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(54) Title: METHODS AND COMPOSITIONS FOR INDUCING AN IMMUNE RESPONSE TO HIV AND MODELS FOR TESTING

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The invention provides methods and compositions for raising an immune response in a subject by administering an HIV antigen. The HIV antigens include HIV clade C polynucleotides and polypeptides. The invention also provides for recombinant HIV viral particles and compositions.

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1157ipenvd123-codon-op	MRVKEKYQHL	WRWGWRWGTM	LLGMLMICS	TEKLWVTVYY	GVPVWKEAKT	50
157ipenvd1234-codon-op	MRVKEKYQHL	WRWGWRWGTM	LLGMLMICS	TEKLWVTVYY	GVPVWKEAKT	50
iv-1157ip-env-codon-op	MRVKEKYQHL	WRWGWRWGTM	LLGMLMICS	TEKLWVTVYY	GVPVWKEAKT	50
hiv1084ienv-codon-op	MRVREGIQRY	PQWNIWGT	LLGFLMRYNG	MGSLLWVTVYY	GVPVWKEAKT	47
Consensus	MRV-----	--W-W-WG--	--LG-LMI---	---LWVTVYY	GVPVWKEAKT	50
1157ipenvd123-codon-op	TLFCASDAKA	YERKEVHNIWA	THACVPTDPN	PQELVLENVT	ENFNMWKDDM	100
157ipenvd1234-codon-op	TLFCASDAKA	YERKEVHNIWA	THACVPTDPN	PQELVLENVT	ENFNMWKDDM	100
iv-1157ip-env-codon-op	TLFCASDAKA	YERKEVHNIWA	THACVPTDPN	PQELVLENVT	ENFNMWKDDM	100
hiv1084ienv-codon-op	TLFCASDAKA	YERKEVHNIWA	THACVPTDPN	PQELVLENVT	ENFNMWKDDM	97
Consensus	TLFCASDAKA	YERKEVHNIWA	THACVPTDPN	PQELVLENVT	ENFNMWKDDM	100
1157ipenvd123-codon-op	VDQMHEDIIS	LWDQSLKPCV	KLTPLCVTLK	CSNFTREGNV	TKREEMDKVK	120
157ipenvd1234-codon-op	VDQMHEDIIS	LWDQSLKPCV	KLTPLCVTLK	CSNFTREGNV	TKREEMDKVK	120
iv-1157ip-env-codon-op	VDQMHEDIIS	LWDQSLKPCV	KLTPLCVTLK	CSNFTREGNV	TKREEMDKVK	150
hiv1084ienv-codon-op	VDQMHEDIIS	LWDQSLKPCV	KLTPLCVTLK	CSNFTREGNV	TKREEMDKVK	142
Consensus	VDQMHEDIIS	LWDQSLKPCV	KLTPLCVTLK	CSNFTREGNV	TKREEMDKVK	150
1157ipenvd123-codon-op	NCSEFNVTTGI	RDKKQKVNAL	FYRLDITEL	DENNNNSSEY	RLINCKSSSTI	121
157ipenvd1234-codon-op	NCSEFNVTTGI	RDKKQKVNAL	FYRLDITEL	DENNNNSSEY	RLINCKSSSTI	121
iv-1157ip-env-codon-op	NCSEFNVTTGI	RDKKQKVNAL	FYRLDITEL	DENNNNSSEY	RLINCKSSSTI	199
hiv1084ienv-codon-op	NCSEFNVTTGI	RDKKQKVNAL	FYRLDITEL	DENNNNSSEY	RLINCKSSSTI	192
Consensus	NCSEFNVTTGI	RDKKQKVNAL	FYRLDITEL	DENNNNSSEY	RLINCKSSSTI	200
1157ipenvd123-codon-op	AGACPKNVFD	PIPIHYCAPA	GYAILKCNKR	TFNGTGPCNN	VSTVQCTHGI	171
157ipenvd1234-codon-op	AGACPKNVFD	PIPIHYCAPA	GYAILKCNKR	TFNGTGPCNN	VSTVQCTHGI	171
iv-1157ip-env-codon-op	AGACPKNVFD	PIPIHYCAPA	GYAILKCNKR	TFNGTGPCNN	VSTVQCTHGI	249
hiv1084ienv-codon-op	AGACPKNVFD	PIPIHYCAPA	GYAILKCNKR	TFNGTGPCNN	VSTVQCTHGI	242
Consensus	AGACPKNVFD	PIPIHYCAPA	GYAILKCNKR	TFNGTGPCNN	VSTVQCTHGI	250

(57) Abstract: The invention provides methods and compositions for raising an immune response in a subject by administering an HIV antigen. The HIV antigens include HIV clade C polynucleotides and polypeptides. The invention also provides for recombinant HIV viral particles and compositions.

WO 2007/126959 A2

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METHODS AND COMPOSITIONS FOR INDUCING AN IMMUNE RESPONSE TO HIV AND MODELS FOR TESTING

5

10 GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by a grant P01 AI48240 from the National Institutes of Health-NIAID. The Government of the United States of America has certain rights in the invention.

15 BACKGROUND

Acquired immune deficiency syndrome (AIDS) is a major health threat throughout the world. HIV is the leading cause of AIDS. To date there are no effective vaccines for AIDS. For these reasons there are great efforts to create vaccines and suitable models for testing the vaccines. One target of therapeutic
20 vaccines in the envelope protein of HIV (gp160). During infection, gp160 is cleaved by host cell proteases to from the gp120 and gp41. Barnett et al. (US2002/0146683) describes numerous HIV Env polypeptides in which at least one of the native B-sheet configurations has been modified. The constructs are taught to be used in vaccine compositions. Vajdy et al. (US2005/0175631) teaches pharmaceutical
25 compositions for the treatment of AIDS in which the compositions have an HIV antigen and a mucosal adjuvant.

SUMMARY OF THE INVENTION

The invention provides methods and compositions for raising an immune
30 response in a subject by administering an HIV antigen. The HIV antigens include HIV clade C polynucleotides and polypeptides. The invention also provides for

recombinant HIV viral particles and compositions. The recombinant HIV viral particles are particularly useful in animal models for studying HIV.

In one aspect, the invention provides for a method of inducing an immune response in a subject comprising administering the polypeptides or polynucleotides encoding an HIV clade C envelope in an amount sufficient to induce an immune response in the subject.

In another aspect, the invention provides an isolated HIV envelope polypeptide. The polypeptide comprises the amino acid sequence of SEQ ID NO:2.

In another aspect, the invention provides a modified HIV clade C envelope polypeptide in which the V1 region is deleted. In yet another aspect, the V1/V2 (SEQ ID NO:4), V1, V2, V3 (SEQ ID NO:6) or V1, V2, V3, V4 (SEQ ID NO:8) regions of the modified HIV clade C envelope polypeptide are deleted.

In another aspect, the present invention provides an isolated polynucleotide encoding an HIV envelope polypeptide. The polynucleotide comprises the nucleotide sequence of SEQ ID NO:1. In another aspect, the invention provides a modified HIV clade C envelope polynucleotide in which the V1 region is deleted. In yet another aspect, the V1/V2 (SEQ ID NO:3), V1, V2, V3 (SEQ ID NO:5) or V1, V2, V3, V4 (SEQ ID NO:7) regions of the modified HIV clade C envelope polynucleotide are deleted.

The invention also provides for vectors and compositions which include the HIV clade C envelope polynucleotides and polypeptides of the invention.

In yet another aspect, the present invention provides an HIV recombinant viral particle having an envelope region with the nucleotide sequence of SEQ ID NO: 9 or SEQ ID NO:10.

In still another aspect, the invention provides a method for assessing the efficacy of a candidate AIDS vaccine in a non-human primate. The method includes the steps of administering a candidate AIDS vaccine to a subject, infecting the non-human primate with the viral particle of the invention, and detecting disease progression.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts an alignment of the amino acid sequence of the HIV envelope consensus region (SEQ ID NO:12) with the envelope regions of strains 1084i (SEQ ID NO:13), full-length 1157ip (SEQ ID NO:2), 1157ip with deletions in envelope variable regions 1, 2 and 3 (SEQ ID NO: 6), and 1157ip with deletions in envelope variable regions 1, 2, 3 and 4 (SEQ ID NO: 8).

Figure 2 depicts an alignment of the nucleic acid sequence of the HIV envelope consensus region (SEQ ID NO: 14) with the envelope regions of strains 1084i (SEQ ID NO: 15), full-length 1157ip (SEQ ID NO:1), 1157ip with deletions in envelope variable regions 1, 2 and 3 (SEQ ID NO: 5), and 1157ip with deletions in envelope variable regions 1, 2, 3 and 4 (SEQ ID NO: 7).

Figure 3 illustrates neutralizing antibody activity against SHIV-1157ip and SHIV89.6P.

Figure 4 illustrates CD4+T cell counts and vial RNA loads in Rhesus Monkeys.

Figure 5 illustrates construction of SHIV-1157ipd3.

Figure 6 illustrates luciferase expression levels in constructs with increasing numbers of NF- κ B sites and replication of SHIV-1157ipd3 v. SHIV-1157ipd3N4.

Figure 7a illustrates coreceptor usage of SHIV-1157ipd3 and SHIV-1157ipd3N4.

Figures 7b and c illustrate virus neutralization with several broadly reactive human nmAbs: b12, 2G12, 2F5 and 4E10, and b12, 2G12, 2F5, 4E10 and 4X, respectively.

Figure 8 depicts the nucleotide sequence of the envelope region of the construct designated 1157ip env (SEQ ID NO: 1).

Figure 9 depicts the amino acid sequence of the envelope region of the construct designated 1157ip env. (SEQ ID NO: 2).

Figure 10 depicts the nucleotide sequence of the envelope region of the construct designated 1157ipdv12 (SEQ ID NO: 3).

Figure 11 depicts the amino acid sequence of the envelope region of the construct designated 1157ipdv12 (SEQ ID NO: 4).

Figure 12 depicts the nucleotide sequence of the envelope region of the construct designated 1157ipdv123 (SEQ ID NO: 5).

Figure 13 depicts the amino acid sequence of the envelope region of the construct designated 1157ipdv123 (SEQ ID NO: 6).

Figure 14 depicts the nucleotide sequence of the envelope region of the construct designated 1157ipdv1234 (SEQ ID NO: 7).

Figure 15 depicts the amino acid sequence of the envelope region of the construct designated 1157ipdv1234 (SEQ ID NO: 8).

5 Figure 16 depicts the nucleotide sequence of the envelope region of the construct designated 1157ipd3 (SEQ ID NO: 9).

Figure 17 depicts the amino acid sequence of the envelope region of the construct designated 1157ipd3 (SEQ ID NO: 10).

10 Figure 18 depicts the amino acid sequence of the Nef region of the construct designated 1157ipd (SEQ ID NO: 11).

Figure 19 illustration of the cloning strategy for SHIV-1157i.

Figure 20 illustration of viremia in vaccinated and control macaques.

DETAILED DESCRIPTION

15 Definitions

As used herein, a “polynucleotide” refers to a covalently linked sequence of nucleotides (i.e., ribonucleotides for RNA and deoxyribonucleotides for DNA) in which the 3' position of the pentose of one nucleotide is joined by a phosphodiester linkage to the 5' position of the pentose of the next nucleotide. The term

20 “polynucleotide” includes single- and double-stranded polynucleotides.

“Polynucleotide” also embraces a short polynucleotide, often referred to as an oligonucleotide (e.g., a primer or a probe). A polynucleotide has a “5'-terminus” and a “3'-terminus” because polynucleotide phosphodiester linkages occur between the 5' carbon and 3' carbon of the pentose ring of the substituent mononucleotides.

25 The end of a polynucleotide at which a new linkage would be to a 5' carbon is its 5' terminal nucleotide. The end of a polynucleotide at which a new linkage would be to a 3' carbon is its 3' terminal nucleotide. As used herein, a polynucleotide sequence, even if internal to a larger polynucleotide (e.g., a sequence region within a polynucleotide), also can be said to have 5'- and 3'- ends.

30 The term “polynucleotide” as it is employed herein embraces chemically, enzymatically, or metabolically modified forms of a polynucleotide. The term also

captures sequences that include any of the known base analogs of DNA and RNA, and includes modifications such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the nucleic acid molecule encodes a therapeutic or antigenic protein. Modifications of polynucleotides may
5 have any number of effects including, for example, facilitating expression of the polypeptide product in a host cell.

The polynucleotides used in the present invention include polynucleotides encoding for an immunogenic fragment or derivative thereof. Such immunogenic fragments or derivatives thereof include fragments encoding for a B-cell epitope or a
10 T-cell epitope. The polynucleotides used in the present invention also include polynucleotides encoding for an env gene or recombinant HIV viral particle.

As used herein a "polypeptide" is a molecular chain of amino acids and does not refer to a specific length of the product. Thus, peptides, oligopeptides dimers, multimers, and the like are included within the definition of polypeptide. This term
15 is also intended to include polypeptide that have been subjected to post-expression modifications such as, for example, glycosylations, acetylations, phosphorylations and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein that includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, as long as
20 the protein maintains the desired activity.

As used herein, "isolated" is meant, when referring to a polynucleotide or a polypeptide, that the indicated molecule is separate and discrete from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological
25 macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

As used herein, "antigen" refers to a molecule containing one or more epitopes (either linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is
30 used interchangeably with the term "immunogen." Normally, a B-cell epitope will

include at least about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a CTL epitope, will include at least about 7-9 amino acids, and a helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids.

- 5 The term "antigen" denotes both subunit antigens, (i.e., antigens which are separate and discrete from a whole organism with which the antigen is associated in nature), as well as, killed, attenuated or inactivated bacteria, viruses, fungi, parasites or other microbes.

Also for purposes of the present invention, an "antigen" refers to a protein
10 that includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the protein maintains the ability to elicit an immunological response, as defined herein. Antigens of the present invention include the polypeptides of SEQ ID NOs: 2, 4, 6, 8, 10 and 11 and variants thereof.

15 As used herein, the term "inducing an immune response" to an antigen or composition is the development in a subject of a humoral and/or a cellular immune response to an antigen present in the composition of interest. A "humoral immune response" refers to an immune response mediated by antibody molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white
20 blood cells. The antigen of interest may also elicit an antibody-mediated immune response. Hence, an immunological response may include one or more of the following effects: the production of antibodies by B-cells; and/or the activation of suppressor, cytotoxic, or helper T-cells and/or T-cells directed specifically to an antigen or antigens present in the composition or vaccine of interest. These
25 responses may serve to neutralize infectivity, and/or mediate antibody-complement, or antibody dependent cell cytotoxicity (ADCC) to provide protection to an immunized host. Such responses can be determined using standard immunoassays and neutralization assays, well known in the art.

A cellular immune response involves an antigen-specific response by
30 cytolytic T-cells ("CTL"s). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility

complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and
5 focus the activity of, specific effector cells, such as B and plasma cells as well as cytotoxic T cells, against cells displaying peptide antigens in association with MHC molecules on their surface. A "cellular immune response" also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4+
10 and CD8+ T-cells. In addition, a chemokine response may be induced by various white blood or endothelial cells in response to an administered antigen.

A composition or vaccine that elicits a cellular immune response may serve to sensitize a vertebrate subject by the presentation of antigen in association with MHC molecules at the cell surface. The cell-mediated immune response is directed
15 at, or near, cells presenting antigen at their surface. In addition, antigen-specific T-lymphocytes can be generated to allow for the future protection of an immunized host.

A cell-mediated immunological response maybe determined by a number of assays known in the art including, lymphoproliferation (lymphocyte activation)
20 assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the antigen in a sensitized subject. Such assays are well known in the art. See, e.g., Erickson et al., J. Immunol. (1993) 151:4189-4199; Doe et al., Eur. J. Immunol. (1994) 24:2369-2376. Cell-mediated immune response may also be determined by the measurement of intracellular cytokines or cytokine secretion by T-cell
25 populations (e.g., by ELISPOT technique), or by measurement of epitope specific T-cells (e.g., by the tetramer technique)(reviewed by McMichael, A. J., and O'Callaghan, C. A., J. Exp. Med. 187(9):1367-1371, 1998; Mcheyzer-Williams, M. G., et al, Immunol. Rev. 150:5-21, 1996; Lalvani, A., et al, J. Exp. Med. 186:859-865, 1997).

30 As used herein, the terms "Env polypeptide" or "envelope polypeptide" refer to a molecule derived from an envelope protein, e.g. from HIV Env (SEQ ID NO:2

or variants thereof). The envelope protein of HIV-1 is a glycoprotein of about 160 kd (gp160). During virus infection of the host cell, gp160 is cleaved by host cell proteases to form gp120 and the integral membrane protein, gp41. The gp41 portion is anchored in (and spans) the membrane bilayer of virion, while the gp120 segment
5 protrudes into the surrounding environment. As there is no covalent attachment between gp120 and gp41, free gp120 is released from the surface of virions and infected cells. Env polypeptides may also include gp140 polypeptides. Env polypeptides can exist as monomers, dimers or multimers.

As used herein, a "gp120 polypeptide" is meant a molecule derived from a
10 gp120 region of the Env polypeptide. The primary amino acid sequence of gp120 is approximately 511 amino acids. The amino acid sequence of gp120 contains five relatively conserved domains interspersed with five variable domains. The variable domains contain extensive amino acid substitutions, insertions and deletions. A "gp120 polypeptide" includes both single subunits or multimers.

15 An "Env polypeptide" or "gp120 polypeptide" as defined herein is not limited to a polypeptide having the exact sequence described herein, but includes proteins having

Additionally, the term "Env polypeptide" (e.g., "gp120 polypeptide") encompasses proteins which include additional modifications to the native sequence,
20 such as additional internal deletions, additions and substitutions. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through naturally occurring mutational events. Thus, for example, the Env polypeptide to be used in vaccine compositions, the modifications must be such that immunological activity (i.e., the ability to elicit an antibody
25 response to the polypeptide) is not lost.

As used herein, the term "modified Env polypeptide" is an Env polypeptide (e.g., gp120 as defined above), which has been manipulated to delete or replace all or a part of variable regions V1, V1/V2, V1-V3 or V1-V4. Generally, modified Env (e.g., gp120) polypeptides have enough of the variable regions removed to expose
30 the CD4 binding site, but leave enough of the structure to allow correct folding.

Although not all possible variable region modifications have been exemplified herein, it is to be understood that other modifications are also encompassed by the present invention. In some embodiments the modified Env polypeptide comprises the amino acid sequence of SEQ ID NO: 4, 6, 8 or 10. In some embodiments the modified Env polypeptide is encoded by the nucleotide sequence of SEQ ID NO: 3, 5, 7 or 9 or a variant thereof.

A variant of a "modified Env polypeptide" or "Env polypeptide" as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, *e.g.*, replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, *e.g.*, replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both as long as the variant is capable of inducing an immune response. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software. Variants of the present invention include "modified Env polypeptides" or "Env polypeptides" that are at least 80%, preferably 85%, more preferably 90%, even more preferably 95% and most preferably 96%, 97%, 98% or 99% homologous to a reference amino acid sequence.

"HIV" is Human Immunodeficiency Virus, a virus that causes immunodeficiency by attacking CD4+ cells in the body. The present invention is primarily focused on HIV clade C. HIV is organized into groups and subtypes (clades). Guidance on the application of the invention to different clades may also be found in HIV Sequence Compendium 2002 Kuiken C, Foley B, Freed E, Hahn B, Marx P, McCutchan F, Mellors J, Wolinsky S, and Korber B, editors. Published by the Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, LA-UR number 03-3564. In particular, consensus sequence data for HIV-1 clades A, B, C and D and a number of isolates can be found on pages 490 to 550; consensus sequence data for HIV-2 clades A, B, C and D and a number of isolates can be found on pages 554 to 578.

One embodiment the HIV clade c env polypeptide is encoded by the polynucleotide sequence of SEQ ID NO:1, 3, 5, 7, or 9 or a variant thereof. In other embodiments the HIV clade C env polypeptide has the amino acid sequence of SEQ ID NO:2, 4, 6, 8, or 10 or a variant thereof.

5 The invention provides methods and compositions for raising an immune response in a subject by administering an HIV antigen. The HIV antigens include HIV clade C polynucleotides and polypeptides. The invention also provides methods and compositions for treating or preventing an HIV infection.

10 The terms "prevent," "preventing," and "prevention," as used herein, refer to a decrease in the occurrence of HIV in an animal. The prevention may be complete, e.g., the total absence of HIV in a subject. The prevention may also be partial, such that the occurrence of HIV in a subject is less than that which would have occurred without the present invention.

15 In one aspect, the invention provides for a method of inducing an immune response in a subject comprising administering an HIV clade C envelope polypeptide in an amount sufficient to induce an immune response in the subject. In one embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO:2. In another embodiment, the HIV clade C envelop polypeptide is modified so that the V1 region is deleted. In some embodiments, the HIV clade C envelope
20 polypeptide is modified so that the V1/V2 regions are deleted. In yet a further, embodiment the polypeptide comprises the amino acid sequence of SEQ ID NO:4. In some embodiments, the HIV clade C envelope polypeptide is modified so that the V1, V2, and V3 regions are deleted. In yet a further, embodiment the polypeptide comprises the amino acid sequence of SEQ ID NO:6. In some embodiments, the
25 HIV clade C envelope polypeptide is modified so that the V1, V2, V3 and V4 regions are deleted. In yet a further, embodiment the polypeptide comprises the amino acid sequence of SEQ ID NO:8. In yet a further embodiment, the method further includes administering a second HIV antigen. The second HIV antigen includes gp120 and gp160.

30 In another aspect, the invention provides for a method of inducing an immune response in a subject comprising administering a polynucleotide encoding a HIV clade C envelope polypeptide in an amount sufficient to induce an immune

response in the subject. In one embodiment, the polynucleotide comprises the nucleotide sequence of SEQ ID NO:1. In another embodiment, the polynucleotide region encoding the V1 region of the HIV clade C envelop polypeptide is modified so that the V1 region is deleted. In some embodiments, the polynucleotide region encoding the V1/V2 regions of the HIV clade C envelop polypeptide are modified so that the V1/V2 regions are deleted. In yet a further, embodiment the polynucleotide comprises the nucleotide sequence of SEQ ID NO:3. In some embodiments, the polynucleotide regions encoding the V1, V2 and V3 regions of the HIV clade C envelop polypeptide are modified so that the V1, V2 and V3 regions are deleted. In yet a further, embodiment the polynucleotide comprises the nucleotide sequence of SEQ ID NO:5. In some embodiments, the polynucleotide region encoding the V1, V2, V3 and V4 regions of the HIV clade C envelop polypeptide are modified so that the V1, V2, V3 and V4 regions are deleted. In yet a further, embodiment the polynucleotide comprises the nucleotide sequence of SEQ ID NO:7. In yet a further embodiment, the method further includes administering a second HIV antigen. The second HIV antigen includes gp120 and gp160.

In another aspect, the invention provides for isolated and modified HIV clade C envelope polypeptides. In one aspect, the invention provides for an isolated HIV envelope polypeptide. In one embodiment, the polypeptide includes the amino acid sequence of SEQ ID NO:2.

In another aspect, the invention provides a modified HIV clade C envelope polypeptide in which the V1 region is deleted. In another aspect, the invention provides a modified HIV clade C envelope polypeptide in which the V1/V2 regions are deleted. In one embodiment, the polypeptide includes the amino acid sequence of SEQ ID NO:4. In another aspect, the invention provides a modified HIV clade C envelope polypeptide in which the V1, V2 and V3 regions are deleted. In one embodiment, the polypeptide includes the amino acid sequence of SEQ ID NO:6. In another aspect, the invention provides a modified HIV clade C envelope polypeptide in which the V1, V2, V3 and V4 regions are deleted. In one embodiment, the polypeptide includes the amino acid sequence of SEQ ID NO:8.

In another aspect, the present invention provides for isolated and modified polynucleotides encoding an HIV envelope polypeptide. In one aspect the invention

provides an isolated polynucleotide encoding an HIV clade C envelope polypeptide. In one embodiment, the polynucleotide comprises the nucleotide sequence of SEQ ID NO:1. In another embodiment, the polynucleotide region encoding the V1 region of the HIV clade C envelop polypeptide is modified so that the V1 region is deleted.

5 In some embodiments, the polynucleotide regions encoding the V1/V2 regions of the HIV clade C envelop polypeptide are modified so that the V1/V2 regions are deleted. In yet a further, embodiment the polynucleotide comprises the nucleotide sequence of SEQ ID NO:3. In some embodiments, the polynucleotide regions encoding the V1, V2 and V3 regions of the HIV clade C envelop polypeptide are

10 modified so that the V1, V2 and V3 regions are deleted. In yet a further, embodiment the polynucleotide comprises the nucleotide sequence of SEQ ID NO:5. In some embodiments, the polynucleotide regions encoding the V1, V2, V3 and V4 regions of the HIV clade C envelop polypeptide are modified so that the V1, V2, V3 and V4 regions are deleted. In yet a further, embodiment the polynucleotide

15 comprises the nucleotide sequence of SEQ ID NO:7.

The invention also provides for vectors and compositions which include the HIV clade C envelope polynucleotides and polypeptides of the invention.

In another aspect, the invention provides for an HIV recombinant viral particle. The viral particle has a 1157 ipd envelope region having the nucleotide

20 sequence of SEQ ID NO: 9. In another aspect, the invention provides an HIV recombinant viral particle which includes a 1157 ipd3 envelope region having the nucleotide sequence of SEQ ID NO: 10. In either of the last two aspects the HIV recombinant viral particle may further include the Nef amino acid sequence of SEQ ID NO:11. In further embodiments of the invention, the recombinant viral particle

25 has two or more NF-kB regions.

The HIV recombinant viral particles may be include in a composition and may be used in a method for assessing the efficacy of a candidate AIDS vaccine in a subject. The method includes the steps of

administering a candidate AIDS vaccine to a non-human primate, infecting the non-human primate with the viral particle and detecting disease progression.

Polypeptides and polynucleotides of the invention

A polynucleotide of the present invention may be employed for producing a polypeptide by recombinant techniques. Such a polypeptide may be useful, for example, as an antigen for the induction of an immune response or as a reagent for the affinity purification of such an antibody.

In one embodiment, the polypeptides are generated using recombinant techniques, well known in the art. In this regard, oligonucleotide probes can be devised based on the known sequences of the Env polypeptide genome and used to probe genomic or cDNA libraries for Env genes. The gene can then be further isolated using standard techniques and, e.g., restriction enzymes employed to truncate the gene at desired portions of the full-length sequence. Similarly, the Env gene(s) can be isolated directly from cells and tissues containing the same, using known techniques, such as phenol extraction and the sequence further manipulated to produce the desired truncations. See, e.g., Sambrook et al., supra, for a description of techniques used to obtain and isolate DNA.

The genes encoding the Env (e.g., full length and variable region deletion) polypeptides can be produced synthetically, based on the known sequences, e.g., sequences disclosed herein. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. The complete sequence is generally assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge (1981) Nature 292:756; Nambair et al. (1984) Science 223:1299; Jay et al. (1984) J. Biol. Chem. 259:6311; Stemmer et al. (1995) Gene 164:49-53.

Recombinant techniques are readily used to clone a gene encoding an Env polypeptide gene which can then be mutagenized in vitro by the replacement of the appropriate base pair(s) to result in the codon for the desired amino acid. Such a change can include as little as one base pair, effecting a change in a single amino acid, or can encompass several base pair changes. Alternatively, the mutations can be effected using a mismatched primer which hybridizes to the parent nucleotide sequence (generally cDNA corresponding to the RNA sequence), at a temperature

below the melting temperature of the mismatched duplex. The primer can be made specific by keeping primer length and base composition within relatively narrow limits and by keeping the mutant base centrally located. See, e.g., Innis et al, (1990) PCR Applications: Protocols for Functional Genomics; Zoller and Smith, Methods
 5 Enzymol. (1983) 100:468. Primer extension is effected using DNA polymerase, the product cloned and clones containing the mutated DNA, derived by segregation of the primer extended strand, selected. Selection can be accomplished using the mutant primer as a hybridization probe. The technique is also applicable for generating multiple point mutations. See, e.g., Dalbie-McFarland et al. Proc. Natl.
 10 Acad. Sci USA (1982) 79:6409.

Once coding sequences for the desired proteins have been isolated or synthesized, they can be cloned into any suitable vector or replicon for expression. A wide variety of vectors encoding modified polypeptides can be generated by creating expression constructs which operably link the polynucleotides of the invention. The
 15 construct is then introduced into a host cell for polypeptide expression. Non-limiting examples of such combinations are discussed in the Examples.

Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform
 20 include the bacteriophage .lambda. (E. coli), pBR322 (E. coli), pACYC177 (E. coli), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-E. coli gram-negative bacteria), pHV14 (E. coli and Bacillus subtilis), pBD9 (Bacillus), pIJ61 (Streptomyces), pUC6 (Streptomyces), YIp5 (Saccharomyces), YCp19 (Saccharomyces) and bovine
 25 papilloma virus (mammalian cells). See, generally, DNA Cloning: Vols. I & II, supra; Sambrook et al., supra; B. Perbal, supra.

Insect cell expression systems, such as baculovirus systems, can also be used and are known to those of skill in the art and described in, e.g., Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Materials and
 30 methods for baculovirus/insect cell expression systems are commercially available in kit form from, inter alia, Invitrogen, San Diego Calif. ("MaxBac™" Kit).

Viral systems, such as a vaccinia based infection/transfection system, as described in Tomei et al., J. Virol. (1993) 67:4017-4026 and Selby et al., J. Gen. Virol. (1993) 74:1103-1113, will also find use with the present invention. In this system, cells are first transfected in vitro with a vaccinia virus recombinant that
5 encodes the bacteriophage T7 RNA polymerase. Following infection, cells are transfected with the DNA of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic
10 production of large quantities of RNA and its translation product(s).

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired HIV
15 clad C envelope polypeptide is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. With the present invention, both the naturally occurring signal peptides or heterologous sequences can be used. Leader sequences can be removed by the host in post-translational processing. See, e.g., U.S. Pat. Nos. 4,431,739; 4,425,437; 4,338,397. Such sequences include, but are not
20 limited to, the TPA leader, as well as the honey bee mellitin signal sequence.

Other regulatory sequences may also be desirable which allow for regulation of expression of the protein sequences relative to the growth of the host cell. Such regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a
25 chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector. Alternatively, the coding sequence
30 can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

In some cases it may be necessary to modify the coding sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the proper reading frame. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a
5 sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are well known to those skilled in the art. See, e.g., Sambrook et al., supra; DNA Cloning, Vols. I and II, supra; Nucleic Acid Hybridization, supra.

The expression vector is then used to transform an appropriate host cell. A
10 number of mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), Vero293 cells, as well as others. Similarly, bacterial hosts such as E.
15 coli, *Bacillus subtilis*, and *Streptococcus* spp., will find use with the present expression constructs. Yeast hosts useful in the present invention include inter alia, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guillermondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells
20 for use with baculovirus expression vectors include, inter alia, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*.

Depending on the expression system and host selected, the proteins of the present invention are produced by growing host cells transformed by an expression
25 vector described above under conditions whereby the protein of interest is expressed. The selection of the appropriate growth conditions is within the skill of the art.

In one embodiment, the transformed cells secrete the polypeptide product into the surrounding media. Certain regulatory sequences can be included in the
30 vector to enhance secretion of the protein product, for example using a tissue plasminogen activator (TPA) leader sequence, a γ -interferon signal sequence or other signal peptide sequences from known secretory proteins. The secreted

polypeptide product can then be isolated by various techniques described herein, for example, using standard purification techniques such as but not limited to, hydroxyapatite resins, column chromatography, ion-exchange chromatography, size-exclusion chromatography, electrophoresis, HPLC, immunoadsorbent techniques, affinity chromatography, immunoprecipitation, and the like.

Alternatively, the transformed cells are disrupted, using chemical, physical or mechanical means, which lyse the cells yet keep the HIV clad C envelope polypeptide substantially intact. Intracellular proteins can also be obtained by removing components from the cell wall or membrane, e.g., by the use of detergents or organic solvents, such that leakage of the Env polypeptides occurs. Such methods are known to those of skill in the art and are described in, e.g., Protein Purification Applications: A Practical Approach, (E. L. V. Harris and S. Angal, Eds., 1990).

For example, methods of disrupting cells for use with the present invention include but are not limited to: sonication or ultrasonication; agitation; liquid or solid extrusion; heat treatment; freeze-thaw; desiccation; explosive decompression; osmotic shock; treatment with lytic enzymes including proteases such as trypsin, neuraminidase and lysozyme; alkali treatment; and the use of detergents and solvents such as bile salts, sodium dodecylsulphate, Triton™, NP40™ and CHAPST™. The particular technique used to disrupt the cells is largely a matter of choice and will depend on the cell type in which the polypeptide is expressed, culture conditions and any pre-treatment used.

Following disruption of the cells, cellular debris is removed, generally by centrifugation, and the intracellularly produced Env polypeptides are further purified, using standard purification techniques such as but not limited to, column chromatography, ion-exchange chromatography, size-exclusion chromatography, electrophoresis, HPLC, immunoadsorbent techniques, affinity chromatography, immunoprecipitation, and the like.

For example, one method for obtaining the intracellular HIV clad C envelope polypeptides of the present invention involves affinity purification, such as by immunoaffinity chromatography using anti-Env specific antibodies, or by lectin affinity chromatography. Particularly preferred lectin resins are those that recognize mannose moieties such as but not limited to resins derived from *Galanthus nivalis*

agglutinin (GNA), *Lens culinaris* agglutinin (LCA or lentil lectin), *Pisum sativum* agglutinin (PSA or pea lectin), *Narcissus pseudonarcissus* agglutinin (NPA) and *Allium ursinum* agglutinin (AUA). The choice of a suitable affinity resin is within the skill in the art. After affinity purification, the Env polypeptides can be further
5 purified using conventional techniques well known in the art, such as by any of the techniques described above.

Relatively small polypeptides, i.e., up to about 50 amino acids in length, can be conveniently synthesized chemically, for example by any of several techniques that are known to those skilled in the peptide art. In general, these methods employ
10 the sequential addition of one or more amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then be either attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complementary (amino or carboxyl) group
15 suitably protected, under conditions that allow for the formation of an amide linkage. The protecting group is then removed from the newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support, if solid phase synthesis
20 techniques are used) are removed sequentially or concurrently, to render the final polypeptide. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide. See, e.g., J.
25 M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis* (Pierce Chemical Co., Rockford, Ill. 1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis. Synthesis. Biology*, editors E. Gross and J. Meienhofer, Vol. 2, (Academic Press, New York, 1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, *Principles of Peptide Synthesis*, (Springer-Verlag, Berlin 1984) and E.
30 Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology*, Vol. 1, for classical solution synthesis.

The polypeptides of the present invention can also be chemically prepared by other methods such as by the method of simultaneous multiple peptide synthesis. See, e.g., Houghten Proc. Natl. Acad. Sci. USA (1985) 82:5131-5135; U.S. Pat. No. 4,631,211.

5 Pharmaceutical preparations

In one embodiment the antigens of the invention are present in pharmaceutical preparations. Generally they are in the form of a polypeptide which can be prepared by the various methods describe herein and known in the art. In some embodiments, the antigens are provided as polynucleotides encoding the
10 antigen.

The HIV clade C envelope polypeptides and the polynucleotides encoding them are useful, for example, as vaccine compositions. Compositions of the invention can be used as vaccine compositions, for example, to enhance or induce an immune response of a subject to a HIV. Immune responses which can be enhanced
15 or induced include both humoral and cell-mediated responses. Compositions comprising particular preparations of HIV clade C envelope molecules can be tested for the ability to induce an immune response using assays well known in the art. For example, a composition can be injected into a laboratory animal, such as a rat or mouse, and the animal can be monitored for the appearance of immune reactants,
20 such as antibodies, directed against the Env polypeptide. Alternatively, assays such as cytotoxic T lymphocyte assays can be performed to determine whether a particular composition of Env molecule is immunogenic.

For use as a vaccine, a composition of the invention preferably comprises a HIV clade C envelope polypeptide or a modified HIV clade C envelope polypeptide.
25 Vaccine compositions of the invention typically comprise a physiologically compatible carrier. Physiologically compatible carriers are well known to those in the art. Such carriers include, but are not limited to, a simple low salt solution which permits preservation of the integrity of the Env polypeptide, e.g., 10 mM NaCl, 0.1 mM EDTA, or to large, slowly metabolized macromolecules, such as proteins,
30 polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Pharmaceutically acceptable salts can also be used in Env compositions, for example, mineral salts such as hydrochlorides,

hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, propionates, malonates, or benzoates. Compositions of the invention can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents.

5 Various methods can be used to administer an HIV clade C envelope molecule vaccine composition to a human, including intravenous, intramuscular, or subcutaneous injection. Vaccine compositions of the invention are administered at a dose effective to induce an immune response against the Env polypeptide. The particular dosages of the composition used to enhance an immune response will
10 vary, for example, according to the Env compositions being used and the mammal to which the composition is administered. Generally, about 5ug to about 50ug of Env polypeptide per kg of patient body weight, about 50ug to about 5 mg/kg, about 100ug to about 500ug/kg, or about 200 to about 250ug/kg will be administered to an adult human. Such ranges by no means preclude use of a higher or lower amount of
15 a component, as might be warranted in a particular application. For example, the actual dose and schedule may vary depending on whether the compositions are administered in combination with other pharmaceutical compositions, or depending on individual differences in pharmacokinetics, drug disposition, and metabolism.

 The antigens and/or adjuvants of the invention can also be delivered using
20 one or more gene vectors, administered via nucleic acid immunization or the like using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466. The constructs can be delivered either subcutaneously, epidermally, intradermally, intramuscularly, intravenous, mucosally (such as nasally, rectally and vaginally), intraperitoneally,
25 orally or combinations thereof.

 An exemplary replication-deficient gene delivery vehicle that may be used in the practice of the present invention is any of the alphavirus vectors, described in, for example, U.S. Pat. Nos. 6,342,372; 6,329,201 and International Publication WO 01/92552.

30 A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. Selected sequences can be inserted into a vector and packaged in

retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems have been described (U.S. Pat. No. 5,219,740; Miller and Rosman, *BioTechniques* (1989) 7:980-990; Miller, A. D., *Human Gene Therapy* (1990) 1:5-14; Scarpa et al., *Virology* (1991) 180:849-852; Burns et al., *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109.

A number of adenovirus vectors have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham, *J. Virol.* (1986) 57:267-274; Bett et al., *J. Virol.* (1993) 67:5911-5921; Mittereder et al., *Human Gene Therapy* (1994) 5:717-729; Seth et al., *J. Virol.* (1994) 68:933-940; Barr et al., *Gene Therapy* (1994) 1:51-58; Berkner, K. L. *BioTechniques* (1988) 6:616-629; and Rich et al., *Human Gene Therapy* (1993) 4:461-476).

Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery. AAV vectors can be readily constructed using techniques well known in the art. See, e.g., U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 Jan. 1992) and WO 93/03769 (published 4 Mar. 1993); Lebkowski et al., *Molec. Cell. Biol.* (1988) 8:3988-3996; Vincent et al. *Vaccines* 90 (1990) (Cold Spring Harbor Laboratory Press); Carter, B. J. *Current Opinion in Biotechnology* (1992) 3:533-539; Muzyczka, N. *Current Topics in Microbiol. and Immunol.* (1992) 158:97-129; Kotin, R. M. *Human Gene Therapy* (1994) 5:793-801; Shelling and Smith, *Gene Therapy* (1994) 1:165-169; and Zhou et al., *J. Exp. Med.* (1994) 179:1867-1875.

Another vector system useful for delivering polynucleotides, mucosally and otherwise, is the enterically administered recombinant poxvirus vaccines described by Small, Jr., P. A., et al. (U.S. Pat. No. 5,676,950, issued Oct. 14, 1997)

as well as the vaccinia virus and avian poxviruses. By way of example, vaccinia virus recombinants expressing the genes can be constructed as follows. The DNA encoding the antigens and/or adjuvants of the invention is first inserted into an appropriate vector so that it is adjacent to a vaccinia

promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK). This vector is then used to transfect cells that are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the coding sequences of interest into
5 the viral genome. The resulting TK recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver genes encoding the antigens and/or adjuvants of the
10 invention. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species; The use of an avipox vector is particularly desirable in human and other mammalian species since members of the avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian
15 cells. Methods for producing recombinant avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, e.g., WO 91/12882; WO 89/03429; and WO 92/03545. Picornavirus-derived vectors can also be used. (See, e.g., U.S. Pat. Nos. 5,614,413 and 6,063,384). Molecular conjugate vectors, such as the adenovirus chimeric
20 vectors described in Michael et al., J. Biol. Chem. (1993) 268:6866-6869 and Wagner et al., Proc. Natl. Acad. Sci. USA (1992) 89:6099-6103, can also be used for gene delivery.

A vaccinia based infection/transfection system can be conveniently used to provide for inducible, transient expression of the coding sequences of interest (for
25 example, sequences encoding the antigens or adjuvants of the invention) in a host cell. In this system, cells are first infected in vitro with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide of interest, driven
30 by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA that is then translated into protein by the host translational machinery. The method provides for high level,

transient, cytoplasmic production of large quantities of RNA and its translation products. See, e.g., Elroy-Stein and Moss, Proc. Natl. Acad. Sci. USA (1990) 87:6743-6747; Fuerst et al., Proc. Natl. Acad. Sci. USA (1986) 83:8122-8126.

As an alternative approach to infection with vaccinia or avipox virus recombinants, or to the delivery of genes using other viral vectors, an amplification system can be used that will lead to high level expression following introduction into host cells. Specifically, a T7 RNA polymerase promoter preceding the coding region for T7 RNA polymerase can be engineered. Translation of RNA derived from this template will generate T7 RNA polymerase that in turn will transcribe more template. Concomitantly, there will be a cDNA whose expression is under the control of the T7 promoter. Thus, some of the T7 RNA polymerase generated from translation of the amplification template RNA will lead to transcription of the desired gene. Because some T7 RNA polymerase is required to initiate the amplification, T7 RNA polymerase can be introduced into cells along with the template(s) to prime the transcription reaction. The polymerase can be introduced as a protein or on a plasmid encoding the RNA polymerase. For a further discussion of T7 systems and their use for transforming cells, see, e.g., International Publication No. WO 94/26911; Studier and Moffatt, J. Mol. Biol. (1986) 189:113-130; Deng and Wolff, Gene (1994) 143:245-249; Gao et al., Biochem. Biophys. Res. Commun. (1994) 200:1201-1206; Gao and Huang, Nuc. Acids Res. (1993) 21:2867-2872; Chen et al., Nuc. Acids Res. (1994) 22:2114-2120; and U.S. Pat. No. 5,135,855.

One or more additional HIV antigens can be used in the vaccine and methods of this invention. For instance, the HIV clad C env antigens can be used with additional HIV antigens. The additional HIV antigens may be administered simultaneously, prior or after administration of the HIV clad C env antigen.

In addition, an immuno-modulatory factor may be added to the pharmaceutical composition. An "immuno-modulatory factor" refers to a molecule, for example a protein that is capable of modulating an immune response or DNA vectors encoding a given immuno-modulatory factor. Non-limiting examples of immunomodulatory factors include lymphokines (also known as cytokines), such as IL-6, TGF-beta, IL-1, IL-2, IL-3, etc.); and chemokines (e.g., secreted proteins such as macrophage inhibiting factor). Certain cytokines, for example TRANCE, flt-3L,

and a secreted form of CD40L are capable of enhancing the immunostimulatory capacity of APCs. Non-limiting examples of cytokines which may be used alone or in combination in the practice of the present invention include, interleukin-2 (IL-2), stem cell factor (SCF), interleukin 3 (IL-3), interleukin 6 (IL-6), interleukin 12 (IL-12), G-CSF, granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin-1 alpha (IL-1.alpha.), interleukin-11 (IL-11), MIP-1.gamma., leukemia inhibitory factor (LIF), c-kit ligand, thrombopoietin (TPO), CD40 ligand (CD40L), tumor necrosis factor-related activation-induced cytokine (TRANCE) and flt3 ligand (flt-3L). Cytokines are commercially available from several vendors such as, for example, Genzyme (Framingham, Mass.), Amgen (Thousand Oaks, Calif.), R&D Systems and Immunex (Seattle, Wash.). The sequences of many of these molecules are also available, for example, from the GenBank database. It is intended, although not always explicitly stated, that molecules having similar biological activity as wild-type or purified cytokines (e.g., recombinantly produced or mutants thereof) and nucleic acid encoding these molecules are intended to be used within the spirit and scope of the invention.

The compositions of the invention will typically be formulated with pharmaceutically acceptable carriers or diluents. As used herein, the term "pharmaceutically acceptable carrier" refers to a carrier for administration of the antigens which does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., Pharm. Res. (1993) 10:362-368; McGee et al. (1997) J Microencapsul. 14(2):197-210; O'Hagan et al. (1993) Vaccine 11(2):149-54. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc., as well as toxins derived from E. coli.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of acceptable excipients is available in the well-known Remington's Pharmaceutical Sciences.

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

Administration

The compositions disclosed herein can be administered to a subject to generate an immune response. Preferably, the composition can be used as a vaccine to treat or prevent HIV infection.

As used herein, "subject" is meant any member of the subphylum chordata, including, without limitation, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. The term does not denote a particular age. Thus, both adult and newborn individuals are intended to be covered. The system described above is intended for use in any of the above vertebrate species, since the immune systems of all of these vertebrates operate similarly.

The compositions will include "immunologically effective amounts" of HIV antigen i.e. amounts sufficient to raise a specific immune response or, more preferably, to treat, reduce, or prevent HIV infection. An immune response can be detected by looking for antibodies to the HIV antigen used (e.g. IgG or IgA) in

patient samples (e.g. in blood or serum, in mesenteric lymph nodes, in spleen, in gastric mucosa, and/or in feces). The precise effective amount for a given patient will depend upon the patient's age, size, health, the nature and extent of the condition, the precise composition selected for administration, the patient's
 5 taxonomic group, the capacity of the patient's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating physician's assessment of the medical situation, and other relevant factors. Thus, it is not useful to specify an exact effective amount in advance, but the amount will fall in a relatively broad range that can be determined through routine trials, and
 10 is within the judgment of the clinician. For purposes of the present invention, an effective dose will typically be from about 0.01 mg/kg to 50 mg/kg in the individual to which it is administered.

EXAMPLES

15 **Example 1. V-loop deleted SHIVenvC induces cross-clade nAb responses in mice.**

A V-loop deleted SHIV-1157ipenv expression vector was prepared as follows: the entire SHIV-1157ip gp160 sequence was codon-optimized and cloned into the pTPS mutagenesis vector . Mutagenic primers were used to
 20 separately delete the V1/V2 loops and the V3 loop. The mutagenic primers were synthesized and included linker sequences GAG to maintain the Env core structure.

Mutagenesis was confirmed by DNA sequencing and the mutant gp160 gene was cloned into the pJW4303 expression vector. The resulting plasmid, pJW1157ipenvΔ123 was tested for purity by restriction digest/gel
 25 electrophoresis and for in vitro expression by transfection into 293T cells followed by Western blot analysis. The Western blot analysis showed a protein band of approximately 135 kb.

A recombinant vaccinia virus was made expressing 1157ipenvΔ123 and used to infect the human osteosarcoma cell line, 143 B. SHIV-1157ipΔ123 Env was
 30 prepared from cell lysates by size-exclusion and lentil-lectin chromatography .

Five BALB/c mice were inoculated subcutaneously 2x with 20 ug of SHIV-1157ipΔ123 Env formulated in MPL + TDM emulsion. Sera from immunized mice were tested for neutralizing antibody activity against homologous SHIV-1157ip and against heterologous SHIV89.6P (Fig 3). Three of 5 mice showed neutralizing antibody activity against SHIV-1157ip and 4 of the 5 mice showed neutralizing antibody activity against heterologous SHIV89.6P clade B.

The results demonstrate that immunization with a multiply V-loop deleted clade C Env, prepared from the full-length gp160 genome by methodology that maintains structural integrity of the multimeric form of the Env glycoprotein, is immunogenic and can induce cross-clade nAb responses.

Example 2. Construction and Adaptation of SHIV-1157i to Rhesus Macaques.

A biologically relevant challenge virus was created to evaluate anti-HIV clade C immune prophylaxis.

15 SHIV-1157i construction.

SHIV-1157i was built upon the backbone of SHIV-vpu⁺ (Li et al., *J. Virol.*(1995), 69; 7061-67) (Fig. 19a) and contained most of gp120 as well as the entire extracellular domain and transmembrane region of gp41 of the primary, original HIV1157i isolate. The HIV1157i isolate is a recently transmitted pediatric HIV clade C isolate from a six-month-old Zambian infant.

The initial cell-free SHIV-1157i stock was generated by transfecting 293T cells with proviral DNA and harvesting the supernatants. The virus replicated in human and rhesus monkey PBMC and in Ghost.CD4.CCR5.GFP cells but did not infect CEMx174 or CEMx174.GFP cells, which do not express CCR5, indicating that SHIV-1157i, like the original HIV1157i, uses CCR5 as coreceptor.

Adaptation of SHIV-1157i to rhesus macaques

To adapt SHIV-1157i to rhesus macaques, infant rhesus monkey RPN-8 was inoculated intravenously (i.v.) with 6 ml of virus. Real-time RT-PCR revealed that

SHIV-1157i replicated in this animal; peak viremia reached 6×10^4 RNA copies/ml at week 4 post-inoculation (p.i.). This first animal never cleared the virus and remained viremic throughout. Four additional animals were subjected to serial blood transfer (Fig. 19b), whereby 1 ml of whole blood collected at peak viremia (week 2 p.i.) was directly transferred into the following monkey recipients: neonates RAo-8 and RII-8, juvenile RTs-7 and infant RKI-8. Compared to the first virus recipient RPn-8, peak viral RNA loads increased between 1 to 2 logs in subsequent recipients (Fig. 19c). All five monkeys in this cohort seroconverted by week 4. At week 6 p.i., the passaged virus, termed SHIV-1157ip, was isolated from PBMC of the last recipient, RKI-8, and a large stock was grown in rhesus monkey PBMC. Virus from this stock was replication-competent in PBMC of other naïve rhesus monkey donors.

SHIV-1157ip replicated neither in CEMx174 or CEMx174-GFP cells nor in U87.CD4, or U87.CD4 cells expressing CCR1, CCR2, CCR3, or CXCR4. SHIV-1157ip also failed to replicate in Ghost-BOB and Ghost-Bonzo cells. Productive infection was only observed in U87.CD4.CCR5 cells, indicating that SHIV-1157ip exclusively used CCR5 as coreceptor, even after several passages in a different species.

Example 3. Pathogenicity of SHIV-1157ip.

Signs of early disease (depletion of memory T cells in peripheral blood and persistently low $CD4^+/CD8^+$ ratios) were observed in two monkeys, RPn-8 and RKI-8, within the first year post-inoculation. This was followed by depletion of absolute $CD4^+$ T cells in peripheral blood. In animal RPn-8, AIDS, as defined by $CD4^+$ T cells persistently <200 cells/ μ l, developed at week 135 p.i.; in two other animals, RKI-8 and RTs-7, absolute $CD4^+$ T-cell counts have persistently been <500 cells/ μ l (Fig. 4). As an additional sign of lentivirus-induced disease, severe, persistent thrombocytopenia has developed in animal RPn-8, although no bleeding has been observed to date.

In order to determine whether a more aggressive virus had evolved in animal RPn-8 after it had developed AIDS, 10 ml of blood was transfused into a naïve

recipient, RBg-9. Three months later, this recipient animal developed thrombocytopenia and depletion of peripheral blood memory T cells. Both of these abnormalities have persisted over the ensuing 18 months. Both donor and recipient have remained persistently viremic.

5 **Example 4. Construction and Mucosal Trans-mission of SHIV-1157ipd3N4.**

After the first SHIV-1157i-infected monkey (RPn-8) had progressed to AIDS, the virus was re-isolated in order to generate another molecular clone. The re-isolated virus, termed SHIV-1157ipd, was isolated four weeks after animal RPn-8's absolute CD4⁺ T-cell count dropped to <200 cells/ μ l. Clone 3 gave rise to the
10 highest level of infectious virus and was selected for further studies (Figs. 4, 5). Genetic analysis of SHIV-1157ipd *env* sequences revealed a number of mutations that resulted in single amino acid substitutions in gp120, especially in V1, V2 and V4. Mutations were also observed in gp41. Five out of five clones sequenced contained a 118-bp deletion in the 3' end of *env*. This 118-bp deletion was located
15 in the intracellular domain of gp41 and led to a loss of 35 of the original amino acids (aa), and due to a frame shift, a gain of 57 aa. The envelope remained infectious.

In order to maximize the replicative capacity and enhance viral loads in vivo, transcriptional control elements in the lentiviral LTR were added. Thus, the LTR of SHIV-1157ipd3 was modified to increase the number of NF- κ B sites. In a set of
20 solo-LTR constructs linked to the luciferase reporter gene, a direct correlation between LTR-mediated gene expression and the number of NF- κ B sites in transient transfection assays was demonstrated (Fig. 6a). The effect was especially pronounced when a *tat* expression plasmid was cotransfected and TNF- α was added to the medium, consistent with the signal transduction cascade activated by this
25 cytokine. After transfection and release of infectious virus, the modifications engineered into the 3' LTR will be copied into the 5' LTR in the subsequent viral replication cycle. The replication kinetics of late-stage viruses encoding two NF- κ B sites produced higher levels of p27 Gag production and/or earlier peaks to stimulation with TNF- α than virus containing only one site (Fig. 6b).

The SHIV-1157ipd3 virus replicated neither in CEMx174 or CEMx174-GFP cells nor in U87.CD4, U87.CD4.CCR1, U87.CD4.CCR2, U87.CD4.CCR3, U87.CD4.CXCR4, GHOST-BOB and GHOST-BONZO cells. Productive infection occurred only in U87.CD4.CCR5 cells (Fig. 7a), indicating that late-stage SHIV-1157ipd3 and its 2 NF- κ B-site counterpart, SHIV-1157ipd3N4, still use CCR5 as coreceptor exclusively.

In order to determine whether late virus was susceptible to neutralization by the broadly reactive human nmAbs b12, 2G12, 2F5 and 4E10 (Fig. 7b) assays were performed in human PBMC. The late virus had lost its sensitivity to 2G12 which was correlated with the loss of a key N-linked glycosylation site at aa position 295 that is required for forming the complex 2G12 epitope (Scanlan et al., 2002); the 2G12 nmAb is unique in that it is targeted to a pure carbohydrate structure that consists of several mannan residues. Nevertheless, the late SHIV-1157ipd3N4 had not developed global neutralization resistance.

Thus, several SHIVenvC strains were prepared that encode one of various forms of the *env* gene that was originally cloned from the R5 HIV1157i, a clade C strain. This virus was isolated from a Zambian child who turned out to be a long-term non-progressor (LTNP) during the prospective follow-up. The SHIV constructs and their designations are as follows:

- SHIV-1157i infectious molecular clone, not yet adapted to rhesus monkeys; “i” designates a virus strain (or *env* gene) isolated from an infant
- SHIV-1157ip biological isolate obtained after passage through several rhesus monkeys; “p” designates a passaged, monkey-adapted virus
- SHIV-1157ipd biological isolate, “d” indicates that the virus was re-isolated from an infected monkey with disease
- SHIV-1157ipd3 infectious molecular clone #3. The 3’ half of the provirus was derived from a monkey with virus-induced disease
- SHIV-1157ipd3N4 identical to SHIV-1157ipd3 except that each LTR has 2 instead of the usual 1 NF- κ B sites.

Example 5. Testing vaccination in animals challenged with SHIV-1157ipd3N4.

Two groups of newborn macaques were vaccinated (Group 1 and Group 3). Group 1 animals were given a combination of DNA expression plasmids encoding
 5 *SIVgag-protease*, *HIVtat* and *HIV1084ienv*. Group 3 animals (controls) were given empty vector DNA. DNA was given at 2 time points - at birth and at 6 weeks. Animals were rested and then given soluble protein boosts consisting of SIV Gag-Pol particles derived from recombinant vaccinia virus infected cells, HIV Tat and HIV1084i gp160. Age-matched Group 2 animals were given soluble proteins with
 10 no DNA priming. Proteins were given at month 9, month 11 and at month 24. Cellular and humoral immunity were assessed 2 weeks after each vaccination time point. The animals were challenged orally with SHIV-1157ip (Fig. 20, panels A, B and C). All RM received a relatively low-dose of 3.7 AID₅₀ which could account for less than 100% infection among controls. In vitro, CD8⁺ T cell-depleted PBMC
 15 from RM that had remained uninfected after oral challenge, were fully susceptible to SHIV-1157ip and SHIV-1157ipd3N4 infection, thus ruling out inherent resistance to these viruses.

Because some vaccinees appeared to have contained SHIV-1157ip, they were re-challenged i.r. with 20 AID₅₀ of SHIV-1157ipd3N4. The rechallenged
 20 animals included one animal from Group 1 (DNA prime/protein boost), 3 animals from Group 2 (protein immunization alone), and one animal from the control group, RAr-9. Plasma vRNA levels for RAr-9 after rechallenge (Fig. 20E, red) and for a group of 8 additional non-vaccinated control animals identically challenged with SHIV-1157ipd3N4 are shown. By week 1, all control animals were infected, and
 25 peak viremia levels ranged from 1.3 - 7.7 x 10⁷ vRNA copies/ml. In contrast, only 1 of 4 previously vaccinated RM had detectable virus by wk 1 (Fig. 20D). Of those that became infected, peak viremia levels were a log lower than in controls (p=0.003). Importantly, one of the protein-only vaccinated animals, RAt-9, still had no evidence of infection after rechallenge with SHIV-1157ipd3N4. At the time of
 30 rechallenge, this RM had strong cellular immunity to SIV Gag and HIV Tat.

To conserve RM, the rechallenge described was piggybacked with i.r. SHIV-1157ipd3N4 challenge of the vaccinees. All RM in the 3 vaccine groups became infected, but mean peak viremia levels for animals of the 3 vaccine groups combined were significantly less than controls (2.4×10^7 vs 4.0×10^7 vRNA copies/ml; $p = 0.006$). Mean plasma viremia levels for the Group 3 RM alone that were DNA primed with clade C *env* and boosted with heterologous clade B gp160 were the lowest at 1.5×10^7 RNA copies/ml ($p = 0.014$).

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

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JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

CLAIMS

1. An isolated HIV envelope polypeptide, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:2.
2. A composition comprising said HIV envelope polypeptide of claim 1 and a pharmacologically acceptable carrier.
3. An isolated polynucleotide encoding an HIV envelope polypeptide, wherein said polynucleotide comprises the nucleotide sequence of SEQ ID NO: 1.
4. A construct comprising said isolated polynucleotide of claim 3.
5. A composition comprising said construct of claim 4 and a pharmacologically acceptable carrier.
6. Use of a composition comprising the amino acid sequence of SEQ ID NO:2 for induction of an immune response in a subject.
7. Use of a composition comprising the amino acid sequence of SEQ ID NO:2, together with a second HIV antigen, for induction of an immune response in a subject.
8. The use of claim 7, wherein said second HIV antigen is gp120 or gp160.
9. Use of a composition comprising the nucleotide sequence of SEQ ID NO:1 for induction of an immune response in a subject.
10. Use of a composition comprising the nucleotide sequence of SEQ ID NO:1, together with a second HIV antigen, for induction of an immune response in a subject.
11. The use of claim 10, wherein said second HIV antigen is gp120 or gp160.
12. A modified HIV clade C envelope polynucleotide, wherein a V1/V2 region of said polynucleotide is deleted, and wherein said polynucleotide comprises the nucleotide sequence of SEQ ID NO:3.
13. A construct comprising said modified HIV clade C envelope polynucleotide of claim 12.

14. A composition comprising the construct of claim 13 and a pharmacologically acceptable carrier.
15. Use of the composition of claim 14 for induction of an immune response in a subject.
16. Use of the composition of claim 14, together with a second HIV antigen, for induction of an immune response in a subject.
17. The use of claim 16, wherein said second HIV antigen is gp120 or gp160.
18. A modified HIV clade C envelope polynucleotide, wherein a V1, V2 and V3 region of said polynucleotide are deleted, and wherein said polynucleotide comprises the nucleotide sequence of SEQ ID NO:5.
19. A construct comprising said modified HIV clade C envelope polynucleotide of claim 18.
20. A composition comprising said modified HIV clade C envelope polynucleotide of claim 18 and a pharmacologically acceptable carrier.
21. Use of the composition of claim 20 for induction of an immune response in a subject.
22. Use of the composition of claim 20, together with a second HIV antigen, for induction of an immune response in a subject.
23. The use of claim 22, wherein said second HIV antigen is gp120 or gp160.
24. A modified HIV clade C envelope polynucleotide, wherein a V1, V2, V3 and V4 region of said polynucleotide are deleted, and wherein said polynucleotide comprises the nucleotide sequence of SEQ ID NO:7.
25. A construct comprising said modified HIV clade C envelope polynucleotide of claim 24.
26. A composition comprising said modified HIV clade C envelope polynucleotide of claim 24 and a pharmacologically acceptable carrier.
27. Use of the composition of claim 26 for induction of an immune response in a subject.

28. Use of the composition of claim 26, together with a second HIV antigen, for induction of an immune response in a subject.
29. The use of claim 28, wherein said second HIV antigen is gp120 or gp160.
30. A modified HIV clade C envelope polypeptide, wherein a V1/V2 region of said polypeptide is deleted, and wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:4.
31. A composition comprising said modified HIV clade C envelope polypeptide of claim 30 and a pharmacologically acceptable carrier.
32. Use of the composition of claim 31 for induction of an immune response in a subject.
33. Use of the composition of claim 31, together with a second HIV antigen, for induction of an immune response in a subject.
34. The use of claim 33, wherein said second HIV antigen is gp120 or gp160.
35. A modified HIV clade C envelope polypeptide, wherein a V1, V2 and V3 region of said polypeptide are deleted, and wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:6.
36. A composition comprising said HIV clade C envelope polypeptide of claim 35 and a pharmacologically acceptable carrier.
37. Use of the composition of claim 36 for induction of an immune response in a subject.
38. Use of the composition of claim 36, together with a second HIV antigen, for induction of an immune response in a subject.
39. The use of claim 38, wherein said second HIV antigen is gp120 or gp160.
40. A modified HIV clade C envelope polypeptide, wherein a V1, V2, V3 and V4 region of said polypeptide are deleted, and wherein said polypeptide comprises the polypeptide sequence of SEQ ID NO:8.
41. A composition comprising said modified HIV clade C envelope polypeptide of claim 40 and a pharmacologically acceptable carrier.

42. Use of the composition of claim 41 for induction of an immune response in a subject.
43. Use of the composition of claim 41, together with a second HIV antigen, for induction of an immune response in a subject.
44. The use of claim 43, wherein said second HIV antigen is gp120 or gp160.
45. An HIV recombinant viral particle comprising a 1157 ipd envelope region having the nucleotide sequence of SEQ ID NO: 9.
46. An HIV recombinant viral particle comprising a 1157 ipd3 envelope region having the amino acid sequence of SEQ ID NO: 10.
47. The HIV recombinant viral particle of claim 45 or 46, wherein said viral particle further comprises the Nef amino acid sequence of SEQ ID NO:11.
48. The recombinant viral particle of any one of claims 45 to 47, wherein said viral particle comprises two or more NF-kB regions.
49. The recombinant viral particle of any one of claims 45 to 47, wherein said viral particle comprises three or more NF-kB regions.
50. A composition comprising the recombinant viral particle of any one of claims 45 to 47 and a pharmacologically acceptable carrier.
51. Use of the isolated HIV envelope of claim 1, the composition of claim 2, or the isolated polynucleotide of claim 3 to assess the efficacy of a candidate AIDS vaccine in a non-human primate.
52. Use of the isolated HIV envelope of claim 1, the composition of claim 2, or the isolated polynucleotide of claim 3 to assess the efficacy of a candidate AIDS vaccine in a non-human primate by detection of disease progression.
53. Use of an immunologically effective amount of a composition comprising a polypeptide having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO: 10 for treatment or prevention of an HIV infection.

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1157ipenvd123-codon-op	M R V	K E K Y Q H L	W R W G W R W G T M	L L G M L M I C S A	T E K L W V T V Y Y	G V P V W K E A K T	50
157ipenvd1234-codon-op	M R V	K E K Y Q H L	W R W G W R W G T M	L L G M L M I C S A	T E K L W V T V Y Y	G V P V W K E A K T	50
iv-1157ip-env-codon-op	M R V	K E K Y Q H L	W R W G W R W G T M	L L G M L M I C S A	T E K L W V T V Y Y	G V P V W K E A K T	50
hiv1084ienv-codon-op	M R V	R G I Q R N Y	P Q R W I W G . .	I L G F L M I Y N G	M G S L W V T V Y Y	G V P V W K E A K T	47
Consensus	M R V	- - - - -	- - W - W - W G - -	- L G - L M I - - -	- - - L W V T V Y Y	G V P V W K E A K T	50
1157ipenvd123-codon-op	T L F C A S D A K A	T L F C A S D A K A	Y E K E V H N I W A	T H A C V P T D P N	P Q E I V L E N V T	E N E N M M W K D D M	100
157ipenvd1234-codon-op	T L F C A S D A K A	T L F C A S D A K A	Y E K E V H N I W A	T H A C V P T D P N	P Q E I V L E N V T	E N E N M M W K D D M	100
iv-1157ip-env-codon-op	T L F C A S D A K A	T L F C A S D A K A	Y E K E V H N I W A	T H A C V P T D P N	P Q E I V L E N V T	E N E N M M W K D D M	100
hiv1084ienv-codon-op	T L F C A S D A K A	T L F C A S D A K A	Y E R E V H N I W A	T H A C V P T D P N	P Q E I V L E N V T	E N E N M M W K D D M	97
Consensus	T L F C A S D A K A	T L F C A S D A K A	Y E - E V H N I W A	T H A C V P T D P N	P Q E - V L E N V T	E N E N M M W - - D M	100
1157ipenvd123-codon-op	V D Q M H E D I I S	V D Q M H E D I I S	L W D Q S L K P C V				120
157ipenvd1234-codon-op	V D Q M H E D I I S	V D Q M H E D I I S	L W D Q S L K P C V				120
iv-1157ip-env-codon-op	V D Q M H E D I I S	V D Q M H E D I I S	L W D Q S L K P C V	K L T P L C V T L K	C S N F T R E G N V	T Y K E E M D K V K	150
hiv1084ienv-codon-op	V D Q M H E D I I S	V D Q M H E D I I S	L W D Q S L K P C V	K L T P L C V T L N	C S N F T R E G N V	S E D M R	142
Consensus	V D Q M H E D I I S	V D Q M H E D I I S	L W D Q S L K P C V	- - - - -	- - - - -	- - - - -	150
1157ipenvd123-codon-op							121
157ipenvd1234-codon-op							121
iv-1157ip-env-codon-op	N C S F N V T T E I	N C S F N V T T G I	R D K K Q K V N A L	F Y R L D I T P L	D E N N N N S S E Y	R L I N C K S S T I	199
hiv1084ienv-codon-op	N C S F N V T T E I	N C S F N V T T E I	K D R K K L E Q A L	F Y R L D I T P L K	N S S S S N F S E Y	R L I N C N T S T V	192
Consensus	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	200
1157ipenvd123-codon-op	A G A C P K V N F D	A G A C P K V N F D	P I P I H Y C A P A	G Y A I L K C N N K	T E N G T G P C H N	V S T V Q C T H G I	171
157ipenvd1234-codon-op	A G A C P K V N F D	A G A C P K V N F D	P I P I H Y C A P A	G Y A I L K C N N K	T E N G T G P C H N	V S T V Q C T H G I	171
iv-1157ip-env-codon-op	T Q A C P K V N F D	T Q A C P K V N F D	P I P I H Y C A P A	G Y A I L K C N N K	T E N G T G P C H N	V S T V Q C T H G I	249
hiv1084ienv-codon-op	S Q A C P K V N F D	S Q A C P K V N F D	P I P I H Y C A P A	G Y A I L K C N N K	T E N G S G P C N N	V S T V Q C T H G I	242
Consensus	- - A C P K V N F D	- - A C P K V N F D	P I P I H Y C A P A	G Y A I L K C N N K	T E N G - G P C - N	V S T V Q C T H G I	250

FIGURE 1

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1157ipenvd123-codon-op	K P V V S T Q L L L	N G S L A E R E I I	I R S E N L T D N V	K T I I V H F N E S	V E I N C T	217
157ipenvd1234-codon-op	K P V V S T Q L L L	N G S L A E R E I I	I R S E N L T D N V	K T I I V H F N E S	V E I N C T	217
iv-1157ip-env-codon-op	K P V V S T Q L L L	N G S L A E R E I I	I R S E N L T D N V	K T I I V H F N E S	V E I N C T R P N N	299
hiv1084ienv-codon-op	K P V V S T Q L L L	N G S L A E R E I I	I R S E N L T D N V	K T I I V H L K D Y	V K I V C T R P N N	292
Consensus	K P V V S T Q L L L	N G S L A E - - I I	I R S E N L T - N V	K T I I V H - - - -	V - I - C T - - - -	300
1157ipenvd123-codon-op		G		I S K E N W N K T L	Q W V R G K L E E H	244
157ipenvd1234-codon-op		G		I S K E N W N K T L	Q W V R G K L E E H	244
iv-1157ip-env-codon-op	N T R K S I R I G P	G Q A F T A T G D I	I G D I R Q A H C N	I S K E N W N K T L	Q W V R G K L E E H	349
hiv1084ienv-codon-op	N T R K S M R I G P	G Q A F Y A T G E I	I G N I R E A H C N	I S G S K W N N T L	Q R V K K K L G E H	342
Consensus	- - - - - G -	G - - - - -	- - - - - A H C M	I S - - - W N - T L	Q - V - - K L - E H	350
1157ipenvd123-codon-op	E P N K T I V . F K	P S S G G D L E I T	T H S F N C R G E F	E Y C N T S K L E N	G T D N S T H M D T	293
157ipenvd1234-codon-op	E P N K T I V . F K	P S S G G D L E I T	T H S F N C R G E F	E Y C N	 G A	279
iv-1157ip-env-codon-op	E P N K T I V . F K	P S S G G D L E I T	T H S F N C R G E F	E Y C N T S K L F N	G T D N S T H M D T	398
hiv1084ienv-codon-op	E P N N T T V D F K	P S S G G D L E I T	T H S F N C R G E F	E Y C N T S K L F N	G T S E S	387
Consensus	F P N - T - V - F K	P S S G G D L E I T	T H S F N C R G E F	F Y C N - - - -	- - - - -	400
1157ipenvd123-codon-op	G N D T V I T I P C	R I K Q I I N M W Q	E V G R A M Y A P P	I E G N I T C K S N	I T G L L L V R D G	343
157ipenvd1234-codon-op	G P C	R I K Q I I N M W Q	E V G R A M Y A P P	I E G N I T C K S N	I T G L L L V R D G	322
iv-1157ip-env-codon-op	G N D T V I T I P C	R I K Q I I N M W Q	E V G R A M Y A P P	I E G N I T C K S N	I T G L L L V R D G	448
hiv1084ienv-codon-op	 N S T I T L P C	K I K Q I I N M W Q	G V G R A M Y A P P	I A G N I T C K S N	I T G L L L T R D G	435
Consensus	 P C	- I K Q I I N M W Q	- V G R A M Y A P P	I - G N I T C K S N	I T G L L L - R D G	450
1157ipenvd123-codon-op	G Q D N S T N N T E	T E R P G G G D M R	N N W R S E L Y K Y	K V V E I K P L G I	A P T K A K R R V V	393
157ipenvd1234-codon-op	G Q D N S T N N T E	T E R P G G G D M R	N N W R S E L Y K Y	K V V E I K P L G I	A P T K A K R R V V	372
iv-1157ip-env-codon-op	G Q D N S T N N T E	T E R P G G G D M R	N N W R S E L Y K Y	K V V E I K P L G I	A P T K A K R R V V	498
hiv1084ienv-codon-op	G N G N G T E	I E R P G G G D M R	D N W R S E L Y K Y	K V V K I E P L G I	A P T K A K R R V V	482
Consensus	G - - - - N - T E	- F R P G G G D M R	- N W R S E L Y K Y	K V V - I - P L G I	A P T K A K R R V V	500

FIGURE 1 (CONTINUED)

3/40

PRETTYBOX of: sa2.ms2(*) February 9, 2006 11:32:04.33

1157ipenvd123-codon-op	ER	KRAVGI	AVFLGFLGAA	GSTMGAAS	IT	LTVQARQLLS	GI V Q Q Q	NNLL	443
157ipenvd1234-codon-op	ER	KRAVGI	AVFLGFLGAA	GSTMGAAS	IT	LTVQARQLLS	GI V Q Q Q	NNLL	422
iv-1157ip-env-codon-op	ER	KRAVGI	AVFLGFLGAA	GSTMGAAS	IT	LTVQARQLLS	GI V Q Q Q	NNLL	548
hiv1084ienv-codon-op	ER	KRAVGI	AVFLGFLGAA	GSTMGAAS	IT	LTVQARQLLS	GI V Q Q Q	NNLL	532
Consensus	ER	KRAVGI	A-FLGFLGAA	GSTMGAAS	-T	LTVQARQLLS	GI V Q Q Q	-NNLL	550
1157ipenvd123-codon-op	RAIEAQQHML	QLTVWGIKQL	QARVLAIER	QARVLAIER	Y	LQDQQLLGII	GC S G K L I C T T		493
157ipenvd1234-codon-op	RAIEAQQHML	QLTVWGIKQL	QARVLAIER	QARVLAIER	Y	LQDQQLLGII	GC S G K L I C T T		472
iv-1157ip-env-codon-op	RAIEAQQHML	QLTVWGIKQL	QARVLAIER	QARVLAIER	Y	LQDQQLLGII	GC S G K L I C T T		598
hiv1084ienv-codon-op	RAIEAQQHML	QLTVWGIKQL	QARVLAIER	QARVLAIER	Y	LQDQQLLGII	GC S G K L I C T T		582
Consensus	RAIEAQQHML	QLTVWGIKQL	QARVLAIER	QARVLAIER	Y	LQDQQLLGII	GC S G K L I C T T		600
1157ipenvd123-codon-op	AVPWNASWSN	KSQTDIWEENN	TWMQWDKEIS	TWMQWDKEIS	S	KHTD	ED S Q N Q Q E K N		543
157ipenvd1234-codon-op	AVPWNASWSN	KSQTDIWEENN	TWMQWDKEIS	TWMQWDKEIS	S	KHTD	ED S Q N Q Q E K N		522
iv-1157ip-env-codon-op	AVPWNASWSN	KSQTDIWEENN	TWMQWDKEIS	TWMQWDKEIS	S	KHTD	ED S Q N Q Q E K N		648
hiv1084ienv-codon-op	DVPWNSSWS	KSQYEDIW	TWMQWDKEIN	TWMQWDKEIN	S	NYT	ED S Q N Q Q E K N		632
Consensus	-VPWN-SWS-	KS--DIW-NM	TWMQWDKEI-	TWMQWDKEI-		--T-TIY-LL	-D S Q - Q Q E K N		650
1157ipenvd123-codon-op	EKDLLALDSW	ENLWNNWFSIT	KWLWYIKIPI	KWLWYIKIPI	I	MI V G G L I G L R	TI F A V L S I V S		593
157ipenvd1234-codon-op	EKDLLALDSW	ENLWNNWFSIT	KWLWYIKIPI	KWLWYIKIPI	I	MI V G G L I G L R	TI F A V L S I V S		572
iv-1157ip-env-codon-op	EKDLLALDSW	ENLWNNWFSIT	KWLWYIKIPI	KWLWYIKIPI	I	MI V G G L I G L R	TI F A V L S I V S		698
hiv1084ienv-codon-op	EKDLLALDSW	ENLWNNWFSIT	KWLWYIKIPI	KWLWYIKIPI	I	MI V G G L I G L R	TI F A V L S I V S		682
Consensus	EK-L L A L D S W	-N L W N N F - I T	-W L W Y I K I F I	-W L W Y I K I F I		MI V G G L I G L R	TI F A V L S - V -		700
1157ipenvd123-codon-op	RVRQGYSPLS	FQTHLPTPRG	PDRPEGIEEE	PDRPEGIEEE	E	GG E R D R D R S I	RL V N G S L A L I		643
157ipenvd1234-codon-op	RVRQGYSPLS	FQTHLPTPRG	PDRPEGIEEE	PDRPEGIEEE	E	GG E R D R D R S I	RL V N G S L A L I		622
iv-1157ip-env-codon-op	RVRQGYSPLS	FQTHLPTPRG	PDRPEGIEEE	PDRPEGIEEE	E	GG E R D R D R S I	RL V N G S L A L I		748
hiv1084ienv-codon-op	RVRQGYSPLS	FQTHLPTPRG	PDRPEGIEEE	PDRPEGIEEE	E	GG E R D R D R S I	RL V N G S L A L I		732
Consensus	RVRQGYSPLS	FQTHLPTPRG	PDR--I EEE	PDR--I EEE		GG E - D R D R S I	RL V - G - L A L -		750

FIGURE 1 (CONTINUED)

PETTYBOX of: aa2.msfc(*) February 9, 2006 11:32:04.33

1157ipenvd123-codon-op	WDDLRSLCLF	SYHRLRLD	LL	IVTRIV	IVELIG	R	WEALXY	WNNL	686
157ipenvd1234-codon-op	WDDLRSLCLF	SYHRLRLD	LL	IVTRIV	IVELIG	R	WEALXY	WNNL	665
iv-1157ip-env-codon-op	WDDLRSLCLF	SYHRLRLD	LL	IVTRIV	IVELIG	R	WEALXY	WNNL	791
hiv1084ienv-codon-op	WDDLRSLCLF	SYHRLRLD	CI	IVAAE	IVELIG	R	WEALXY	WNNL	782
Consensus	WDDLRSLCLF	SYHRLRLD	-L	IV-R--E	IVELIG	R--	WEALXY	-L	800
1157ipenvd123-codon-op	LQYWSQELKN	SAVSLRLNA	TA	IAVAEGTDRV	IVELIG	IE	IRHH	IRRR	736
157ipenvd1234-codon-op	LQYWSQELKN	SAVSLRLNA	TA	IAVAEGTDRV	IVELIG	IE	IRHH	IRRR	715
iv-1157ip-env-codon-op	LQYWSQELKN	SAVSLRLNA	TA	IAVAEGTDRV	IVELIG	IE	IRHH	IRRR	841
hiv1084ienv-codon-op	VQYWGELKK	BAVSLRLDT	TA	IAVAEGTDRV	IVELIG	IE	IRHH	IRRR	832
Consensus	-QYW--ELK-	SAVSLRL--TA		IAVAEGTDRV	IVELIG	IE-VQ--CRA	IRHH	IRRR-RR	850
1157ipenvd123-codon-op	GLERIIL*	743							
157ipenvd1234-codon-op	GLERIIL*	722							
iv-1157ip-env-codon-op	GLERIIL*	848							
hiv1084ienv-codon-op	GLERIIL*	839							
Consensus	GLERIIL--	856							

FIGURE 1 (CONTINUED)

[illegible]

FIGURE 2

6/40

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251      dna2.msf{1157ipenvd123-codon-op}
      dna2.msf{1157ipenvd1234-codon-op}
      dna2.msf{shiv-1157ip-env-codon-op}
      dna2.msf{hiv1084ienv-codon-op}
      Consensus
300      agtacCaGcA cctgtgGgc tGgGgCTggc gCtgGgGcac catgctgcTG
      agtacCaGcA cctgtgGgc tGgGgCTggc gCtgGgGcac catgctgcTG
      tcgtgCtGgA gaacgtGacC gaGaaCTtca aCatGtGgaa ggacgacaTG
      tggtgCtGgA gaacgtGacC gaGaaCTtca aCatGtGgaa gaacgacaTG
      -----C-G-A -----G--C --G--CT--- -C--G-G--- -----TG
301      GgcatgCtGA TGatCtGctc CgcCAcCgag aaGctGtggg tGaCCgTGtA
      GgcatgCtGA TGatCtGctc CgcCAcCgag aaGctGtggg tGaCCgTGtA
      GtggacCaGA TGcaCgagga CatCAtCtcc ctGtgGgacc aGtCCcTGaA
      GtggacCaGA TGcaCgagga CatCAtCtcc ctGtgGgacc aGtCCcTGaA
      G-----C-GA TG--C----- C--CA-C--- --G--G---- -G-CC-TG-A
351      ctaCgGCGTG cccgTGtggg aggaGgcCaa GACCaccctg TtctgcgcCt
      ctaCgGCGTG cccgTGtggg aggaGgcCaa GACCaccctg TtctgcgcCt
      gccCtGCGTG aagcTGacc cccTgtGcgt GACCctgaag TgctccaaCt
      gccCtGCGTG aagcTGacc cccTgtGcgt GACCctgaag Tg.....Ca
      ---C-GCGTG ---TG--- ----G--C--- GACC----- T-----C-
401      cCgaCgccaA GgcCtaCgag aaggaggtgc AcaAcATctg ggcacccac
      cCgaCgccaA GgcCtaCgag aaggaggtgc AcaAcATctg ggcacccac
      tCacCcgcgA GggCaaCgtg acctacaagg AggAgATgga caaggtgaag
      cCgaCgtgaA GtcCgcCaac tccacctccg AggAcATgcg caactgctcc
      -C--C-----A G--C--C--- -----A--A-AT--- -----
451      gcCtgCgtgc cCacCGacc CAacccCcag gagatcgtGc tGgAGaacgT
      gcCtgCgtgc cCacCGacc CAacccCcag gagatcgtGc tGgAGaacgT
      aaCtgCtcct tCAaCGtgac CAccggCata cgcgacaaGa aGcAGaaggT
      ttCaaCgtga cCacCGagat CAaggaCcgc aagaagctGg aGcAGgcccT
      --C--C----- -CA-CG---- CA-----G- -G-AG-----T

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FIGURE 2 (CONTINUED)

7/40

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dna2.msf{1157ipenvd123-codon-op}
dna2.msf{1157ipenvd1234-codon-op}
dna2.msf{shiv-1157ip-env-codon-op}
  dna2.msf{hiv1084ienv-codon-op}
Consensus
501 GacCgagaac tTcaACatgt gGaaggacga CatggtGgAC cagatgcaCg 550
GacCgagaac tTcaACatgt gGaaggacga CatggtGgAC cagatgcaCg
GaaCgcccctg tTctACcgcc tGgacatcac CcccctGgAC gagaacaaCa
GttCtaccgc cTggACatcg tG.....cc CctgaaGaAC tcctcctcCt
G--C----- -T--AC---- -G----- C-----G-AC -----C-

dna2.msf{1157ipenvd123-codon-op}
dna2.msf{1157ipenvd1234-codon-op}
dna2.msf{shiv-1157ip-env-codon-op}
  dna2.msf{hiv1084ienv-codon-op}
Consensus
551 aggACatCat CtccttgtGg gaccagtcCc tgAAgcCCTg CgtgggcgCC 600
aggACatCat CtccttgtGg gaccagtcCc tgAAgcCCTg CgtgggcgCC
acaACtcCtc CgagtaccGc ttgatcaaCt gCAAgcCCTc CaccatcaCC
ccaACttCtc CgagtaccGc ctgatcaaCt gCAAcacCCTc CaccgtgtCC
---AC--C-- C-----G- -----C- --AA--CCT- C-----CC

dna2.msf{1157ipenvd123-codon-op}
dna2.msf{1157ipenvd1234-codon-op}
dna2.msf{shiv-1157ip-env-codon-op}
  dna2.msf{hiv1084ienv-codon-op}
Consensus
601 ggcGCCCTGCC CCAAGGTGAA CTTCGACCCC ATCCCCATCC ACTACTGCCG 650
ggcGCCCTGCC CCAAGGTGAA CTTCGACCCC ATCCCCATCC ACTACTGCCG
cagGCCCTGCC CCAAGGTGAA CTTCGACCCC ATCCCCATCC ACTACTGCCG
cagGCCCTGCC CCAAGGTGAA CTTCGACCCC ATCCCCATCC ACTACTGCCG
---GCCTGCC CCAAGGTGAA CTTCGACCCC ATCCCCATCC ACTACTGCCG

dna2.msf{1157ipenvd123-codon-op}
dna2.msf{1157ipenvd1234-codon-op}
dna2.msf{shiv-1157ip-env-codon-op}
  dna2.msf{hiv1084ienv-codon-op}
Consensus
651 CCCC GCCCGC TACGCCATCC TGAAGTGCAA CAACAAGACC TTCAACGGCa 700
CCCC GCCCGC TACGCCATCC TGAAGTGCAA CAACAAGACC TTCAACGGCa
CCCC GCCCGC TACGCCATCC TGAAGTGCAA CAACAAGACC TTCAACGGCa
CCCC GCCCGC TACGCCATCC TGAAGTGCAA CAACAAGACC TTCAACGGCt
CCCC GCCCGC TACGCCATCC TGAAGTGCAA CAACAAGACC TTCAACGGC-

dna2.msf{1157ipenvd123-codon-op}
dna2.msf{1157ipenvd1234-codon-op}
dna2.msf{shiv-1157ip-env-codon-op}
  dna2.msf{hiv1084ienv-codon-op}
Consensus
701 CCGGCCCCCTG CcACAACGTG TCCACCGTGC AGTGCACCCA CGGCATCAAG 750
CCGGCCCCCTG CcACAACGTG TCCACCGTGC AGTGCACCCA CGGCATCAAG
CCGGCCCCCTG CcACAACGTG TCCACCGTGC AGTGCACCCA CGGCATCAAG
CCGGCCCCCTG CcACAACGTG TCCACCGTGC AGTGCACCCA CGGCATCAAG
CCGGCCCCCTG C-ACAACGTG TCCACCGTGC AGTGCACCCA CGGCATCAAG
```

FIGURE 2 (CONTINUED)

751	dna2.msf{1157ipenvd123-codon-op}	800	CCCGTGGTGT	CCACCCAGCT	GCTGCTGAAC	GGCTCCCTGG	CCGAGcgCGA
	dna2.msf{1157ipenvd1234-codon-op}		CCCGTGGTGT	CCACCCAGCT	GCTGCTGAAC	GGCTCCCTGG	CCGAGcgCGA
	dna2.msf{shiv-1157ip-env-codon-op}		CCCGTGGTGT	CCACCCAGCT	GCTGCTGAAC	GGCTCCCTGG	CCGAGcgCGA
	dna2.msf{hiv1084ienv-codon-op}		CCCGTGGTGT	CCACCCAGCT	GCTGCTGAAC	GGCTCCCTGG	CCGAGgagGA
	Consensus		CCCGTGGTGT	CCACCCAGCT	GCTGCTGAAC	GGCTCCCTGG	CCGAG---GA
		801					
	dna2.msf{1157ipenvd123-codon-op}		GATCATCATC	CGCTCCGAGA	ACCTGACCgA	CAACGTGAAG	ACCATCATCG
	dna2.msf{1157ipenvd1234-codon-op}		GATCATCATC	CGCTCCGAGA	ACCTGACCgA	CAACGTGAAG	ACCATCATCG
	dna2.msf{shiv-1157ip-env-codon-op}		GATCATCATC	CGCTCCGAGA	ACCTGACCgA	CAACGTGAAG	ACCATCATCG
	dna2.msf{hiv1084ienv-codon-op}		CATCATCATC	CGCTCCGAGA	ACCTGACCaa	CAACGTGAAG	ACCATCATCG
	Consensus		-ATCATCATC	CGCTCCGAGA	ACCTGACC-A	CAACGTGAAG	ACCATCATCG
		851					
	dna2.msf{1157ipenvd123-codon-op}		TGCACtTcAA	cGAgTcCGTG	gAGATCaact	GCACCgGCgC	CggC.....
	dna2.msf{1157ipenvd1234-codon-op}		TGCACtTcAA	cGAgTcCGTG	gAGATCaact	GCACCgGCgC	CggC.....
	dna2.msf{shiv-1157ip-env-codon-op}		TGCACtTcAA	cGAgTcCGTG	gAGATCaact	GCACCcGCcC	CaaCaacaac
	dna2.msf{hiv1084ienv-codon-op}		TGCACcTgAA	gGAcTaCGTG	aAGATCgtgT	GCACCcGCcC	CaaCaacaac
	Consensus		TGCAC-T-AA	-GA-T-CGTG	-AGATC---T	GCACC-GC-C	C--C-----
		901					
	dna2.msf{1157ipenvd123-codon-op}						
	dna2.msf{1157ipenvd1234-codon-op}						
	dna2.msf{shiv-1157ip-env-codon-op}		accgcaagt	ccatccgcat	cggccccggc	caggccttct	acgccaccgg
	dna2.msf{hiv1084ienv-codon-op}		accgcaagt	ccatgcgcat	cggccccggc	caggccttct	acgccaccgg
	Consensus		-----	-----	-----	-----	-----
		951					
	dna2.msf{1157ipenvd123-codon-op}				GCCCA	CTGCAACATC	TCCaaggagA
	dna2.msf{1157ipenvd1234-codon-op}				GCCCA	CTGCAACATC	TCCaaggagA
	dna2.msf{shiv-1157ip-env-codon-op}		cgacatcatc	ggcgacatcc	gccagGCCCC	CTGCAACATC	TCCaaggagA
	dna2.msf{hiv1084ienv-codon-op}		cgagatcatc	ggcaacatcc	gcgagGCCCC	CTGCAACATC	TCCgggtcca
	Consensus		-----	-----	-----GCCCA	CTGCAACATC	TCC-----A

FIGURE 2 (CONTINUED)

FIGURE 2 (CONTINUED)

1251	dna2.msf{1157ipenvd123-codon-op}	GTGGCAGGag	GTGGGgCGCG	CCATGTACGC	CCCCCCATC	GagGGCAACA
	dna2.msf{1157ipenvd1234-codon-op}	GTGGCAGGag	GTGGGgCGCG	CCATGTACGC	CCCCCCATC	GagGGCAACA
	dna2.msf{shiv-1157ip-env-codon-op}	GTGGCAGGag	GTGGGgCGCG	CCATGTACGC	CCCCCCATC	GagGGCAACA
	dna2.msf{hiv1084ienv-codon-op}	GTGGCAGGgc	GTGGGcCGCG	CCATGTACGC	CCCCCCATC	GccGGCAACA
	Consensus	GTGGCAGG--	GTGGG-CGCG	CCATGTACGC	CCCCCCATC	G--GGCAACA
1301	dna2.msf{1157ipenvd123-codon-op}	TCACCTGCAA	GTCCAACATC	ACCGGCCTGC	TGCTGgtgCG	CGACGGCGGC
	dna2.msf{1157ipenvd1234-codon-op}	TCACCTGCAA	GTCCAACATC	ACCGGCCTGC	TGCTGgtgCG	CGACGGCGGC
	dna2.msf{shiv-1157ip-env-codon-op}	TCACCTGCAA	GTCCAACATC	ACCGGCCTGC	TGCTGgtgCG	CGACGGCGGC
	dna2.msf{hiv1084ienv-codon-op}	TCACCTGCAA	GTCCAACATC	ACCGGCCTGC	TGCTGaccCG	CGACGGCGGC
	Consensus	TCACCTGCAA	GTCCAACATC	ACCGGCCTGC	TGCTG---CG	CGACGGCGGC
1351	dna2.msf{1157ipenvd123-codon-op}	cAggacaact	cCacCAACaa	CACCGAGAcC	TTCCGCCCCCG	GCGGCGGCGA
	dna2.msf{1157ipenvd1234-codon-op}	cAggacaact	cCacCAACaa	CACCGAGAcC	TTCCGCCCCCG	GCGGCGGCGA
	dna2.msf{shiv-1157ip-env-codon-op}	cAggacaact	cCacCAACaa	CACCGAGAcC	TTCCGCCCCCG	GCGGCGGCGA
	dna2.msf{hiv1084ienv-codon-op}	aA.....	.CggCAACgg	CACCGAGAtC	TTCCGCCCCCG	GCGGCGGCGA
	Consensus	-A-----	-C--CAAC--	CACCGAGA-C	TTCCGCCCCCG	GCGGCGGCGA
1401	dna2.msf{1157ipenvd123-codon-op}	CATGCGCaAC	AACTGGCGCT	CCGAGCTGTA	CAAGTACAAG	GTGGTGgAGA
	dna2.msf{1157ipenvd1234-codon-op}	CATGCGCaAC	AACTGGCGCT	CCGAGCTGTA	CAAGTACAAG	GTGGTGgAGA
	dna2.msf{shiv-1157ip-env-codon-op}	CATGCGCaAC	AACTGGCGCT	CCGAGCTGTA	CAAGTACAAG	GTGGTGgAGA
	dna2.msf{hiv1084ienv-codon-op}	CATGCGCgAC	AACTGGCGCT	CCGAGCTGTA	CAAGTACAAG	GTGGTGaAGA
	Consensus	CATGCGC-AC	AACTGGCGCT	CCGAGCTGTA	CAAGTACAAG	GTGGTG-AGA
1451	dna2.msf{1157ipenvd123-codon-op}	TCaAGCCCCCT	GGGCATCGCC	CCCACCAAGG	CCAAGCGCCG	CGTGGTGGAG
	dna2.msf{1157ipenvd1234-codon-op}	TCaAGCCCCCT	GGGCATCGCC	CCCACCAAGG	CCAAGCGCCG	CGTGGTGGAG
	dna2.msf{shiv-1157ip-env-codon-op}	TCaAGCCCCCT	GGGCATCGCC	CCCACCAAGG	CCAAGCGCCG	CGTGGTGGAG
	dna2.msf{hiv1084ienv-codon-op}	TCgAGCCCCCT	GGGCATCGCC	CCCACCAAGG	CCAAGCGCCG	CGTGGTGGAG
	Consensus	TC-AGCCCCCT	GGGCATCGCC	CCCACCAAGG	CCAAGCGCCG	CGTGGTGGAG

FIGURE 2 (CONTINUED)

11/40

dna2.msf{1157ipenvd123-codon-op}	1501	CGCGagAAGC	GC GCCGTGGG	CATCGGCGCC	gTGTTCCCTGG	1550	GCTTCCCTGGG
dna2.msf{1157ipenvd1234-codon-op}		CGCGagAAGC	GC GCCGTGGG	CATCGGCGCC	gTGTTCCCTGG		GCTTCCCTGGG
dna2.msf{shiv-1157ip-env-codon-op}		CGCGagAAGC	GC GCCGTGGG	CATCGGCGCC	gTGTTCCCTGG		GCTTCCCTGGG
dna2.msf{hiv1084ienv-codon-op}		CGCGgCAAGC	GC GCCGTGGG	CATCGGCGCC	cTGTTCCCTGG		GCTTCCCTGGG
Consensus		CGCG--AAGC	GC GCCGTGGG	CATCGGCGCC	-TGTTCCCTGG		GCTTCCCTGGG
dna2.msf{1157ipenvd123-codon-op}	1551	CGCCGCCGGC	TCCACCATGG	GC GCCGCCCTC	CaTcACCCCTG	1600	ACCGTGCCAGG
dna2.msf{1157ipenvd1234-codon-op}		CGCCGCCGGC	TCCACCATGG	GC GCCGCCCTC	CaTcACCCCTG		ACCGTGCCAGG
dna2.msf{shiv-1157ip-env-codon-op}		CGCCGCCGGC	TCCACCATGG	GC GCCGCCCTC	CaTcACCCCTG		ACCGTGCCAGG
dna2.msf{hiv1084ienv-codon-op}		CGCCGCCGGC	TCCACCATGG	GC GCCGCCCTC	CcTgACCCCTG		ACCGTGCCAGG
Consensus		CGCCGCCGGC	TCCACCATGG	GC GCCGCCCTC	C-T-ACCCCTG		ACCGTGCCAGG
dna2.msf{1157ipenvd123-codon-op}	1601	CCCGCCAGCT	GCTGTCCGGC	ATCGTGCCAGC	AGCAGgACAA	1650	CCTGCTGCCG
dna2.msf{1157ipenvd1234-codon-op}		CCCGCCAGCT	GCTGTCCGGC	ATCGTGCCAGC	AGCAGgACAA		CCTGCTGCCG
dna2.msf{shiv-1157ip-env-codon-op}		CCCGCCAGCT	GCTGTCCGGC	ATCGTGCCAGC	AGCAGgACAA		CCTGCTGCCG
dna2.msf{hiv1084ienv-codon-op}		CCCGCCAGCT	GCTGTCCGGC	ATCGTGCCAGC	AGCAGaACAA		CCTGCTGCCG
Consensus		CCCGCCAGCT	GCTGTCCGGC	ATCGTGCCAGC	AGCAG-ACAA		CCTGCTGCCG
dna2.msf{1157ipenvd123-codon-op}	1651	GCCATCGAGG	CCCAGCAGCA	CATGCTGCAG	CTGACCCGTGT	1700	GGGGCATCAA
dna2.msf{1157ipenvd1234-codon-op}		GCCATCGAGG	CCCAGCAGCA	CATGCTGCAG	CTGACCCGTGT		GGGGCATCAA
dna2.msf{shiv-1157ip-env-codon-op}		GCCATCGAGG	CCCAGCAGCA	CATGCTGCAG	CTGACCCGTGT		GGGGCATCAA
dna2.msf{hiv1084ienv-codon-op}		GCCATCGAGG	CCCAGCAGCA	CATGCTGCAG	CTGACCCGTGT		GGGGCATCAA
Consensus		GCCATCGAGG	CCCAGCAGCA	CATGCTGCAG	CTGACCCGTGT		GGGGCATCAA
dna2.msf{1157ipenvd123-codon-op}	1701	aCAGCTGCAG	GCCCGCGTGC	TGGCCATCGA	GCGCTACCTG	1750	CAGGACCAGC
dna2.msf{1157ipenvd1234-codon-op}		aCAGCTGCAG	GCCCGCGTGC	TGGCCATCGA	GCGCTACCTG		CAGGACCAGC
dna2.msf{shiv-1157ip-env-codon-op}		aCAGCTGCAG	GCCCGCGTGC	TGGCCATCGA	GCGCTACCTG		CAGGACCAGC
dna2.msf{hiv1084ienv-codon-op}		gCAGCTGCAG	GCCCGCGTGC	TGGCCATCGA	GCGCTACCTG		CAGGACCAGC
Consensus		-CAGCTGCAG	GCCCGCGTGC	TGGCCATCGA	GCGCTACCTG		CAGGACCAGC

FIGURE 2 (CONTINUED)

12/40

```
1251          dna2.msf{1157ipenvd123-codon-op}  
          dna2.msf{1157ipenvd1234-codon-op}  
          dna2.msf{shiv-1157ip-env-codon-op}  
          dna2.msf{hiv1084ienv-codon-op}  
          Consensus  
1300          GTGGCAGGag GTGGGgCGG CCATGTACGC CCCCCCATC GagGCAACA  
          GTGGCAGGag GTGGGgCGG CCATGTACGC CCCCCCATC GagGCAACA  
          GTGGCAGGag GTGGGgCGG CCATGTACGC CCCCCCATC GagGCAACA  
          GTGGCAGGgc GTGGGcCGG CCATGTACGC CCCCCCATC GcGCAACA  
          GTGGCAGG-- GTGGG-CGG CCATGTACGC CCCCCCATC G--GCAACA  
1301          TCACCTGCAA GTCCAACATC ACCGGCCTGC TGCTGgtgCG CGACGGCGGC  
          TCACCTGCAA GTCCAACATC ACCGGCCTGC TGCTGgtgCG CGACGGCGGC  
          TCACCTGCAA GTCCAACATC ACCGGCCTGC TGCTGgtgCG CGACGGCGGC  
          TCACCTGCAA GTCCAACATC ACCGGCCTGC TGCTGaccCG CGACGGCGGC  
          TCACCTGCAA GTCCAACATC ACCGGCCTGC TGCTG---CG CGACGGCGGC  
1351          cAggacaact cCacCAACaa CACCGAGAcC TTCCGCCCCCG GCGGCGCGA  
          cAggacaact cCacCAACaa CACCGAGAcC TTCCGCCCCCG GCGGCGCGA  
          cAggacaact cCacCAACaa CACCGAGAcC TTCCGCCCCCG GCGGCGCGA  
          aA..... .CggCAACgg CACCGAGAcC TTCCGCCCCCG GCGGCGCGA  
          -A----- -C--CAAC-- CACCGAGA-C TTCCGCCCCCG GCGGCGCGA  
1401          CATGCGCaAC AACTGGCGCT CCGAGCTGTA CAAGTACAAG GTGGTGgAGA  
          CATGCGCaAC AACTGGCGCT CCGAGCTGTA CAAGTACAAG GTGGTGgAGA  
          CATGCGCaAC AACTGGCGCT CCGAGCTGTA CAAGTACAAG GTGGTGgAGA  
          CATGCGCgAC AACTGGCGCT CCGAGCTGTA CAAGTACAAG GTGGTGaAGA  
          CATGCGC-AC AACTGGCGCT CCGAGCTGTA CAAGTACAAG GTGGTG-AGA  
1451          TCaAGCCCCCT GGGCATCGCC CCCACCAAGG CCAAGCGCCG CGTGGTGGAG  
          TCaAGCCCCCT GGGCATCGCC CCCACCAAGG CCAAGCGCCG CGTGGTGGAG  
          TCaAGCCCCCT GGGCATCGCC CCCACCAAGG CCAAGCGCCG CGTGGTGGAG  
          TCgAGCCCCCT GGGCATCGCC CCCACCAAGG CCAAGCGCCG CGTGGTGGAG  
          TC-AGCCCCCT GGGCATCGCC CCCACCAAGG CCAAGCGCCG CGTGGTGGAG
```

FIGURE 2 (CONTINUED)

13/40

dna2.msf{1157ipenvd123-codon-op}	1751	AGCTGCTGGG	CATCTGGGGC	TGCTCCGGCA	AGCTGATCTG	CACCACCGcC	1800
dna2.msf{1157ipenvd1234-codon-op}		AGCTGCTGGG	CATCTGGGGC	TGCTCCGGCA	AGCTGATCTG	CACCACCGcC	
dna2.msf{shiv-1157ip-env-codon-op}		AGCTGCTGGG	CATCTGGGGC	TGCTCCGGCA	AGCTGATCTG	CACCACCGcC	
dna2.msf{hiv1084ienv-codon-op}		AGCTGCTGGG	CATCTGGGGC	TGCTCCGGCA	AGCTGATCTG	CACCACCGaC	
Consensus		AGCTGCTGGG	CATCTGGGGC	TGCTCCGGCA	AGCTGATCTG	CACCACCG-C	
dna2.msf{1157ipenvd123-codon-op}	1801	GTGCCCTGGA	ACgCCTCCTG	GTCCaaCAAG	TCCcAgaccG	ACATCTGGga	1850
dna2.msf{1157ipenvd1234-codon-op}		GTGCCCTGGA	ACgCCTCCTG	GTCCaaCAAG	TCCcAgaccG	ACATCTGGga	
dna2.msf{shiv-1157ip-env-codon-op}		GTGCCCTGGA	ACgCCTCCTG	GTCCaaCAAG	TCCcAgaccG	ACATCTGGga	
dna2.msf{hiv1084ienv-codon-op}		GTGCCCTGGA	ACTCCTCCTG	GTCCtcCAAG	TCCtAcgagG	ACATCTGGac	
Consensus		GTGCCCTGGA	AC-CCTCCTG	GTCC--CAAG	TCC-A---G	ACATCTGG--	
dna2.msf{1157ipenvd123-codon-op}	1851	gAACATGACC	TGGATGCAGT	GGGACAAGGA	GATctcCAAg	cACACCGaCA	1900
dna2.msf{1157ipenvd1234-codon-op}		gAACATGACC	TGGATGCAGT	GGGACAAGGA	GATctcCAAg	cACACCGaCA	
dna2.msf{shiv-1157ip-env-codon-op}		gAACATGACC	TGGATGCAGT	GGGACAAGGA	GATctcCAAg	cACACCGaCA	
dna2.msf{hiv1084ienv-codon-op}		cAACATGACC	TGGATGCAGT	GGGACAAGGA	GATCaaCAAC	tACACCaACA	
Consensus		-AACATGACC	TGGATGCAGT	GGGACAAGGA	GATC--CAA-	-ACACC-ACA	
dna2.msf{1157ipenvd123-codon-op}	1901	CCATCTACCg	cCTGCTGGaG	GACTCCCAGA	aCCAGCAGGA	GAAGAACCgAG	1950
dna2.msf{1157ipenvd1234-codon-op}		CCATCTACCg	cCTGCTGGaG	GACTCCCAGA	aCCAGCAGGA	GAAGAACCgAG	
dna2.msf{shiv-1157ip-env-codon-op}		CCATCTACCg	cCTGCTGGaG	GACTCCCAGA	aCCAGCAGGA	GAAGAACCgAG	
dna2.msf{hiv1084ienv-codon-op}		CCATCTACCg	cCTGCTGGtG	GACTCCCAGA	cCCAGCAGGA	GAAGAACCgAG	
Consensus		CCATCTACC-	-CTGCTGG-G	GACTCCCAGA	-CCAGCAGGA	GAAGAACCgAG	
dna2.msf{1157ipenvd123-codon-op}	1951	AAGGAcCTGC	TGGCCCTGGA	CTCCTGGgAG	AACCTGTGGA	ACTGGTTctc	2000
dna2.msf{1157ipenvd1234-codon-op}		AAGGAcCTGC	TGGCCCTGGA	CTCCTGGgAG	AACCTGTGGA	ACTGGTTctc	
dna2.msf{shiv-1157ip-env-codon-op}		AAGGAcCTGC	TGGCCCTGGA	CTCCTGGgAG	AACCTGTGGA	ACTGGTTctc	
dna2.msf{hiv1084ienv-codon-op}		AAGGAgCTGC	TGGCCCTGGA	CTCCTGGaAG	AACCTGTGGA	ACTGGTTca	
Consensus		AAGGA-CTGC	TGGCCCTGGA	CTCCTGG-AG	AACCTGTGGA	ACTGGTTc-	

FIGURE 2 (CONTINUED)

14/40

dna2.msf{1157ipenvd123-codon-op}	2001	CATCACCAAG	TGGCTGTGGT	ACATCAAGAT	CTTCATCATG	ATCGTGGCG	2050
dna2.msf{1157ipenvd1234-codon-op}		CATCACCAAG	TGGCTGTGGT	ACATCAAGAT	CTTCATCATG	ATCGTGGCG	
dna2.msf{shiv-1157ip-env-codon-op}		CATCACCAAG	TGGCTGTGGT	ACATCAAGAT	CTTCATCATG	ATCGTGGCG	
dna2.msf{hiv1084ienv-codon-op}		CATCACCAAC	TGGCTGTGGT	ACATCAAGAT	CTTCATCATG	ATCGTGGCG	
Consensus		CATCACCAA~	TGGCTGTGGT	ACATCAAGAT	CTTCATCATG	ATCGTGGCG	
dna2.msf{1157ipenvd123-codon-op}	2051	GCCTGATCGG	CCTGCGCATC	ATCTTCGCCG	TGCTGTCCAT	cGTGtccCGC	2100
dna2.msf{1157ipenvd1234-codon-op}		GCCTGATCGG	CCTGCGCATC	ATCTTCGCCG	TGCTGTCCAT	cGTGtccCGC	
dna2.msf{shiv-1157ip-env-codon-op}		GCCTGATCGG	CCTGCGCATC	ATCTTCGCCG	TGCTGTCCAT	cGTGtccCGC	
dna2.msf{hiv1084ienv-codon-op}		GCCTGATCGG	CCTGCGCATC	ATCTTCGCCG	TGCTGTCCAT	gGTGaaCCGC	
Consensus		GCCTGATCGG	CCTGCGCATC	ATCTTCGCCG	TGCTGTCCAT	-GTG--CCGC	
dna2.msf{1157ipenvd123-codon-op}	2101	GTGCGCCAGG	GCTACTCCCC	CCTGTCCCTTC	CAGACCCacc	tgCCCCACCCC	2150
dna2.msf{1157ipenvd1234-codon-op}		GTGCGCCAGG	GCTACTCCCC	CCTGTCCCTTC	CAGACCCacc	tgCCCCACCCC	
dna2.msf{shiv-1157ip-env-codon-op}		GTGCGCCAGG	GCTACTCCCC	CCTGTCCCTTC	CAGACCCacc	tgCCCCACCCC	
dna2.msf{hiv1084ienv-codon-op}		GTGCGCCAGG	GCTACTCCCC	CCTGTCCCTTC	CAGACCCctga	ccCCCCAaCCC	
Consensus		GTGCGCCAGG	GCTACTCCCC	CCTGTCCCTTC	CAGACCC---	--CCCA-CCC	
dna2.msf{1157ipenvd123-codon-op}	2151	CCGCGGCCCC	GACCGCCccg	aggGCATCGA	GGAGGAGGGC	GGCGAGCgcg	2200
dna2.msf{1157ipenvd1234-codon-op}		CCGCGGCCCC	GACCGCCccg	aggGCATCGA	GGAGGAGGGC	GGCGAGCgcg	
dna2.msf{shiv-1157ip-env-codon-op}		CCGCGGCCCC	GACCGCCccg	aggGCATCGA	GGAGGAGGGC	GGCGAGCgcg	
dna2.msf{hiv1084ienv-codon-op}		CCGCGGCCCC	GACCGCctg	gccGCATCGA	GGAGGAGGGC	GGCGAGCagG	
Consensus		CCGCGGCCCC	GACCGCC--G	---GCATCGA	GGAGGAGGGC	GGCGAGC--G	
dna2.msf{1157ipenvd123-codon-op}	2201	ACCGCGACCG	CTCCATCCGC	CTGGTGaaCG	GCTcCCTGGC	CCTGatCTGG	2250
dna2.msf{1157ipenvd1234-codon-op}		ACCGCGACCG	CTCCATCCGC	CTGGTGaaCG	GCTcCCTGGC	CCTGatCTGG	
dna2.msf{shiv-1157ip-env-codon-op}		ACCGCGACCG	CTCCATCCGC	CTGGTGaaCG	GCTcCCTGGC	CCTGatCTGG	
dna2.msf{hiv1084ienv-codon-op}		ACCGCGACCG	CTCCATCCGC	CTGGTGtcCG	GCTtCCTGGC	CCTGgcCTGG	
Consensus		ACCGCGACCG	CTCCATCCGC	CTGGTG--CG	GCT-CCTGGC	CCTG--CTGG	

FIGURE 2 (CONTINUED)

15/40

```

2251      dna2.msf{1157ipenvd123-codon-op}      2300
      dna2.msf{1157ipenvd1234-codon-op}      GACGACCTGC GCTCCCTGTG CCTGTCTCTCC TACCACCGCC TGC GCGACct
      dna2.msf{shiv-1157ip-env-codon-op}      GACGACCTGC GCTCCCTGTG CCTGTCTCTCC TACCACCGCC TGC GCGACct
      dna2.msf{hiv1084ienv-codon-op}          GACGACCTGC GCTCCCTGTG CCTGTCTCTCC TACCACCGCC TGC GCGACctg
      Consensus                               GACGACCTGC GCTCCCTGTG CCTGTCTCTCC TACCACCGCC TGC GCGAC--

2301      dna2.msf{1157ipenvd123-codon-op}      2350
      dna2.msf{1157ipenvd1234-codon-op}      gCTgCTGATC GTGaCCCCGCa tCGtgGAGCT GCTGGGCCGC cgc.....
      dna2.msf{shiv-1157ip-env-codon-op}      gCTgCTGATC GTGaCCCCGCa tCGtgGAGCT GCTGGGCCGC cgc.....
      dna2.msf{hiv1084ienv-codon-op}          caTcCTGATC GTGgCCCCGcg cCGccGAGCT GCTGGGCCGC tcCtccctgc
      Consensus                               --T-CTGATC GTG-CCCCG- -CG--GAGCT GCTGGGCCGC --C-----

2351      dna2.msf{1157ipenvd123-codon-op}      2400
      dna2.msf{1157ipenvd1234-codon-op}      .....GGCTGG GAGGCCCTGA AGTActgGtG gaaCCTGcTG
      dna2.msf{shiv-1157ip-env-codon-op}      .....GGCTGG GAGGCCCTGA AGTActgGtG gaaCCTGcTG
      dna2.msf{hiv1084ienv-codon-op}          gcggcctgca gaagGGCTGG GAGGCCCTGA AGTActgGtG ctCCTGgTG
      Consensus                               -----GGCTGG GAGGCCCTGA AGTAC--G-G ---CCTG-TG

2401      dna2.msf{1157ipenvd123-codon-op}      2450
      dna2.msf{1157ipenvd1234-codon-op}      CAGTACTGGt cCCaGGAGCT GAAGAAcTCC GCCgTgTCCC TGCTGaACgC
      dna2.msf{shiv-1157ip-env-codon-op}      CAGTACTGGt cCCaGGAGCT GAAGAAcTCC GCCgTgTCCC TGCTGaACgC
      dna2.msf{hiv1084ienv-codon-op}          CAGTACTGGg gCctGGAGCT GAAGAAgTCC GCCaTcTCCC TGCTGgACaC
      Consensus                               CAGTACTGG- -CC-GGAGCT GAAGAA-TCC GCC-T-TCCC TGCTG-AC-C

2451      dna2.msf{1157ipenvd123-codon-op}      2500
      dna2.msf{1157ipenvd1234-codon-op}      CACCGCCATC GCCGTGGCCG AGGGCACCGA CCGCgTgATC GAGgTGgTgC
      dna2.msf{shiv-1157ip-env-codon-op}      CACCGCCATC GCCGTGGCCG AGGGCACCGA CCGCgTgATC GAGgTGgTgC
      dna2.msf{hiv1084ienv-codon-op}          CACCGCCATC GCCGTGGCCG AGGGCACCGA CCGCgTgATC GAGgTGgTgC
      Consensus                               CACCGCCATC GCCGTGGCCG AGGGCACCGA CCGC-T-ATC GAG-TG-T-C

```

FIGURE 2 (CONTINUED)

dna2.msf{1157ipenvd123-codon-op}	2501	AGgGCgcCTG	CCGCGCCATC	cGcACATCC	CCCGCCGCAT	gCGCCAGGGC	2550
dna2.msf{1157ipenvd1234-codon-op}		AGgGCgcCTG	CCGCGCCATC	cGcACATCC	CCCGCCGCAT	gCGCCAGGGC	
dna2.msf{shiv-1157ip-env-codon-op}		AGgGCgcCTG	CCGCGCCATC	cGcACATCC	CCCGCCGCAT	gCGCCAGGGC	
dna2.msf{hiv1084ienv-codon-op}		AGcGCatCTG	CCGCGCCATC	tGCaACATCC	CCCGCCGCAT	cCGCCAGGGC	
Consensus		AG-GC--CTG	CCGCGCCATC	-GC-ACATCC	CCCGCCGCAT	-CGCCAGGGC	
dna2.msf{1157ipenvd123-codon-op}	2551	cTgGAGcgCa	tCCTGctGTg	A			2571
dna2.msf{1157ipenvd1234-codon-op}		cTgGAGcgCa	tCCTGctGTg	A			
dna2.msf{shiv-1157ip-env-codon-op}		cTgGAGcgCa	tCCTGctGTg	A			
dna2.msf{hiv1084ienv-codon-op}		tTcGAGgCcG	cCCTGCaGta	A			
Consensus		-T-GAG--C-	-CCTGC-GT-	A			

FIGURE 2 (CONTINUED)

17/40

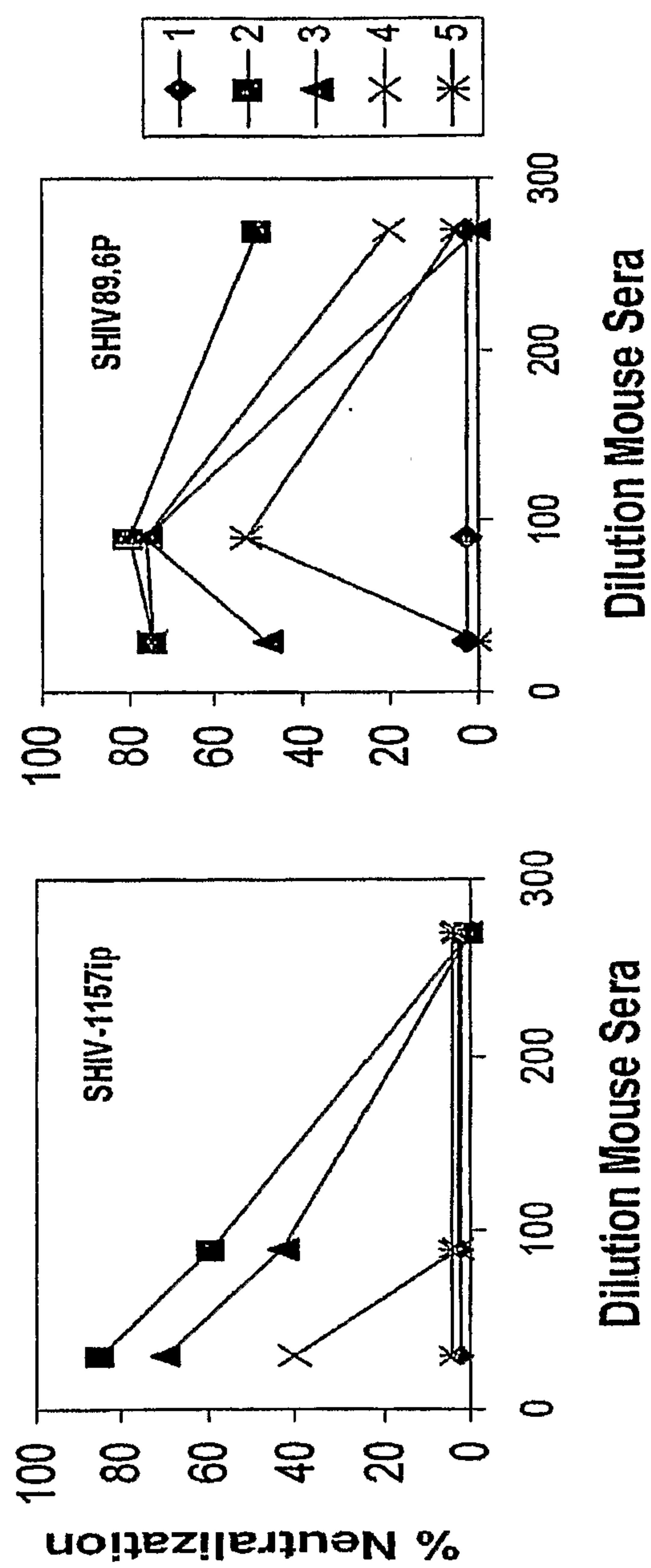


FIGURE 3

18/40

FIGURE 4A

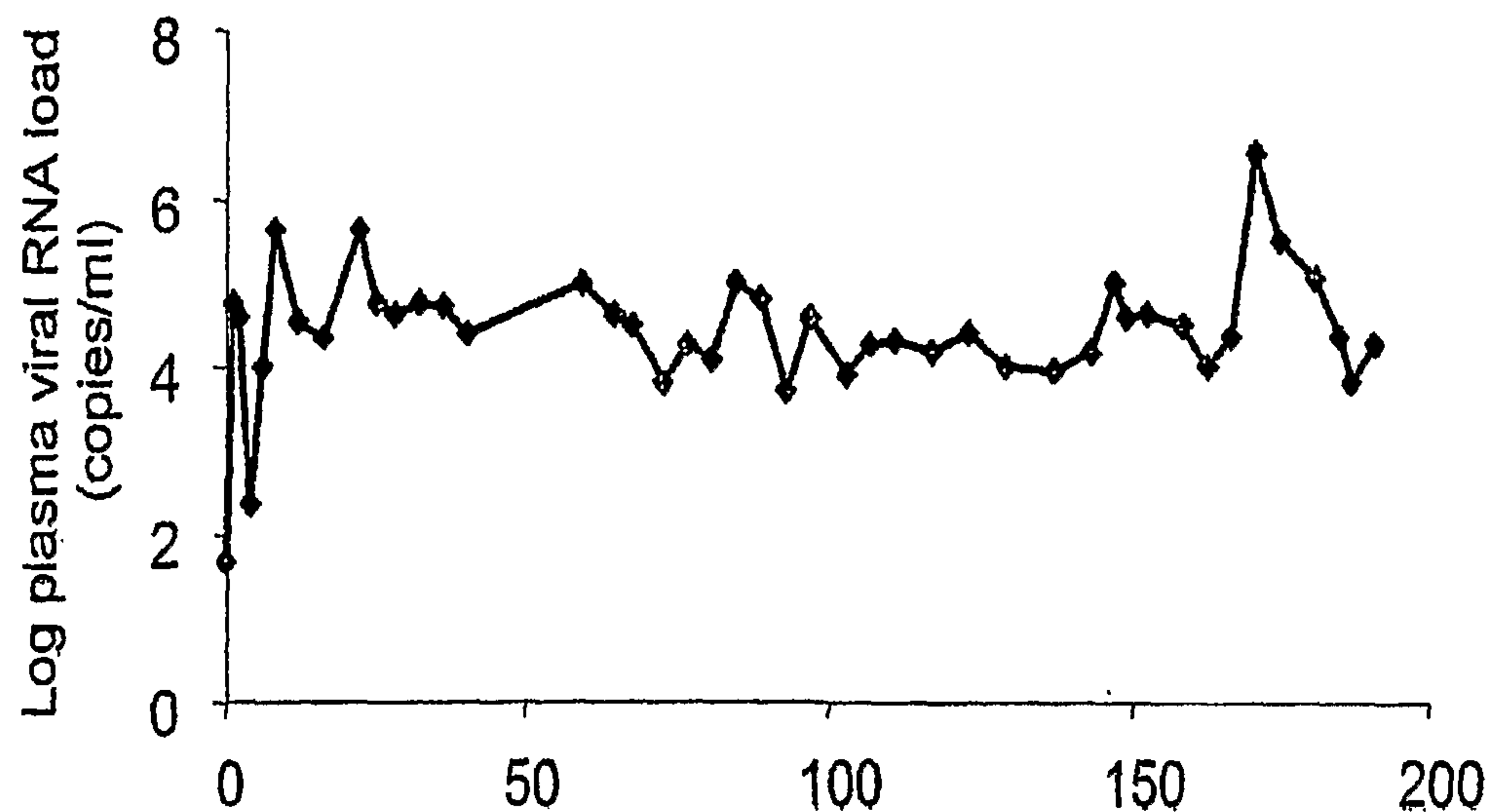
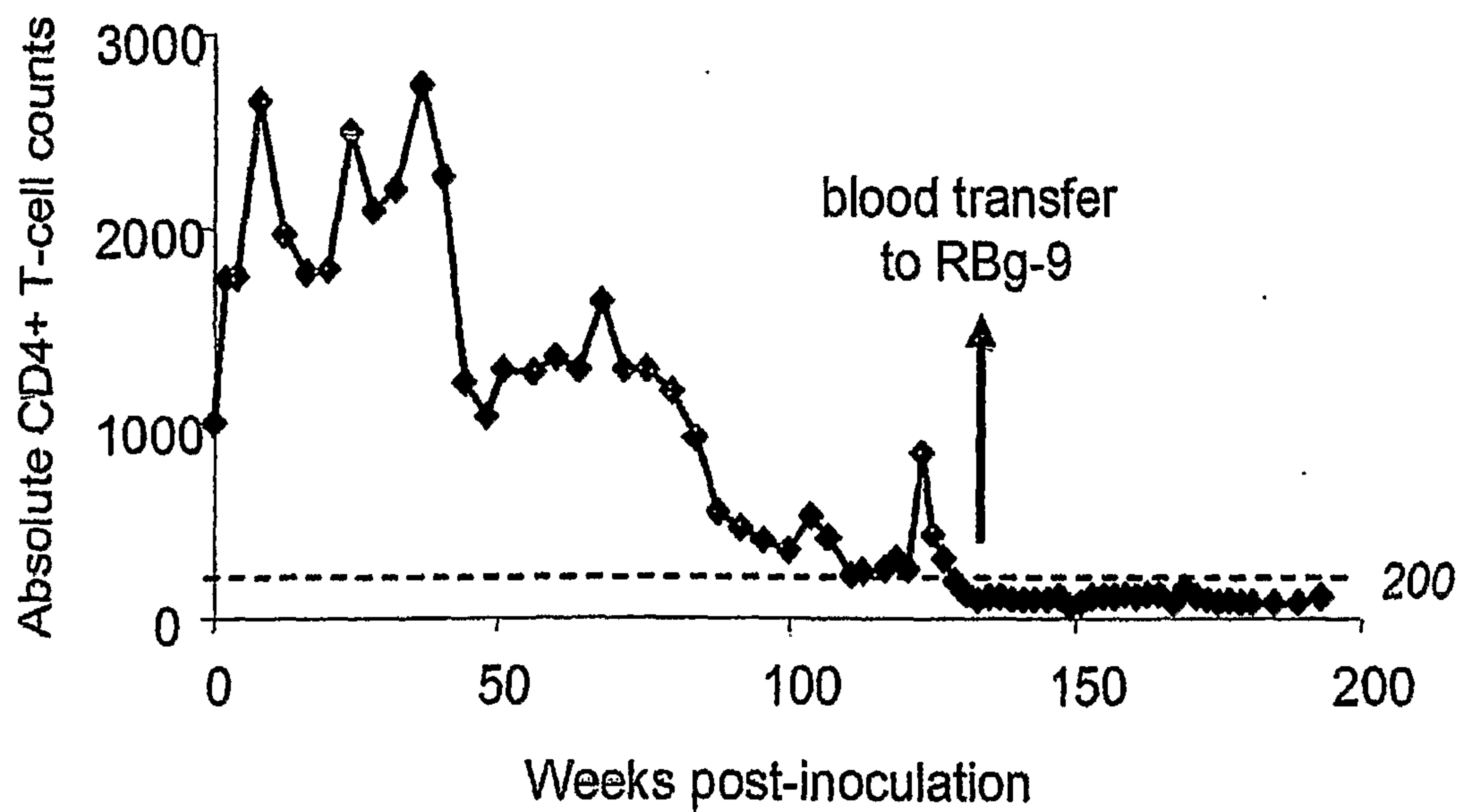


FIGURE 4B



19/40

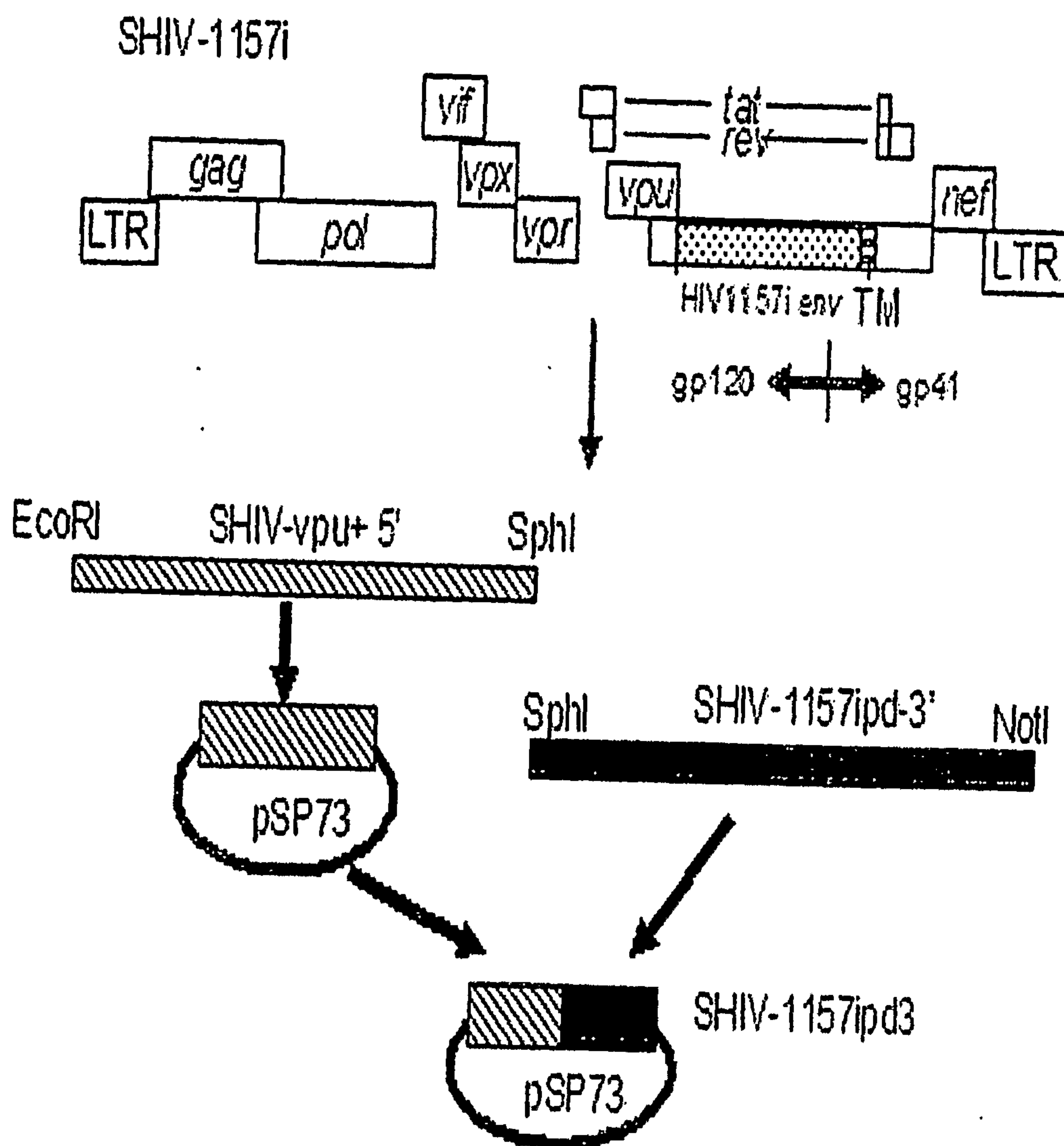


FIGURE 5

20/40

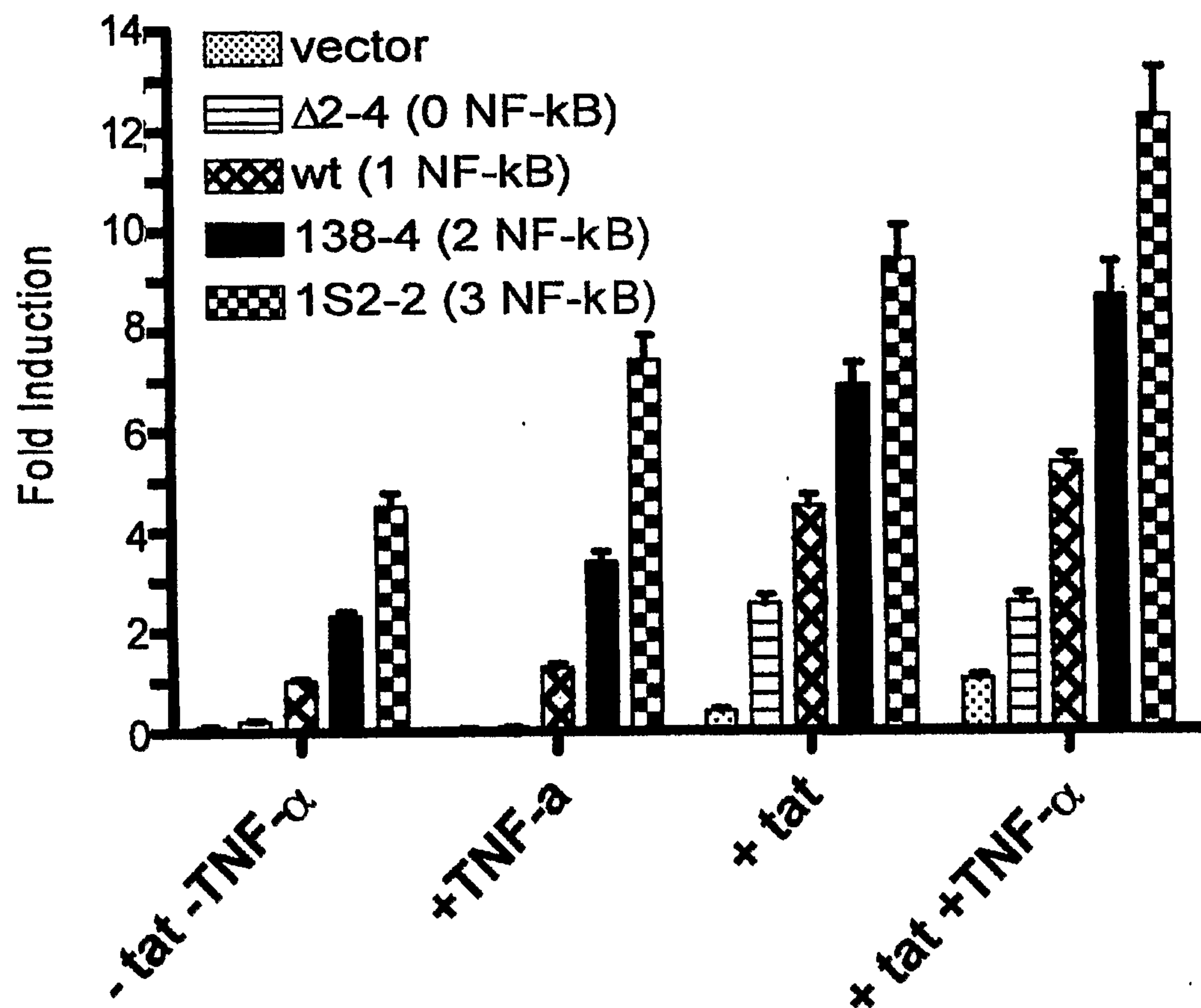


FIGURE 6A

21/40

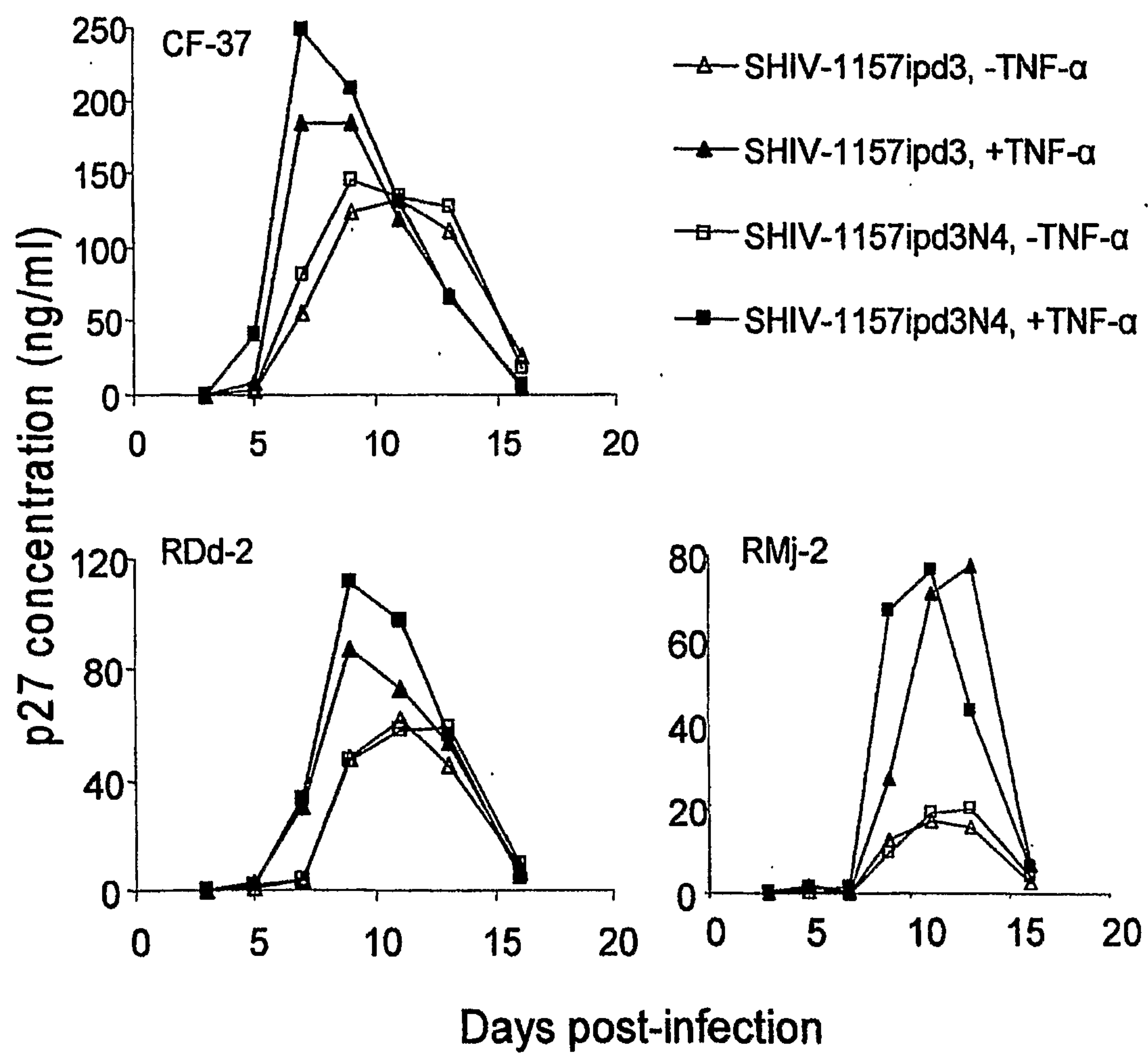


FIGURE 6B

22/40

FIGURE 7A

U87.CD4.CCR5 cells

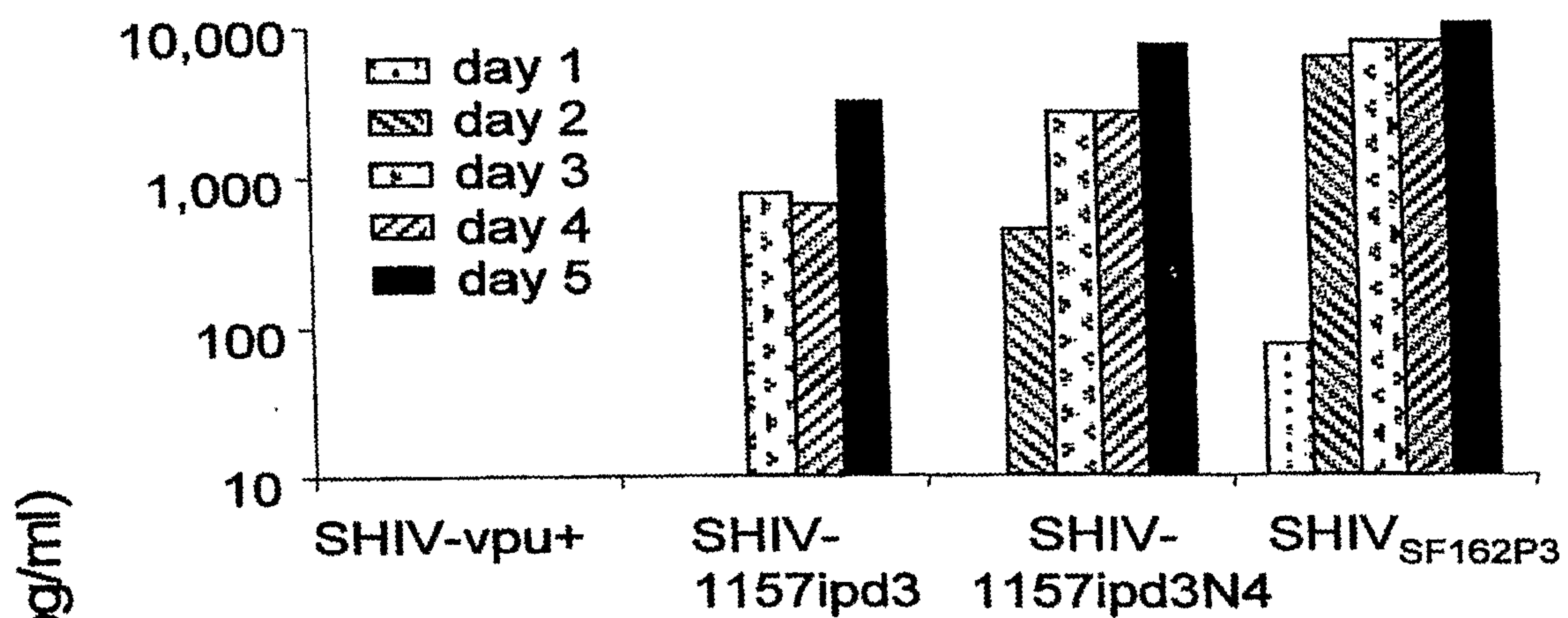
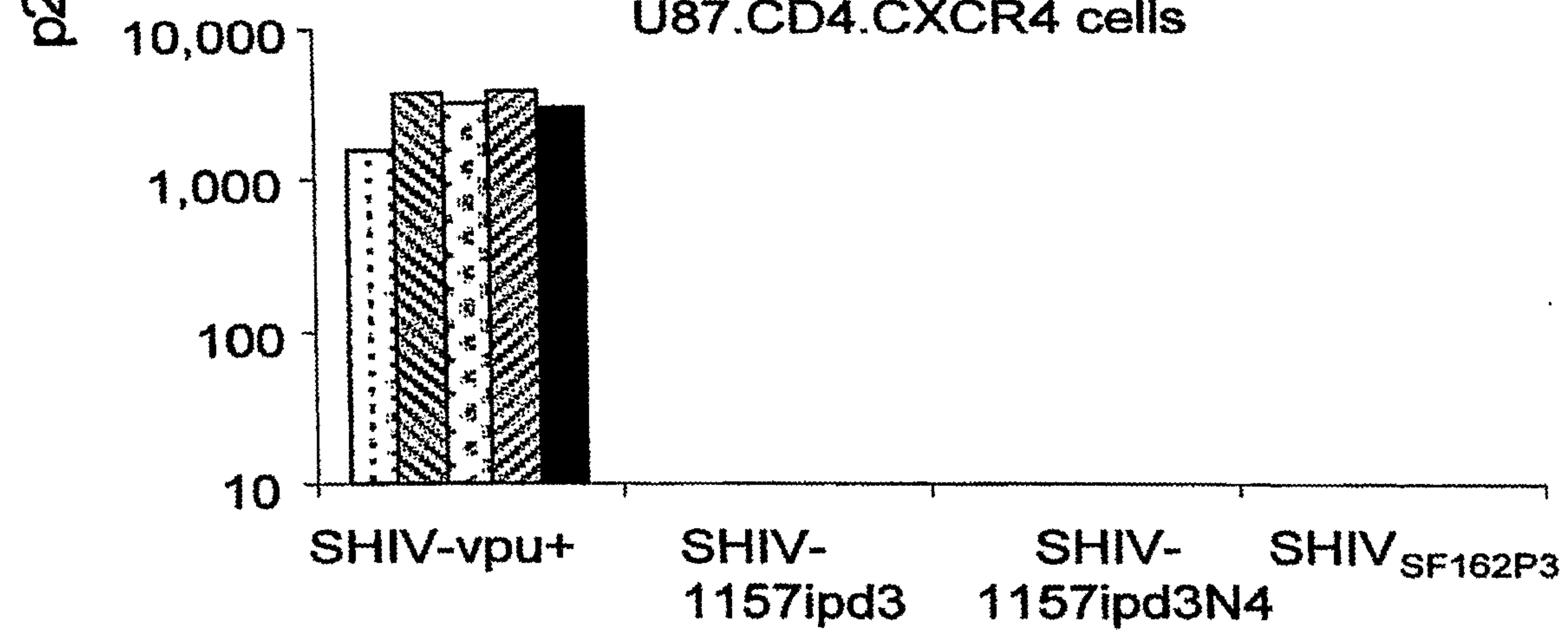


FIGURE 7B

U87.CD4.CXCR4 cells



23/40

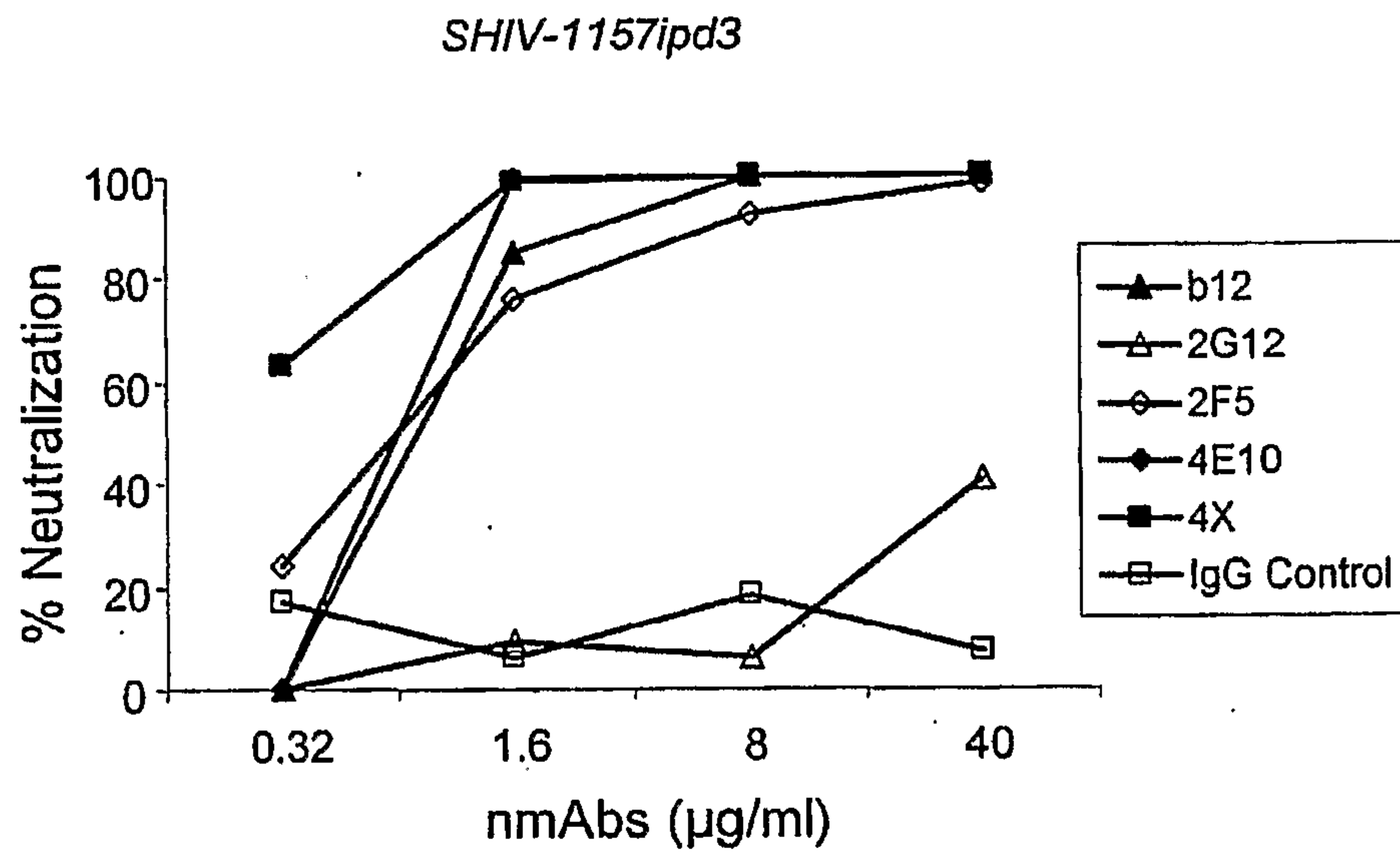


FIGURE 7C

24/40

ATGCGCGTGAAGGAGAAGTACCAGCACCTGTGGCGCTGGGGCTGGCG
CTGGGGCACCATGCTGCTGGGCATGCTGATGATCTGCTCCGCCACCGA
GAAGCTGTGGGTGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGC
CAAGACCACCCTGTTCTGCGCCTCCGACGCCAAGGCCTACGAGAAGGA
GGTGCAACAACATCTGGGCCACCCACGCCTGCGTGCCACCGACCCCAA
CCCCCAGGAGATCGTGCTGGAGAACGTGACCGAGAACTTCAACATGTG
GAAGGACGACATGGTGGACCAGATGCACGAGGACATCATCTCCCTGTG
GGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGAC
CCTGAAGTGCTCCAACCTTCACCCGCGAGGGCAACGTGACCTACAAGGA
GGAGATGGACAAGGTGAAGAACTGCTCCTTCAACGTGACCACCGGCAT
CCGCGACAAGAAGCAGAAGGTGAACGCCCTGTTCTACCGCCTGGACAT
CACCCCCCTGGACGAGAACAACAACAACTCCTCCGAGTACCGCTTGATC
AACTGCAAGTCCTCCACCATCACCCAGGCCTGCCCAAGGTGAACTTCG
ACCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTACGCCATCCTGAA
GTGCAACAACAAGACCTTCAACGGCACCGGCCCTGCCACAACGTGTC
CACCGTGCAGTGCACCCACGGCATCAAGCCCGTGGTGTCCACCCAGCT
GCTGCTGAACGGCTCCCTGGCCGAGCGCGAGATCATCATCCGCTCCGA
GAACCTGACCGACAACGTGAAGACCATCATCGTGCACTTCAACGAGTCC
GTGGAGATCAACTGCACCCGCCCAACAACAACACCCGCAAGTCCATCC
GCATCGGCCCCCGGCCAGGCCTTCTACGCCACCGGCGACATCATCGGCG
ACATCCGCCAGGCCCACTGCAACATCTCCAAGGAGAACTGGAACAAGAC
CCTGCAGTGGGTGCGCGGCAAGCTGGAGGAGCACTTCCCCAACAAGAC
CATCGTGTTCAAGCCCTCCTCCGGCGGGCGACCTGGAGATCACCACCCA
CTCCTTCAACTGCCGCGGGCGAGTTCTTCTACTGCAACACCTCCAAGCTG
TTCAACGGCACCGACAACCTCCACCCACATGGACACCGGCAACGACACC
GTGATCACCATCCCCTGCCGCATCAAGCAGATCATCAACATGTGGCAGG
AGGTGGGGCGCGCCATGTACGCCCCCCCCATCGAGGGCAACATCACCT
GCAAGTCCAACATCACCGGCCTGCTGCTGGTGC GCGACGGCGGGCCAG
GACAACTCCACCAACAACACCGAGACCTTCCGCCCCCGCGGGCGGGCGAC
ATGCGCAACAACCTGGCGCTCCGAGCTGTACAAGTACAAGGTGGTGGAG
ATCAAGCCCCTGGGCATCGCCCCCACC AAGGCCAAGCGCCGCGTGGTG
GAGCGCGAGAAGCGCGCCGTGGGCATCGGCGCCGTGTTCTGGGCTT
CCTGGGCGCCGCGGGCTCCACCATGGGCGCCGCTCCATCACCTGAC
CGTGCAAGGCCCGCCAGCTGCTGTCCGGCATCGTGCAAGCAGCAGGACAA
CCTGCTGCGCGCCATCGAGGCCAGCAGCACATGCTGCAGCTGACCGT
GTGGGGCATCAAACAGCTGCAGGCCCGCGTGCTGGCCATCGAGCGCTA
CCTGCAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCTCCGGCAAGCT
GATCTGCACCACCGCCGTGCCCTGGAACGCCTCCTGGTCCAACAAGTC
CCAGACCGACATCTGGGAGAACATGACCTGGATGCAGTGGGACAAGGA
GATCTCCAAGCACACCGACACCATCTACCGCCTGCTGGAGGACTCCCA
GAACCAGCAGGAGAAGAACGAGAAGGACCTGCTGGCCCTGGACTCCTG
GGAGAACCTGTGGAACCTGGTTCTCCATCACC AAGTGGCTGTGGTACATC
AAGATCTTCATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCATCT
TCGCCGTGCTGTCCATCGTGTCCCGCGTGCGCCAGGGCTACTCCCCC
TGTCTTCCAGACCCACCTGCCACCCCCCGCGGGCCCCGACCGCCCCG
AGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCTCCATC

FIGURE 8

25/40

CGCCTGGTGAACGGCTCCCTGGCCCTGATCTGGGACGACCTGCGCTCC
CTGTGCCTGTTCTCCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGA
CCCGCATCGTGGAGCTGCTGGGCCGCCGCGGCTGGGAGGCCCTGAAG
TACTGGTGGAACCTGCTGCAGTACTGGTCCCAGGAGCTGAAGAACTCC
GCCGTGTCCCTGCTGAACGCCACCGCCATCGCCGTGGCCGAGGGCACC
GACCGCGTGATCGAGGTGGTGCAGGGCGCCTGCCGCGCCATCCGCCA
CATCCCCCGCCGCATGCGCCAGGGCCTGGAGCGCATCCTGCTGTGA
SEQ ID NO: 1

FIGURE 8 (CONTINUED)

SUBSTITUTE SHEET (RULE 26)

26/40

MRVKEYQHLWRWGWRWGTMLLGMLMICSATEKLWVTVYYGVPVWKEAKTT
LFCASDAKAYEKEVHNIWATHACVPTDPNPQEIVLENTENFNMWKDDMVDQ
MHEDIISLWDQSLKPCVKLTPLCVTLKCSNFTREGNVTYKEEMDKVKNCSEFNT
TGIRDKKQKVNALFYRLDITPLDENNNNSSEYRLINCKSSTITQACPKVNFDPPI
HYCAPAGYAILKCNNKTFNGTGPCHNVSTVQCTHGIKPVSTQLLNGSLAERE
IIRSENLTDNVKTIVHFNESVEINCTRPNNNTRKSIRIGPGQAFYATGDIIGDIRQ
AHCNISKENWNKTLQWVRGKLEEHFPNKTIVFKPSSGGDLEITTHSFNCRGEFF
YCNTSKLFNGTDNSTHMDTGNDTVITPCRIKQIINMWQEVGRAMYAPPIEGNIT
CKSNITGLLLVRDGGQDNSTNNTETFRPGGGDMRNNWRSELYKYKVVEIKPLG
IAPTAKRRVVEREKRAVGIGAVFLGFLGAAGSTMGAASITLTVQARQLLSGIVQ
QQDNLLRAIEAQQHMLQLTVWGIKQLQARVLAIERYLQDQQLLGIWGCSEGLIC
TTAVPWNASWSNKSQTDIWENMTWMQWDKEISKHTDTIYRLLEDSSQNQQEKN
EKDLLALDSWENLWNWFSITKWLYIKIFIMIVGGLIGLRIFAVLSIVSRVRQGY
PLSFQTHLPTPRGPDRPEGIEEEGGERDRDRSIRLVNGSLALIWDLLRSLCLFS
YHRLRDLILLIVTRIVELLGRRGWEALKYWWNLLQYWSQELKNSAVSLLNATAIA
VAEGTDRVIEVVQGACRAIRHIPRRMRQGLERILL*

SEQ ID NO: 2

FIGURE 9

27/40

ATGCGCGTGAAGGAGAAGTACCAGCACCTGTGGCGCTGGGGCTGGCGCTG
GGGCACCATGCTGCTGGGCATGCTGATGATCTGCTCCGCCACCGAGAAGCT
GTGGGTGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCAAGACCA
CCCTGTTCTGCGCCTCCGACGCCAAGGCCTACGAGAAGGAGGTGCACAACA
TCTGGGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGAGATC
GTGCTGGAGAACGTGACCGAGAACTTCAACATGTGGAAGGACGACATGGTG
GACCAGATGCACGAGGACATCATCTCCCTGTGGGACCAGTCCCTGAAGCCC
TGCGTGGGCGCCGGCGCCTGCCCAAGGTGAACTTCGACCCCATCCCCATC
CACTACTGCGCCCCCGCCGGCTACGCCATCCTGAAGTGCAACAACAAGACC
TTCAACGGCACCGGCCCTGCCACAACGTGTCCACCGTGCAGTGCACCCAC
GGCATCAAGCCCGTGGTGTCCACCCAGCTGCTGCTGAACGGCTCCCTGGCC
GAGCGCGAGATCATCATCCGCTCCGAGAACCTGACCGACAACGTGAAGACC
ATCATCGTGCACTTCAACGAGTCCGTGGAGATCAACTGCACCCGCCCAACA
ACAACACCCGCAAGTCCATCCGCATCGGCCCCGGCCAGGCCTTCTACGCCA
CCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCTCCAAGG
AGAACTGGAACAAGACCCTGCAGTGGGTGCGCGGCAAGCTGGAGGAGCAC
TTCCCCAACAAGACCATCGTGTTCAAGCCCTCCTCCGGCGGCGACCTGGAG
ATCACCACCCACTCCTTCAACTGCCGCGGCGAGTTCTTCTACTGCAACACCT
CCAAGCTGTTCAACGGCACCGACAACCTCCACCCACATGGACACCGGCAACG
ACACCGTGATCACCATCCCCTGCCGCATCAAGCAGATCATCAACATGTGGCA
GGAGGTGGGGCGCGCCATGTACGCCCCCCCCCATCGAGGGCAACATCACCT
GCAAGTCCAACATCACCGGCCTGCTGCTGGTGC GCGACGGCGGCCAGGAC
AACTCCACCAACAACACCGAGACCTTCCGCCCCGGCGGGCGGCGACATGCG
CAACAACCTGGCGCTCCGAGCTGTACAAGTACAAGGTGGTGGAGATCAAGCC
CCTGGGCATCGCCCCCACCAAGGCCAAGCGCCGCGTGGTGGAGCGCGAGA
AGCGCGCCGTGGGCATCGGCGCCGTGTTCTTCTGGGCTTCTGGGCGCCGCC
GGCTCCACCATGGGGCGCCGCCTCCATCACCTGACCGTGCAGGCCCGCCA
GCTGCTGTCCGGCATCGTGCAGCAGCAGGACAACCTGCTGCGCGCCATCGA
GGCCCAGCAGCACATGCTGCAGCTGACCGTGTGGGGCATCAAACAGCTGCA
GGCCCGCGTGCTGGCCATCGAGCGCTACCTGCAGGACCAGCAGCTGCTGG
GCATCTGGGGCTGCTCCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGGA
ACGCCTCCTGGTCCAACAAGTCCCAGACCGACATCTGGGAGAACATGACCT
GGATGCAGTGGGACAAGGAGATCTCCAAGCACACCGACACCATCTACCGCC
TGCTGGAGGACTCCCAGAACCAGCAGGAGAAGAACGAGAAGGACCTGCTG
GCCCTGGACTCCTGGGAGAACCTGTGGAACCTGGTTCTCCATACCAAGTGG
CTGTGGTACATCAAGATCTTCATCATGATCGTGGGCGGCCTGATCGGCCTGC
GCATCATCTTCGCCGTGCTGTCCATCGTGTCCCGCGTGCGCCAGGGCTACT
CCCCCCTGTCCTTCCAGACCCACCTGCCACCCCCCGCGGCCCCGACCGC
CCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCTCCA
TCCGCCTGGTGAACGGCTCCCTGGCCCTGATCTGGGACGACCTGCGCTCCC
TGTGCCTGTTCTCCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGACCC
GCATCGTGGAGCTGCTGGGCGCCGCGGCTGGGAGGCCCTGAAGTACTGG
TGGAACCTGCTGCAGTACTGGTCCCAGGAGCTGAAGAACTCCGCCGTGTCC
CTGCTGAACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCGTGAT
CGAGGTGGTGCAGGGCGCCTGCCGCGCCATCCGCCACATCCCCCGCCGCA
TGCGCCAGGGCCTGGAGCGCATCCTGCTGTGA

SEQ ID NO: 3

FIGURE 10

28/40

MRVKEYQHLWRWGWRWGTMLLGMLMICSATEKLWVTVYYGVPVWKEAKTT
LFCASDAKAYEKEVHNIWATHACVPTDPNPQEIVLENTENFNMWKDDMVDQ
MHEDIISLWDQSLKPCVGAGACPKVNFDPPIHYCAPAGYAILKCNNKTFNGTG
PCHNVSTVQCTHGIKPVVSTQLLLNGSLAEREIIIRSENLTDNVKTIVHFNESVEI
NCTRPNNNTRKSIRIGPGQAFYATGDIIGDIRQAHCNISKENWNKTLQWVRGKL
EEHFPNKTIVFKPSSGGDLEITTHSFNCRGEFFYCNTSKLFNGTDNSTHMDTGN
DTVITIPCRIKQIINMWQEVGRAMYAPPIEGNITCKSNITGLLLVRDGGQDNSTNN
TETFRPGGGDMRNNWRSELYKYKVVEIKPLGIAPTAKARRVVEREKRAVGIGA
VFLGFLGAAGSTMGAASITLVQARQLLSGIVQQQDNLLRAIEAQQHMLQLTVW
GIKQLQARVLAIERYLQDQQLGIWGC SGKLICTTAVPWNASWSNKSQTDIWEN
MTWMQWDKEISKHTDTIYRLLED SQNQKEKNEKDLLALDSWENLWNWFSITK
WLWYIKIFIMIVGGLIGLRIIFAVLSIVSRVRQGYSPLSFQTHLPTPRGPDRPEGIE
EEGGERDRDRSIRLVNGSLALIWD DLRSCLFSYHRLRDL LLIVTRIVELLGRRG
WEALKYWWNLLQYWSQELKNSAVSLLNATAIAVAEGTDRVIEWVQGACRAIRHI
PRRMRQGLERILL*
SEQ ID NO: 4

FIGURE 11

29/40

ATGCGCGTGAAGGAGAAGTACCAGCACCTGTGGCGCTGGGGCTGGCGCTG
GGGCACCATGCTGCTGGGCATGCTGATGATCTGCTCCGCCACCGAGAAGC
TGTGGGTGACCGTGTACTACGGCGT.GCCCCGTGTGGAAGGAGGCCAAGACC
ACCCTGTTCTGCGCCTCCGACGCCAAGGCCTACGAGAAGGAGGTGCACAA
CATCTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGA
TCGTGCTGGAGAACGTGACCGAGAACTTCAACATGTGGAAGGACGACATGG
TGGACCAGATGCACGAGGACATCATCTCCCTGTGGGACCAGTCCCTGAAGC
CCTGCGTGGGCGCCGGCGCCTGCCCAAGGTGAACTTCGACCCCATCCCC
ATCCACTACTGCGCCCCCGCCGGCTACGCCATCCTGAAGTGCAACAACAAG
ACCTTCAACGGCACCGGCCCTGCCACAACGTGTCCACCGTGCAGTGCAC
CCACGGCATCAAGCCCGTGGTGTCCACCCAGCTGCTGCTGAACGGCTCCC
TGGCCGAGCGCGAGATCATCATCCGCTCCGAGAACCTGACCGACAACGTG
AAGACCATCATCGTGCACCTTCAACGAGTCCGTGGAGATCAACTGCACCGGC
GCCGGCGCCCACTGCAACATCTCCAAGGAGAACTGGAACAAGACCCTGCA
GTGGGTGCGCGGCAAGCTGGAGGAGCACTTCCCCAACAAGACCATCGTGT
TCAAGCCCTCCTCCGGCGGGCGACCTGGAGATCACCACCCACTCCTTCAACT
GCCGCGGGCGAGTTCTTCTACTGCAACACCTCCAAGCTGTTCAACGGCACCG
ACAACCTCCACCCACATGGACACCGGCAACGACACCGTGATCACCATCCCCT
GCCGCATCAAGCAGATCATCAACATGTGGCAGGAGGTGGGGCGCGCCATG
TACGCCCCCCCCATCGAGGGCAACATCACCTGCAAGTCCAACATCACCGGC
CTGCTGCTGGTGC GCGACGGCGGCCAGGACAACCTCCACCAACAACACCGA
GACCTTCCGCCCCGGCGGGCGGCGACATGCGCAACAACCTGGCGCTCCGAG
CTGTACAAGTACAAGGTGGTGGAGATCAAGCCCCTGGGCATCGCCCCCAC
CAAGGCCAAGCGCCGCGTGGTGGAGCGCGAGAAAGCGCGCCGTGGGCATC
GGCGCCGTGTTCTGTTCTGGGCTTCTGGGCGCCGCGGCTCCACCATGGGCG
CCGCCTCCATCACCTGACCGTGCAGGCCCGCCAGCTGCTGTCCGGCATC
GTGCAGCAGCAGGACAACCTGCTGCGCGCCATCGAGGCCCAGCAGCAT
GCTGCAGCTGACCGTGTGGGGCATCAAACAGCTGCAGGCCCGCGTGTGG
CCATCGAGCGCTACCTGCAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC
TCCGGCAAGCTGATCTGCACCAACCGCCGTGCCCTGGAACGCCTCCTGGTC
CAACAAGTCCCAGACCGACATCTGGGAGAACATGACCTGGATGCAGTGGG
ACAAGGAGATCTCCAAGCACACCGACACCATCTACCGCCTGCTGGAGGACT
CCCAGAACCAGCAGGAGAAGAACGAGAAGGACCTGCTGGCCCTGGACTCC
TGGGAGAACCTGTGGAACCTGGTTCTCCATCACCAAGTGGCTGTGGTACATC
AAGATCTTCATCATGATCGTGGGCGGGCCTGATCGGCCTGCGCATCATCTTC
GCCGTGCTGTCCATCGTGTCCCGCGTGCGCCAGGGCTACTCCCCCTGTC
CTTCCAGACCCACCTGCCACCCCCCGCGGCCCGGACCGCCCCGAGGGC
ATCGAGGAGGAGGGCGGGCGAGCGCGACCGCGACCGCTCCATCCGCCTGG
TGAACGGCTCCCTGGCCCTGATCTGGGACGACCTGCGCTCCCTGTGCCTG
TTCTCCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGACCCGCGATCGTG
GAGCTGCTGGGCGCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAAACC
TGCTGCAGTACTGGTCCCAGGAGCTGAAGAACTCCGCCGTGTCCCTGCTGA
ACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCGTGATCGAGGT
GGTGCAGGGCGCCTGCCGCGCCATCCGCCACATCCCCCGCCGCATGCGC
CAGGGCCTGGAGCGCATCCTGCTGTGA

SEQ ID NO: 5

FIGURE 12

SUBSTITUTE SHEET (RULE 26)

30/40

MRVKEYQHLWRWGWRWGTMLLGMLMICSATEKLWVTVYYGVPVWKEAKTT
LFCASDAKAYEKEVHNIWATHACVPTDPNPQEIVLENTENFNMWKDDMVDQ
MHEDIISLWDQSLKPCVGAGACPKVNFDPIPIHYCAPAGYAILKCNNKTFNGTG
PCHNVSTVQCTHGIKPVVSTQLLLNGSLAEREIIIRSENLTDNVKTIVHFNESVEI
NCTGAGAHCNISKENWNKTLQWVRGKLEEHFPNKTIVFKPSSGGDLEITTHSF
NCRGEFFYCNTSKLFNGTDNSTHMDTGNDTVITIPCRIKQIINMWQEVGRAMYA
PPIEGNITCKSNITGLLLVRDGGQDNSTNNTETFRPGGGDMRNNWRSELYKYK
VVEIKPLGIAPTKAKRRVVEREKRAVGIGAVFLGFLGAAGSTMGAASITLTVQAR
QLLSGIVQQQDNLLRAIEAQQHMLQLTVWGIKQLQARVLAIERYLQDQQLGIW
GCSGKLICTTAVPWNASWSNKSQTDIWENMTWMQWDKEISKHTDTIYRLLDS
QNQQEKNEKDLLALDSWENLWNWFSITKWLWYIKIFIMIVGGLIGLRRIIFAVLSIV
SRVRQGYSPLSFQTHLPTPRGPDRPEGIEEEGGERDRDRSIRLVNGSLALIWD
DLRSLCLFSYHRLRDLLLIVTRIVELLGRRGWEALKYWWNLLQYWSQELKNSAV
SLLNATAIAVAEGTDRVIEVVQGACRAIRHIPRRMRQGLERILL*
SEQ ID NO: 6

FIGURE 13

31/40

ATGCGCGTGAAGGAGAAGTACCAGCACCTGTGGCGCTGGGGCTGGCGCT
GGGGCACCATGCTGCTGGGCATGCTGATGATCTGCTCCGCCACCGAGAAG
CTGTGGGTGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCAAGA
CCACCCTGTTCTGCGCCTCCGACGCCAAGGCCTACGAGAAGGAGGTGCAC
AACATCTGGGCCACCCACGCCTGCGTGCCCAACCGACCCCAACCCCCAGGA
GATCGTGCTGGGAGAACGTGACCGAGAACTTCAACATGTGGAAGGACGACA
TGGTGGACCAGATGCACGAGGACATCATCTCCCTGTGGGACCAGTCCCTG
AAGCCCTGCGTGGGGCGCCGGCGCCTGCCCCAAGGTGAACTTCGACCCCA
TCCCCATCCACTACTGCGCCCCCGCCGGCTACGCCATCCTGAAGTGCAAC
ACAAGACCTTCAACGGCACCCGGCCCCCTGCCACAACGTGTCCACCGTGCA
GTGCACCCACGGCATCAAGCCCGTGGTGTCCACCCAGCTGCTGCTGAACG
GCTCCCTGGCCGAGCGCGAGATCATCATCCGCTCCGAGAACCTGACCGAC
AACGTGAAGACCATCATCGTGCATTCAACGAGTCCGTGGAGATCAACTGC
ACCGGCGCCGGCGCCCACTGCAACATCTCCAAGGAGAACTGGAACAAGAC
CCTGCAGTGGGTGCGCGGCAAGCTGGAGGAGCACTTCCCCAACAAGACC
ATCGTGTTCAAGCCCTCCTCCGGCGGGCGACCTGGAGATCACCACCCACTC
CTTCAACTGCCGCGGGCGAGTTCTTCTACTGCAACGGCGCCGGCCCCCTGCC
GCATCAAGCAGATCATCAACATGTGGCAGGAGGTGGGGCGCGCCATGTAC
GCCCCCCCCATCGAGGGCAACATCACCTGCAAGTCCAACATCACCGGCCT
GCTGCTGGTGC GCGACGGCGGGCCAGGACAACCTCCACCAACAACACCGAG
ACCTTCCGCCCCCGGGCGGGCGGCGACATGCGCAACAACCTGGCGCTCCGAGC
TGACAAGTACAAGGTGGTGGAGATCAAGCCCCTGGGCATCGCCCCCACC
AAGGCCAAGCGCCGCGTGGTGGAGCGCGAGAAGCGCGCCGTGGGCATC
GGCGCCGTGTTCTGCGGCTTCTGGGCGCCGCGGCTCCACCATGGGCG
CCGCCTCCATCACCTGACCGTGCAAGGCCCGCCAGCTGCTGTCCGGCATC
GTGCAGCAGCAGGACAACCTGCTGCGCGCCATCGAGGCCCAGCAGCACA
TGCTGCAGCTGACCGTGTGGGGCATCAAACAGCTGCAGGCCCGCGTGCT
GGCCATCGAGCGCTACCTGCAGGACCAGCAGCTGCTGGGCATCTGGGGC
TGCTCCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGGAACGCCTCCTG
GTCCAACAAGTCCCAGACCGACATCTGGGAGAACATGACCTGGATGCAGT
GGGACAAGGAGATCTCCAAGCACACCGACACCATCTACCGCCTGCTGGAG
GACTCCCAGAACCAGCAGGAGAAGAACGAGAAGGACCTGCTGGCCCTGG
ACTCCTGGGAGAACCTGTGGAACCTGGTTCTCCATCACCAAGTGGCTGTGG
TACATCAAGATCTTCATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATC
ATCTTCGCCGTGCTGTCCATCGTGTCCCGCGTGCGCCAGGGCTACTCCCC
CCTGTCCTTCCAGACCCACCTGCCCAACCCCCCGCGGCCCGACCGCCCC
GAGGGCATCGAGGAGGAGGGCGGGCGAGCGCGACCGCGACCGCTCCATC
CGCCTGGTGAACGGCTCCCTGGCCCTGATCTGGGACGACCTGCGCTCCCT
GTGCCTGTTCTCCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGACCC
GCATCGTGGAGCTGCTGGGCCGCGCGGCTGGGAGGCCCTGAAGTACTG
GTGGAACCTGCTGCAGTACTGGTCCCAGGAGCTGAAGAACTCCGCCGTGT
CCCTGCTGAACGCCACCGCCATCGCCGTGGCCGAGGGGCACCGACCGCGT
GATCGAGGTGGTGCAGGGCGCCTGCCGCGCCATCCGCCACATCCCCCGC
CGCATGCGCCAGGGCCTGGAGCGCATCCTGCTGTGA

SEQ ID NO: 7

FIGURE 14

SUBSTITUTE SHEET (RULE 26)

32/40

MRVKEYQHLWRWGWRWGTMLLGMLMICSATEKLWVTVYYGVPVWKEAKTT
LFCASDAKAYEKEVHNIWATHACVPTDPNPQEIVLENTENFNMWKDDMVDQ
MHEDIISLWDQSLKPCVGAGACPKVNFDPPIHYCAPAGYAILKCNNKTFNGTG
PCHNVSTVQCTHGIKPVVSTQLLNGSLAEREIIIIRSENLTDNVKTIVHFNESVEI
NCTGAGAHCNISKENWNKTLQWVRGKLEEHFPNKTIVFKPSSGGDLEITTHSF
NCRGEFFYCNGAGPCRIKQIINMWQEVGRAMYAPPIEGNITCKSNITGLLLVRD
GGQDNSTNNTETFRPGGGDMRNNWRSELYKYKVVEIKPLGIAPTKAKRRVVE
REKRAVGIGAVFLGFLGAAGSTMGAASITLTVQARQLLSGIVQQQDNLLRAIEA
QQHMLQLTWGQIKQLQARVLAIERYLQDQQLLGIWGC SGKLICTTAVPWNASW
SNKSQTDIWENMTWMQWDKEISKHTDTIYRLLED SQNQQEKNEKDLLALDSW
ENLWNWFSITKWLWYIKIFIMIVGGLIGLRIIFAVLSIVSRVRQGYSPLSFQTHLPT
PRGPDRPEGIEEEGGERDRDRSIRLVNGSLALIWD DLRSLCLFSYHRLRDL LLIV
TRIVELLGRRGWEALKYWWNLLQYWSQELKNSAVSLLNATAI AVAEGTDRVIEV
VQGACRAIRHIPRRMRQGLERILL*

SEQ ID NO: 8

FIGURE 15

33/40

GCATGCTGTAGAGCAAGAAATGGAGCCAGTAGATCCTAGACTAGAGCCCTG
GAAGCATCCAGGAAGCAGGCCTAAACTGCTTGTACCAATTGCTATTGTAAA
AAGTGTTGCTTTTCATTGCCAAGTTTGTTTCATAACAAAAGCCCTAGGCATCTC
CTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGTC
AGACTCATCAAGCTTCTCTATCAAAGCAGTAAGTAGTACATGTAATGCAATC
TATACAAATAGAAATAGTAGCATTAGTAGTAGCAATAATAATAGCAATAGTTG
TGTGGTCCATAGTAATCATAGAATATAGGAAAATATTAAGACAAAGAAAAATA
GACAGGTTAATTAATAGACTAATAGAAAGAGCAGAAGACAGTGGCAATGAG
AGTGAAGGAGAAATATCAGCACTTGTGGAGATGGGGGTGGAGATGGGGCA
TCATGCTCCTTGGGATGTTGATGATCTGTAGTGCTACAGAAAAATTGTGGGT
CACAGTCTATTATGGGGTACCTGTATGGAAAGAAGCAAAAACACTACTTTATTC
TGTGCATCAAATGCTAAAGCATATGAGAAAGAAGTACATAACATCTGGGCTA
CACATGCCTGTGTACCCACAGACCCCAACCCACAAGAAATAGTTTTGGGAA
ATGTAACAGAAAATTTTAACATGTGGAAAAATGACATGGTGGATCAGATGCA
TGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAGTTG
ACTTCACTCTGTGTCACCTTTAAAGTGTAGTAATTTTACCGGGAAGAGTAATGT
TACCTACAAAGGGGATATGGAAGTAAAAAATTGCTCTTTCAATGTAACCACA
GAAATAAGAGATAAGAAGCAGAAAGTGTATGCTCTTTTTTATAGACTTGATAT
AACACCACTTGATGACAACTCTAGTGAGTATATATTAATAAATTGCAATTCCT
CAACCATAACACAAGCCTGTCCAAAGGTCAATTTTGACCCAATTCCTATACA
TTATTGTGCTCCAGCTGGTTATGCGATTCTAAAGTGTAAATAATAAGACATTTA
ATGGGACAGGACCATGCCATAATGTCAGTACAGTACAATGTACACATGGAAT
TAAGCCAGTGGTATCAACTCAACTACTGTAAACGGTAGCCTAGCAGAAGG
GGAGATAATAATTAGATCTGAAAATCTGACAGACAATGTCAAACAATAATA
GTACACTTTAATGAATCTGTAGAAATTACTTGTACAAGACCCAACAATAATAC
AAGAAAAAGTATAAGCATAGGACCAGGACAAGCAATCTATGCCACAGGTGA
TATAATAGGAGACATAAGACAAGCACACTGTAACATTAGTAAAGAAAATTGG
AACAAAACCTTTACAATGGGTAAAGGGGAAAATTAAGAACAACCTTCCCTAATA
AAACAATAGTATTTAAACCATCCTCAGGAGGGGATCTAGAAATTACAACACA
TAGCTTTAATTGTAGAGGAGAATTTTCTATTGCAACACATCAAACTGTTTA
ATAGTACAGACAATAGTACACACATGGGTACAGAAAATAATACAATCATCAC
AATCCCATGTAGAATAAAACAAATTATAAACATGTGGCAGGAGGTAGGACGA
GCAATGTATGCCCCCCCCCATAGAAGGAAACATAACATGTAAATCAAATATCA
CAGGACTACTACTGGTACGTGATGGAGGATGGGACAACAGTACAAATGACA
CAGAAACATTCAGGCCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGT
GAATTATATAAATATAAGGTGGTAGAAGTCAAGCCATTGGGAATAGCACCCA
CTAAGGCAAAAAGGAGAGTGGTGGAGAGAGAAAAAAGAGCAGTGGGAATA
GGAGCTGTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGC
GGCGTCAATAACGCTGACGGTACAGGCCAGACAACCTGTTGTCTGGTATAGT
GCAGCAGCAAGACAATTTGCTGAGAGCTATAGAGGCGCAACAACATATGTT
GCAACTCACAGTCTGGGGCATTAAAGCAGCTCCAGGCGAGAGTCCTGGCTAT
AGAAAGATACCTACAGGATCAACAGCTCCTAGGGATTTGGGGCTGCTCTGG
AAAACCTCATCTGCACCACTGCTGTGCCTTGGAACGACAGTTGGAGTAATAAA
TCTCAAACAGATATTTGGGAGAACATGACCTGGATGCAGTGGGATAGAGAA
ATTAGTAGACACACAGACACAATATACAGGTTGCTTGAAGACTCACAAAACC
AGCAGGAGAAAAATGAAAAAGATTTATTAGCATTGGACAGTTGGAAAAATTT
GTGGAATTGGTTTAGCATACAAGGTGGCTGTGGTATATAAAAATATTCATA

FIGURE 16

34/40

ATGATAGTAGGAGGCCTGATAGGTTTGAGAATAATTTTTGCTGTGCTCTCGA
TAGTGAATAGAGTTAGGCAGGGATACTCACCATTATCGTTTCAGACCCACCT
CCCACCTCCGAGGGGAGCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTG
GAGAGAGAGACAGAGACAGATCCATTGATTAGTGACCGGATCCTTAGCAC
TTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGA
GAGACTTACTCTTGATTGTAACGAGGACTGTGGAACCTCCTGGGACGCAGAG
GGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTACTGTATTGGAGTCAGG
AACTAAAGAATAGTGCTGTTAGCTTGCTCAACGCCACAGCCATAGCAGTAAG
ACAATATGGGTGGAGCTATTTCCATGAGGCGGTCCAGGCCGTCTGGAGATC
TGCGACAGAGACTCTTGCGGGCGCGTGGGGAGACTTATGGGAGATTCTTA
GGAGAGGTGGAAGATGGATACTCGCAATCCCCAGGAGGATTAGACAAGGG
CTCGAGCTCACTCTCTTGAGAGGACAGAAATACAATCAGGGACAGCATAT
GAATACTCCATGGAGAAACCCAGCTGAAGAGGGAGAAAAATTAGCATAACAG
AAAACAAAATATGGATGATATAGATGAGGAAGATGATGACTTGGTAGGGGTA
TCAGTGAGGCCAAAAGTTCTCCTAAGAACAATGAGTTACAAATTGGCAATAG
ACATGTCTCATTTTATAAAAGAAAAGGGGGGACTGGAAGGGATTTATTACAG
TGCAAGAAGACATAGAATCTTAGACATATACTTAGAAAAGGAAGAAGGCATC
ATACCAGATTGGCAGGATTACACCTCAGGACCAGGAATTAGATACCCAAAG
ACATTTGGCTGGCTATGGAAATTAGTCCCTGTAGATGTATCAGATGAGGCAC
AGGAGGATGAGGAGCATTACTTAATGCATCCAGCTCAAACCTTCCCAGTGGG
ATGACCCTTGGGGAGAGGTTCTAGCATGGAAGTTTGATCCAACCTCTGGCCT
ACACTTATGAGGCATATGTTAGATACCCAGAAGAGTTTGGAAGCAAGTCAG
GCCTGTCAGAGGAAGAGGTTAGAAGAAGGCTAACCGCAAGAGGCCTTCTTA
ACATGGCTGACAAGAAGGAACTCGCTGAAACAGCAGGGACTTTCCACAAG
GGGATGTTACGGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACT
TTCTTGATGTATAAATATCACTGCATTTTCGCTCTGTATTAGTCGCTCTGCGG
AGAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTA
GAGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCGGTGCTGG
GCAGAGTGACTCCACGCTTGCTTACTTAAAGCCCTCTTCAATAAAGCTGCCA
TTTAGAAGTA

SEQ ID NO:9

FIGURE 16 (CONTINUED)

SUBSTITUTE SHEET (RULE 26)

35/40

MRVKEYQHLLWRWGWRWGIMLLGMLMICSATEKLWVTVYYGVPVWKEAKT
TLFCASNAKAYEKEVHNIWATHACVPTDPNPQEIVLGNVTENFNMWKNDMVD
QMHEDIISLWDQSLKPCVKLTSLCVTLKCSNFTGKSNVTYKGDMEVKNCSFN
VTTEIRDKKQKVYALFYRLDITPLDDNSSEYILINCNSSTITQACPKVNFDPIPIH
YCAPAGYAILKCNNKTFNGTGPCHNVTSTVQCTHGIKPVVSTQLLLNGSLAEGE
IIIRSENLTDNVKTIIVHFNESVEITCTRPNNNTRKSISIGPGQAIYATGDIIGDIRQ
AHCNISKENWNKTLQWVRGKLKEHFPNKTIVFKPSSGGDLEITTHSFNCRGEF
FYCNTSKLFNSTDNSTHMGTEENNTIITPCRIKQIINMWQEVGRAMYAPPIEGNI
TCKSNITGLLLVRDGGWDNSTNDTETFRPGGGDMRDNRSELYKYKVVEVK
PLGIAPTKAKRRVVEREKRAVGIGAVFLGFLGAAGSTMGAASITLTVQARQLL
SGIVQQQDNLLRAIEAQQHMLQLTVWGIKQLQARVLAIERYLQDQQLLGIWGC
SGKLICTTAVPWNDSSWSNKSQTDIWENMTWMQWDREISRHTDTIYRLLED
QNQQEKNEKDLLALDSWKNLWNWFSITRWLWYIKIFIMIVGGLIGLRIIFAVLSI
VNRVRQGYSPLSFQTHLPLPRGADRPEGIEEEGGERDRDRSIRLVGTGSLALI
DDLRLCLFSYHRLRDLLIVTRTVELLGRRGWEALKYWWNLLLYWSQELKN
SAVSLLNATAIAVRQYGWSYFHEAVQAVWRSATETLAGAWGDLWEILRRGG
RWILAIPRRIRQGLELTLL*

SEQ ID NO: 10

FIGURE 17

SUBSTITUTE SHEET (RULE 26)

SHIV-1157i Nef MGGAI SMRRSRPSGDLRQRLLRARGETYGRLLGEVEDG
SHIV-1157ipd Nef -----F-----

YSQSPGGLDKGLSSLSCGQKYNQGYMNTPWRNPAEEREKLAYRKQNMDDIDEEDDDL
-----S-----H-----G-----

VGVSVRPKVPLRTMSYKLAIDMSHFKEKGGLEGIYSARRHRILDIYLEKEEGIIPDW
-----L-----

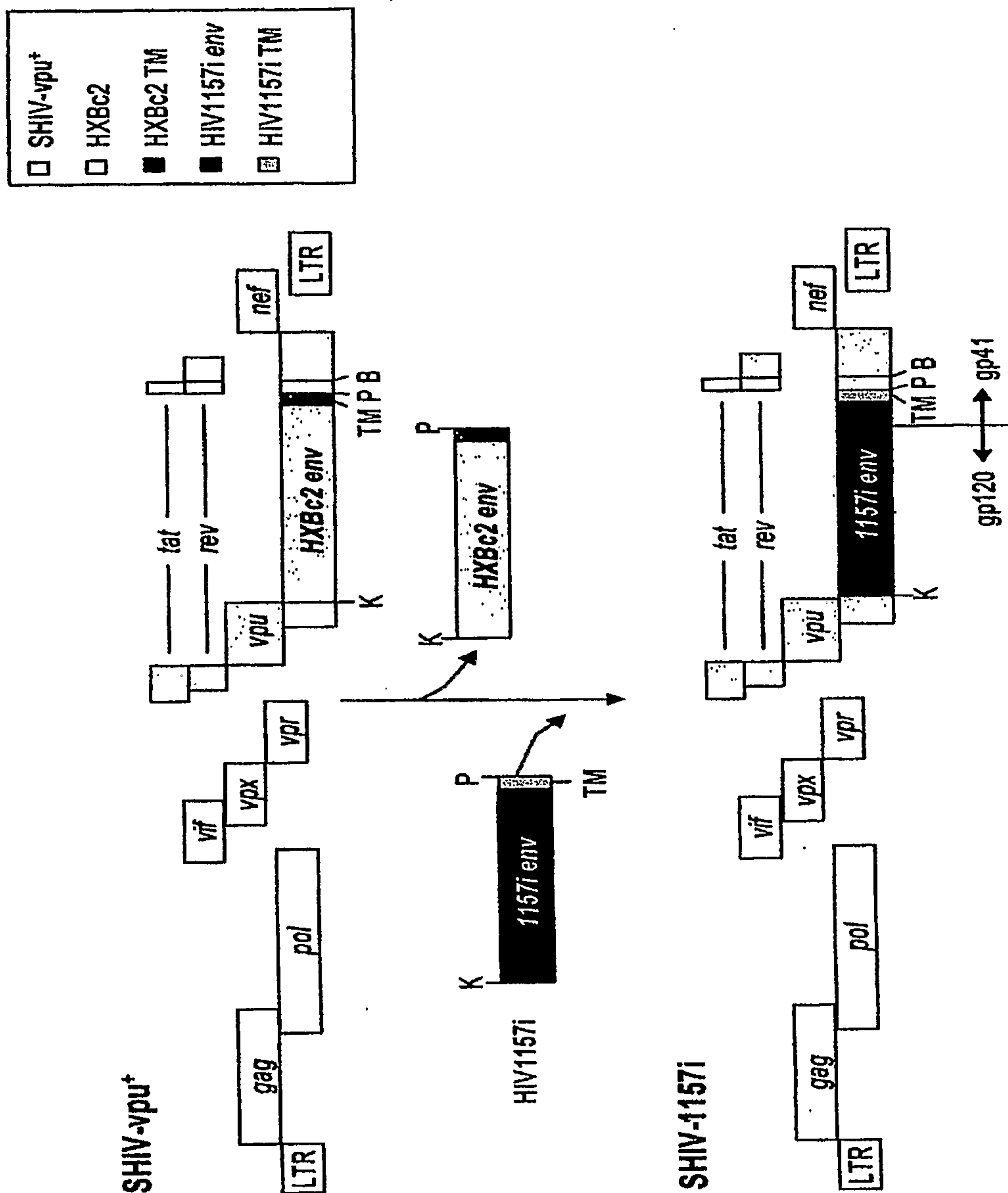
QDYTSGPGIRYPKTFGWLWKLVPVNVSDAQEDEEHYLMHPAQTSQWDDPWGEVLAWKF
-----D-----

DPTLAYTYEAYVRYPEEFGSKSGLSEEEVRRRLTARGLLNMAKKETR

SEQ ID NO: 11

FIGURE 18

37/40



38/40

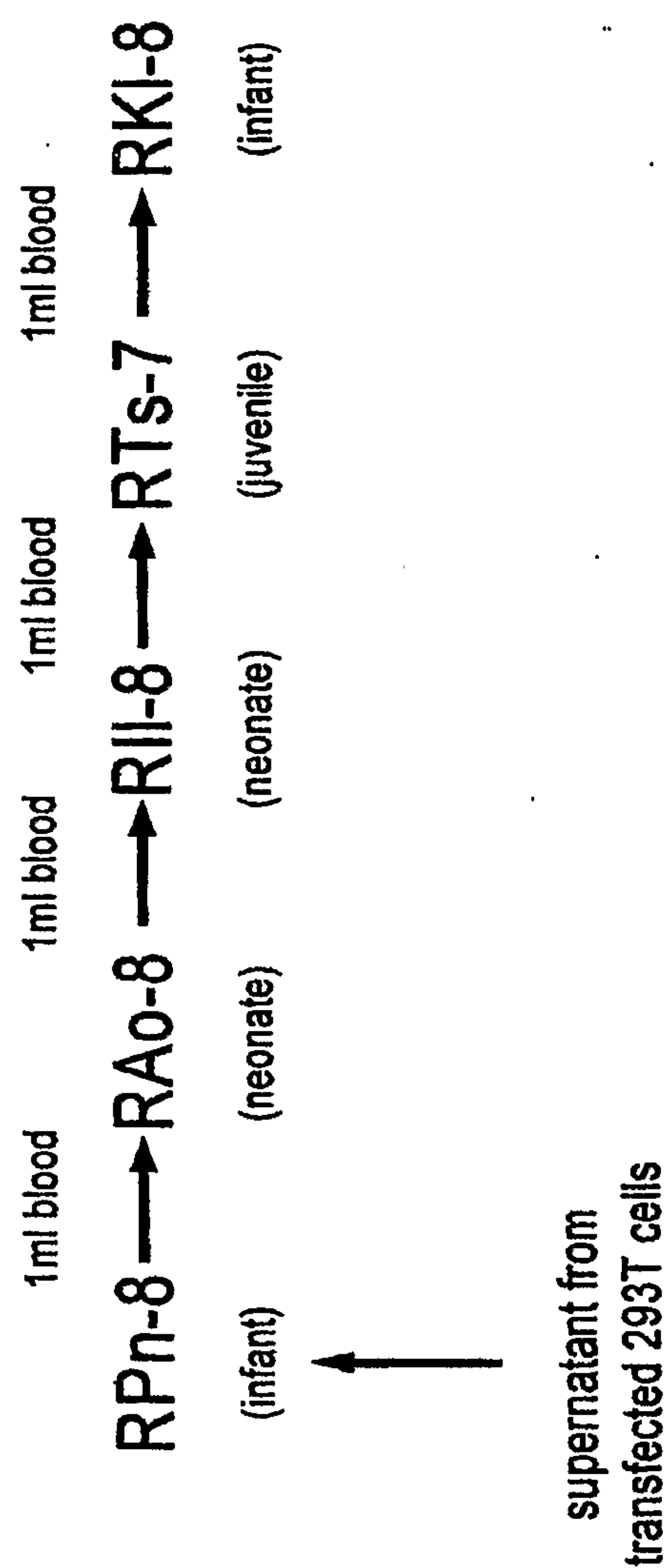


FIGURE 19B

39/40

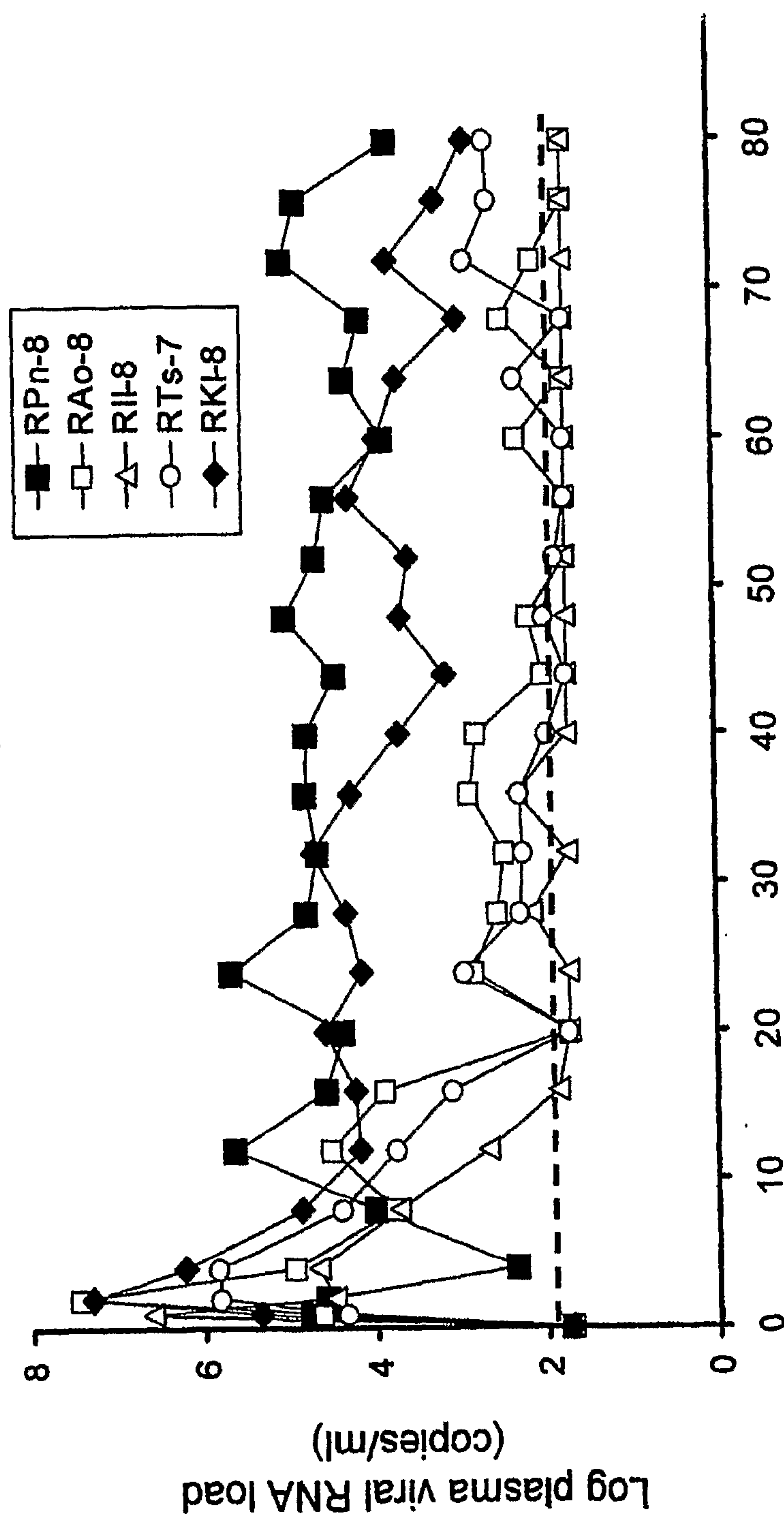


FIGURE 19C

40/40

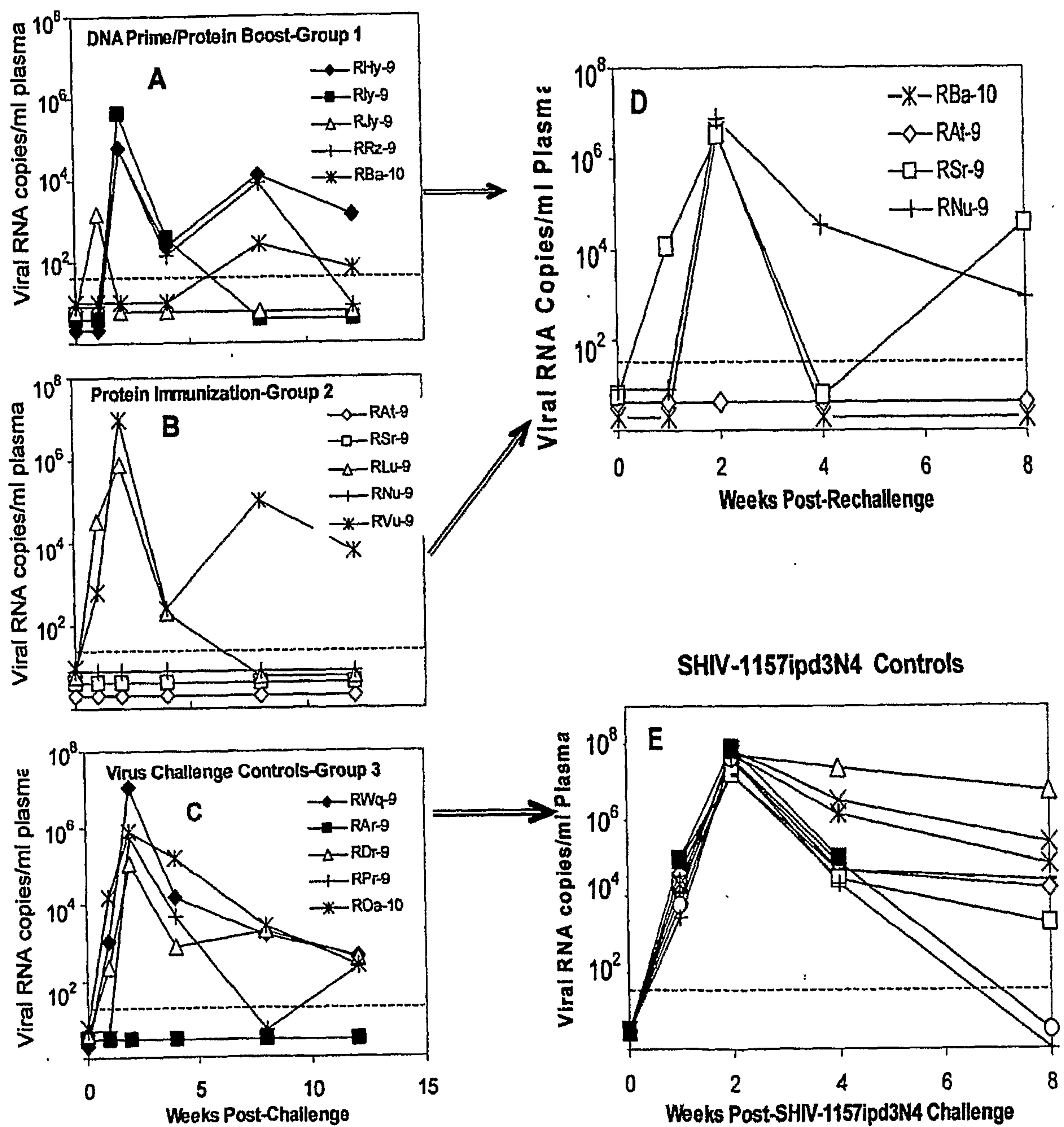


FIGURE 20

[illegible]