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Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(54) Title: VACCINE

(57) Abstract: The present invention relates, in general, to human immunodeficiency virus (HIV) and, in particular, to HIV-I envelope (Env) immunogens.

WO 2008/127596 A1

VACCINE

This application claims priority from U.S. Provisional Application No. 60/907,719, filed April 13, 2007, the entire content of which is incorporated herein by reference.

This invention was made with government support under Grant No. AI067854 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

The present invention relates, in general, to human immunodeficiency virus (HIV) and, in particular, to HIV-1 envelope (Env) immunogens.

BACKGROUND

It has been hypothesized that some of the quantitative and qualitative abnormalities in immune responses in HIV-1 infection may be due to the presence of immunosuppressive activity of gp160 mediated by Env superantigen (SA) activity (Karray et al, Proc. Natl. Acad. Sci. USA 94(4):1356-1360 (1997)) or by immunosuppressive effects of gp120 binding to CD4 on T cells, macrophages or DCs (Pantaleo et al, N. Engl. J. Med. 328(5):327-335 (1993), Vingerhoets et al, Clin. Exp. Immunol. 111(1):12-19 (1998)). The present invention results, at least in part, from studies designed to test this hypothesis. These studies included the production of HIV-1 Envs that express epitopes to which broadly neutralizing antibodies can bind and the mutation of such Envs such that they have no superantigen activity and/or they cannot bind immune cell CD4 in an immunosuppressive manner. The present invention relates to such mutated envelopes and to methods of inducing an immune response using same.

SUMMARY OF THE INVENTION

The present invention relates generally to HIV and, more specifically, to immunogenic compositions and methods of inducing an immune response against HIV using same.

Objects and advantages of the present invention will be clear from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic structure of HIV-1 JRFL Env and mutant JRFL Envs with mutation at CD4 binding site and superantigen motif.

Figure 2. Western blot analysis and ELISA assay of HIV-1 JRFL mutant gp140 Envs.

Figure 3. Surface plasma resonance analysis of HIV-1 JRFL mutant gp140 Envs.

DETAILED DESCRIPTION OF THE INVENTION

The invention is exemplified below with respect to HIV-1 envelope (Env) which contains various antigenic epitopes such as CD4 binding site, variable loops, MPER 4E10 and 2F5 neutralizing epitopes as well as other neutralizing epitopes. HIV-1 Envs used as immunogens to date induce antibodies that only neutralize selected HIV-1 primary isolates. To test the hypothesis that one reason that broadly neutralizing antibodies cannot be made is due to SAg activity and or CD4 binding immunosuppressive activity, a strategy has been developed for: 1) removing the

SAg-binding motif on HIV-1 Env gp140CF oligomer, and 2) disrupting the CD4 binding site of HIV Env oligomer.

HIV-1 subtype B primary isolate JRFL is a tier 2 virus that is a relatively difficult isolate to neutralize, yet has both MPER 4E10 and 2F5 gp41 broadly neutralizing epitopes expressed well on this oligomer (Liao et al, Virology 353:268-282 (2006)). A JRFL gp140 WT immunogen induced antibodies that neutralized only a select few subtype B isolates but did not neutralize its autologous JRFL isolate (Liao et al, Virology 353:268-282 (2006)). Experiments were performed using JRFL Env 140 oligomer as a prototype (see Example below).

Three mutant JRFL gp140 expression constructs were designed and generated (Fig. 1) using pcDNA3.1 plasmid (Invitrogen, Carlsbad, CA). Stably transfected 293T cell lines have been established to produce recombinant JRFL gp140 with CD4 binding site mutated (JRFLΔCD4BS), JRFL gp140 with deletions of SA binding motif (JRFLΔSAg) and JRFL gp140 with both CD4 binding site and superantigen motif mutated (JRFLΔCD4BS-SAg). Recombinant proteins of all three were expressed and purified from the supernatants of the stably transfected 293T cell lines by lectin columns (Fig. 2A). Western blot analysis using HIV-1 gp120 MAb T8, JRFL mutant Envs with or without deglycosylation with PNGase digestion showed no differences in apparent migration patterns in SDS-PAGE under reducing or non- reducing conditions in comparison with the wild-type JRFL Env (Fig. 2A). ELISA assays demonstrated that mutation either at the CD4 binding site or at the SA motif maintained the ability to bind gp120 MAb T8 and MPER MAbs 2F5 and 4E10, while abrogated the ability of these mutant Envs to bind CD4 and CD4 binding site MAb, 1B12 (Fig. 2B). JRFLΔSAg mutant Env also lost the ability to bind to CD4i MAb A32 (Fig. 2B).

Functional and antigenic epitopes on JRFL Env mutants were further characterized by surface plasma resonance analysis (Fig. 3). It has been found that JRFLΔCD4BS Env strongly bound HIV gp120 MAb T8 and bound MAb A32 at

low levels (Fig. 3A), while no constitutive binding of MAb 17B, or anti-gp41 MAb 7B2 binding to JRFLΔCD4BS Env was observed. Substitution of amino acids DPE with APA at one of CD4 binding touch points completely abolished the ability of JRFLΔCD4BS Env to bind CD4 (Fig. 3A). Various anti-HIV-1 V3 antibodies also bound to both JRFL gp140 Env (Fig. 3B, solid lines) as well as to JRFLΔCD4BS gp140 Env (Fig. 3B, broken lines). HIV-1 MPER neutralizing epitopes were preserved as HIV-1 MPER mAbs 2F5 and 4E10 bound in comparable levels to both JRFL gp140 (Fig. 3C, solid line) and JRFLΔCD4BS gp140 (Fig. 3C, broken line). However JRFLΔCD4BS gp140 did not bind to the non-neutralizing murine MPER MAb 5A9, which bound to JRFL gp140 with low avidity, while strong binding of human cluster II MAb 98-6 and 126-6 to both JRFL gp140 (Fig. 3D, solid lines) and JRFLΔCD4BS gp140 (Fig. 3D, broken lines) was observed. A study of the functional and immunogenic properties of JRFL Env with mutations at both CD4 binding site and SA motif are in progress.

The immunogen of one aspect of the invention comprises an envelope either in soluble form or anchored, for example, in cell vesicles or in liposomes containing translipid bilayer envelope. To make a more native envelope, sequences can be configured in lipid bilayers for native trimeric envelope formation. Alternatively, the invention, in the form of gp160, can be used as an immunogen.

The immunogen of the invention can be formulated with a pharmaceutically acceptable carrier and/or adjuvant (such as alum or oCpG) using techniques well known in the art. Suitable routes of administration of the present immunogen include systemic (e.g., intramuscular or subcutaneous). Alternative routes can be used when an immune response is sought in a mucosal immune system (e.g., intranasal).

The immunogens of the invention can be chemically synthesized or synthesized using well-known recombinant DNA techniques. Nucleic acids

encoding the immunogens of the invention can be used as components of, for example, a DNA vaccine wherein the encoding sequence is administered as naked DNA or, for example, a minigene encoding the immunogen can be present in a viral vector. The encoding sequence can be present, for example, in a replicating or non-replicating adenoviral vector, an adeno-associated virus vector, an attenuated mycobacterium tuberculosis vector, a Bacillus Calmette Guerin (BCG) vector, a vaccinia or Modified Vaccinia Ankara (MVA) vector, another pox virus vector, recombinant polio and other enteric virus vector, Salmonella species bacterial vector, Shigella species bacterial vector, Venezuelean Equine Encephalitis Virus (VEE) vector, a Semliki Forest Virus vector, or a Tobacco Mosaic Virus vector. The encoding sequence, can also be expressed as a DNA plasmid with, for example, an active promoter such as a CMV promoter. Other live vectors can also be used to express the sequences of the invention. Expression of the immunogen of the invention can be induced in a patient's own cells, by introduction into those cells of nucleic acids that encode the immunogen, preferably using codons and promoters that optimize expression in human cells. Examples of methods of making and using DNA vaccines are disclosed in U.S. Pat. Nos. 5,580,859, 5,589,466, and 5,703,055.

The invention further relates to a composition comprising an immunologically effective amount of the immunogen of this invention, or nucleic acid sequence encoding same, in a pharmaceutically acceptable delivery system. The compositions can be used for prevention and/or treatment of immunodeficiency virus infection. The compositions of the invention can be formulated using adjuvants, emulsifiers, pharmaceutically-acceptable carriers or other ingredients routinely provided in vaccine compositions. Optimum formulations can be readily designed by one of ordinary skill in the art and can include formulations for immediate release and/or for sustained release, and for induction of systemic immunity and/or induction of localized mucosal immunity

(e.g, the formulation can be designed for intranasal administration). The present compositions can be administered by any convenient route including subcutaneous, intranasal, oral, intramuscular, or other parenteral or enteral route. The immunogens can be administered as a single dose or multiple doses. Optimum immunization schedules can be readily determined by the ordinarily skilled artisan and can vary with the patient, the composition and the effect sought.

The invention contemplates the direct use of both the immunogen of the invention and/or nucleic acids encoding same and/or the immunogen expressed as minigenes in the vectors indicated above. For example, a minigene encoding the immunogen can be used as a prime and/or boost.

Certain aspects of the invention are described in greater detail in the non-limiting Example that follows. (See also US Appln. No. 10/572,638.)

EXAMPLE 1

Cloning of JRFL Env gp140CF with mutation at the CD4 binding site. The amino acid sequence at position 358 to 360 (DPE) was one of touch points when HIV-1 Env binds to CD4 (Kwong et al, Nature 398:648-659 (1998)). To mutate CD4 binding site on JRFL Env, 2 pairs of the mutagenic primers were designed and synthesized for use in PCR (Table 1) to introduce mutations in gene sequence by changing the coding sequence for DPE to the coding sequence for APA by PCR. HIV-1 JRFL gp140CF gene construct (Liao et al, Virology 353:268-282 (2006)) was used as template in PCR amplification to produce JRFL Env mutant genes. Two sets of the first round PCR were performed to introduce the site-specific mutations and generate the first half and the second half of the JRFL140 DNA fragments. The first half JRFL 140 DNA fragment was amplified by using the primer pair of the forward primer (JRFL-F1) and reverse primer (JRFL-mut1165). The second half JRFL 140 DNA fragment was amplified by

using the primer pair of the forward primer (JRFL-mutF1142) and reverse primer (JRFL-R1978) (Table 1). The amplified two JRFL DNA fragments from these 2 sets of PCR (10ng of each) were used as templates for the second round of PCR to produce the full-length JRFL 140 gene using the primer pair of JRFL-F1 and JRFL-R1978. All PCRs were carried out in total volume of 50:1 using 1 unit of AccuPrime Taq Polymerase High Fidelity (Invitrogen; Carlsbad, CA), and 50 pmol of each primer. The PCR thermocycling conditions were as follows: one cycle at 94°C for 1 min; 25 cycles of a denaturing step at 94°C for 30 sec, an annealing step at 55°C for 30 sec, an extension step at 68°C for 2min; and one cycle of an additional extension at 68°C for 5 min. The resulting full-length JRFL 140 DNA fragment was purified with PCR purification column (Qiagen) and enzymatic digestion with restriction enzyme SalI and BamHI, and then cloned to expression vector pcDNA3.1 (-)/Hygro (Invitrogen Co, CA) via Xba I and BamH I site. The resulting DNA clones of JRFL with the CD4 BS mutated (pJRFLΔCD4 BS) were validated by DNA sequencing of full-length of the gene construct.

List of **Table 1**PCR primers used in PCR.

Primer Name	Primer Sequence (5′ to 3′)	Purpose
JRFL-F1	TTCAGCTAGC GTCGACGACCATGCCCATGGGGTCTCTGC	
JRFL-R1978	GTGTGTGGATCCGGTACCCTACCACAGCCACTTGGTGATGTC	
JRFL-mutF1142	GGTGGTGCCCCTGCCATTGTGATGCACAGCTTCAACTGTGGTGGTGAGTTCTTC	CD4 BS mutant
JRFL-mutR1165	CATCACAATGGCAGGGGCACCACCAGAGCTGTGATTGAACAC	
JRFL-mutF1128	CAGCACCCAGGCGGCCGCCAGCACCTGGAACAACAACACTGAGGGCAGCAACAACACTGA GGCAACACCATCACCCTGCCTTGCAGGGCCGCGGCGATCATCAACATGTGGCAG	Super antiger mutant
JRFL-mutR1237	CATGTTGATGATCGCCGCGGCCCTGCAAGGCAGGGTGATGGTGTTGCCCTCAGTGTTGTT CTGCCCTCAGTGTTGTTGTTCCAGGTGCTGGCGGCCGCCTGGGTGCTGTTGCAG	Super antiger mutant

Cloning of JRFL Env gp140CF with mutation at the superantigen (SAg) motif. The superantigen-binding site is formed by protein sequences from two regions of HIV-1 gp120. The core motif is a discontinuous epitope spanning the V4 variable region and the amino-terminal region flanking the C4 constant domain. The amino acid sequence at position 358 to 360 (APA) was one of touch points when HIV-1 Env binds to CD4 (Karray et al, Proc. Natl. Acad. Sci. USA 94(4):1356-1360 (1997)). To disrupt the superantigen binding site, a primer pair (Table 1, JRFL-F1128 and JRFL-R1237) was designed to change the coding sequence for LFN at the SAg1 region to the coding sequence for AAA and change the coding sequence for IKQ at the SAg2 region to the coding sequence for AAA (Fig. 1). HIV-1 JRFL gp140CF gene construct (Liao et al, Virology 353:268-282 (2006)) was used as template in PCR amplification to produce JRFL Env mutant genes. Two sets of the first round PCR were performed to introduce the site-specific mutations and generate the first half and the second half of the JRFL140 DNA fragments. The first half JRFL 140 DNA fragment was amplified by using the primer pair of the forward primer (JRFL-F1) and reverse primer (JRFL-mut-R1237). The second half JRFL 140 DNA fragment was amplified by using the primer pair of the forward primer (JRFL-mut F1128) and reverse primer (JRFL-R1978) (Table 1). The amplified two JRFL DNA fragments from these 2 sets of PCR (10ng of each) were used as templates for the second round of PCR to produce the full-length JRFL 140 gene using the primer pair of JRFL-F1 and JRFL-R1978. All PCRs were carried out in total volume of 50μl using 1 unit of AccuPrime Taq Polymerase High Fidelity (Invitrogen; Carlsbad, CA), and 50 pmol of each primer. The PCR thermocycling conditions were as follows: one cycle at 94°C for 1 min; 25 cycles of a denaturing step at 94°C for 30 sec, an annealing step at 55°C for 30 sec, an extension step at 68°C for 2min; and one cycle of an additional extension at 68°C for 5 min. The resulting full-length JRFL

140 DNA fragment was purified with PCR purification column (Qiagen) and enzymatic digestion with restriction enzyme SalI and BamHI, and then cloned to expression vector pcDNA3.1 (-)/Hygro (Invitrogen Co, CA) via Xba I and BamH I site. The resulting DNA clones of JRFL with the superantigen binding region mutated (pJRFLΔSAg) were validated by DNA sequencing of full-length of the gene construct.

Cloning of JRFL Env gp140CF with mutations at both CD4BS and the superantigen (SAg) motif. To disrupt both CD4BS and the superantigen binding site, HIV-1 JRFLΔCD4SAg DNA construct was used as template in PCR amplification to produce JRFL Env mutant genes. Two sets of the first round PCR were performed to introduce the site-specific mutations and generate the first half and the second half of the JRFL140 DNA fragments. The first half JRFL 140 DNA fragment was amplified by using the primer pair of the forward primer (JRFL-F1) and reverse primer (JRFL-mut1237). The second half JRFL 140 DNA fragment was amplified by using the primer pair of the forward primer (JRFL-mutF1128) and reverse primer (JRFL-R1978) (Table 1). The amplified two JRFL DNA fragments from these 2 sets of PCR (10ng of each) were used as templates for the second round of PCR to produce the full-length JRFL 140 gene using the primer pair of JRFL-F1 and JRFL-R1978. All PCRs were carried out in total volume of 50μl using 1 unit of AccuPrime Taq Polymerase High Fidelity (Invitrogen; Carlsbad, CA), and 50 pmol of each primer. The PCR thermocycling conditions were as follows: one cycle at 94°C for 1 min; 25 cycles of a denaturing step at 94°C for 30 sec, an annealing step at 55°C for 30 sec, an extension step at 68°C for 2min; and one cycle of an additional extension at 68°C for 5 min. The resulting full-length JRFL 140 DNA fragment were purified with PCR purification column (Qiagen) and enzymatic digestion with restriction enzyme SalI and BamHI , and then cloned to expression vector pcDNA3.1 (-)/Hygro

(Invitrogen Co, CA) via Xba I and BamH I site. The resulting DNA clones of JRFL with the CD4 BS mutated (pJRFLΔCD4BS-SAg) were validated by DNA sequencing of full-length of the gene construct.

Generation of Stable Cell Lines and Expression: A human cell line 293T was used for establishing a stably transfected cell lines for expressing mutant JRFL Envs. 293T cells in tissue culture plates were transfected with either pJRFLΔCD4BS, pJRFLΔCDBS-SAg, or pJRFLΔCD4BS-SAg plasmid. Stabley transfected 293T cell clones that were resistant to hygromycin were selected in culture medium containing 20% fetal bovine serum and hygromycin (200 μg/ml). Hygromycin-resistant clones were further cloned by the limiting dilution to select single colonies under hygromycin pressure (200 μg/ml). The individual cell lines that express JRFLΔCD4BS, JRFLΔCDBS-SAg, or JRFLΔCD4BS-SAg gene constructs were confirmed to being correct by DNA sequencing.

* * *

All documents and other information sources cited above are hereby incorporated in their entirety by reference.

WHAT IS CLAIMED IS:

1. An immunogen comprising an HIV-1 envelope (Env) protein comprising a CD4 binding site mutation or a superantigen (SAg) binding motif mutation.

2. The immunogen according to claim 1 wherein said Env protein comprises gp140.

3. The immunogen according to claim 1 wherein said immunogen comprises a CD4 binding site mutation and a SAg binding motif mutation.

4. The immunogen according to claim 1 wherein said Env protein comprises said CD4 binding site mutation and wherein said immunogen does not bind CD4.

5. The immunogen according to claim 2 wherein said mutation is at one or more of the amino acids at positions 358 to 360.

6. The immunogen according to claim 5 wherein the mutation results in amino acid sequence APA at positions 358 to 360.

7. The immunogen according to claim 1 wherein said Env protein comprises said SAg binding motif mutation.

8. The immunogen according to claim 7 wherein the sequence LFN at the SAg1 motif is mutated to AAA and the sequence IKQ at the SAg2 motif is mutated to AAA.

9. The immunogen according to claim 1 wherein monoclonal antibodies 2F5 or 4E10 bind said immunogen.

10. A composition comprising said immunogen according to claim 1 and a carrier.

11. A nucleic acid construct comprising a sequence encoding the immunogen according to claim 1.

12. A composition comprising the nucleic acid according to claim 11 and a carrier.

13. A method of inducing an immune response in a mammal comprising administering to said mammal an amount of the immunogen according to claim 1 sufficient to effect said induction.

14. A method of inducing an immune response in a mammal comprising administering to said mammal said nucleic acid according to claim 11 under conditions such that said sequence is expressed, said immunogen is produced and said induction is effected.

15. The method according to claim 13 or 14 wherein said mammal is a human.

Figure 1.

Schematic structure of HIV-1 JRFL Env and mutant JRFL Envs with mutation at CD4 binding site and superanitigen motif.

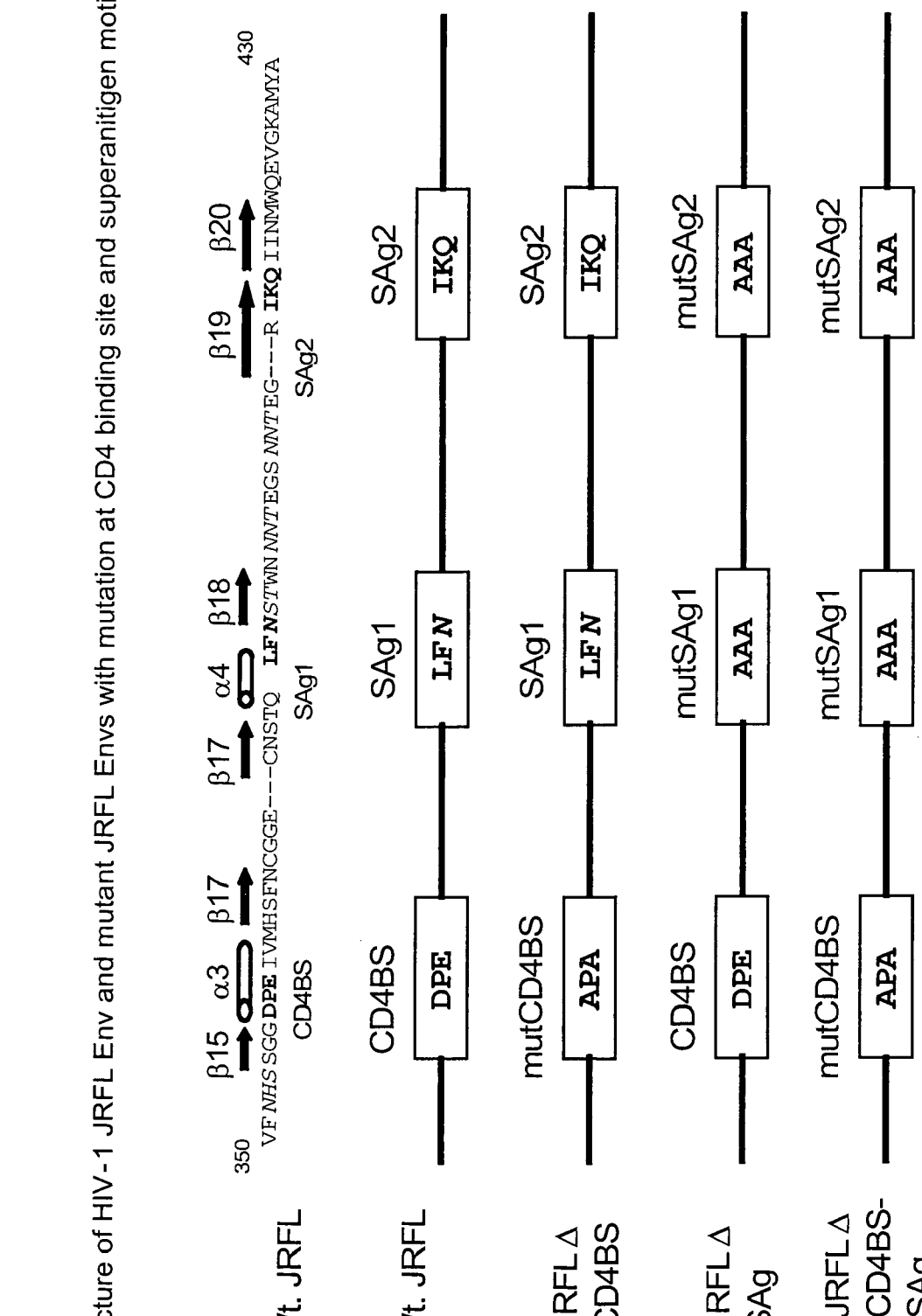
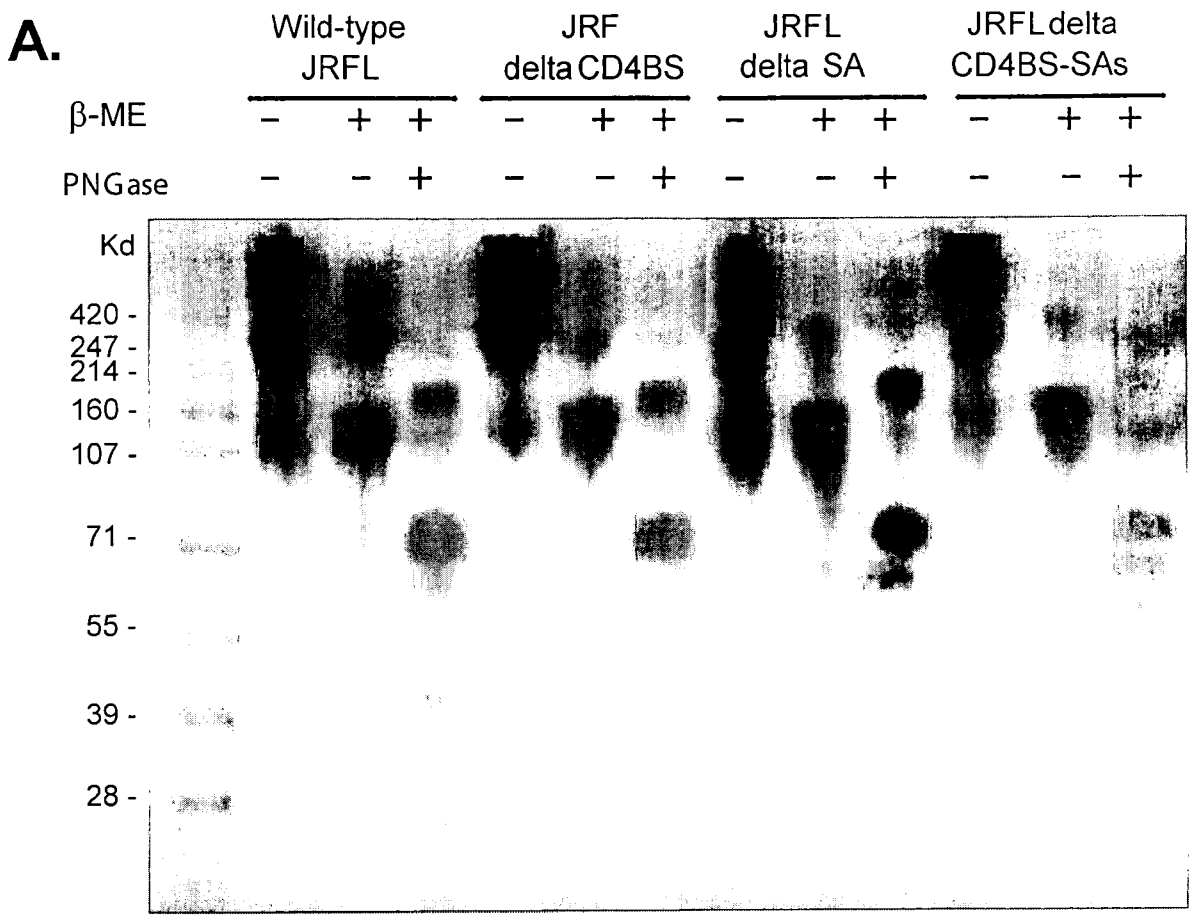
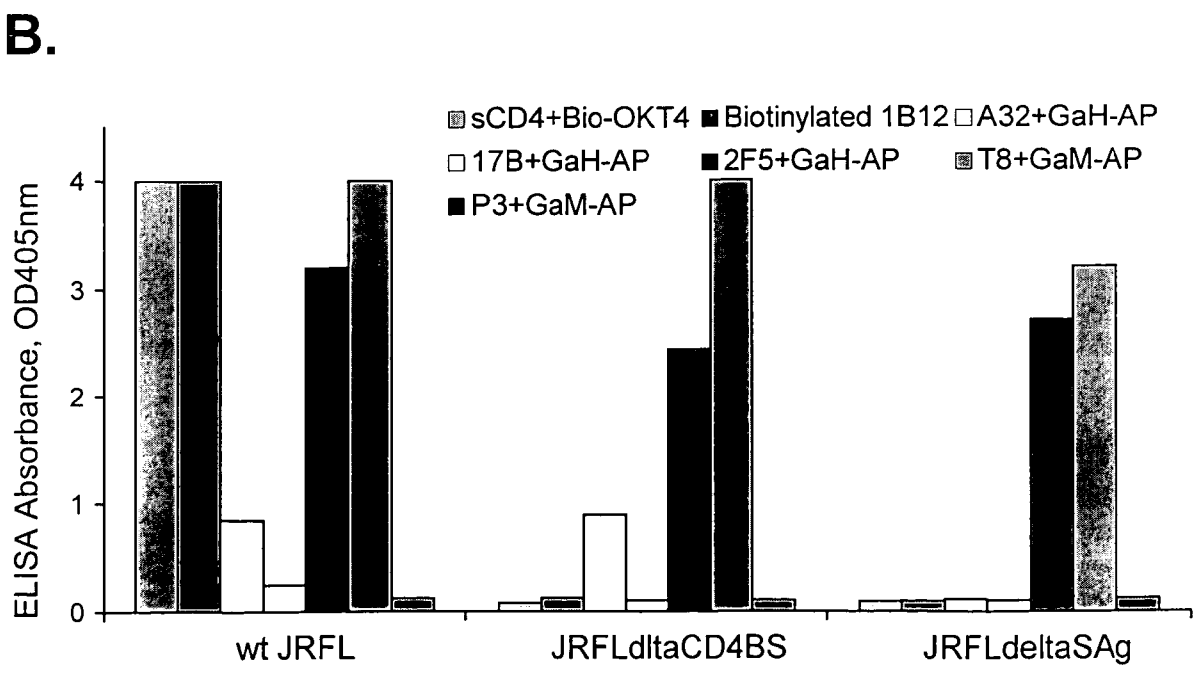


Figure 2.

Western blot analysis and ELISA assay of HIV-1 JRFL mutant gp140 Envs.





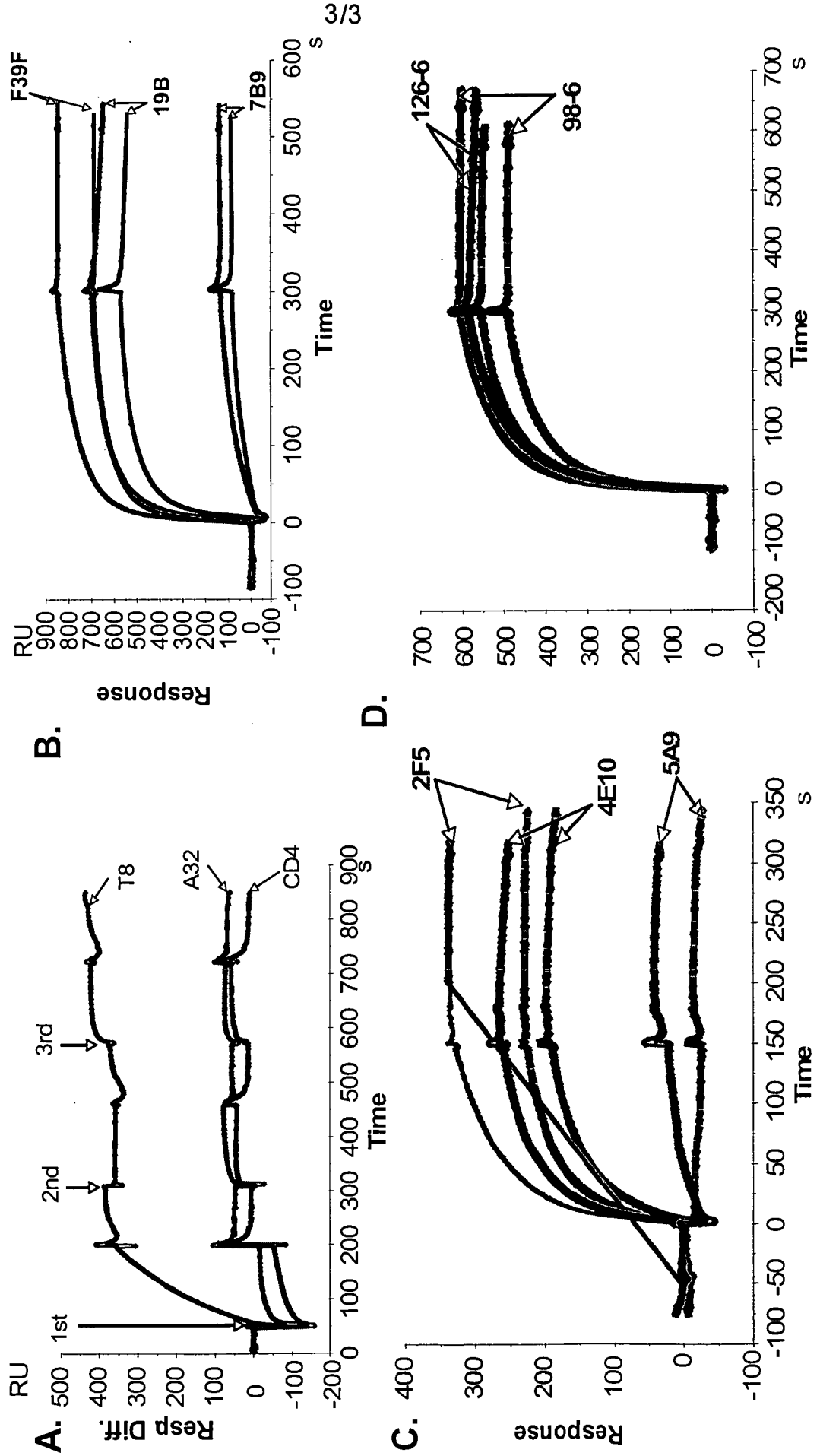


Figure 3. Schematic structure of HIV-1 JRFL Env and mutant JRFL Env with mutation at CD4 binding site and superantigen motif.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US08/04579

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **A61K 39/00**(2006.01)

USPC: 424/184.1,188.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/184.1,188.1

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	DUNFEE et al. The HIV Env variant N283 enhances macrophage tropism and is associated with brain infection and dementia. PNAS. 10 October 2006, Vol. 130, No. 41, pages 15160-15165, entire document.	1,2,10-12 ---------- 9,13-15
X --- Y	BOLMSTEDT et al. Effects of mutations in glycosylation sites and disulphide bonds on processing, CD4-binding and fusion activity of human immunodeficiency virus envelope glycoproteins. Journal of General Virology. 1991, Vol. 72, pages 1269-1277, entire document.	1,2,4,10-12 ---------- 9,13-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 July 2008 (03.07.2008)	04 SEP 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer NICOLE KINSEY WHITE Telephone No. (571) 272-1600

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US08/04579

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-12

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

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

Group I, claims 1-12, drawn to an immunogen comprising a CD4 binding site mutation or a SAg binding motif mutation and compositions comprising the immunogen.

Group II, claims 13-15, drawn to methods of inducing an immune response in a mammal.

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

The technical feature shared among the inventions listed as Groups I and II is an immunogen comprising a CD4 binding site mutation or a SAg binding motif mutation. The noted shared technical feature does not provide a contribution over the prior art, as evidenced by the teachings of Dunfee et al. (PNAS, 2006, 103(41):15160-15165). Dunfee et al. discloses mutant HIV envelope genes and proteins comprising a mutation in the CD4 binding site (see, for example, the abstract). Hence, in the absence of a contribution over the prior art, the noted shared technical feature is not a shared special technical feature. Without a shared special technical feature, the inventions listed as Groups I and II lack unity with one another.

Continuation of B. FIELDS SEARCHED Item 3:
Dialog database
In house patent database
Search terms: HIV, gp120, gp140, gp160, CD4, superantigen, SAg, mutation