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(71) Applicant *(for all designated States except US)*: **DUKE UNIVERSITY** [US/US]; P.O. Box 90083, Durham, NC 27708-0083 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HAYNES, Barton, F.** [US/US]; c/o Duke University, P.O. Box 90083, Durham, NC 27708-0083 (US). **ALAM, Munir, S.** [US/US]; c/o Duke University, P.O Box 90083, Durham, NC 27708-0083 (US). **LIAO, Hua-Xin** [US/US]; c/o Duke University, P.O. Box 90083, Durham, NC 27708-0083 (US).

(74) Agent: **WILSON, Mary, J.**; Nixon & Vanderhye P.C., 901 North Glebe Road, 11th Floor, Arlington, VA 22201-4714 (US).

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(54) **Title:** METHOD OF INDUCING NEUTRALIZING ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS

(57) **Abstract:** The present invention relates, in general, to human immunodeficiency virus (HIV), and, in particular, to a method of inducing neutralizing antibodies to HIV and to compounds and compositions suitable for use in such a method.

METHOD OF INDUCING NEUTRALIZING ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS

This application claims priority from U.S. Prov. Appln. No. 60/670,243, filed April 12, 2005, U.S. Prov. Appln. No. 60/675,091, filed
5 April 27, 2005, U.S. Prov. Appln. No. 60/697,997, filed July 12, 2005, and U.S. Prov. Appln. No. 60/757,478, filed January 10, 2006, the entire contents of which are incorporated herein by reference.

TECHNICAL FIELD

The present invention relates, in general, to human
10 immunodeficiency virus (HIV), and, in particular, to a method of inducing neutralizing antibodies to HIV and to compounds and compositions suitable for use in such a method.

BACKGROUND

The first antibodies that are made in acute HIV-1 infection are
15 against the CD4 binding site (Moore et al, J. Virol. 68(8) 5142 (1994)), the CCR5 co-receptor binding site (Choe et al, Cell 114(2):161-170 (2003)), and the V3 loop (Moore et al, J. Acquir. Immun. Def. Syn. 7(4):332 (1994)). However, these antibodies do not control HIV-1 and are easily escaped (Burton et al, Nature Immun. 5:233-236 (2004), Wei et al, Nature
20 422(6929):307-312 (2003)). Neutralizing antibodies against autologous virus develop fifty to sixty days after infection, but antibodies capable of neutralizing heterologous HIV-1 strains do not arise until after the first year of infection (Richman et al, Proc. Natl. Acad. Sci. USA 100(7):4144-4149 (2003), Wei et al, Nature 422(6929):307-312 (2003)).

The four epitopes on HIV-1 envelope to which rare broadly reactive neutralizing antibodies bind are the CD4 binding site (CD4BS) (mab (monoclonal antibody) IgG1b12) (Zwick et al, J. Virol. 77(10):5863-5876 (2003)), the membrane proximal external region (MPER) epitopes defined
5 by human mabs 2F5 and 4E10 (Armbruster et al, J. Antimicrob. Chemother. 54:915-920 (2004), Stiegler and Katinger, J. Antimicrob. Chemother. 51:757-759 (2003), Zwick et al, Journal of Virology 79:1252-1261 (2005), Purtscher et al, AIDS 10:587 (1996)) (Fig. 1), and the mannan glycan epitope defined by human mab 2G12 (Scanlan et al, Adv.
10 Exper. Med. Biol. 535:205-218 (2003)). These four rare human mabs are all unusual: two are IgG3 (2F5 and 4E10), one has a unique Ig dimer structure (2G12), one has a very hydrophobic CDR3 (2F5) (Ofek et al, J. Virol. 198:10724 (2004)), and, in all four, the CDR3 is unusually long (Burton et al, Nature Immunol. 5(3):233-236 (2004), Kunert et al, AIDS
15 Res. Hum. Retroviruses 20(7):755-762 (2004), Zwick et al, J. Virol. 78(6):3155-3161 (2004), Cardoso et al, Immunity 22:163-172 (2005)). Of these, 2F5- and 4E10-like human mabs are quite rare. Acute HIV-1 patients do not make antibodies against the MPER or 2G12 epitopes (Robinson, unpublished (2005), Shaw, unpublished (2005), MPER can be
20 defined as amino acids 652 to 683 of HIV envelope (Cardoso et al, Immunity 22:163-173 (2005) (e.g., QQEKNEQELLELDKWASLWNWFDITNWLWYIK). CD4 binding site (BS) antibodies are commonly made early in HIV-1 infection, but these antibodies generally do not have the broad spectrum of neutralization
25 shown by mab IgG1b12 (Burton et al, Nat. Immunol. 5(3):233-236 (2004)).

A number of epitopes of the HIV-1 envelope have been shown to cross-react with host tissues (Pinto et al, AIDS Res. Hum. Retrov. 10:823-828 (1994), Douvas et al, AIDS Res. Hum. Retrov. 10:253-262 (1994),

Douvas et al, AIDS Res. Hum. Retrov. 12:1509-1517 (1996)), and autoimmune patients have been shown to make antibodies that cross-react with HIV proteins (Pinto et al, AIDS Res. Hum. Retrov. 10:823-828 (1994), Douvas et al, AIDS Res. Hum. Retrov. 10:253-262 (1994), Douvas
5 et al, AIDS Res. Hum. Retrov. 12:1509-1517 (1996), Barthel et al, Semin. Arthr. Rheum. 23:1-7 (1993)). Similarly, induction of immune responses to self-epitopes has been suggested to be a cause of the autoimmune abnormalities and T cell depletion in AIDS (Douvas et al, AIDS Res. Hum. Retrov. 12:1509-1517 (1996), Ziegler et al, Clin. Immunol. Immunopath.
10 41:305-313 (1986)).

High affinity peptide ligands for the 2F5 mab have been made that induce high levels of antibody against the peptide but do not broadly neutralize HIV-1 primary isolates (McGaughey et al, Biochemistry 42(11):3214-3223 (2003), Zhang et al, J. Virol. 78(15):8342-8348 (2004),
15 rev. in Zwick et al, J. Virol. 79:1252-1261 (2005)). These results have been interpreted to mean that the peptide ligands for 2F5 are not in the appropriate conformation for induction of anti-MPER antibodies (Burton et al, Nature Immunology 5(3):233-236 (2004), Zwick et al, J. Virol. 79:1252-1261 (2005)). A series of highly constrained HIV-1 Env immunogens have
20 been made with the IgG1b12, 2G12, 2F5 and 4E10 epitopes stably expressed, and it has been demonstrated that these immunogens do not induce broadly reactive neutralizing antibodies in guinea pigs or rabbits, and, specifically, do not make neutralizing antibodies to the MPER epitopes (Liao et al, J. Virol. 78(10):5270-5278 (2004); Haynes,
25 unpublished (2005)). These results have raised the question as to whether broadly reactive neutralizing antibodies to HIV-1 envelope are not made in normal animals and humans because they cannot be made.

Because long, hydrophobic CDR3 regions are typical of natural polyreactive autoantibodies (Meffre et al, J. Clin. Invest. 108:879-886 (2001), Ramsland et al, Exp. Clin. Immun. 18:176-198 (2001)), and HIV-1-infected patient B lymphocytes are polyclonally driven to make cardiolipin
5 antibodies (Weiss et al, Clin. Immunol. Immunopathol. 77:69-74 (1995), Grunewald et al, Clin. Exp. Immunol. 15:464-71 (1999)), studies were undertaken to assay these and other anti-HIV-1 mabs for cardiolipin and other autoantigen reactivities. The present invention results, at least in part, from the realization that two broadly reactive HIV-1 envelope gp 41
10 human mabs, 2F5 and 4E10, are polyspecific autoantibodies reactive with cardiolipin.

SUMMARY OF THE INVENTION

The present invention relates generally to human HIV. More specifically, the invention relates to a method of inducing neutralizing
15 antibodies to HIV and to compounds and compositions suitable for use in such a method. In a specific embodiment, the present invention provides immunogens that present MPER epitopes in their native membrane bound environment, and immunization methods using such immunogens that break tolerance.

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

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Broadly neutralizing antibodies (2F5, 4E10) bind to epitopes that lie proximal to the host membrane. Both 2F5 and 4E1 mAbs are IgG3,

have long CDR3s, and bind to epitopes that lie within HIV-1 gp41 (aa 660-683) membrane proximal external region (MPER).

Figures 2A-2D. Reactivity of 2F5, 4E10, IgG1b12 Mabs with human Hep-2 epithelial cells. Fig. 2A shows Mab 2F5 reacting with Hep-2 cells in a diffuse cytoplasmic and nuclear pattern, Fig. 2B shows Mab 4E10 reacting with HEp-2 cells in a pattern similar to 2F5. Fig. 2C shows Mab IgG1b12 reacting with Hep-2 cells in a diffuse cytoplasmic pattern, with nucleoli reactive in the nucleus. Fig. 2C insert shows higher magnification of cells showing the nucleolar reactivity of IgG1b12 (arrows). Fig. 2D shows negative reactivity of Mab 1.9F on Hep-2 cells. Antibody amounts per slide assayed in Figs. 2A-2D were 3.75 μg per slide of Mab. Mab 2F5 was positive on HEp-2 cells at 0.125 μg per slide (5 μg/ml). Mab 4E10 was positive on HEp-2 at 0.125 μg per slide (5 μg/ml), and IgG1b12 was positive at 1.25 μg per slide (50 μg/ml). All Figs. X200.; Fig. 2C insert X400. Images shown are from an experiment representative of three performed.

Figures 3A-3D. Assay of Mabs 2F5 and 4E10 against lipids and specificity of binding. Fig. 3A shows ELISA reactivity of MAbs 4E10 (solid bars) and 2F5 (open bars) to cardiolipin (CL), phosphatidylserine (PS), phosphatidylcholine (PC), phophatidylethanolamine (PE), and sphingomyelin (SM). Whereas both 4E10 and 2F5 reacted with cardiolipin, only 4E10 reacted with the other lipids tested. Reactivity of control human anti-CCR5 binding site MAb 1.7b was negative (data not shown). Reactivity of MAbs against empty coated plate was similarly negative (not shown). To show specificity of binding of MAb 2F5 to cardiolipin, 150-300 μg/ml of 2F5 and 1000 μg/ml of anti-2F5 idiotype

murine MAb 3H6, which blocks the neutralization of HIV-1 by MAb 2F5 (Kunert et al, AIDS 16:667 (2002)), were used. The 2F5 anti-idiotype significantly blocked the binding of MAb 2F5 to cardiolipin by a mean of 70% in 3 separate experiments ($p<0.03$) (Fig. 3B). In a separate ELISA, MAb 2F5 bound to cardiolipin in half-maximal (EC50) response of 660 nM (not shown). Fig. 3C shows the dose response curve of 4E10 MAb binding to cardiolipin. The half-maximal (EC50) response of 4E10 binding (80nM) was calculated from a four parametric, sigmoidal curve fitting analysis. Binding data was acquired from an ELISA of 4E10 MAb binding (0.5nM-1000nM) to cardiolipin coated on ELISA plate (1.35μg/well). Fig. 3D shows soluble HIV-1 Env gp140 oligomers (CON-S) expressing the 4E10 epitope inhibits binding of 4E10 MAb to cardiolipin. The IC50 of inhibition of 4E10 binding to cardiolipin was calculated to be 145nM. The inhibition assay was carried out by using varying concentrations of gp140 (19.25-1230 nM) mixed with 10 μg/ml of 4E10 MAb, which were then added to wells containing 1.35 μg of cardiolipin. MAb 3H6 (1 mg/ml) (but not control MAb) also blocked the binding of MAb 2F5 to SSA/Ro, centromere B, and histones (not shown). All data in Figs. 3A-3D are representative of at least two experiments performed.

Figures 4A and 4B. Amino acid (Fig. 4A) and nucleic acid (Fig. 4B) sequences of CON-S Env gp160. A CFI form of the protein of Fig. 4A was used in Example 2. (Gp140CFI refers to an HIV-1 envelope design with the cleavage site (C), fusion site (F), and gp41 immunodominant region (I) deleted in addition to the deletion of the transmembrane and cytoplasmic domains.)

Figure 5. Structures of phosphospholipids used in immunization regimens and resulting neutralization titers.

Figures 6A and 6B. Peptide sequences used in the generation of peptide-
5 liposome conjugates. The nominal epitopes of mAbs 2F5 and 4E10 binding epitopes include sequences ELDKWAS and WFNITNW, respectively, and are underlined. The V3 sequences were derived from gp120 of HIV-1 MN strain and were used as a control construct. Scrambled sequences are used controls.

10

Figure 7. Schematic presentation of various designs of MPER gp41 constructs. The functional regions are indicated above the schematic constructs. Amino acid sequences are indicated below each of schematic constructs. Initiation and maturation signal sequences are highlighted in
15 blue; immunodominant regions are highlighted in bold; MPER regions are highlighted in brown and GTH1 domains are highlighted in red and transmembrane domains are underlined. His-tags were added to the C-terminal ends of the constructs for easy purification and are highlighted in green.

20

Figure 8. Binding of mAb 4E10 to peptide-liposome conjugates. BIAcore binding curves show specific and markedly higher binding of mAb 4E10 to GTH1-4E10 liposomes. Low levels of binding with fast kinetics to GTH1-2F5 liposomes were also detected.

25

Figure 9. Binding of 2F5 mAb to peptide-liposomes. MAb 2F5 bound specifically to GTH1-2F5 liposomes and showed no binding to GTH1-4E10 liposomes.

Figure 10. A32 mAb binding to peptide-liposomes. A control anti-gp120 Mab, A32, showed no binding to any of the liposome conjugates. 17b, a CD4-inducible mAb, also showed no binding to the above liposome conjugates (data not shown).

5

Figure 11. Generation of fluorescein conjugated peptide-liposomes. Peptide-liposomes were conjugated with a fluorescein tag by incorporating fluorescein-POPE in the lipid composition. Binding assays show that the specificity of mAb 4E10 binding is retained in fluorescein conjugated
10 liposomes. Fluorescein-conjugated GTH1-2F5 liposomes gave similar results.

Figure 12. Reactivity of immunized guinea pig sera with 4E10 peptide. ELISA binding assay show strong positive reactivity of sera to 4E10
15 peptide from two guinea pigs immunized with GTH1-4E10 liposomes. All pre-bleed sera gave background binding while a low level of binding was observed in a serum from an animal immunized with 4E10 peptide. Both the positive sera from the peptide-liposome immunized animals also showed neutralizing activity (Table 2). One serum (1102) showed
20 neutralization of MN and SS1196 strains with antibody titers at 1:209 and 1:32 respectively. The second serum (1103) was only effective against the MN virus (1:60).

DETAILED DESCRIPTION OF THE INVENTION

25 The present invention results, at least in part, from studies demonstrating that certain broadly neutralizing HIV-1 antibodies are autoantibodies. A large number of HIV^+ patients transiently make low

levels of such antibodies, however, the studies described herein indicate that gp41 epitopes do not induce these antibody specificities but, rather, that cross-reactive autoantigens, including cardiolipin, are the priming antigen.

5 The present invention provides a method of inducing antibodies that neutralize HIV. The method comprises administering to a patient in need thereof an amount of at least one heterologous (e.g., non-human) or homologous (e.g., human) cross-reactive autoantigen sufficient to effect the induction. Cross-reactive autoantigens suitable for use in the instant 10 invention include cardiolipin, SS-A/RO, dsDNA from bacteria or mammalian cells, centromere B protein and RiBo nucleoprotein (RNP).

Suitable autoantigens also include phospholipids in addition to cardiolipin, such as phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, phosphotidylinositol, sphingomyelin, and derivatives 15 thereof, e.g., 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-L-serine] (POPS), 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE), and dioleoyl phosphatidylethanolamine (DOPE). Use of hexagonal II phases of phospholipids can be advantageous and phospholipids that readily form hexagonally packed cylinders of the hexagonal II tubular phase (e.g., 20 under physiological conditions) are preferred, as are phospholipids that can be stabilized in the hexagonal II phase. (See Rauch et al, Proc. Natl. Acad. Sci. USA 87:4112-4114 (1990); Aguilar et al et al, J. Biol. Chem. 274: 25193-25196 (1999)).

Fragments of such autoantigens comprising the cross-reactive 25 epitopes can also be used.

The autoantigen, or fragment thereof, can be used, for example, in prime boost regimens that can be readily optimized by one skilled in the art (DNA sequences encoding proteinaceous components of such

regimens can be administered under conditions such that the proteinaceous component is produced *in vivo*). By way of example, cross-reactive autoantigen can be used as a first vaccine prime to boost natural auto-antibodies (e.g., anti-cardiolipin 4E10- and 2F5-like antibodies).
5 Either autoantigen (e.g., cardiolipin (or fragment thereof)), or an HIV-envelope protein/polypeptide/peptide comprising a cross-reactive epitope(s), such as the 2F5 and/or 4E10 epitopes (which epitopes can include at least the sequences ELDKWA and NWFDIT, respectively), can be used as the boost. (See sequences disclosed in PCT/US04/30397.)
10 (It will be appreciated that HIV-envelope is not an autoantigen.)

The mode of administration of the autoantigen and/or HIV-protein/polypeptide/peptide, or encoding sequence, can vary with the immunogen, the patient and the effect sought, similarly, the dose administered. Optimum dosage regimens can be readily determined by
15 one skilled in the art. Typically, administration is subcutaneous, intramuscular, intravenous, intranasal or oral.

The immunogenic agents can be administered in combination with an adjuvant. While a variety of adjuvants can be used, preferred adjuvants include CpG oligonucleotides and other agents (e.g., TRL9
20 agonists) that can break tolerance to autoantigens without inducing autoimmune disease (Tran et al, Clin. Immunol. 109:278-287 (2003), US Appln Nos. 20030181406, 20040006242, 20040006032, 20040092472, 20040067905, 20040053880, 20040152649, 20040171086, 20040198680, 200500059619).
25 The invention includes compositions suitable for use in the instant method, including compositions comprising the autoantigen, and/or HIV protein/polypeptide/peptide comprising one or more cross-reactive epitopes (e.g., 4E10 and/or 2F5 epitopes), or 4E10 or 2F5 epitope mimics,

and a carrier. When a DNA prime or boost can be used, suitable formulations include a DNA prime and a recombinant adenovirus boost and a DNA prime and a recombinant mycobacteria boost, where the DNA or the vectors encode, for example, either HIV envelope or a protein autoantigen, such as SS-A/Ro. Other combinations of these vectors can be used as primes or boosts, either with or without HIV protein/polypeptide/peptide and/or autoantigen. The composition can be present, for example, in a form suitable for injection or nasal administration. Advantageously, the composition is sterile. The composition can be present in dosage unit form.

The present invention also relates to a passive immunotherapy approach wherein B cells from patients with a primary autoimmune disease, such as systemic lupus erythematosis (SLE) or anti-phospholipid antibody syndrome or patients with infectious diseases such as syphilis, leishmaniasis, and leprosy, are used in the production of cross-reactive antibodies (including monoclonal antibodies other than 4E10 and 2F5). That is, the invention includes the use of B cells from SLE patients, as well as other patients with disordered immunoregulation (that is, patients with a primary autoimmune disease, or a non-HIV infection such as those noted above, that produce autoantibodies cross-reactive with HIV envelope), in the production of immortal cell lines that provide a source of antibodies that cross-react with HIV envelope (such as 2F5-like and 4E10-like antibodies) (see Stiegler et al, AIDS Res. Hum. Retroviruses 17:1757-1765 (2001), Armbruster et al, J. Antimicrob. Chemother. 54:915-920 (2004), USP 5,831,034). Advantageously, the B cells are from an SLE patient (or patient with another primary autoimmune disease) that is HIV infected or that has received an envelope-based HIV vaccine (while not wishing to be bound by theory, HIV infection or vaccination may serve to

"boost" primed B1 cells (e.g., cardiolipin–primed B1 cells) to produce 2F5- and/or 4E10-like antibodies and escape deletion (which would occur in a normal subject) – the "boost" may trigger somatic hypermutation so that the resulting Ig genes encode antibodies that fit 2F5 and or 4E10-like epitopes – or that fit other gp160 epitopes that induce broadly neutralizing antibodies but are deleted in normal subjects). The production of immortal cell lines from B cells can be effected using any of a variety of art recognized techniques, including, but not limited to, fusing such B cells with myeloma cells to produce hybridomas.

Once selected, sequences encoding such cross-reactive antibodies (or binding fragments thereof) can be cloned and amplified (see, for example, Huse et al, Science 246:1275-1281 (1989), and phage–display technology as described in WO 91/17271, WO 92/01047, USPs 5,877,218, 5,871,907, 5,858,657, 5,837,242, 5,733,743 and 5,565,332). Soluble antibodies for therapy can then be designed and produced using art recognized techniques (Stiegler et al, AIDS Res. Hum. Retroviruses 17:1757-1765 (2001), Armbruster et al, J. Antimicrob. Chemother. 54:915-920 (2004)).

In accordance with this approach, the antibody (or binding fragment thereof) can be administered in doses ranging from about 10 to 100 mg/dose, preferably 25 mg/dose. The dosage and frequency can vary with the antibody (or binding fragment thereof), the patient and the effect sought (see Armbruster et al, J. Antimicrob. Chemother. 54:915-920 (2004)). The antibodies described above can be used prophylactically or therapeutically.

The antibodies (or binding fragments thereof), or DNA encoding the antibodies or binding fragments, can be formulated with a carrier (e.g., pharmaceutically acceptable carrier) and can be administered by, for

example, parenteral, intravenous, subcutaneous, intramuscular or intranasal routes.

Finally, animal species such as camels (Ramsland et al, Exp. Clin. Immunogenet. 18:176-198 (2001), Litman et al, Annu. Rev. Immunol. 7:109-147 (1999)), cows (Ramsland et al, Exp. Clin. Immunogenet. 18:176-198 (2001), Litman et al, Annu. Rev. Immunol. 7:109-147 (1999)) and sharks (Ramsland et al, Exp. Clin. Immunogenet. 18:176-198 (2001), Litman et al, Annu. Rev. Immunol. 7:109-147 (1999), Hohman et al, Proc. Natl. Acad. Sci. U S A. 90:9882-9886 (1993)) have very long CDR3 lengths, and their antibodies show polyreactivitiy. These engineered CDR3s that show polyreactivity to HIV envelope can be utilized for making potent therapeutic antibodies (e.g, monoclonal antibodies, including, for example, chimeric and humanized antibodies, and antigen binding fragments thereof) to HIV and to many infectious agents.

In a specific embodiment, the present invention further relates to synthetic liposome-peptide conjugates and to methods of using same as immunogens for the generation of broadly neutralizing antibodies against HIV-1. This embodiment of the invention provides compositions and methods for embedding into synthetic liposomes nominal epitope peptides of broadly neutralizing antibodies that bind to the MPER of HIV-1 gp41. Also provided are immunization strategies and protocols for the generation of anti-HIV-1 neutralizing antibodies and for the detection of antigen specific B cell responses.

In accordance with this embodiment of the invention, peptide sequences that include a nominal epitope of a broadly neutralizing anti-HIV antibody and a hydrophobic linker, such as GTH1 (see Figure 6 for sequence), are embedded into synthetic liposomes. In a preferred aspect, the nominal epitope is that of mAbs 2F5 (ELDKWAS) or 4E10

(WFNITNW), which, as noted above, lie in the MPER of HIV-1 envelope gp41. The epitope can be present in the peptide such that antibodies specific therefor have relatively unconstrained access or, alternatively, the epitope can be present in the peptide in relation to the hydrophobic linker so as to mimic the native orientation of the MPER region. Specific examples of peptide sequences suitable for use in the invention are set forth in Figure 6. In addition, the MPER gp41 region can be expressed as recombinant proteins in recombinant vaccinia virus, in human cell expression systems, and formulated with amphipathic alpha helices at the N or C termini of the gp41 component for ease in association with liposomes (Figure 7).

Liposomes suitable for use in the invention include, but are not limited to, those comprising POPC, POPE, DMPA (or sphingomyelin (SM)) and cholesterol (Ch). While optimum ratios can be determined by one skilled in the art, examples include POPC:POPE:SM:Ch or POPC:POPE:DMPA:Ch at ratios of 45:25:20:10. Alternative formulations of liposomes that can be used include DMPC (1,2-dimyristoyl-sn-glycero-3-phoshphocholine), cholesterol (Ch) and DMPG (1,2-dimyristoyl-sn-glycero-3-phoshpho-rac-(1-glycerol) formulated at a molar ratio of 9:7.5:1 (Wassef et al, ImmunoMethods 4:217-222 (1994); Alving et al, G. Gregoriadis (ed.), Liposome technology 2nd ed., vol. III CRC Press, Inc., Boca Raton, FL (1993); Richards et al, Infect. Immun. 66(6):285902865 (1998)).

The optimum ratio of peptide to total lipid can vary, for example, with the peptide and the liposome. For the peptides of Example 3, a ratio 1:420 was advantageous.

The liposome-peptide conjugates can be prepared using standard techniques (see too Example 3 that follows).

The peptide-liposome immunogens of the invention can be formulated with, and/or administered with, adjuvants such as lipid A, oCpGs, TRL4 agonists or TLR 7 agonists that facilitate robust antibody responses (Rao et al, Immunobiol. Cell Biol. 82(5):523 (2004)). Other adjuvants that can be used include alum and Q521 (which do not break existing B cell tolerance). Preferred formulations comprise an adjuvant that is designed to break forms of B cell tolerance, such as oCpGs in an oil emulsion such as Emulsigen (an oil in water emulsion) (Tran et al, Clin. Immunol. 109(3):278-287 (2003)). Additional suitable adjuvants include those described in 11/302,505, filed December 14, 2005, including the TRL agonists disclosed therein.

The peptide-liposome immunogens can be administered, for example, IV, intranasally, subcutaneously, intraperitoneally, intravaginally, or intrarectally. The route of administration can vary, for example, with the patient, the conjugate and/or the effect sought, likewise the dosing regimen. The peptide-liposome immunogens are preferred for use prophylactically, however, their administration to infected individuals may reduce viral load.

As described in Example 3 that follows, the peptide-liposome conjugates can be used as reagents for the detection of MPER-specific B cell responses. For example, the peptide-liposome constructs can be ~~conjugated with a detectable label, e.g., a fluorescent label, such as~~ fluorescein. The fluorescein-conjugated liposomes can be used in flow cytometric assays as a reagent for the detection of anti-MPER specific B cell responses in hosts immunized with HIV-1 Env proteins that present exposed MPER region. These reagents can be used to study peripheral blood B cells to determine the effectiveness of immunization for anti-

MPER antibody induction by measuring the number of circulating memory B cells after immunization.

It will be appreciated from a reading of the foregoing that if HIV has evolved to escape the host immune response by making the immune
5 system blind to it, other infectious agents may have evolved similarly. That is, this may represent a general mechanism of escape. That being the case, approaches comparable to those described herein can be expected to be useful in the treatment of such other agents well.

Certain aspects of the invention are described in greater detail in
10 the non-limiting Examples that follow (see also Maksyutov et al, J. Clin. Virol. Dec; 31 Suppl 1:S26-38 (2004), US Appln. 20040161429, and Haynes et al, Science 308:1906 (2005)).

EXAMPLE 1

Design of an HIV-1 immunogen that can induce broadly reactive
15 neutralizing antibodies is a major goal of HIV-1 vaccine development. While rare human mabs exist that broadly neutralize HIV-1, HIV-1 envelope immunogens do not induce these antibody specificities. In this study, it was demonstrated that the two most broadly reactive HIV-1 envelope gp41 human mabs, 2F5 and 4E10, are polyspecific,
20 autoantibodies reactive with cardiolipin. Thus, current HIV-1 vaccines may not induce antibodies against membrane proximal gp41 epitopes because of gp41 membrane proximal epitopes mimicry of autoantigens.

Experimental Details

25 *Monoclonal Antibodies.* Mabs 2F5, 2G12, and 4E10 were produced as described (Steigler et al, AID Res. Human Retroviruses 17:1757 (2001), Purtscher et al, AIDS 10:587 (1996), Trkola et al, J. Virol. 70:1100

(1996)). IgG1b12 (Burton et al, Science 266:1024-1027 (1994)) was the generous gift of Dennis Burton, Scripps Institute, La Jolla, CA. Mab 447-52D (Zolla-Pazner et al, AIDS Res. Human Retrovirol. 20:1254 (2004)) was obtained from the AIDS Reagent Repository, NIAID, NIH. The
5 remainder of the mabs in Table 1 were produced from HIV-1 infected subjects and used as described (Robinson et al, AIDS Res. Human Retrovirol. 6:567 (1990), Binley et al, J. Virol. 78:13232 (2004)).

Autoantibody Assays. An anti-cardiolipin ELISA was used as
10 described (DeRoe et al, J. Obstet. Gynecol. Neonatal Nurs. 5:207 (1985), Harris et al, Clin. Exp. Immunol. 68:215 (1987)). A similar ELISA was adapted for assay for mab reactivity to phosphatidylserine, phosphatidylcholine, phosphatidyethanolamine, and sphingomyelin (all purchased from Sigma, St. Louis, MO.). The Luminex AtheNA Multi-Lyte
15 ANA Test (Wampole Laboratories, Princeton, NJ) was used for mab reactivity to SS-A/Ro, SS-B/La, Sm, ribonucleoprotein (RNP), Scl-70, Jo-1, double stranded (ds) DNA, centromere B, and histone. Mab concentrations assayed were 150 μg, 50 μg, 15 μg, and 5 μg/ml. Ten μl of each concentration (0.15 μg, 0.05 μg, 0.015 μg, and 0.005 μg,
20 respectively, per assay) were incubated with the Luminex fluorescence beads and the test performed per manufacturer's specifications. Values in Table 1 are results of assays with 0.15 μg added per test. In addition, an ELISA for SS-A/Ro (ImmunoVision, Springdale, AR) and dsDNA (Inova Diagnostics, San Diego, CA) was also used to confirm these autoantigen
25 specificities. Reactivity to human epithelial Hep-2 cells was determined using indirect immunofluoresence on Hep-2 slides using Evans Blue as a counterstain and FITC-conjugated goat anti-human IgG (Zeus Scientific, Raritan N.J.). Slides were photographed on a Nikon Optiphot fluorescence

microscope. Rheumatoid factor was performed by nephelometry (Dade Behring, Inc (Newark, DE). Lupus anticoagulant assay was performed by activated partial thromboplastin (aPTT) and dilute Russell viper venom testing, as described (Moll and Ortel, Ann. Int. Med. 127:177 (1997)).
5 Fourty μl of 1 mg/ml of 2F5, 4E10 and control mabs were added to pooled normal plasma (final mab concentration, 200 μg/ml) for lupus anticoagulant assay. Anti-β2 glycoprotein-1 assay was an ELISA (Inova Diagnostics, Inc.). Serum antibodies to dsDNA, SS-A/Ro, SS-B/La, Sm, RNP and histone occur in patients with SLE; serum antibodies to
10 centromere B and scl-70 (topoisomerase I) are found in systemic sclerosis; and antibodies to Jo-1 are found in association with polymyositis (Rose and MacKay, The Autoimmune Diseases, Third Ed. Academic Press, Sand Diego, CA (1998)).

Results

15 The reactivity of mabs 2F5 and 4E10, two additional rare broadly reactive neutralizing mabs (2G12 and IgG1b12), and thirty-one common anti-HIV-1 Env human mabs, with cardiolipin (Robinson et al, AIDS Res. Human Retrovirol. 6:567 (1990)) was determined (Table 1). Both 2F5 and 4E10 reacted with cardiolipin, whereas all 33 of the other mabs were
20 negative. Mab 2F5 also reacted with SS-A/Ro, histones and centromere B autoantigen, while mab 4E10 reacted with the systemic lupus erythematosus (SLE) autoantigen, SS-A/Ro. Both 2F5 and 4E10 reacted with Hep-2 human epithelial cells in a diffuse cytoplasmic and nuclear pattern (Robinson et al, AIDS Res. Human Retrovirol. 6:567 (1990))
25 (Fig. 2). Thus, both 2F5 and 4E10 are characterized by polyspecific autoreactivity.

Table 1.

Mab Type and Antibody Name	Cardiolipin	Hep-2 Cell Reactivity	Ro(SSA)	dsDNA	Centromere B	Histones
Membrane Proximal External Region (2F5)	47	+Cytoplasmic nuclear	290	-	1,776	1,011
Membrane Proximal External Region (4E10)	15,434	+Cytoplasmic nuclear	221	-	-	-
CD4 Binding Site (IgG1b12)	-	+Cytoplasmic nucleolar	-	513	479	185
CD4 Binding Site (F1.5E, 25G)	-	-	-	-	-	-
Adjacent CD4 Binding Site (A32)	-	-	-	-	1,131	-
Adjacent CD4 Binding Site (1.4G)	-	-	-	768	1,422	539
Adjacent CD4 Binding Site (1.4C, 4.6H, 4.11C)	-	-	-	-	-	-
Third variable loop (CO11, F2A3, F3.9F, LA21, 447-52D)	-	-	-	-	-	-
gp41 immunodominant region (7B2, KU32)	-	-	-	-	-	-
gp41 immunodominant region (2.2B)	-	+intermediate filament	-	-	314	-
C1-C4 gp120 (8.2A, 2.3B)	-	-	-	-	-	-
C1-C4 gp120 (EH21, C11)	-	-	-	-	-	-
Glycan-dependent (2G12)	-	-	-	-	-	-
CCR5 binding site (1.7B, 2.1C, LF17, E51 1.9F, LA15, 4.8E, LA28, 1.9E, E047, 2.5E, ED10)	-	-	-	-	-	-
Positive control serum	34	+homogeneous nuclear	1365	228	624	34
Negative controls	<16	-	<120	<120	<120	<120

All Mabs were negative in assays for reactivity with La (SSB), Sm, Scl-70 and Jo-1, except for Ku32 mab that reacted with Sm. Ro (SSA), dsDNA, centromere B, histone and cardiolipin antibody values are in relative units based on a standard curve. - = negative

5 Of the two other rare neutralizing mabs, one mab, 2G12, was not autoreactive, while another mab against the CD4 binding site, IgG1b12

(Stiegler et al, AIDS Res. Hum. Retroviruses 17:1757 (2001)), reacted with ribonucleoprotein, dsDNA, and centromere B as well as with Hep-2 cells in a cytoplamic and nucleolar pattern (Table 1 and Fig. 2). Of the 31 more common anti-HIV-1 mabs studied, only two mabs with specificity for binding near the CD4 binding site (A32, 1.4G) and a mab to a non-neutralizing gp41 epitope (2.2 B) showed evidence of polyreactivity (Table 1).

To determine if 2F5 and 4E10 were similar to prothrombotic anti-cardiolipin antibodies found in SLE-associated anti-phospholipid syndrome (Burton et al, Science 266:1024-1027 (1994)), both mabs were tested for lupus anticoagulant activity, and for the ability to bind to prothombin (PT), beta-2 glycoprotein-1, phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM) (Robinson et al, AIDS Res. Human Retrovirol. 6:567 (1990)). Whereas 2F5 was negative for these reactivities, 4E10 had lupus anticoagulant reactivity, and reacted strongly with PS, PC, PE, weakly with SM and PT, and negatively with β2 glycoprotein-1. (See Fig. 3.)

Anti-cardiolipin antibodies can be found in patients with disordered immunoregulation due to autoimmune disease or infection (Burton et al, Science 266:1024-1027 (1994)). Anti-cardiolipin autoantibodies are induced by syphilis, leprosy, leishmaniasis, Epstein Barr virus, and HIV-1 (Burton et al, Science 266:1024-1027 (1994)). Unlike anti-cardiolipin antibodies found in SLE, "infectious" anti-cardiolipin antibodies are rarely prothrombotic, and are transient. Thus, 4E10 is similar to anti-cardiolipin antibodies in autoimmune disease, and 2F5 is similar to anti-cardiolipin antibodies in infectious diseases.

Autoreactive B cell clones with long CDR3 lengths are normally deleted or made tolerant to self antigens ((Zolla-Pazner et al, AIDS Res.

Human Retrovirol. 20:1254 (2004)). Thus, HIV-1 may have evolved to escape membrane proximal antibody responses by having conserved neutralizing epitopes as mimics of autoantibody epitopes. These data suggest that current HIV-1 vaccines do not routinely induce robust membrane proximal anti-envelope neutralizing antibodies because antibodies targeting these epitopes are derived from autoreactive B cell clones that are normally deleted or made tolerant upon antigenic stimulation by HIV-1 Env. These observations may also explain the rare occurrence of HIV-1 in SLE patients who may be unable to delete such clones (Fox et al, Arth. Rhum. 40:1168 (1997)).

EXAMPLE 2

The ability of autoantigens of the invention to induce the production of neutralizing antibodies was studied using, as autoantigen, cardiolipin (lamellar and hexagonal phases), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-L-serine] (POPS) (lamellar and hexagonal phases), 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) (lamellar phase) and dioleoyl phosphatidylethanolamine (DOPE) (hexagonal phase). Guinea pigs (4 per group) were immunized with phospholopid (cardiolipin lamellar phase, cardiolipin hexagonal phase, POPS lamellar phase, POPS hexagonal phase, POPE lamellar phase or DOPE hexagonal phase) in 10 μg of oCpGs, four times, with each immunization being two weeks apart. Following the four phospholipid immunizations, a final immunization was made IP with 10 μg of oCpGs with 100 μg of group M consensus Env, CON-S gp140CFI oligomer (that is, the CFI form of the protein shown in Fig. 4A).

Neutralization assays were performed using an Env pseudotype neutralization assay in TMZ cells (Wei et al, Nature 422:307-312 (2003), Derdeyn et al, J Virol 74:8358-8367 (2000), Wei et al, Antimicrob Agents Chemother 46:1896-1905 (2002), Platt et al, J Virol 72:2855-2864 (1998), Mascola et al, J. Virol. 79:10103-10107 (2005)), as described below:

Cell Culture

TZM-bl is an adherent cell line and is maintained in T-75 culture flasks. Complete growth medium (GM) consists of D-MEM supplemented with 10% fetal bovine serum (FBS, heat-inactivated) and gentamicin (50 μg/ml). Cell monolayers are disrupted and removed by treatment with trypsin/EDTA:

Trypsin-EDTA Treatment for Disruption of TZM-bl Cell Monolayers:

Cell monolayers maintained in T-75 culture flasks are disrupted and removed by treatment with trypsin/EDTA at confluency when splitting cells for routine maintenance and when preparing cells for assay.

1. Decant the culture medium and remove residual serum by rinsing monolayers with 6 ml of sterile PBS.
2. Slowly add 2.5 ml of an 0.25% Trypin-EDTA solution to cover the cell monolayer. Incubate at room temp for 30-45 seconds. Decant the trypsin solution and incubate at 37°C for 4 minutes. Do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
3. Add 10 ml of GM and suspend the cells by gentle pipet action. Count cells.
4. Seed new T-75 culture flasks with approximately 10^6 cells in 15 ml of GM. Cultures are incubated at 37°C in a 5% CO_2/95% air environment. Cells should be split approximately every 3 days.

Virus Stocks

Stocks of uncloned viruses may be produced in either PBMC or T cell lines. Pseudoviruses may be produced by transfection in an appropriate cell type, such as 293T cells. All virus stocks should be made cell free by low speed centrifugation and filtration (0.45-micron) and stored at -80°C in GM containing 20% FBS.

TCID50 Determination

It is necessary to determine the TCID50 of each virus stock in a single-cycle infection assay (2-day incubation) in TZM-bl cells prior to performing neutralization assays. A cut-off value of 2.5-times background RLU is used when quantifying positive infection in TCID50 assays.

Too much virus in the neutralization assay can result in strong virus-induced cytopathic effects that interfere with accurate measurements. Most virus stocks must be diluted at least 10-fold to avoid cell-killing. A standard inoculum of 200 TCID50 was chosen for the neutralization assay to minimize virus-induced cytopathic effects while maintaining an ability to measure a 2-log reduction in virus infectivity. It should be noted that different strains vary significantly in their cytopathicity. Virus-induced cytopathic effects may be monitored by visual inspection of syncytium formation under light microscopy. Cytopthic effects may also be observed as reductions in luminescence at high virus doses in the TCID50 assay.

Neutralizing Antibody Assay Protocol

NOTE 1: All incubations are performed in a humidified 37°C, 5% CO_2 incubator unless otherwise specified.

NOTE 2: Assays with replication-competent viruses are performed in DEAE-GM containing 1 μM indinavir.

1. Using the format of a 96-well flat-bottom culture plate, place 150 μl of GM in all wells of column 1 (cell control). Place 100 μl in all wells of columns 2-11 (column 2 will be the virus control). Place an additional 40 μl in all wells of columns 3-12, row H (to receive test samples).

2. Add 11 μl of test sample to each well in columns 3 & 4, row H. Add 11 μl of a second test sample to each well in columns 5 & 6, row H. Add 11 μl of a third test sample to each well in columns 7 & 8, row H. Add 11 μl of a fourth test sample to each well in columns 9 & 10, row H. Add 11 μl of a fifth test sample to each well in columns 11 & 12, row H. Mix the samples in row H and transfer 50 μl to row G. Repeat the transfer and dilution of samples through row A (these are serial 3-fold dilutions). After final transfer and mixing is complete, discard 50 μl from the wells in columns 3-12, row A into a waste container of disinfectant.

3. Thaw the required number of vials of virus by placing in an ambient temperature water bath. When completely thawed, dilute the virus in GM to achieve a concentration of 4,000 $TCID_{50}$/ml.

Cell-free stocks of virus should be prepared in advance and cryopreserved in working aliquots of approximately 1 ml.

4. Dispense 50 μl of cell-free virus (200 $TCID_{50}$) to all wells in columns 2-12, rows A through H. Mix by pipet action after each transfer. Rinse pipet tips in a reagent reservoir containing 40 ml sterile PBS between each transfer to avoid carry-over.

5. Cover plates and incubate for 1 hour.

6. Prepare a suspension of TZM-bl cells (trypsinize aproximately 10-15 minutes prior to use) at a density of 1 x 10^5 cells/ml in GM containing

DEAE dextran (37.5 μg/ml). Dispense 100 μl of cell suspension (10,000 cells per well) to each well in columns 1-12, rows A though H. Rinse pipet tips in a reagent reservoir filled with sterile PBS between each transfer to avoid carry-over. The final concentration of DEAE dextran is 15 μg/ml.

5 7. Cover plates and incubate for 48 hours.

8. Remove 150 μl of culture medium from each well, leaving approximately 100 μl. Dispense 100 μl of Bright Glo™ Reagent to each well. Incubate at room temperature for 2 minutes to allow complete cell lysis. Mix by pipet action (at least two strokes) and transfer 150 μl to a
10 corresponding 96-well black plate. Read the plate immediately in a luminometer.

9. Percent neutralization is determined by calculating the difference in average RLU between test wells (cells + serum sample + virus) and cell control wells (cells only, column 1), dividing this result by the difference in
15 average RLU between virus control (cell + virus, column 2) and cell control wells (column 1), subtracting from 1 and multiplying by 100. Neutralizing antibody titers are expressed as the reciprocal of the serum dilution required to reduce RLU by 50%.

20 As shown in Fig. 5, animals receiving DOPE (hexagonal phase) had a neutralization titer of 170.

EXAMPLE 3

Immunogen design

25 Peptide sequences that include the nominal epitopes of mAbs 2F5 and 4E10, respectively, linked to a hydrophobic linker (GTH1) were

synthesized and embedded into synthetic liposomes (Figure 6). The first generation of immunogens was designed with the 2F5 and 4E10 epitope sequences at the distal end of the lipid bilayer (Figure 6A). These constructs provided unconstrained access of mAbs to their respective
5 epitopes. The second generation constructs have been designed to mimic the native orientation of the MPER region with the 2F5 and 4E10 mAb epitope sequences linked proximal to the hydrophobic linker (Figures 6A, 6B).

The composition of the synthetic liposomes comprised the following
10 phospholipids, POPC (1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine), POPE (1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphoethanolamine), DMPA (1,2-Dimyristoyl-sn-Glycero-3-Phosphate), and Cholesterol dissolved in chloroform (purchased from Avanti Polar Lipids (Alabaster, AL).).

15 Synthetic liposomes were prepared by dispensing appropriate molar amounts of phospholipids (POPC:POPE:DMPA:Ch = 45:25:20:10) in chloroform resistant tubes. The phospholipids were mixed by vortexing and the mixture was dried in the fume hood under a gentle stream of nitrogen. Any residual chloroform was removed by storing the lipids under
20 a high vacuum (15h). Aqueous suspensions of phospholipids were prepared by adding PBS or TBS buffer, pH 7.4, and incubating at 37°C for 10-30 minutes, with intermittent, vigorous vortexing to resuspend the phospholipids. The milky, uniform suspension of phospholipids was then sonicated in a bath sonicator (Misonix Sonicator 3000, Misonix Inc.,
25 Farmingdale, NY). The sonicator was programmed to run 3 consecutive cycles of 45 seconds of total sonication per cycle. Each cycle included 5 seconds of sonication pulse (70 watts power output) followed by a pulse

off period of 12 seconds. At the end of sonication, the suspension of lamellar liposomes was stored at 4°C.

HIV-1 MPER peptides GTH1-2F5 and GTH1-4E10 (Figure 6) were dissolved in 70% chloroform, 30% methanol. Chloroform solutions of lipids were added to the peptide solution, in the molar ratios of 45:25:20:10 (POPC:POPE :DMPA :Cholesterol). Each peptide was added to a ratio of peptide:total phospholipids of 1:420. The mixture was vortexed, then dried and resuspended as described above.

Binding assays to test specificity of mAb binding to each peptide-lipid conjugate were performed following capture of the liposomes on a BAcore L1 sensor chip, which allows immobilization of lipid bilayer via a hydrophobic linker. 2F5, 4E10 and control mAbs (A32 or 17b) were injected over each of the sensor surfaces with either synthetic liposomes, or peptide-lipid conjugates and the binding monitored on a BIAcore 3000 instrument (Figures 8-11).

Immunization strategy

The immunization strategy incorporated a regimen that allows temporary breaks in tolerance. The protocol involves the use of oCpGs, the TLR9 ligand that has been used to break tolerance for the production of anti-dsDNA antibodies in mice (Tran et al, Clin. Immunol. 109(3):278-287 (2003)). The peptide-liposome conjugates were mixed (1:1) with the adjuvant, Emulsigen plus oCpG. The Emulsigen mixed adjuvant (2x) was prepared by mixing 375μL of Emulsigen, 250 μL of oCpG and 625 μL of saline. Each guinea pig was immunized on a 21-day interval with 250 μg of either peptide alone or peptide-liposome conjugates with equivalent amount of peptide. Serum samples were harvested as pre-bleed prior to first immunization and at each subsequent immunizations. Serum samples

were analyzed by ELISA assay (Figure 12) for binding to peptide epitopes and for viral neutralization assay (Table 2). Data in Figure 12, show strong reactivity to 4E10 peptide of sera from two guinea pigs immunized with GTH1-4E10 liposomes, while only low level of reactivity was observed in a
5 serum from 4E10 peptide immunized animal. Both the positive sera also neutralized HIV-1 MN strain (Table 2).

Table 2. Induction of neutralizing antibodies in guinea pigs immunized with 4E10 peptide-liposomes

Animal No.	HIV-1 Strain/antibody titer		
	MN		SS1196
1102 Bleed 4	209		32
1103 Bleed 4	60		<20

Application of peptide-liposome conjugates in the detection of antigen
10 specific B cell responses.

The above peptide-liposome conjugates have been utilized as a reagent for the detection of MPER specific B cell responses. The peptide-liposome constructs (2F5 and 4E10) were conjugated with fluorescein by incorporating fluorescein-POPE in the lipid composition. The flourescein-
15 POPE was mixed with unonjugated POPE at a ratio of 45:55 and then mixed with the rest of the lipids in the molar ratio as described above. In

BIAcore binding assays, both fluorescein conjugated 2F5 and 4E10-peptide-liposomes retained their specificity in binding to their respective mAbs (Figure 11).

5

* * *

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

WHAT IS CLAIMED IS:

1. A method of inducing the production in a patient of anti-human immunodeficiency virus (HIV) antibodies comprising administering to a patient in need thereof an amount of at least one autoantigen cross-
5 reactive with HIV envelope sufficient to effect said induction.

2. The method according to claim 1 wherein said autoantigen is cardiolipin, SS-A/RO, double stranded (ds)DNA, centromere B protein or RiBo nucleoprotein (RNP), or fragment thereof that induces production of said antibodies.

10 3. The method according to claim 2 wherein said autoantigen is cardiolipin or fragment thereof that induces production of said antibodies.

4. The method according to claim 1 wherein said antibodies bind a gp41 membrane proximal external region (MPER) epitope.

5. The method according to claim 4 wherein said MPER
15 epitope comprises the sequence NWFDIT or ELDKWA.

6. The method according to claim 1 wherein said method further comprises administering to said patient an HIV envelope protein, polypeptide or peptide comprising an epitope cross-reactive with said autoantigen.

7. The method according to claim 6 wherein a DNA sequence encoding said HIV protein, polypeptide or peptide is administered under conditions such that said DNA sequence is expressed and said HIV protein, polypeptide or peptide is thereby produced in said patient.

5 8. The method according to claim 1 further comprising administering to said patient an adjuvant that breaks tolerance to said autoantigen.

9. The method according to claim 8 wherein said adjuvant comprises a TRL9 agonist.

10 10. The method according to claim 9 wherein said TRL9 agonist comprises a CpG oligonucleotide.

11. A composition comprising an autoantigen that is cross-reactive with HIV envelope and an agent that breaks tolerance to said autoantigen.

15 12. The composition according to claim 11 wherein said autoantigen is cardiolipin, SS-A/RO, dsDNA, centromere B protein or RiBo RNP, or fragment thereof comprising an epitope cross-reactive with HIV envelope.

13. The method according to claim 11 wherein said agent is a 20 TRL9 agonist.

14. The method according to claim 13 wherein said TRL9 agonist comprises a CpG oligonucleotide.

15. A method of producing autoantibodies that are cross-reactive with HIV envelope comprising isolating B cells from a patient with primary autoimmune disease or from a non-HIV infected patient with an infectious disease selected from the group consisting of syphilis, leishmaniasis and leprosy, and creating therefrom an immortal cell line that produces said autoantibodies.

16. The method according to claim 15 wherein said patient is a primary autoimmune patient that is HIV infected or that has received an envelope-based HIV vaccine.

17. The method according to claim 15 wherein said B cells are fused with myeloma cells to form hybridomas that produce said autoantibodies.

18. The method according to claim 15 wherein said patient is a systemic lupus erythematosus (SLE) patient, an anti-phospholipid antibody syndrome patient or a non-HIV infected patient with an infectious disease selected from the group consisting of syphilis, leishmaniasis and leprosy.

19. The method according to claim 18 wherein said patient is a systemic lupus erythematosus (SLE) patient that is HIV infected or that has received an envelope-based HIV vaccine, or said patient is a non-HIV

infected patient with an infectious disease selected from the group consisting of syphilis, leishmaniasis and leprosy that has received an envelope-based HIV vaccine.

20. A method of inducing the production in a patient of anti-HIV
5 antibodies comprising administering to a patient in need thereof an amount of at least one autoantigen cross-reactive with an HIV virion sufficient to effect said induction.

21. The method according to claim 20 wherein said autoantigen is a phospholipid or a derivative thereof.

10 22. The method according to claim 21 wherein said phospholipid is cardiolipin, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, phosphotidylinositol or sphingomyelin, or derivative thereof.

23. The method according to claim 22 wherein said phospholipid
15 is dioleoyl phosphatidylethanolamine (DOPE) (hexagonal phase).

24. The method according to claim 20 wherein said autoantigen is cross-reactive with HIV envelope.

25. The method according to claim 20 wherein said autoantigen is centromere F protein, or fragment thereof comprising an epitope cross-
20 reactive with the HIV virion.

26. The method according to claim 25 wherein said autoantigen is cross-reactive with HIV envelope.

27. A composition comprising an autoantigen that is cross-reactive with an HIV virion and an agent that breaks tolerance to said
5 autoantigen.

28. The composition according to claim 27 wherein said autoantigen is a phospholipid or a deriviative thereof.

29. The composition according to claim 28 wherein said phospholipid is cardiolipin, phosphatidylserine, phosphatidylethanolamine,
10 phosphatidylcholine, phosphotidylinositol or sphingomyelin, or derivative thereof.

30. The composition according to claim 29 wherein said phospholipid is dioleoyl phosphatidylethanolamine (DOPE) (hexagonal phase).

15 31. The composition according to claim 27 wherein said autoantigen is cross-reactive with HIV envelope.

32. The method according to claim 1 or 20 wherein said patient is not infected with HIV.

33. A method of inducing the production in a patient of anti-HIV
20 antibodies comprising administering to a patient in need thereof an

amount of at least one liposome-peptide conjugate in an amount sufficient to effect said induction, wherein said peptide comprises a membrane external proximal region (MPER) epitope.

34. The method according to claim 33 wherein said peptide
5 comprises the sequence ELDKWAS or WFNITNW.

35. The method according to claim 33 wherein said liposome-peptide conjugate comprises a hydrophobic linker.

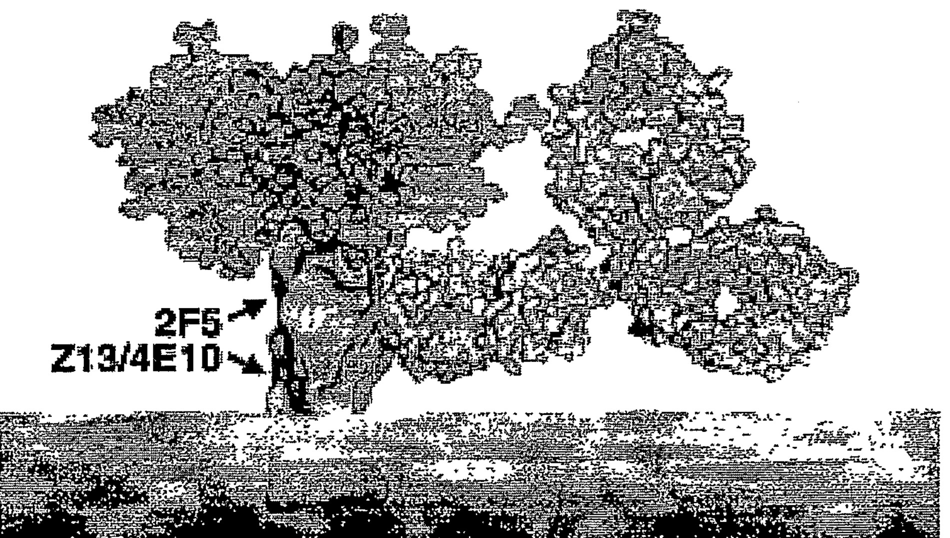
36. An immunogen comprising an MPER epitope embedded in a liposome.

10 37. The immunogen according to claim 36 bound to a detectable label.

Figure 1

Broadly Neutralizing Antibodies (2F5, 4E10) bind to epitopes that lie proximal to the host membrane

- Against epitopes that lie within HIV-1 gp41 (aa 660-683) membrane proximal external region (MPER)
- 2F5 -ELDKWAS; 4E10-WFNITNW
- Both IgG3s; Long hydrophobic CDR3s



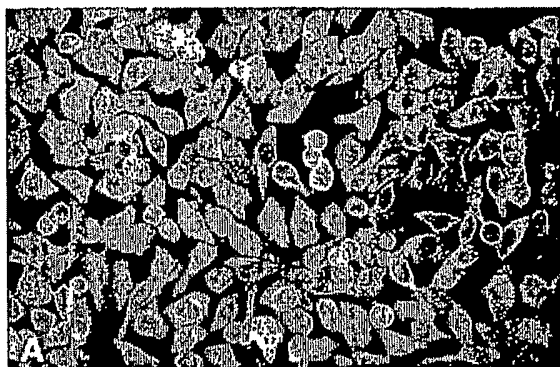


Figure 2A

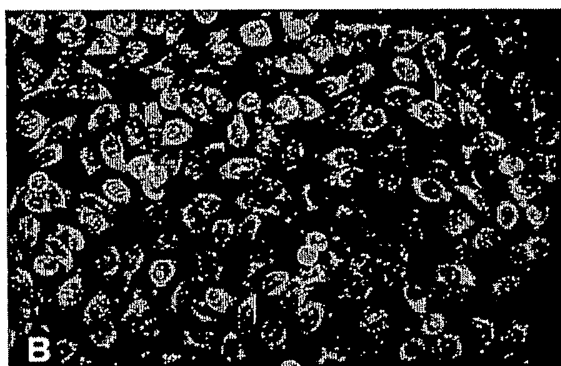


Figure 2B

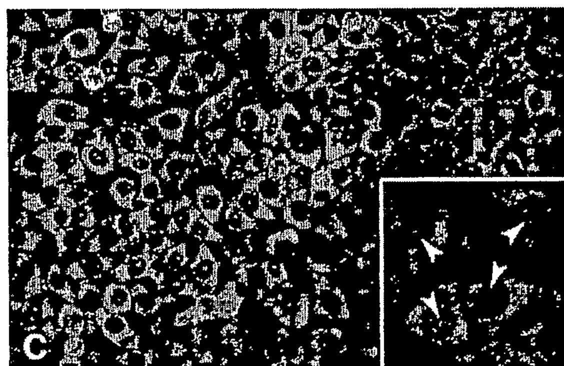


Figure 2C

Figure 2D

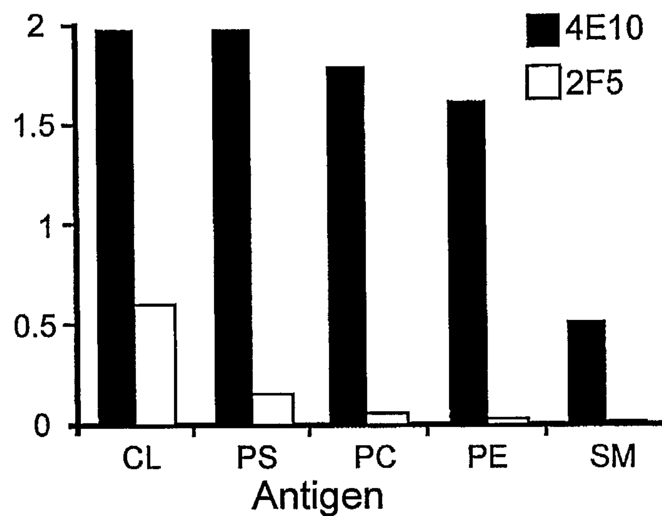


Figure 3A

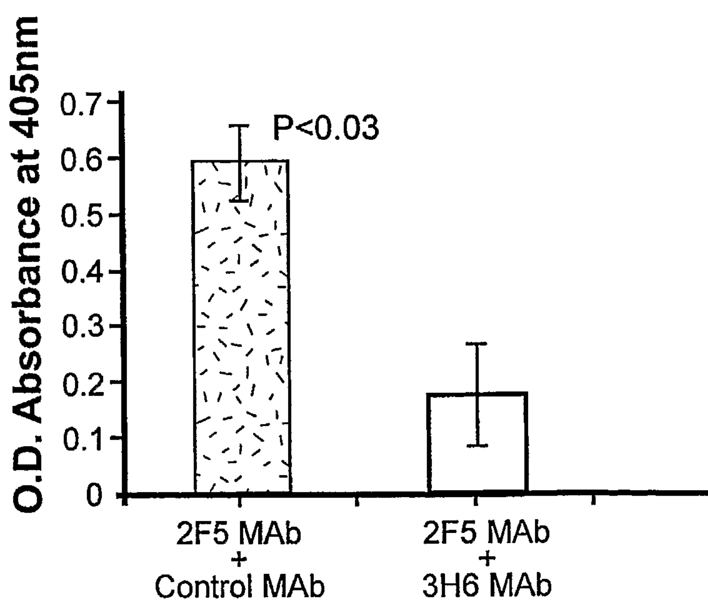


Figure 3B

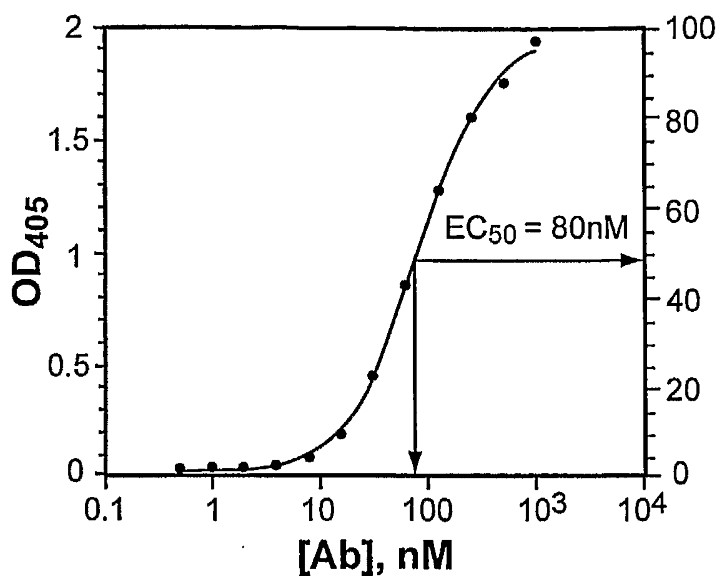


Figure 3C

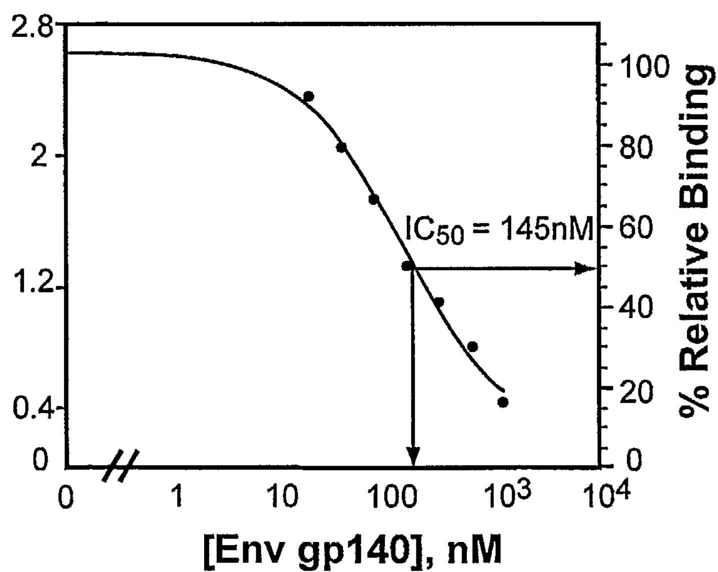


Figure 3D

Amino Acid and Nucleotide Sequences of CON-S Env gp160*

MRVRGIQRNCQHLWRWGTLILGMLMICSAAENLWVTVYYGVPVWKEANTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEIVLENVTENFNMWKNNMVEQMHEDII
SLWDQSLKPCVKLTPLCVTLNCTNVNVTNTTNNTEEKGEIKNCSFNITTEIRDKKQKVYALFYRLDVVPIDDNNNNSSNYRLINCNTSAITQACPKVSFEPIPIHYCA
PAGFAILKCNDKKFNGTGPCKNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENITNNAKTIIVQLNESVEINCTRPNNNTRKSIRIGPGQAFYATGDIIGDIRQ
AHCNISGTKWNKTLQQVAKKLREHFNNKTIIFKPSSGGDLEITTHSFNCRGEFFYCNTSGLFNSTWIGNGTKNNNNTNDTITLPCRIKQIINMWQGVGQAMYAPPIEG
KITCKSNITGLLLTRDGGNNNTNETEIFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAK*RRVVEREKRAVGIGAVFLGFLGAAGSTMGAASIT*LTVQARQLLSGI
VQQQSNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQ**LLGIWGCSGKLICTTTVPWNSSWSNKSQ**DEIWDNMTWMEWEREINNYTDIIYSLIEESQNQQEKN
EQELLALDKWASLWNWFDITNWLWYIKIFIMIVGGLIGLRIVFAVLSIVNRVRQGYSPLSFQTLIPNPRGPDRPEGIEEEGGEQDRDRSIRLVNGFLALAWDDLRSLC
LFSYHRLRDFILIAARTVELLGRKGLRRGWEALKYLWNLLQYWGQELKNSAISLLDTTAIAVAEGTDRVIEVVQRACRAILNIPRRIRQGLERALL

Figure 4A

Nucleotide sequence:

ATGCGCGTGCGCGGCATCCAGCGCAACTGCCAGCACCTGTGGCGCTGGGGCACCCTGATCCTGGGCATGCTGATGATCTGCTCCGCCGCCGAGAACCTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCAACACCACCCTGTTCTGCGCCTCCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTGGGCCACCCACGCC
TGCGTGCCCACCGACCCCAACCCCCAGGAGATCGTGCTGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCACGAGGACATCATC
TCCCTGTGGGACCAGTCCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTGACCCTGAACTGCACCAACGTGAACGTGACCAACACCACCAACAACACCGAGGAG
AAGGGCGAGATCAAGAACTGCTCCTTCAACATCACCACCGAGATCCGCGACAAGAAGCAGAAGGTGTACGCCCTGTTCTACCGCCTGGACGTGGTGCCCATCGACGAC
AACAACAACAACTCCTCCAACTACCGCCTGATCAACTGCAACACCTCCGCCATCACCCAGGCCTGCCCCAAGGTGTCCTTCGAGCCCATCCCCATCCACTACTGCGCC
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GAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCAACAACTACACCGACATCATCTACTCCCTGATCGAGGAGTCCCAGAACCAGCAGGAGAAGAAC
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GTGCAGCGCGCCTGCCGCGCCATCCTGAACATCCCCCGCCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAA

*Cleavage site and fusion domain are in italic font and underlined; the immunodominant region is highlighted in bold. The transmembrane and cytoplsmic domains are underlined

Figure 4B

Phospholipid	Structure	Phase	Neutralization Titer (Geometric Mean) HIV-1 SF162
Cardiolipin		Lamellar	<20
		Hexagonal $_{II}$	<20
POPS		Lamellar	<20
		Hexagonal $_{II}$	<20
POPE		Lamellar	<20
DOPE		Hexagonal $_{II}$	170

Figure 5

Peptide sequences used in the generation of peptide-liposome conjugates

1st generation conjugates

GTH1-2F5: YKRWIILGLNKIVRMYS-QQEKNEQELLELDKWASLWN
GTH1-4E10: YKRWIILGLNKIVRMYS-SLWNWFNITNWLWYIK
GTH1-V3 (Control): YKRWIILGLNKIVRMYS-KQIINMWQEVGKAMYACTRPNYNKRK
RIHIGPGRAFYTTK

2nd generation conjugates (native orientation)

MPER652-GTH1:
QQEKNEQELLELDKWASLWNWFNITNWLW-YIKYKRWIILGLNKIVRMYS

MPER656-GTH1:
NEQELLELDKWASLWNWFNITNWLW-YIKYKRWIILGLNKIVRMYS

MPER652 Scr.-GTH1:
EAWLWDLLIWNLQFEWKNNWTEQNQLEKS-YIKYKRWIILGLNKIVRMYS

Figure 6A

Additional 2nd Generation Peptides for Incorporation Into Liposomes

- 1). 4E10-GGG-GTH1:
- SLWNWFNITNWLWYIK-GGG-YKRWIILGLNKIVRMYS

- 2). Scr.4E10-GGG-GTH1:
- KNIWLSNYFWLINWWT-GGG-YKRWIILGLNKIVRMYS

- 3). 2F5-GGG-GTH1:
- QQEKNEQELLELDKWASLWN-GGG-YKRWIILGLNKIVRMYS

- 4). Scr.2F5-GGG-GTH1:
- NKEQDQAEESLQLWEKLNWL-GGG-YKRWIILGLNKIVRMYS

- 5). 4E10-GTH1:
- SLWNWFNITNWLWYIK-YKRWIILGLNKIVRMYS

- 6). Scr.4E10-GTH1:
- KNIWLSNYFWLINWWT-YKRWIILGLNKIVRMYS

- 7). 2F5-GTH1:
- QQEKNEQELLELDKWASLWN-YKRWIILGLNKIVRMYS

- 8). Scr.2F5-GTH1:
- NKEQDQAEESLQLWEKLNWL-YKRWIILGLNKIVRMYS

Figure 6B

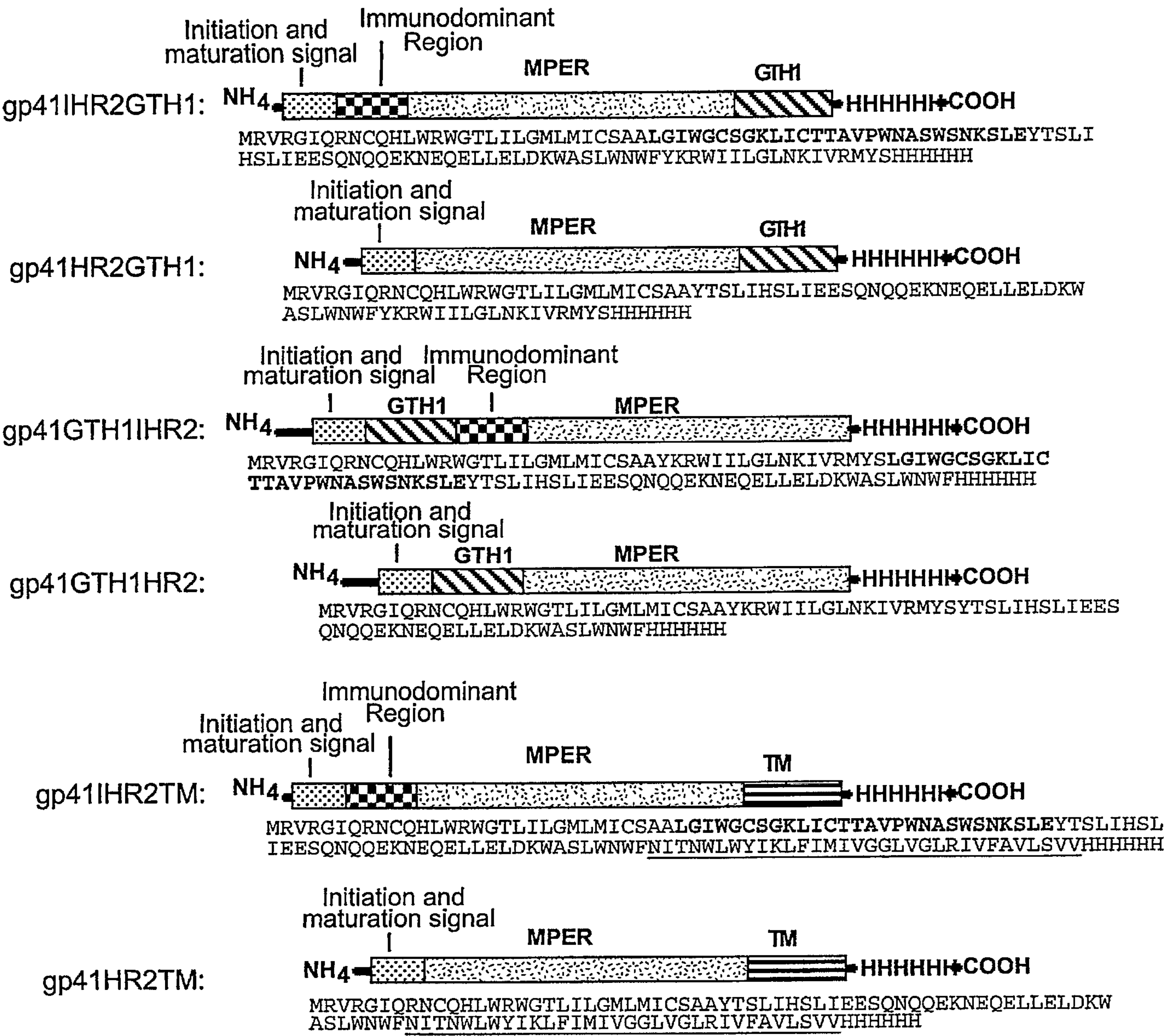


Figure. 7
Schematic presentation of various designs of MPER gp41 constructs

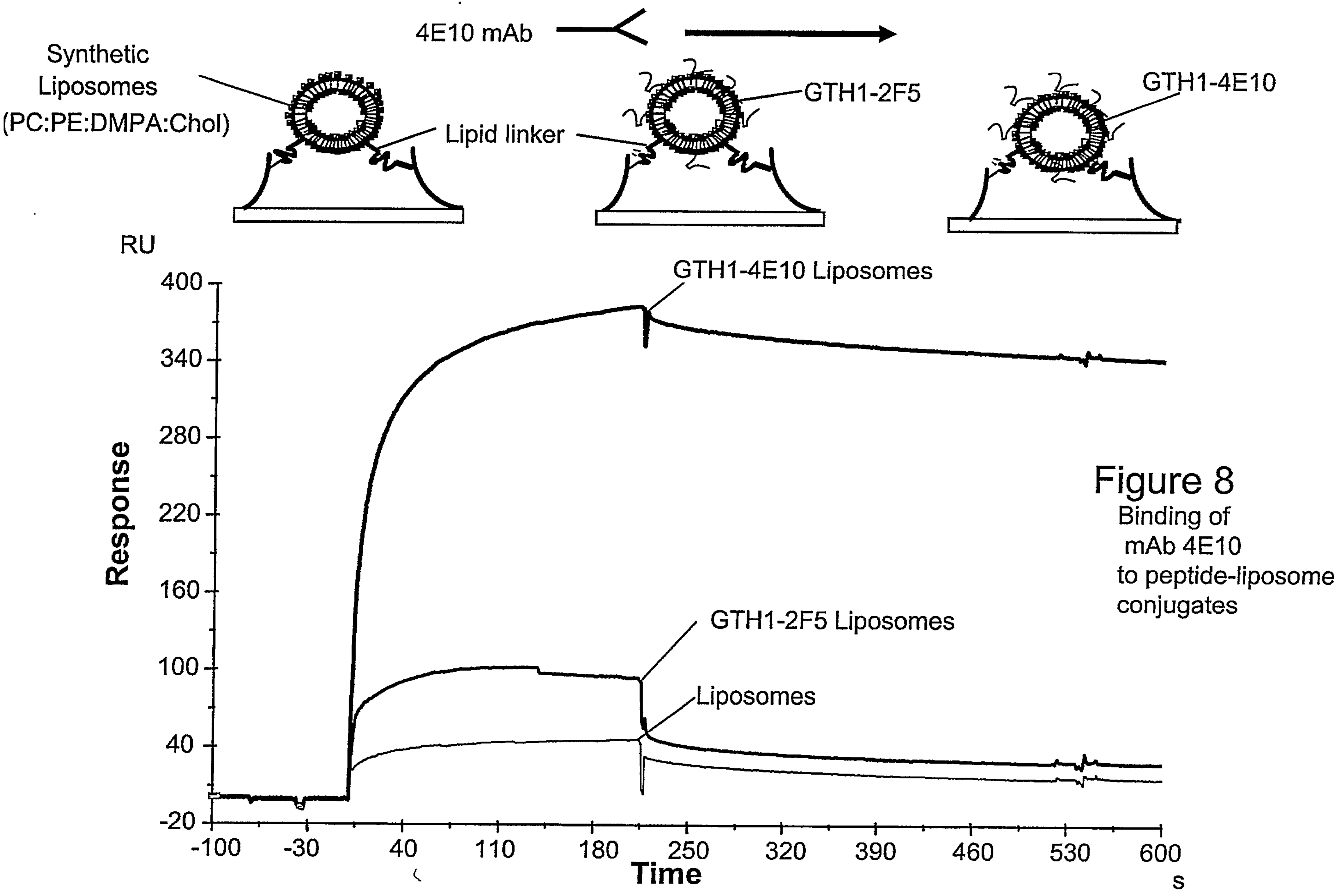
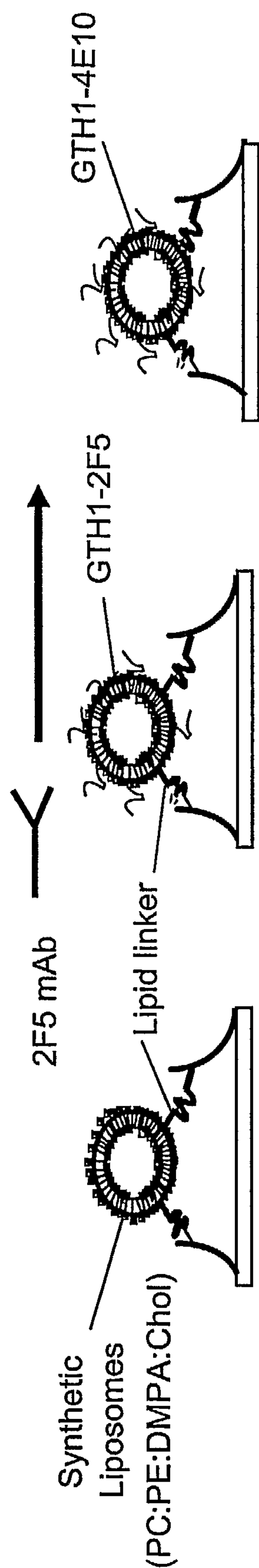


Figure 8
Binding of mAb 4E10 to peptide-liposome conjugates

Figure 9 Binding of 2F5 mAb to peptide-liposomes



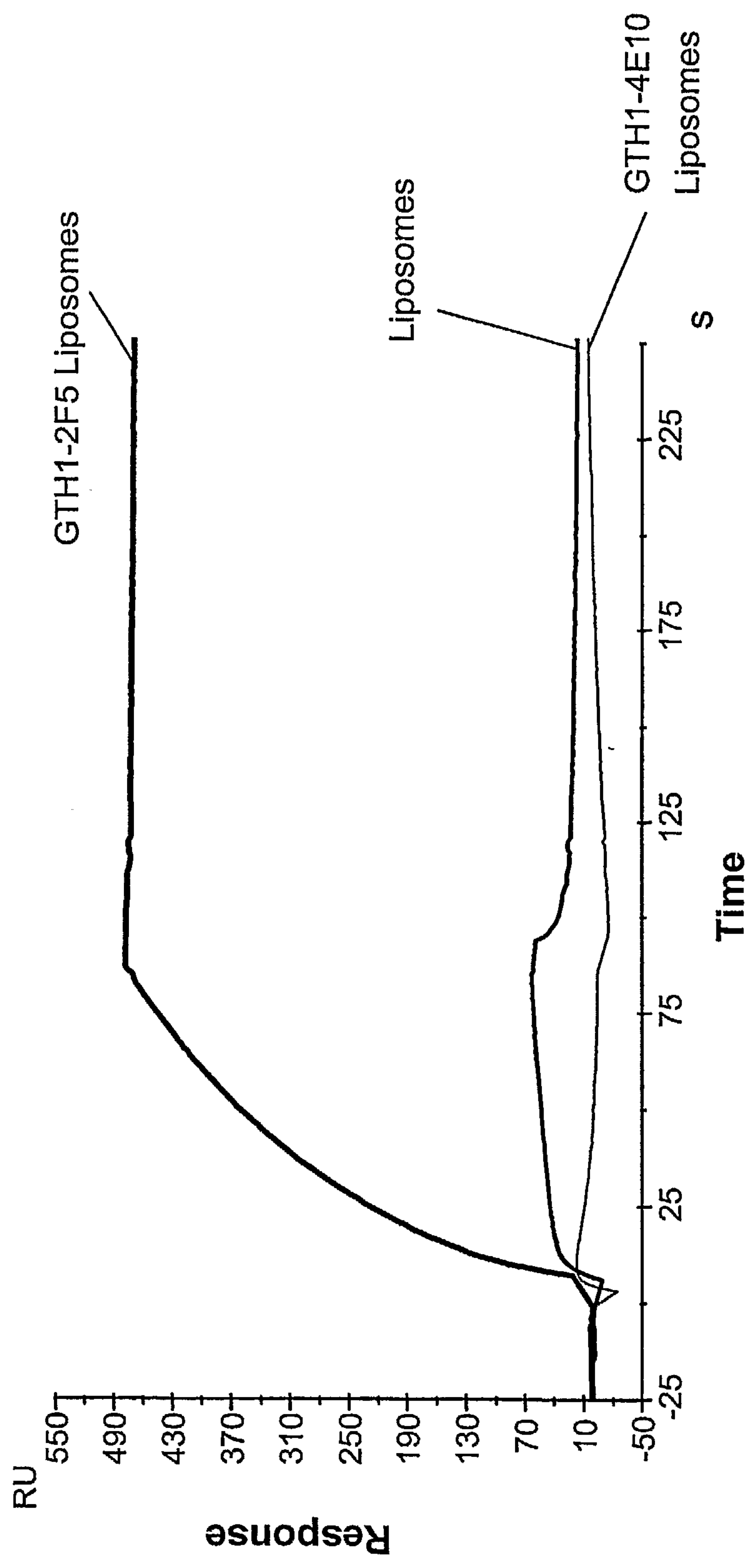
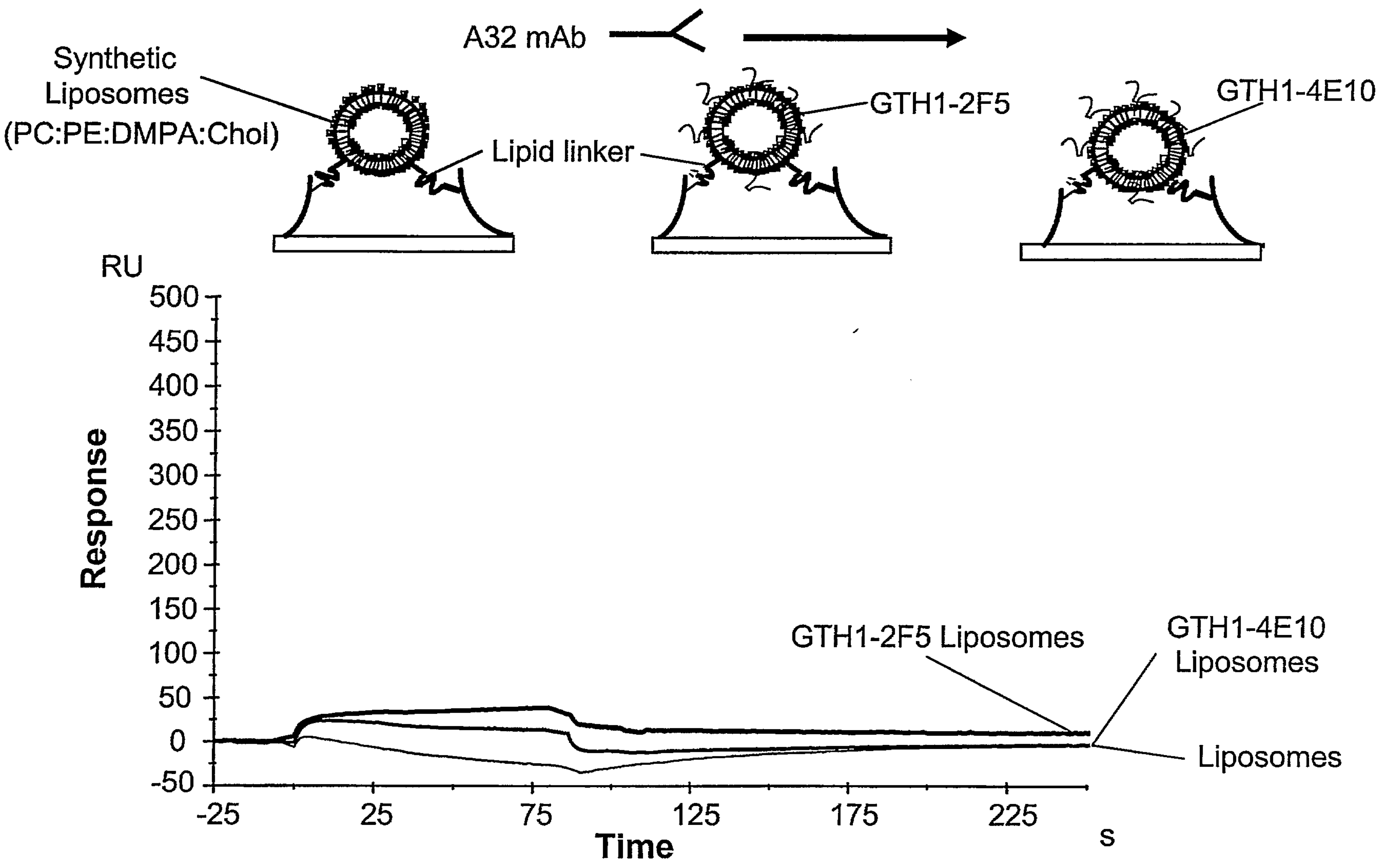


Figure 10 **A32 mAb (control) binding to peptide-liposomes**



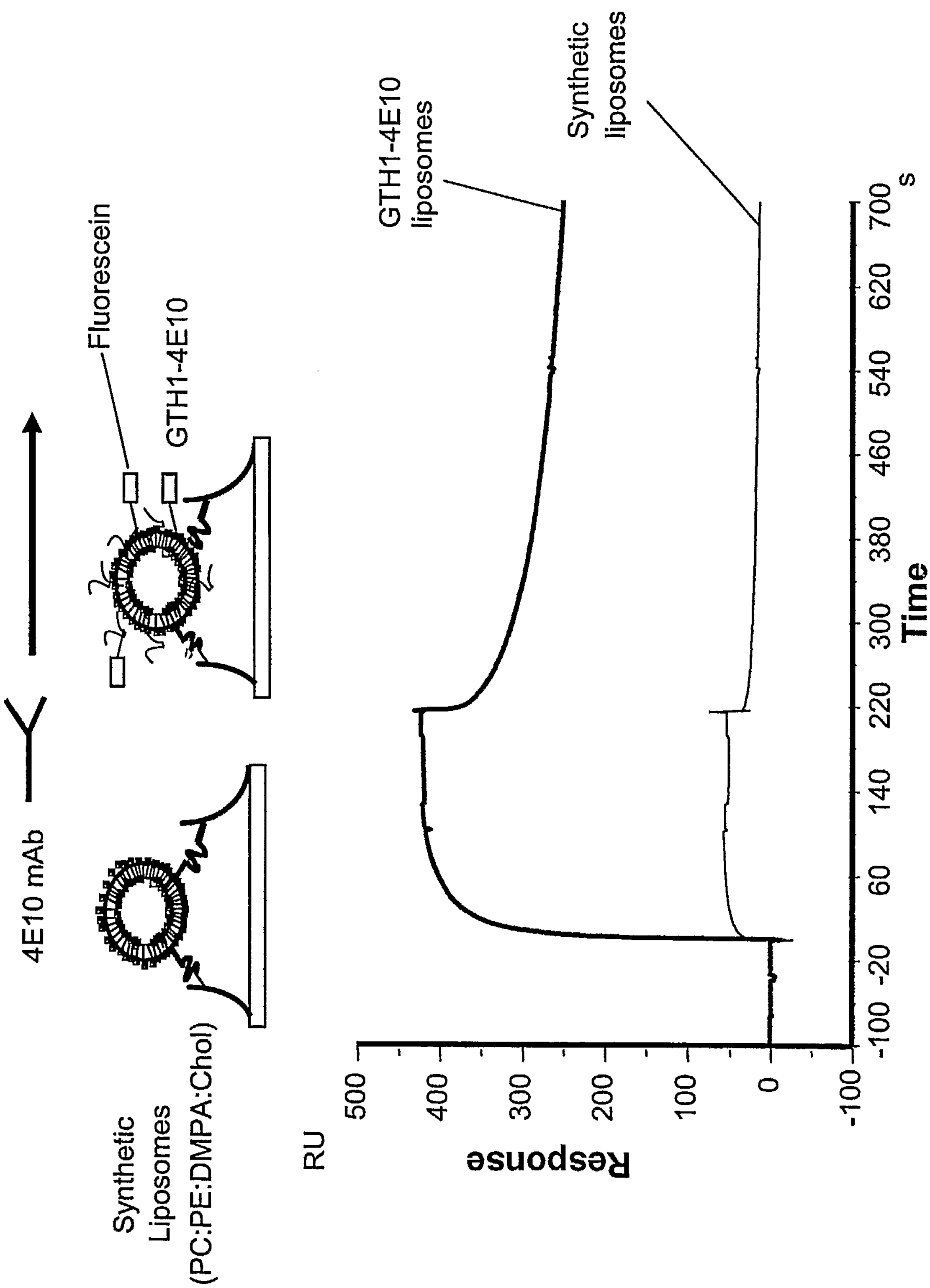


Figure 11 Generation of Fluorescein conjugated peptide-liposomes

Figure 12 Reactivity of immunized guinea pig sera with 4E10 peptide