. Nucleic acid molecules within the invention can be cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (i.e., either a sense or an antisense strand). Fragments of these molecules, which are also considered within the scope of the invention, can be produced, for example, by the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can
10 be produced by in vitro transcription.

As used herein, both "protein" and "polypeptide" mean any chain of amino acid residues, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). The polypeptide can be a naturally occurring, synthetic, or a recombinant molecule consisting of a hybrid with one portion, for example, encoding all or a portion of a V1/V2 domain, and a
15 second portion being encoded by all or part of a second gene.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety.

20 EXAMPLES

EXAMPLE 1

Isolated V1/V2 domain of HIV-1 Env exists in at least two structurally and immunologically distinct forms

The native V1/V2 domain of a clade B sequence (CaseA2) was expressed by fusion to
25 the C-terminus of a 273 amino acid sequence fragment of the MuLV gp70 domain. This fusion glycoprotein was characterized by SDS-PAGE under various conditions, by radioimmunoprecipitation experiments with a panel of mAbs directed against V1/V2-specific epitopes, and by MALDI-TOF analysis of immunologically fractionated forms.

The CaseA2 gp70-V1/V2 sequence is set forth in Fig. 14.

30 The sequence of P565 plasmid is set forth in Fig. 15.

Plasmid has 1 HindIII site at the KL site in the V2 region

CAG AAA GAA TAT GCA CTT TTT TAT AAG CTT GAT ATA GTA CCA ATA GAT AAT
(SEQ ID NO. 49)

5 Q K E Y A L F Y K L D I V P I D N
(SEQ ID NO. 50)

This can be mutated and a unique HindIII site can be inserted by silent mutations into the highly conserved KL site at the beginning of the V1 sequence

10 CCC CCC GCT AGC GTA AAA TTA ACC CCA CTC (SEQ ID NO. 51)
P P A S V K L T P L (SEQ ID NO. 52)
CCC CCC GCT AGC GTA A/Ag cTt ACC CCA CTC (SEQ ID NO. 6)
HindIII

15 The gp70-V1/V2 sequence of P565 is set forth in Fig. 16.

The V1/V2 domain of a number of HIV-1 Envs was expressed in isolated form as a disulfide-bonded glycoprotein joined to the C-terminus to a 273 amino acid fragment of the Friend MuLV gp70 protein. The CaseA2 V1/V2 fusion protein was radiolabeled and
20 immunoprecipitated with monoclonal antibodies (mAbs) directed against a target in the gp70 carrier domain by capture of the antibody Fc regions with PANSORBIN®, a commercial preparation of crosslinked Staph A. The immunoprecipitated proteins were removed from PANSORBIN® by boiling in the presence of 1% SDS, and analyzed by SDS-PAGE before and
25 after reduction of disulfide bonds with dithiothreitol (DTT). Under non-reducing conditions a closely-spaced doublet was resolved on 10% gels at the position expected for the deglycosylated protein. These two bands coalesced into a single band with slightly lower mobility than the doublet after reduction, indicating that the doublet represented different disulfide-bonded conformeric forms. Analysis of samples immunoprecipitated with a variety of
30 mAbs directed against sites in the V1/V2 domain contained either the upper or lower band, indicating that these represented two antigenically distinct conformeric forms. Analysis of the

structure of the immunofractionated forms by MALDI-TOF mass spectrometry confirmed that these possessed distinct disulfide-bonded structures and allowed the assignment of the structures represented by the upper and lower band form.

These results demonstrate that the isolated V1/V2 domain of HIV-1 Env exists in at least two structurally and immunologically distinct forms.

EXAMPLE 2

Heterogeneous forms of V1/V2 domains are not limited to the Case-A2 sequences.

The biochemical method described above (SDS-PAGE of deglycosylated gp70-V1/V2 proteins under non-reducing conditions) allowed good resolution of structurally distinct V1/V2 forms of the Case-A2 sequence. However, applying the similar method to the BaL sequence resulted in a smaller separation between the two bands, and a modified SF162 sequence gave two bands that were barely distinguishable. Furthermore, for several other gp70-V1/V2 fusion proteins (e.g., the ConC sequence, or the Th023 clade A/E sequence) only a single band was observed. However, sequential immunoprecipitation assays revealed the presence of distinct conformational forms, indicating that the diverse forms were present, but could not be separated by gel electrophoresis.

EXAMPLE 3

Direct binding studies with rgp120/rgp140 proteins and functional studies

Evidence was also obtained for immunological heterogeneity in recombinant and native gp120 and gp140 immunogens. Sequential immunoprecipitations with monoclonal antibodies specific for distinct conformation epitopes in V1/V2 showed that these antibodies recognized only a fraction of the molecules (Fig. 1). Native and recombinant gp120 and gp140 proteins were recognized by MAbs specific for either the upper band-specific or lower band-specific epitopes, indicating the presence of both structures in these antigens. Finally, evidence has been presented that MAbs reactive with either upper band-specific or lower band-specific epitopes neutralized viral infectivity, indicating that both forms were present in functional Env proteins and suggesting that both contribute to viral binding and/or entry.

Analyses by sequential immunoprecipitations revealed that a substantial fraction (~25%) of three rgp120s tested were non-reactive with 697D, directed against a conformational V2

epitope. (Fig. 1A). C108g is a mAb isolated from an HIV-infected chimp that recognizes a glycan-dependent V1/V2 epitope overlapping with the PG9/PG16 site and possesses potent neutralizing activity against a limited set of viruses. C108g also recognized only a fraction of BaL rgp120 containing the correctly-folded V2 domain. (Fig. 1B).

5 To facilitate the structural analysis of the native V1/V2 domain, this region of a primary clade B Env (CaseA2) sequence was expressed as a fusion protein, joined to an N-terminal fragment of the MuLV Env protein, gp70 (Fig. 2). This fusion protein system allowed the expression of the V1/V2 domain as a native glycosylated and disulfide-bonded protein. Separation of the V1/V2 domain from the gp70 domain by cleavage at a proteolytic site
10 engineered between the two domains showed that these regions form distinct and independent folded domains (Fig. 3). The deglycosylated gp70-V1/V2_{CaseA2} protein was resolved as a doublet by SDS-PAGE under non-reducing conditions, indicating distinct conformational forms (Fig. 4; see also Figure 6 for three distinct and possible confirmations of the V1/V2 domain). The two V1/V2 conformational forms possess distinct immunoreactivities with V1/V2-specific mAbs.
15 Mabs isolated from infected humans and mice immunized with rgp120 all specifically recognize the V1/V2 lower band form. Mabs from rats immunized with V1/V2 fusion protein recognize upper band or both bands (Fig. 5).

Homogeneous conformational forms of the V1/V2 fusion protein were isolated by immunoaffinity chromatography and analyzed by mass spectrometry. The two forms were
20 fractionated by immunoaffinity on columns containing lower band-specific mab 238. The flow-through contained upper band forms, while the column eluate contained the lower band form. The upper band form contained peptides at mass of 1573 and 2634 predicted for the C form, while the lower band form contained the complex peptide with mass of 4206 characteristic of forms A and B (Fig. 7).

25 Alternate V1/V2 conformeric forms of the CaseA2 and BaL V1/V2 fusion proteins were analyzed (Fig. 8). For the CaseA2 protein, rat Mab K10A11 (directed against a site in the gp70 carrier domain) pulled down both forms; mouse Mab SC-258 recognized only lower form; and human Mab 8.22.2 (from immunized Xenomouse, which contained only human Ig genes) recognized predominantly upper band form. The two forms were also seen for BaL V1/V2 fusion
30 protein, although they were not resolved as well. Again, K10A11 recognized both bands, rat Mab

10/76b, directed against a linear V2 epitope, recognized both upper and lower band equally well, while chimp Mab C108g recognized only the lower band form. The C108g epitope was not well-expressed in this protein, perhaps because of its glycan-dependence.

Figure 9 shows an analysis of reactivity of human vaccine sera with conformers of the
 5 gp70-V1/V2_{CaseA2} protein. Titrations of HIV-1-positive human immune sera show different patterns against specific V1/V2 conformers (Fig. 10).

The wide variety of various pg 120 V1/V2 sequence sequences exemplified herein are aligned in Fig. 11. The respective SEQ ID NO., plasmid number and corresponding names of Envs from which these sequences represent are provided within Fig. 11. The respective
 10 sequences are divided to indicate different regions of the V1/V2 domain; the conserved flanking regions, the V1 hypervariable region, the semi-conserved V2 region, the V2 hypervariable region and the V2 flank. It is also noted that these sequences are arranged by decreasing size of the V1 hypervariable region, and this order is reflected in the gels shown in both Fig. 12 ((+PngaseF, Non-Reduced)) and Fig. 13 ((+PngaseF, Reduced - DTT). Figures 12 and 13 show an analysis
 15 of the conformational heterogeneity in V1/V2 domain, as demonstrated by SDS-PAGE analysis of deglycosylated gp70-fusion proteins, in both non-reduced (Fig. 12) and reduced (Fig. 13) form. The plasmid number and sequence name are indicated above each sample in both Fig. 12 and Fig. 13. Samples are arranged according to the listing of Fig. 11, described above, as according to size of the V1 hypervariable region. The CaseA2 sequence (p3020) is repeated in each gel to
 20 serve as a standard. Protein markers are also included as size standards. All samples represented in both Fig. 12 and 13 were deglycosylated by treatment with PNGaseF before analysis; again, with the gels of Fig. 12 being run in unreduced form and the gels of Fig. 13 representing gels showing samples analyzed after reduction of disulfide bonds by treatment with DTT. Many of the samples demonstrate multiple bands, similar in complexity to those seen for the CaseA2
 25 protein. These bands coalesce to a single form after reduction (bottom gels). In general, samples with longer V1 hypervariable regions (left-hand gels) tend to display greater conformational diversity than samples with short V1 regions (right-hand gels).

INDUSTRIAL APPLICABILITY

The invention has applications in the treatment and diagnosis of HIV-1 virus infection
 30 disease. All publications cited in the specification, both patent publications and non-patent

publications, are indicative of the level of skill of those skilled in the art to which this invention pertains. All these publications are herein fully incorporated by reference to the same extent as if each individual publication were specifically and individually indicated as being incorporated by reference.

5 Although the invention herein has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the principles and applications of the present invention. It is therefore to be understood that numerous modifications may be made to the illustrative embodiments and that other
10 invention as defined by the following claims.

CLAIMS

What is claimed is:

1. An isolated and purified polypeptide comprising a V1/V2 domain of a HIV envelope protein having a structural configuration of a "C" form, as shown in Figure 6 and Figure 7.
2. The polypeptide of claim 1 wherein the HIV envelope protein is native gp120.
3. The polypeptide of claim 1 wherein the HIV envelope protein is native gp140.
4. The polypeptide of claim 1 wherein the HIV envelope protein is recombinant gp120.
5. The polypeptide of claim 1 wherein the HIV envelope protein is recombinant gp140.
6. The polypeptide of claim 4 wherein recombinant gp120 comprises a portion of the gp120 coding region, such that the antigenicity of the V1/V2 structural configuration of the "C" form is maintained.
7. The polypeptide of claim 1 which is a fusion protein comprising an amino-terminal sequence directly linked to a V1/V2 domain of an HIV envelope protein having a structural configuration of a "C" form, as shown in Figure 6 and Figure 7.
8. The polypeptide of claim 7 wherein the amino-terminal sequence represents a portion of MuLV gp70.
9. The polypeptide of claim 8 having the amino acid sequence as set forth in Figure 14 (SEQ ID NO:2).
10. An isolated nucleic acid molecule encoding a polypeptide with at least 90% amino acid sequence identity to the polypeptide of claim 9, wherein said polypeptide maintains substantial antigenicity of the V1/V2 structural configuration of the "C" form, as shown in Figure 6 and Figure 7.
11. A recombinant DNA molecule comprising the nucleic acid sequence of claim 10.
12. A cell line transfected with a recombinant DNA molecule of claim 11.

13. A method of treating a HIV positive subject comprising administering to the subject a pharmaceutical composition comprising a polypeptide of claim 1, said pharmaceutical composition optionally containing a pharmaceutically active carrier, wherein administration of the pharmaceutical composition results in a reduction in HIV viral load.

14. A method of vaccinating a human subject against infection or progression of HIV which comprises administration of a pharmaceutical composition which comprises a polypeptide of claim 1, said pharmaceutical composition optionally containing a pharmaceutically active carrier, wherein administration of the pharmaceutical composition results in generation of an immune response against HIV infection.

15. A pharmaceutical composition, comprising: (a) an effective amount of a polypeptide of claim 1; and, (b) optionally, a pharmaceutically acceptable carrier.

16. A method for inducing an enhanced immunological response against a HIV antigen in a mammalian host, said method comprising inoculating the mammalian host with a polypeptide of claim 1.

17. A method of identifying a HIV antiviral compound, comprising: (a) combining component (i) -- a test compound, component (ii) -- a "C" form-specific antibody, and component (iii) -- a polypeptide of claim 1; (b) measuring the effect the test compound has on the affinity of component (ii) for component (iii) of step (a); and, (c) comparing the effect the test compound has on the affinity of component (ii) for component (iii) versus the affinity of component (ii) for component (iii) in the absence of the test compound.

18. The method of claim 17 wherein component (i) is selected from the group consisting of a peptide, a protein, a non-proteinaceous organic or inorganic molecule, DNA and RNA.

19. An isolated and purified polypeptide comprising a V1/V2 domain of an HIV envelope protein having a structural configuration of a "B" form, as shown in Figure 6 and Figure 7.

20. The polypeptide of claim 19 wherein the HIV envelope protein is native gp120.

21. The polypeptide of claim 19 wherein the HIV envelope protein is native gp140.

22. The polypeptide of claim 19 wherein the HIV envelope protein is recombinant gp120.
23. The polypeptide of claim 19 wherein the HIV envelope protein is recombinant gp140.
24. The polypeptide of claim 22 wherein recombinant gp120 comprises a portion of the gp120 coding region, such that the antigenicity of the V1/V2 structural configuration of the "B" form is maintained.
25. The polypeptide of claim 19 which is a fusion protein comprising an amino-terminal sequence directly linked to a V1/V2 domain of an HIV envelope protein having a structural configuration of a "B" form, as shown in Figure 6 and Figure 7.
26. The polypeptide of claim 25 wherein the amino-terminal sequence represents a portion of MuLV gp70.
27. The polypeptide of claim 26 having the amino acid sequence as set forth in Figure 14 (SEQ ID NO:2).
28. An isolated nucleic acid molecule encoding a polypeptide with at least 90% amino acid sequence identity to the polypeptide of claim 27, wherein said polypeptide maintains substantial antigenicity of the V1/V2 structural configuration of the "B" form, as shown in Figure 6 and Figure 7.
29. A recombinant DNA molecule comprising the nucleic acid sequence of claim 28.
30. A cell line transfected with a recombinant DNA molecule of claim 29.
31. A method of treating a HIV positive subject comprising administering to the subject a pharmaceutical composition comprising a polypeptide of claim 19, said pharmaceutical composition optionally containing a pharmaceutically active carrier, wherein administration of the pharmaceutical composition results in a reduction in HIV viral load.
32. A method of vaccinating a human subject against infection or progression of HIV which comprises administration of a pharmaceutical composition which comprises a polypeptide of claim 19, said pharmaceutical composition optionally containing a pharmaceutically active

carrier, wherein administration of the pharmaceutical composition results in generation of an immune response against HIV infection.

33. A pharmaceutical composition, comprising: (a) an effective amount of a polypeptide of claim 19; and, (b) optionally, a pharmaceutically acceptable carrier.

34. A method for inducing an enhanced immunological response against an HIV antigen in a mammalian host, said method comprising inoculating the mammalian host with a polypeptide of claim 19.

35. A method of identifying a HIV antiviral compound, comprising: (a) combining component (i) -- a test compound, component (ii) -- a "B" form-specific antibody, and component (iii) -- a polypeptide of claim 19; (b) measuring the effect the test compound has on the affinity of component (ii) for component (iii) of step (a); and, (c) comparing the effect the test compound has on the affinity of component (ii) for component (iii) versus the affinity of component (ii) for component (iii) in the absence of the test compound.

36. The method of claim 35 wherein component (i) is selected from the group consisting of a peptide, a protein, a non-proteinaceous organic or inorganic molecule, DNA or RNA.

37. An isolated and purified polypeptide comprising a V1/V2 domain of a HIV envelope protein having a structural configuration of a "B"/"A" combined form, as shown in Figure 6 and Figure 7.

38. The polypeptide of claim 37 wherein the HIV envelope protein is native gp120.

39. The polypeptide of claim 37 wherein the HIV envelope protein is native gp140.

40. The polypeptide of claim 37 wherein the HIV envelope protein is recombinant gp120.

41. The polypeptide of claim 37 wherein the HIV envelope protein is recombinant gp140.

42. The polypeptide of claim 40 wherein recombinant gp120 comprises a portion of the gp120 coding region, such that the antigenicity of the V1/V2 structural configuration of the "B"/"A" combined form is maintained.

43. The polypeptide of claim 37 which is a fusion protein comprising an amino-terminal sequence directly linked to a V1/V2 domain of an HIV envelope protein having a structural configuration of a "B"/"A" combined form, as shown in Figure 6 and Figure 7.

44. The polypeptide of claim 43 wherein the amino-terminal sequence represents a portion of MuLV gp70.

45. The polypeptide of claim 44 having the amino acid sequence as set forth in Figure 14 (SEQ ID NO:2).

46. An isolated nucleic acid molecule encoding a polypeptide with at least 90% amino acid sequence identity to the polypeptide of claim 45, wherein said polypeptide maintains substantial antigenicity of the V1/V2 structural configuration of the "B"/"A" combined form, as shown in Figure 6 and Figure 7.

47. A recombinant DNA molecule comprising the nucleic acid sequence of claim 46.

48. A cell line transfected with a recombinant DNA molecule of claim 47.

49. A method of treating a HIV positive subject comprising administering to the subject a pharmaceutical composition comprising a polypeptide of claim 37, said pharmaceutical composition optionally containing a pharmaceutically active carrier, wherein administration of the pharmaceutical composition results in a reduction in HIV viral load.

50. A method of vaccinating a human subject against infection or progression of HIV which comprises administration of a pharmaceutical composition which comprises a polypeptide of claim 37, said pharmaceutical composition optionally containing a pharmaceutically active carrier, wherein administration of the pharmaceutical composition results in generation of an immune response against HIV infection.

51. A pharmaceutical composition, comprising: (a) an effective amount of a polypeptide of claim 37; and, (b) optionally, a pharmaceutically acceptable carrier.

52. A method for inducing an enhanced immunological response against a HIV antigen in a mammalian host, said method comprising inoculating the mammalian host with a polypeptide of claim 37.

53. A method of identifying a HIV antiviral compound, comprising: (a) combining component (i) -- a test compound, component (ii) -- a "B"/"A" combined form-specific antibody, and component (iii) -- a polypeptide of claim 37; (b) measuring the effect the test compound has on the affinity of component (ii) for component (iii) of step (a); and, (c) comparing the effect the test compound has on the affinity of component (ii) for component (iii) versus the affinity of component (ii) for component (iii) in the absence of the test compound.

54. The method of claim 53 wherein component (i) is selected from the group consisting of a peptide, a protein, a non-proteinaceous organic or inorganic molecule, DNA and RNA.

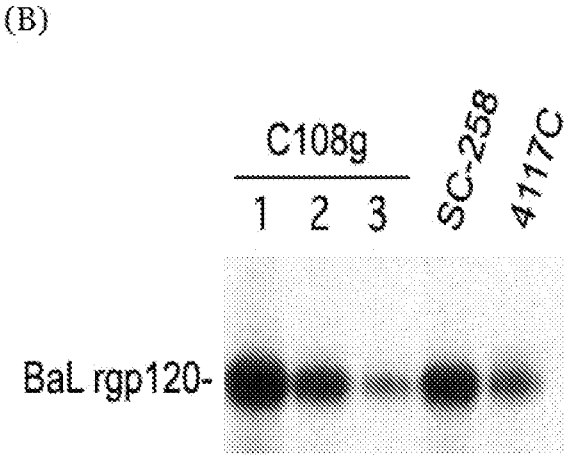
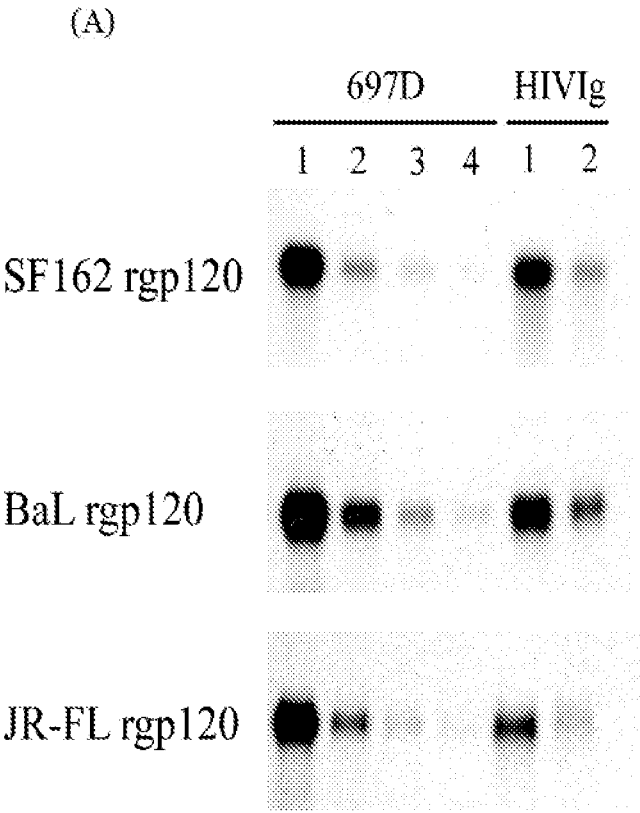


FIGURE 1

FIGURE 2

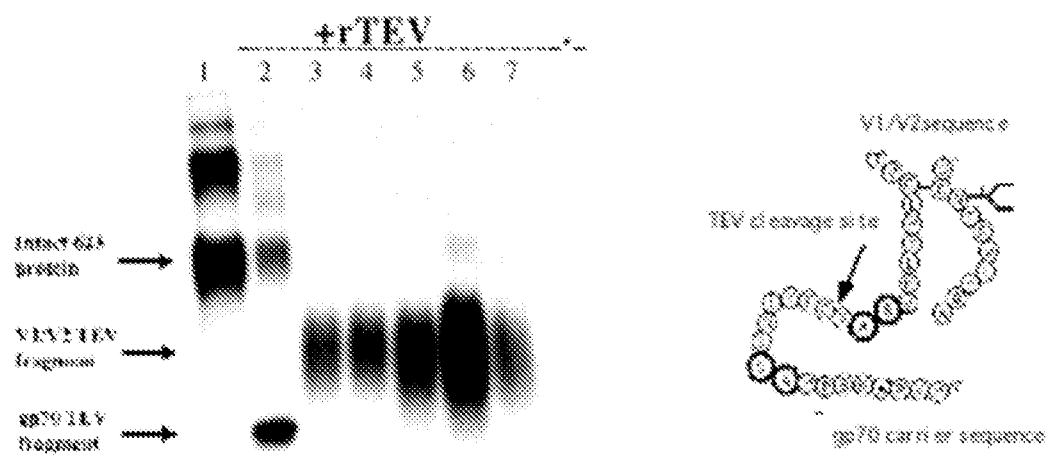


FIGURE 3

FIGURE 4

FIGURE 5

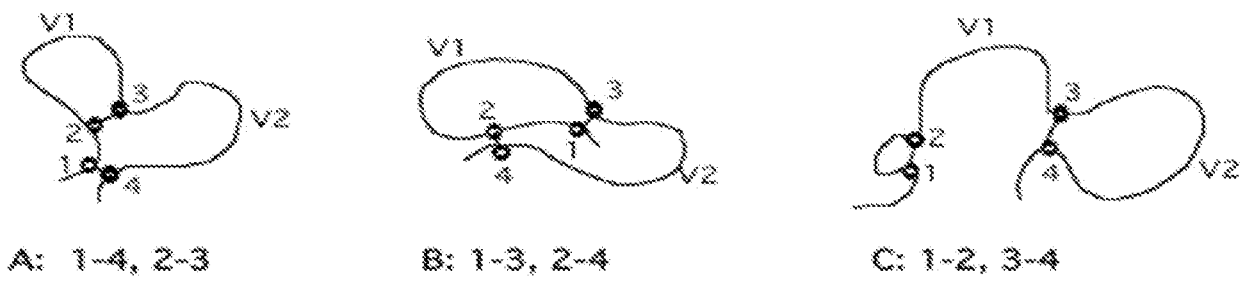


FIGURE 6

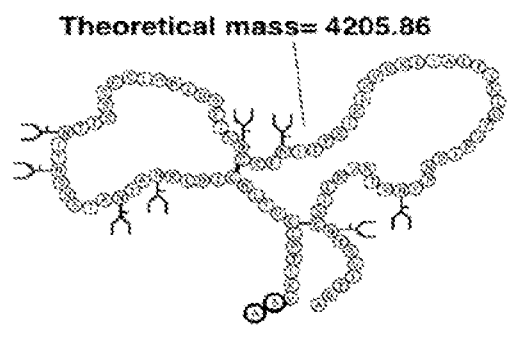
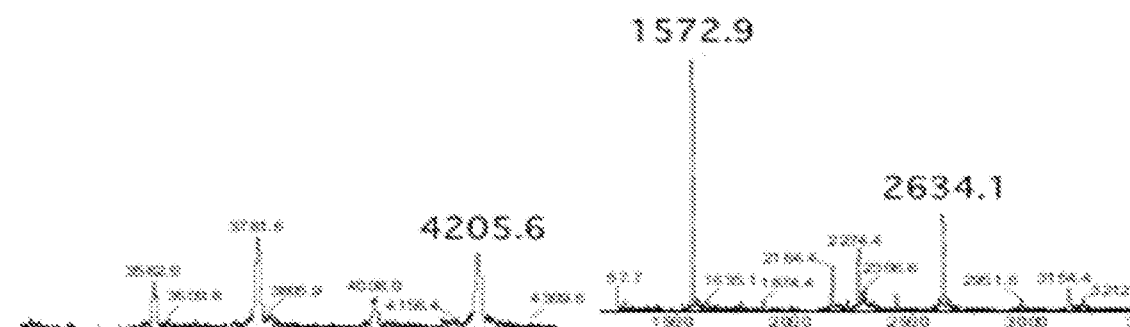
7/22

Lower Band

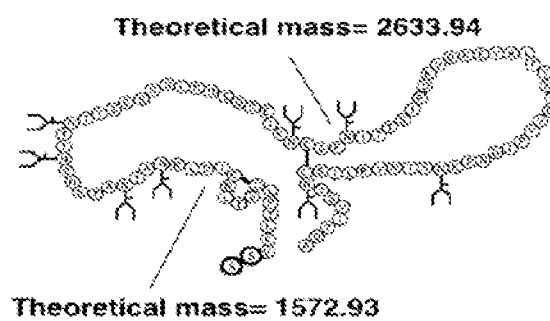
(mAb 238 eluate)

Upper Band

(mAb 238 flow through)



"A" or "B" Form



"C" Form

FIGURE 7

8/22

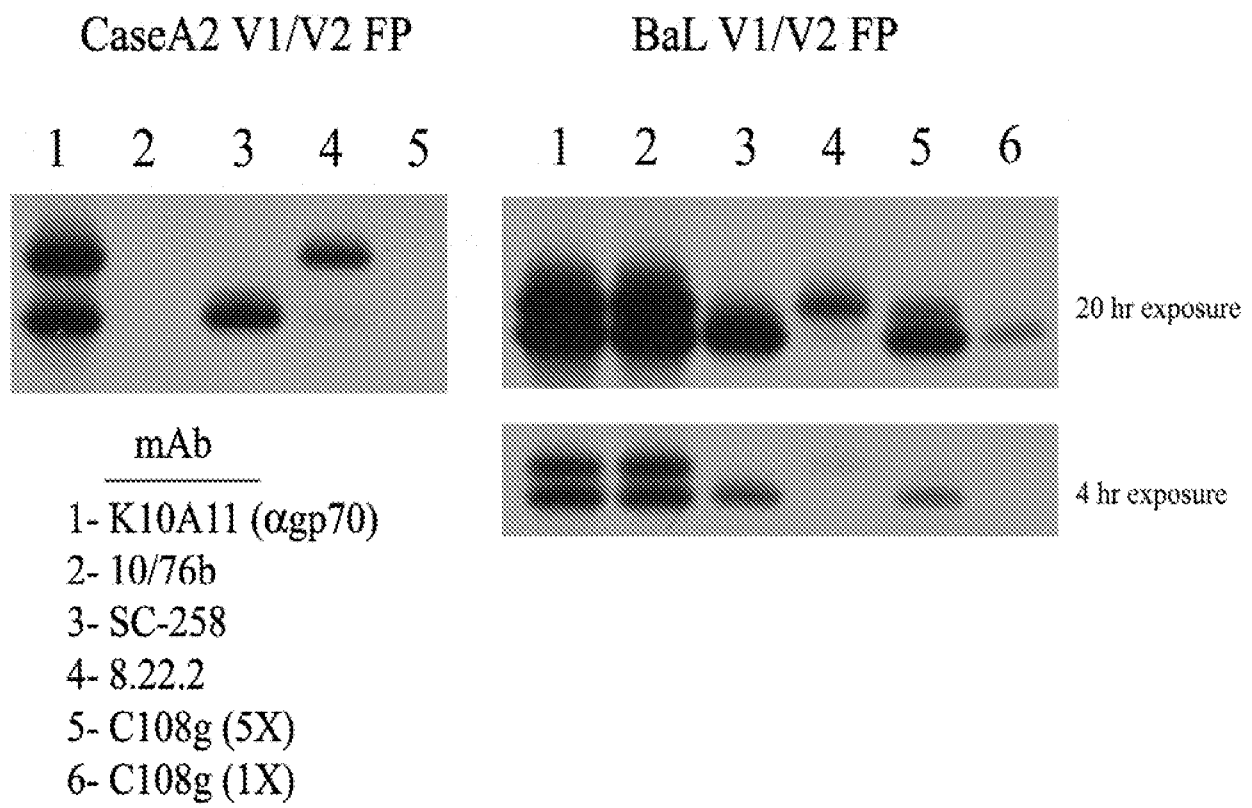


FIGURE 8

9/22

Vaccine sera, obtained from the AIDS Vaccine Evaluation Group (AVEG), were from subjects immunized with the following antigens:

<u>Serum#</u>	<u>Antigen</u>	<u>Serum#</u>	<u>Antigen</u>
8, 12, 14	HIVAC + rgp120 IIIB in Alum	5, 6, 13, 16, 17, 19	300 mg rgp120 MN/IIIB in alum
7	300 mg rgp120 MN in alum	4, 20	300 mg rgp120 IIIB/ in alum
10, 18	300 mg rgp120 IIIB in alum	9, 11	100 mg QS21 + 300 mg rgp120 MN in alum
15	LWS (HIV-IIIB infected)		

Lanes 1-3 contain rodent Mabs SC-238 (lane 1), K10-A11 (lane 2) and K19B3 (lane 3)

35S Cys-labeled CaseA2 V1/V2 fusion protein was immunoprecipitated with sera at a dilution of 1:25 and precipitates were digested with PNGaseF prior to analysis by SDS-PAGE under non-reducing conditions.

FIGURE 9

10/22

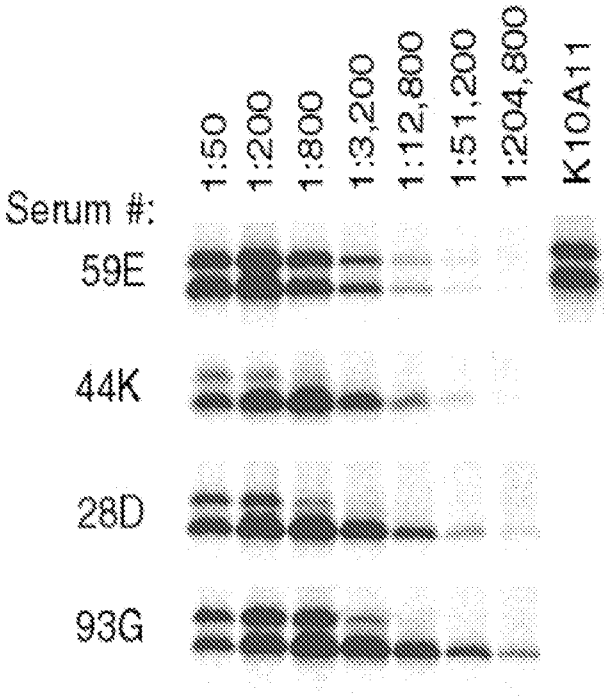


FIGURE 10

[illegible]

FIGURE 11

12/22

FIGURE 12

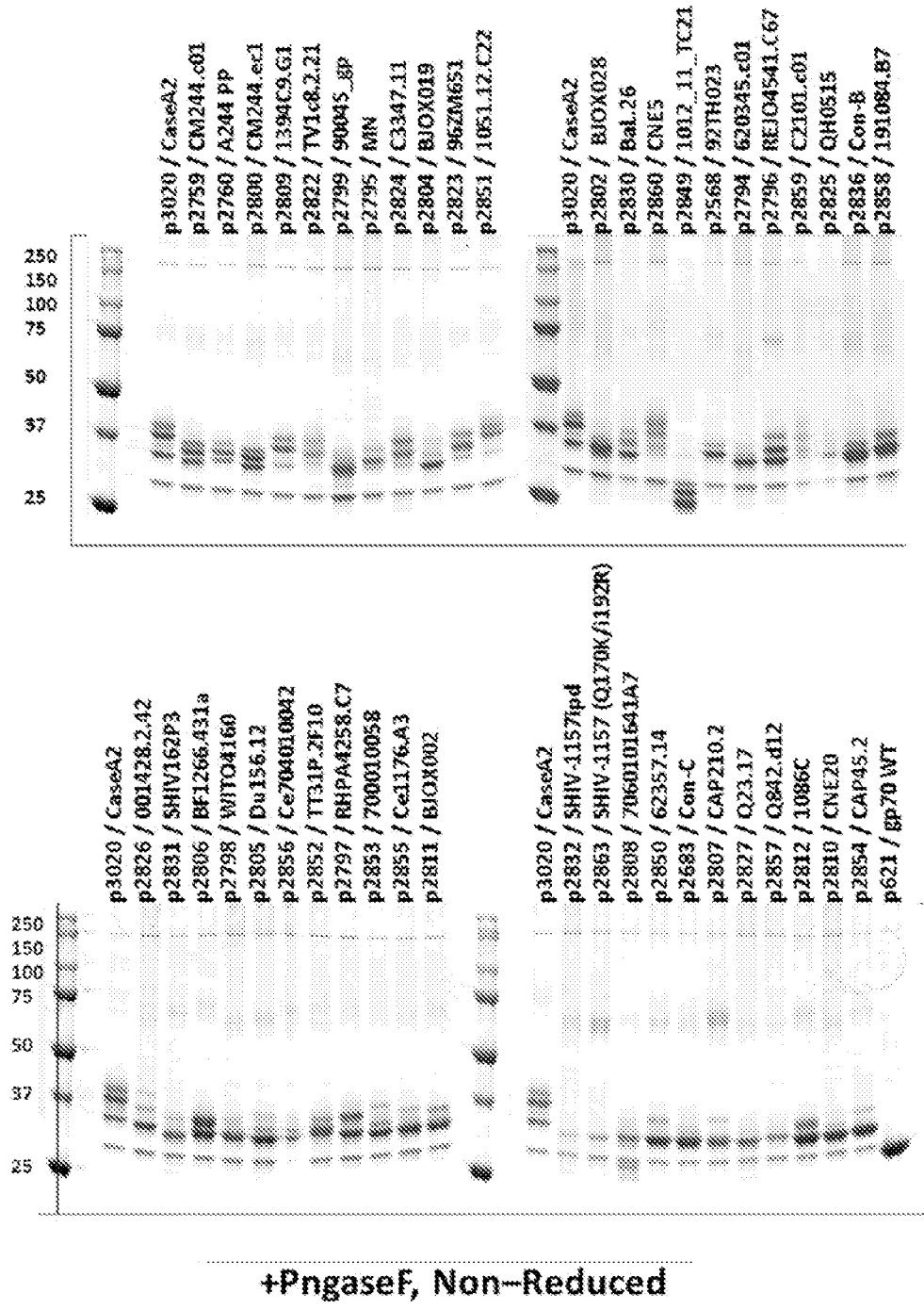
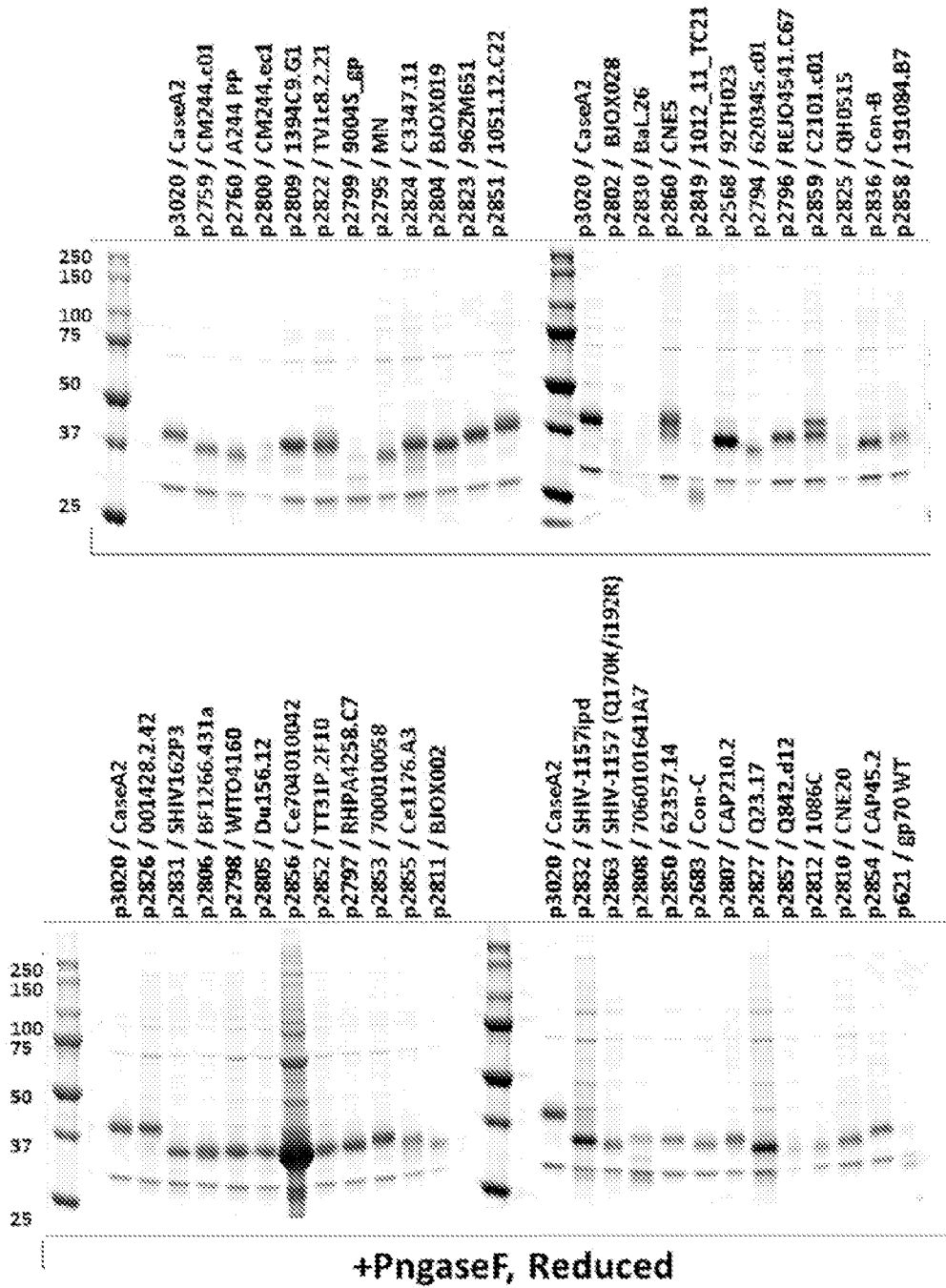


FIGURE 13



1/1
1
ATG GGG TGT TCA ACG CTC CCA AAA TCC CTT AAA GAT AAG ATT GAC CCG CCG GAC CTC CTA AAT CCC TTA ATT CTC TTC CTG TCT CTC AAA
M A C S T L F K S P K D K I D F R D L L I F L I L F L S L K
61/21
1
151/51
1
121/41
1
211/71
1
301/101
1
391/131
1
481/161
1
571/191
1
661/221
1
751/251
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841/291
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931/331
1
1021/371
1
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1201/451
1
1291/491
1
1381/531
1
1471/571
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1561/611
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1651/651
1
1741/691
1
1831/731
1
1921/771
1
2011/811
1
2101/851
1
2191/891
1
2281/931
1
2371/971
1
2461/1011
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2551/1051
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9571/4171
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9661/4211
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9751/4251
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9841/4291
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15871/6971
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16141/7091
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16501/7251
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16591/7291
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16681/7331
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16771/7371
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16861/7411
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16951/7451
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17041/7491
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17131/7531
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17221/7571
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17401/7651
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17671/7771
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17761/7811
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20371/8971
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20461/9011
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FIGURE 14

15/22

901/301		931/311	961/321
CCC GCT AGC GTA AAA TTA ACC CCA CTC TGT GTT ACT TTA AAT TGC ATT GAT TTA AGS AAT GCT ACT AAT GCC ACT AGT AAT AGC AAT ACT			
P A S V E L T P L C V T L N C I L D L K N A T N A T S N S N S N S N T			
991/331		1021/341	1051/351
ACT AAT ACC ACT AGT AGC GCG GGA CTG ATG ATG GAA CAA GGA GAA ATA AAA AAC TGC TCT TTC AAT ATC ACC ACA AGC ATA AGA GAT			
T N T T S S S S S G G L L M M E Q Q G E I K N C S F N I T T S I R D			
1081/361		1111/371	1141/381
AAG GTA CAG AAA GAA TAT GCA CTT TTT TAT AAG CTT GAT ATA GTA CCA ATA GAT AAT CCT AAA AAT AGT ACC AAC TAT AGG TTG ATA AGT			
K V Q K E Y A L E F Y K L D I V P I D N F K N S T N Y R L I S			
1171/391		1201/401	
TGT AAC ACC TCA GTC ATT ACA CAG GCC GGC GCC TAA TAG ATC GAT TAG TTC AAT TTG TTA AA (SEQ ID No. 1)			
C N T S V I T Q A G A * * I D * F N L L (SEQ ID No. 2)			

FIGURE 14 (continued)

FIGURE 15

P565 plasmid (11,256 bp)

TTGGGCTGCAGGTCGATCGACTCTAGAACCcaaaacgcgtatttcggacaaacacacCCGCTG
GAAAGGACCCTATACCGTCCTACTGACTACCCCCACCGCTCTCAAAGTAGACGGC
ATTGCAGCGTGGATCCACGCTGCCCACGTAAAGGCTGCCGACACCAGGATTGAGC
CACCAGCAGAATCGACATGGCGTGTTCAACGCTCCCAAATCCCTTAAAGATAAGA
TTGACCCGCGGGACCTCCTAATCCCTTAAATTCTCTTCCTGTCTCTCAAAGGGGGCC
AGATCCGCGAGCACCCGGCTCCAGCCCTCACCATCACCACCATCACGTCTACAACA
TTACCTGGGAAGTGACCAATGGGGATCGGGAGACAGTATGGGCAATATCAGGCAA
CCACCCTCTGTGGACTTGGTGGCCAGTCCTCACCCACAGATTTGTGTATGTTAGCTC
TCAGTGGGCGCGCCCACTGGGGGCTAGAGTATCAGGCCCCCTATTCTCGCCCC
CGGGGCCCCCTTGTTGCTCAGGGAGCAGCGGGAGCAGTGCAGGCTGTTCCAGAG
ACTGCGACGAGCCCTTGACCTCCCTCACCCCTCGGTGCAACACTGCCTGGAACAG
ACTTAAGCTAGACCAGGTAACCTCATAAATCAAGTGAGGGATTTTATGTCTGCCCCG
GGTCACATCGCCCCCGGGAAGCCAAGTCCTGTGGAGGTCCAGACTCCTTCTACTG
TGCTCTTGGGGCTGCGAGACAACCGGTAGAGTATACTGGAAGCCCTCCTCCTCT
TGGGACTACATCACAGTGGACAACAATCTCACCAGTCCAGGCTGTCCAGGTAT
GCAAAGACAATAAGTGGTGCAATCCCTTGGCTATCCAGTTTACAAACGCCGGGAAA
CAGGTCACTCATGGACAACCTGGACACTATTGGGGTCTACGTCTTTATGTCTCTGG
GCGGGACCCGGGGCTTACTTTCGGGATCCGACTCAGATATCAAATCTAGGACCT
CGGGTCCCGATAGGACCGAACCCCGTCCTGGCAGACCAACTTTCGCTCCCGCGA
CCTAATCCCTACCCAAACCTGCCAAGTCTCCCCCGCTAGCGTAA~~AAATTA~~ACCCC
ACTCTGTGTTACTTTAAATTGCATTGATTTAAGGAATGCTACTAATGCCACTAGTAAT
AGCAATACTACTAATACCACTAGTAGTAGCGGGGGACTGATGATGGAACAAGGAG
AAATAAAAACTGCTCTTTCAATATCACCACAAGCATAAGAGATAAGGTACAGAAAG
AATATGCACTTTTTTAT~~AAGCTT~~GATATAGTACCAATAGATAATCCTAAAAATAGTAC
CAACTATAGGTTGATAAGTTGTAACACCTCAGTCATTACACAGGCCGGCGCCTAAT
AGATCGATTAGTTCAATTTGTTAAAGACAGGATCTCAGTAGTCCAGGCTTTAGTCCT
GACTCAACAATAACCACAGCTAAAACCACTAGAATACGAGCCATGATAAATAAAG
ATTTTATTTAGTTTCCAGAAAAAGGGGGGAATGAAAGACCCACCAAGTTGCTTAG
GGCGAGCTCGAATTCATTGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACT
TGCTTTAAAAAACCTCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAAT
TGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATC
ACAAATTTACAAATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAAC
TCATCAATGTATCTTATCATGTCTGGATCCTCTACGCCGGACGCATCGTGGCCGGC
ATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCTATATCGCCGACATCACCGATG
GGGAAGATCGGGCTCGCCACTTCGGGCTCATGAGCGCTTGTTTCGGCGTGGGTAT
GGTGGCAGGCCCGTGCGCGGGGGACTGTTGGGCGCCATCTCCTTGCAATGCACC
ATTCCTTGCGGCGGCGGTGCTCAACGGCCTCAACCTACTACTGGGCTGCTTCCTA
ATGCAGGAGTCGCATAAGGGAGAGCGTCGACCTCGGGCCGCGTTGCTGGCGTTT
TTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGA
GGTGGCGAAACCCGACAGGACTATAAAGATAACAGGCGTTTCCCCCTGGAAGCTC
CCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTT
CTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTC

FIGURE 15 (continued)

GGTGTAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCC
GACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACG
ACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGT
AGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGG
ACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTtAcCTTCGAAAAAGAGTTGGT
AGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAA
GCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTA
CGGGGTCTGACGCTCAGTGGAAACGAAAACTCACGTTAAGGGATTTTGGTCATGAG
ATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTTAAATCA
ATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAG
GCACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCATAGTTGCCTGACTCCCCGT
CGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATG
ATACCGCGAGACCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACCAGCCAG
CCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTC
TATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCA
ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCT
TCATTAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTG
CAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCC
GCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCA
TCCGTAAGATGCTTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATA
GTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCG
CCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAA
ACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCAC
CCAAGTATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAG
GAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACT
CATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGC
GGATACATATTTGAATGTATTTAGAAAAATAAACAATAGGGGTTCCGCGCACATTT
CCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTAT
AAAAATAGGCGTATCACGAGGCCCTGATGGCTCTTTGCGGCACCCATCGTTCGTA
ATGTTCCGTGGCACCGAGGACAACCCTCAAGAGAAAATGTAATCACACTGGCTCA
CCTTCGGGTGGGCCTTTCTGCGTTTATAAGGAGACACTTTATGTTTAAGAAGGTTG
GTAAATTCCTTGCGGCTTTGGCAGCCAAGCTAGATCCAGCTTTTTGCAAAAGCCTA
GGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGGC
CTCGGCCTCTGCATAAATAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCG
GAACTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGACTATGGT
TGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGG
GACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCT
GCTGGGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACAGCCAAG
CTAGCTTGAATTAATTCCCGAGCCCTTCCAATACAAAACTAATTAGACTTTGAGTG
ATCTTGAGCCTTTCTAGTTTTTGTATTGGAAGGGCTCGTCGCCAGTCTCATTGAG
AAGGCATGTGCGGACGATGGCTTCTGTCACTGCAAAGGGGTCACAATTGGCAGAG
GGGCGGCGGTCTTCAAAGTAACCTTTCTTCTCCTGGCCGAGCCGAGAATGGGAGT
AGAGCCGACTGCTTGATTCCACACCAATCTCCTCGCCGCTCTCACTTCGCCTCGT
TCTCGTGGCTCGTGGCCCTGTCCACCCCGTCCATCATCCCGCCGGCCACCGCTCA

FIGURE 15 (continued)

GAGCACCTTCCACCATGGCCACCTCAGCAAGTTCCCACTTGAACAAAAACATCAAG
CAAATGTACTTGTGCCTGCCCCAGGGTGAGAAAGTCCAAGCCATGTATATCTGGGT
TGATGGTACTGGAGAAGGACTGCGCTGCAAAACCCGCACCCTGGACTGTGAGCCC
AAGTGTGTAGAAGAGTTACCTGAGTGAATTTTGTATGGCTCTAGTACCTTTTCAGTCT
GAGGGCTCCAACAGTGACATGTATCTCAGCCCTGTTGCCATGTTTCGGGACCCCTT
CCGCAGAGATCCCAACAAGCTGGTGTCTGTGAAGTTTTCAAGTACAACCGGAAG
CCTGCAGAGACCAATTTAAGGCACTCGTGTAAACGGATAATGGACATGGTGAGCA
ACCAGCACCCCTGGTTTGAATGGAACAGGAGTATACTCTGATGGGAACAGATGG
GCACCCTTTTGGTTGGCCTTCCAATGGCTTTCCTGGGCCCCAAGGTCCGTATTACT
GTGGTGTGGGCGCAGACAAAGCCTATGGCAGGGATATCGTGGAGGCTCACTACC
GCGCCTGCTTGTATGCTGGGGTCAAGATTACAGGAACAAATGCTGAGGTCATGCC
TGCCCAGTGGGAGTTCCAAATAGGACCCTGTGAAGGAATCCGCATGGGAGATCAT
CTCTGGGTGGCCCGTTTCATCTTGCATCGAGTATGTGAAGACTTTGGGGTAATAGC
AACCTTTGACCCCAAGCCCATTCTGGGAACTGGAATGGTGCAGGCTGCCATACC
AACTTTAGCACCAAGGCCATGCGGGAGGAGAATGGTCTGAAGTAAGTAGCTTCCT
CTGGAGCCATCTTTATTCTCATGGGGTGGAAAGGGCTTTGTGTTAGGGTTGGGAAA
GTTGGACTTCTCACAACTACATGCCATGCTCTTCGTGTTTGTGATAAGCCTATCGT
TTTGTACCCGTTGGAGAAGTGACAGTACTCTAGGAATAGAATTACAGCTGTGATAT
GGGAAAGTTGTCACGTAGGTTCAAGCATTAAAGGTCTTTAGTAAGAACTAAATACA
CATACAAGCAAGTGGGTGACTTAATTCTTACTGATGGGAAGAGGCCAGTGATGGG
GGTCTTCCCATCCAAAAGATAATTGGTATTACATGTTGAGGACTGGTCTGAAGCAC
TTGAGACATAGGTCACAAGGCAGACACAGCCTGCATCAAGTATTTATTGGTTTCTT
ATGGAACATGCCTGCTCCTGCCCTTGAAGGACAGGTTTCTAGTGACAAGGTCA
GACCCTCACCTTTACTGCTTCCACCAGGCACATCGAGGAGGCCATCGAGAACTA
AGCAAGCGGCACCGGTACCACATTCGAGCCTACGATCCCAAGGGGGGCGCTGGAC
AATGCCCGTCTGCTGACTGGGTTCACGAAACGTCCAACATCAACGACTTTTCTGC
TGGTGTGCGCAATCGCAGTGCCAGCATCCGCATTCCCCGGAAGTGTGCGGCCAGGA
GAAGAAAGGTTACTTTGAAGACCGCGCCCTCTGCCAATTGTGACCCCTTTGCA
GTGACAGAAGCCATCGTCCGCACATGCCTTCTCAATGAGACTGGCGACGAGCCCT
TCCAATACAAAACTAATTAGACTTTGAGTGATCTTGAGCCTTTCCTAGTTTCATCCC
ACCCCGCCCCAGCTGTCTCATTGTAACCTCAAAGTAGTTTCATCCACCCCGCCCCAG
CTGTCTCATTGTAACCTCAAAGGGATGGAATATCAAGGTCTTTTTATTCTCGTGCCC
AGTTAATCTTGCTTTTATTGGTCAAGATAGAGGAGTCAAGTTCTTAATCCCTATACA
CCCAACCCTCATTTCTTTTCTATTTAGCTTTCTAGTGGGGGTGGGAGGGGTAGGGG
AAGGGAACGTAACTGCTTCATCTCATCAGGAATGCATGTCCAGTAGGCAGAG
CTGCCACAGAGTGGGTGTATTTGTGGAGGAGGACTTTTTCTTCAGGACAGTTAAAA
GAGCAGGTCCACTGCTTGGATTGACAATCCCCTATAGGTAGAGAGCTGCTAGTTC
TTCAGGTAAAACCAACTTTCTATTCCAAATGGAAGTTAGGTGAGGAGTAGTGGGAG
GAGTTCATGCCCTCCATGAAGACAGCTCAGTGTATCACCTGACAGATGGGTAGCC
CTACTGTAAAAGAAGGAAAAGTTATTTCTGGGTCTCCATTTATAACACAAAGCAGA
GTAGTATTTTTATATTTAAATGTAAAAACAAAAGTTATATATATGGATATGTGGATAT
ATGTGTATTTCTAATTGAGGAAACCATCCTAGTTACTGGGTTTGCCAAGTTTGAAGA
GCTTGGTTAACAAGAAAGGATCTCTTGAGTAGAGGTGGGGGTGCAGTACCAGGAA
AGGTGGTTATCTGGGGCTCAGCGCTTTATTACTATGTGGGGTTTCCCTGCCCACTC

FIGURE 15 (continued)

TGCAGGAGCAGATGCTGGACAGGTAGCAGGGTGGGACACCAGTGCTGCCACCAC
CTGTCCCTGTGCTTAGGCTAAGATGCATATGTATCCACACAGAGTTAGCAGGATGG
AGTTGGCTGGTCAACTTGAACATTGTTACTGATAGGGGTGGGTGGGGTTTTATTTTT
TGGTGGGACTAGCATGTCATAAAGCAGGCCTTTTGATATATTAAATTTTTTAAAGC
AAAACAAGTTCAGCTTTTAATCAACTTTGTAGGGTTTCTAACTTTACAGAATTGCCT
GTTTGTTCAGTGTCTCCATCCACTTTGCTCTTGGAGGAACGGAGGACAGGCAGAC
CTGGAGTTAAACATTTGTCATTTTGTGTCATAGTGTCTACTTTCTCCCAGCAGAAT
ATTCCTTTCCTTCTTAGGAGTCCTATGGAGTTTTGTTTTTGTTTTTTCTATTACGAT
AAACATACCCACCTCCATTCTGGCTTGCCCTGCTGTTCTCTGGTTGTTTGTGTGC
TGTCCGCAGCAGGCTGCCTGTGGTTTTCTCTTGCCATGACGACTTCTAATTGCCAT
GTACAGTATGTTCAAGTTAGATAACTCCTCATTGTAAACAGACTGTAAGTCCAGAG
CAGCGCTTATAAATCAACCTAACATTTATAAGATTTCTCTTGACTTGTTTCTTTGTG
GTTGGGGGAGGAAGAAAAAAGCGTGCAGTATTTTTTGTTCCTTCATTTCTTA
TCAAAGAAAGGGGAGTGTTTCTGTTTTGTTTACTCGCAAATAAGCTAGCTTATCT
ATTGGCTTTTCTTTTTTTTTTTTTTAAACGGGCTTTTCTTGACCTATAATTTGG
GGTAAGGTGTGAGAGTTTTATAGTTTTTGAGACAGGGTCTTGGTGTATACCCTTG
GCTGGCCTGGAGCTAACTATGTAGACTGGGCTAGCCTTTAACTTGCAGTTCTGCTT
TCAATTAGGGTTTATACATTTAGTCTTGGAATTCCTAGTTCCACGTTTAACTCTTT
ACATTTCAAAGCAGTGTTATCTGAAGAGTTCAGGCGCAGAGTCAATTCAATAGAGT
TACACAAAACCTAAAAACAAGTTTTAAATACCAAGTTATGTTGGCCTGGCCACTT
TTCACAGCTGTCCACAACTCAATGTGACAAGGCTACAAATTGGATATACTAGAATTT
CCTGGTGATTTGGAACCCCTGCTTCATTTCCCGGAACCAGGGCTTTTGGTGACAGT
CCTAGCTTATCAGATTATTTAAACAGTTACTCTTCCTGCCCTTCTTCCTGAGACCT
TTGTCCAGCTGCCATGAGCCATCTACACAGTACTTGCTTCCCTGTTGAAGTCACTG
AAGGCACATCAGCCCAAGACATAAAGGCTTGCCCGGATTCACTAGCCTGGTGAA
CTTGTGGTTCTCTGATGTTTTGTCCTGTTTTGTTGTGATTTAGTCTCAAATTTCCAG
CCTGGTTTGAAATCTGGGCTCCCAGCCTTCAATAAGGAGGACTACAGATATGTAC
GACTGAGCCTTGATTCCAGCCTCATGTTTATACGTCTGTGCTCAGCTCCCTGAAGG
TTCCAGTTTGAACTCAATAATCCAGGGGTGAGAAAGTCTTGATCTTATCCCCACA
GTATGGCACCAAGCCTGGCTGAGCCTTCTGACTTAGTCTGCCCTGTTGCTATTTAA
GCACTTTTCTTCACTAGGCTAAAAATAAAGGAGCTTCCTCCTTTGCCATGGCGCT
GTGCATGATAGGAAAAGGTAGCTATCTACTAGCATATTAAGTCCACTGTTTTGCTT
TGTGTGTTTGGTTTTTGAGGAAGGGTCTCAACTGTGTATCCCTGGCTGGCCTGGCC
GGATCTAGCTTCGTGTCAAGGACGGTGACTGCAGTGAATAATAAATGTGTGTTTG
TCCGAAATACGCGTTTTGAGATTTCTGTGCGCGACTAAATTCATGTGCGCGGATAG
TGGTGTATATCGCCGATAGAGATGGCGATATTGGAATAATCGATATTTGAAATATG
GCATATTGAAATGTGCGCGATGTGAGTTTCTGTGTAAGTATCGCCATTTTTCC
AAAAGTGATTTTTGGGCATACGCGATATCTGGCGATAGCGCTTATATCGTTTACGG
GGGATGGCGATAGACGACTTTGGTGACTTGGGCGATTCTGTGTGTGCGCAAATATC
GCAGTTTCGATATAGGTGACAGACGATATGAGGCTATATCGCCGATAGAGGCGAC
ATCAAGCTGGCACATGGCCAATGCATATCGATCTATACATTGAATCAATATTGGCC
ATTAGCCATATTATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTG
CATACGTTGTATCCATATCATAATATGTACATTTATATTGGCTCATGTCCAACATTAC
CGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAT

FIGURE 15 (continued)

TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCG
CCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATGTTT
CCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGG
TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTAT
TGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTAT
GGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGA
TGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGACTCACGGGGATT
TCCAAGTCTCCACCCCATGACGTCAATGGGAGTTTGTGTTTTGGCACCAAATCAAC
GGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAG
GCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATC
GCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGAT
CCAGCCTCCGCGGCGCGGAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGA
GTGACGTAAGTACCGCCTATAGAGTCTATAGGCCACCCCCCTTGGCTTCTTATGCA
TGCTATACTGTTTTTGGCTTGGGGTCTATACACCCCCGCTTCCTCATGTTATAGGTG
ATGGTATAGCTTAGCCTATAGGTGTGGGTATTGACCATTATTGACCACTCCCCTAT
TGGTGACGATACTTTCCATTACTAATCCATAACATGGCTCTTTGCCACAACTCTCTT
TATTGGCTATATGCCAATACACTGTCCTTCAGAGACTGACACGGACTCTGTATTTTT
ACAGGATGGGGTCTCATTTATTATTACAAATTCACATATACAACACCACCGTCCCC
AGTGCCCGCAGTTTTTTATTAAACATAACGTGGGATCTCCACGCGAATCTCGGGTAC
GTGTTCCGGACATGGGCTCTTCTCCGGTAGCGGCGGAGCTTCTACATCCGAGCCC
TGCTCCCATGCCTCCAGCGACTCATGGTCGCTCGGCAGCTCCTTGCTCCTAACAG
TGGAGGCCAGACTTAGGCACAGCACGATGCCACCACCACAGTGTGCCGCACA
AGGCCGTGGCGGTAGGGTATGTGTCTGAAAATGAGCTCGGGGAGCGGGCTTGCA
CCGCTGACGCATTTGGAAGACTTAAGGCAGCGGCAGAGAAGAAGATGCAGGCAGCT
GAGTTGTTGTGTTCTGATAAGAGTCAGAGGTAACCTCCGTTGCGGTGCTGTTAACG
GTGGAGGGCAGTGTAGTCTGAGCAGTACTCGTTGCTGCCGCGCGCGCCACCAGA
CATAATAGCTGACAGACTAACAGACTGTTTCTTCCATGGGTCTTTTCTGCAGTCAC
CGTCCTTGACACGAAGC (Seq. ID No. 3)

[illegible]

FIGURE 16

FIGURE 16 (continued)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/58433

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/21; C12Q 1/70 (2013.01)

USPC - 424/208.1, 435/5

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)

USPC - 424/208.1, 435/5

IPC(8) - A61K 39/21; C12Q 1/70 (2013.01)

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

USPC - 424/208.1, 184.1, 188.1; 435/5

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

PatBase (PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD), FreePatentsOnline (US Pat, PgPub, EPO, JPO, WIPO, NPL), GoogleScholar (PL, NPL); search terms: HIV-1 Envelope protein conformational forms disulfide exchange reshuffling V1/V2 domain vaccine immunogenic composition structural configuration ?C form?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003/0105282 A1 (PINTER) 05 June 2003 (05.06.2003) FIG 2, 6; para [0015]; [0032], [0037]	1-8, 13-18
A	Go et al. , Analysis of the disulfide bond arrangement of the HIV-1 envelope protein CON-S gp140 Delta-CFI shows variability in the V1 and V2 regions, Journal of Proteome Research, 10, 578-591 (04 February 2011) Abstract, pg 579, col 1, para 4, Fig. 5a, 5b, 7	1-8, 13-18
A	N-butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with changes in antibody recognition of the V1/V2 region of gp120. J Virol. October 1996 5 Vol 70 No 10 Pages 7143-7152. Especially abstract, pg 7146 Table 1	1-8, 13-18
A	US 2004/0224308 A1 (Binley et al.) 11 November 2004 (11.11.2004) para [0018] , [0107]-[0111], [0264]- [0268], [0268]- [0304]	1-8, 13-18

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

21 January 2014 (21.01.2014)

Date of mailing of the international search report

10 FEB 2014

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 13/58433

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 9-12, 27-30, 45-48
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 9-12, 27-30, 45-48 are unsearchable because Applicant failed to submit a valid CRF to the ISA/225 mailed on 12 September 2013. Accordingly, the USPTO cannot supply a search for the sequences listed in this application.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid

Group I: Claims 1-8, 13-18, drawn to a composition/method comprising an isolated and purified polypeptide comprising a V1/V2 domain of a HIV envelope protein having a structural configuration of a "C" form and its application for treating/vaccinating/inducing an enhanced immunologic response in a mammal host against HIV infection and a method of identifying a HIV antiviral compound.

Group II: claims 19-26, 31-44 and 49-54 drawn to a composition/method comprising an isolated and purified polypeptide comprising a V1/V2 domain of a HIV envelope protein having a structural configuration of a "B" form or an isolated and purified polypeptide comprising a V1/V2 domain of a HIV envelope protein having a mixture of structural configurations comprising "B" and "A" forms, as shown in Figure 6, and their application for treating/vaccinating/inducing an enhanced immunologic response in a mammal host against HIV infection and a method of identifying a HIV antiviral compound.

Continued in supplemental box

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1-8, 13-18

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box III

The groups of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Group I required V1/V2 domain having a structural configuration of conformer C, not required by Group II.

The inventions of Groups I-II share a composition/method comprising an isolated and purified polypeptide comprising a V1/V2 domain of a HIV envelope protein and its application for treating/vaccinating/inducing an enhanced immunologic response in a mammal host against HIV infection and a method of identifying a HIV antiviral compound.

However, these shared technical features do not represent a contribution or improvement over the prior art, where it is obvious over US 2003/0105282 A1 (PINTER) [published 5 June 2003] in view of the publication titled "Analysis of the disulfide bond arrangement of the HIV-1 envelope protein CON-S gp140 Delta-CF1 shows variability in the V1 and V2 regions" by GO et al. (hereinafter "Go") [published 4 February 2011], in final view of the publication titled "N-butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with changes in antibody recognition of the V1/V2 region of gp120" by FISCHER et al. (hereinafter "Fischer") [published October 1996 in J Virol Vol 70 No 10 Pages 7143-7152].

Pinter discloses an isolated polypeptide comprising a V1/V2 domain of a HIV envelope protein (fusion peptide of gp120 V1/V2 domain fused to MuLV gp70 fragment; FIG 2; para [0015]; showing 'A' form [as depicted in instant application Fig 6]) and also showing resolution of 2 conformational forms together (para [0032], [0037]; FIG 6; SDS-PAGE doublet due to two conformational forms under non-reducing conditions but identical mobility under reducing conditions; para [0070]). Case-A2 fusion protein prepared as described above was purified by affinity chromatography on a Ni-NTA column, utilizing a His6 tag incorporated near the N-terminus of the carrier gp70 sequence. The protein was shown by SDS-PAGE to be >90% pure. Case-A2 fusion protein exists in at least two conformational forms. A radioimmunoprecipitation analysis of 35S-cysteine labeled Case-A2 fusion protein demonstrated the presence of two conformational forms of the antigen, which differed both in their reactivity with different monoclonal antibodies and their mobility on SDS gels (approximately 2 kD apparent molecular weight difference) when analyzed under non-reducing conditions (FIG. 6). The presence of multiple structural configurations in V1/V2 domain co-existent in one solution is further taught by Go, who shows heterogeneity in the disulfide bonding in the V1/V2 region and suggest that V1/V2 region does not have a single conserved disulfide bonding pattern, and that variability could impact immunogenicity of expressed envelope protein (abstract and pg 579, col 1, para 4). Go demonstrates that ESI-MS/MS can be employed to identify disulfide-linked tryptic peptide (Fig. 7) and in addition to conventional 'A' conformer, other disulfide bonding patterns could also exist (Fig 5A shows conformer A and Fig. 5B show different disulfide bonding patterns). Since Pinter teaches that different monoclonal antibodies can specifically distinguish between the upper and lower bands of Case A2 fusion protein indicated in the SDS-PAGE (-DTT) (para [0070]); it would have been obvious for an artisan of ordinary skill to have used the ESI-MS/MS to elucidate the different structural configurations of V1/V2 regions, because Go teaches that variability in disulfide-bonding patterns could impact immunogenicity of expressed envelope protein.

In addition to structural characterization of V1/V2, Pinter teaches using the isolated purified V1/V2 for vaccinating/treatment to generate an enhanced immunological response against the HIV virus (para [0012]; "The invention also features a method for stimulating the formation of antibodies capable of neutralizing infection by an HIV viral isolate in at least one mammalian species, which method includes immunizing a mammalian subject with a composition comprising a protein which includes a gp120 V1/V2 domain of an HIV-1 strain").

Finally, concerning a method of identifying a HIV antiviral compound, Fischer teaches combining a test compound (abstract; N-butyldeoxynojirimycin (NB-DNJ)) with an anti-HIV antibody directed against the V1/V2 domain (in part), and the HIV envelope containing the V1/V2 domain and comparing the effect of the compound on the affinity component on antibody binding to the target containing the V1/V2 domain (abstract: "In this study we have examined the effect of NB-DNJ on the structure of recombinant gp120 (rgp120), expressed in CHO cells, by using a panel of 40 monoclonal antibodies. The levels of binding of antibodies to rgp120 produced in the presence [rgp120(+)] and absence [rgp120(-)] of NB-DNJ were compared by enzyme-linked immunosorbent assay and surface plasmon resonance (BIAcore; Pharmacia). The results showed an increase in the binding to rgp120(+) of antibodies directed against the C1 and C2 regions and a decrease in the binding of antibodies directed against the V1/V2 loops compared with antibody binding to rgp120(-). A decrease in the binding to rgp120(+) of antibodies directed against discontinuous epitopes was also observed"; pg 7146 Table 1; TABLE 1. BIAcore analysis of the binding of antibodies to rgp120(+) and rgp120(-) [note effect on V1/V2 affinity for antibody indicated]).

As the common technical features were known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I-II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Comment on Item 4:

Claims 9-12, 27-30 and 45-48 are held to be unsearchable because no electronic sequence was submitted in response to ISA/225 on 12 September 2013.

Observation

The description in Claims 37-44 and 49-54 of a V1/V2 domain having a structural configuration B/A combined form lacks clarity, and is unsupported by the description. There is no "B"/"A" combined form shown in Figure 6 or 7, or elsewhere in the description, contrary to the recitations in these claims. For purposes of this invitation, the limitation in question is interpreted as "An isolated and purified polypeptide comprising a V1/V2 domain of a HIV envelope protein having a mixture of structural configurations comprising "B" and "A" forms, as shown in Figure 6."