

Description**Technical Field**

5 The present invention is directed to novel analogs (muteins) of the envelope proteins from human immunodeficiency virus type 1 (HIV-1), methods of making the analogs, DNA sequences encoding the analogs, and methods of using the analogs, for example, in immunoassays and vaccine compositions.

Background

10 HIV-1, and a recently identified related virus named HIV-2, are the known causative agents of Acquired Immune Deficiency Syndrome (AIDS). Many isolates of HIV-1 have been identified and sequenced. A striking feature of these independent isolates is substantial genomic and amino acid variation, centered particularly in the envelope gene and proteins. This variation among HIV-1 isolates has important implications for both AIDS diagnostics and potential vaccines.

15 Starcich et al., (1986) *Cell* 45:637-648, reports on the genetic variation in five independent HIV-1 isolates, as well as variations in deduced amino acid sequences. Both conserved and variable regions were observed.

20 Coffin, (1986) *Cell* 46:1-4, is a review directed to the variation in the envelope of HIV-1, and the possible mechanism which brings about the variation. Coffin hypothesized that the highly variable domains, termed "hypervariable" domains, are masking epitopes within the conserved domains from being available for neutralizing antibodies or cell-mediated immune responses. It is concluded that vaccination strategy for HIV-1 should be directed towards developing the ability to provoke an immune response directed against the conserved regions of the envelope despite the presence of masking variable domains.

25 Modrow et al., (1987) *J. Virol.* 61:570-578, is also directed to comparison of the amino acid sequences of various HIV-1 isolates. Computer analysis was employed to predict epitopes in the envelope protein, and it was found that the majority of predicted epitopes were located in the hypervariable regions. See, e.g., Figure 1 and Tables 1 & 2, incorporated herein by reference.

30 Hahn et al., (1986) *Science* 232:1548-1553, discloses the sequence variations in a series of HIV-1 isolates from a single individual, particularly in the envelope. See also Saag et al., (1988) *Nature* 334:440-444; Fisher et al., (1988) *Nature* 334:444-447.

35 Rusche et al., (1988) *Proc. Natl. Acad. Sci. USA* 85:3198-3202, discloses that a short peptide having the sequence of an HIV-1 isolate in the third hypervariable domain of the envelope protein was able to absorb isolate-specific neutralizing antibodies from antisera, and that another group was able to elicit isolate-specific neutralizing antibodies by immunization with a peptide from the same domain. It is suggested that since this neutralizing epitope is found in one of the hypervariable domains, that a possible vaccination strategy is to prepare a subunit antigen made up of the critical epitope from multiple isolates. See also Looney et al. (1988) *Science* 241:357-359.

40 Goudsmit et al., (1988) *Proc. Natl. Acad. Sci. USA* 85:4478-4482, reports on the neutralizing ability of antibodies raised in chimpanzees infected with various HIV-1 isolates, and a rabbit immunized with an envelope subunit. The epitope identified by Rusche et al. is reported to be an isolate-specific neutralizing epitope, and that it is immunodominant in HIV-1 chimpanzees.

45 Due in large part to the generally accepted view that attenuated or killed virus vaccines for AIDS are not feasible, the primary focus for the development of vaccines has been the subunit antigens. Several groups have suggested that short oligopeptides from the envelope domain would make suitable subunit antigens. Other groups are pursuing live recombinant virus vaccines, natural or recombinant viral polypeptide vaccines, or anti-idiotypic vaccines. See, e.g., Koff et al., (1988) *Science* 241:426-432 (and references cited therein).

50 A continuing need exists to develop new and better HIV-1 envelope analogs, as well as polypeptides that avoid isolate-specific immune interactions for use in diagnostics and as potential vaccines.

Summary of the Invention

55 The present invention is directed to HIV-1 envelope analogs (muteins) comprising the constant domains of gp120env or gp160env, but lacking at least one epitope from a hypervariable domain. Despite the reports in the literature suggesting the importance of the epitopes in the hypervariable domains (e.g., immunodominant and/or neutralizing), it has surprisingly been discovered that the muteins of the present invention are useful as diagnostic reagents exhibiting at least as great or greater reactivity to antibodies

raised against diverse isolates, and as antigens in raising nonisolate-specific antibodies upon immunization of a mammal.

In one embodiment, the present invention comprises an improved analog of HIV-1 gp120env or gp160env wherein the improvement comprises the deletion of at least one epitope within a hypervariable domain, while retaining the domains conserved among HIV-1 isolates.

In another embodiment, the present invention is directed to a polypeptide comprising epitopes that bind antibodies to HIV-1 gp120env or gp160env within an amino acid sequence according to the formula:

C1-V1-V2-C2-V3-C3-V4-C4-V5-C5

wherein:

C1 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Ser29 through Cys130 of HIV-1 SF2;

C2 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Cys199 through Leu291 of HIV-1 SF2;

C3 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Ser366 through Cys387 of HIV-1 SF2;

C4 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Cys415 through Gly456 of HIV-1 SF2;

C5 comprises an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Phe466 through Arg509 or Leu855 of HIV-1 SF2;

V1 is an amino acid sequence of 0 to a maximum of about 30 residues;

V2 is an amino acid sequence of 0 to a maximum of about 50 residues;

V3 is an amino acid sequence of 0 to a maximum of about 90 residues;

V4 is an amino acid sequence of 0 to a maximum of about 30 residues; and

V5 is an amino acid sequence of 0 to a maximum of about 10 residues;

with the proviso that at least one of the V domains selected from the group consisting of V1, V2, V3, V4 and V5 contains no more than about one-third of the said maximum number of residues for the V domain.

Still another embodiment of the present invention is directed to a polypeptide analog of HIV-1 gp120env or gp160env comprising (a) about 300 to about 850 amino acid residues in length; (b) constant domains, in the N-terminal to the C-terminal direction, Ser29-Cys130 of HIV-1 SF2 (C1), Cys199-Leu291 of HIV-1 SF2 (C2), Ser366-Cys387 (C3), Cys415-Gly456 (C4) of HIV-1 SF2, and Phe466-Arg509 or Phe466-Leu855 of HIV-1 SF2 (C5), or domains substantially homologous to said C1, C2, C3, C4 or C5; and (c) the intervening domains, if any, located between said constant domains comprising sequences found between substantially homologous constant domains in native HIV-1 gp120env, with the proviso that at least one of said intervening domains between said constant domains is either missing or missing an epitope.

The present invention is also directed to an immunoassay for the detection of antibodies to HIV-1 comprising: (a) providing a liquid sample to be tested for the presence of anti-HIV-1 antibodies; (b) contacting said sample with a polypeptide as described above under conditions whereby any anti-HIV-1 antibodies present in said sample may bind to an epitope to provide sample-contacted polypeptide; and (c) detecting any antibody bound to said sample-contacted polypeptide.

In yet another embodiment, the present invention is directed to a method of selectively raising antibodies in a mammal to epitopes in the constant domains of human immunodeficiency virus type 1 (HIV-1) gp120env or gp160env comprising administering to said mammal a polypeptide as described above, whereby antibodies to said polypeptide are produced in response to said administration.

The present invention is also directed to a composition useful in such method comprising the polypeptide described above in combination with a pharmaceutically acceptable carrier.

The present invention is also directed in another embodiment to a DNA sequence encoding the above polypeptide, as well as a cellular host comprising the DNA sequence under the control of transcriptional and translational control sequences whereby the polypeptide encoded by the DNA sequence is expressed by the cellular host. The present invention is also directed to methods of producing the above polypeptide by growing a culture of the above cellular host under conditions whereby the polypeptide encoded by the above DNA sequence is expressed, and recovering the polypeptide from the culture.

These and other embodiments of the present invention will be apparent to those of ordinary skill in the art.

Brief Description of the Figures

Figure 1 is a schematic diagram of the wild-type gp120env gene from HIV-SF2.

Figure 2 shows the alignment of amino acid sequences for various HIV-1 isolates, including SF2, with the constant and variable domains indicated. Potential N-linked glycosylation sites, for the HXB2 sequence only, are indicated by "[]"; cysteine residues have "" above them.

Figure 3 is a restriction map of mammalian expression vector pSV7dARV120tpa.

Figure 4 is a restriction map of mammalian expression vector pCMV6ARV120tpa.

Figure 5 is a restriction map of yeast expression vector pHL15.

Figure 6 contains graphs showing the result of an ELISA testing the reactivity of HIV-1 envelope mutants of the present invention and controls against a North American serum panel.

Figure 7 contains graphs showing the result of an ELISA testing the reactivity of HIV-1 envelope mutants of the present invention and controls against an African serum panel.

Figure 8 shows the results of an ELISA using sera from guinea pigs immunized with envelope mutants according to the present invention or various controls.

Figure 9 shows the results of an ELISA using sera from goats immunized with envelope mutants according to the present invention or various controls.

Detailed Description

HIV-1 is a known virus, of which many isolates have been observed. See, e.g., Levy et al., (1984) *Science* 225:840; Barre-Sinoussi et al., (1983) *Science* 220:868; Popovic et al., (1984) *Science* 224:497; *AIDS: Papers from Science, 1982-1985* (R. Kulstad ed. 1986); *Current Topics in AIDS* (M. Gottlieb et al. eds. 1987). The envelope gene of HIV-1 produces a precursor glycoprotein of approximately 160 mw, referred to as gp160 or gp160env. The precursor is cleaved during expression to provided gp120 (gp120env), the major exterior envelope glycoprotein, and gp41 (gp41env), the transmembrane protein. Various HIV-1 isolates have been reported in the literature, including, BH10, PV22, BRU, HXB2, WMJ1, WMJ2, WMJ3, CDC4, HTLV-III_{RF}, HTLV-III_B, Z3, Z6, Z321, MAL, NY5, ELI, JY1, LAV1A, and HAT3. Of particular interest to the present invention is HIV-1 isolate SF2, originally designated ARV2. See, e.g., Levy, U.S. Patent No. 4,716,102; Levy et al., (1984) *Science* 225:84; EPO Pub. No. 181,150.

The conserved and hypervariable domains of HIV-1 envelope proteins have been described previously. See, e.g., Modrow et al., *supra*. Figure 1 herein is a schematic diagram showing the location of the five hypervariable regions (V1, V2, V3, V4 and V5) and the five constant regions (C1, C2, C3, C4 and C5) in the envelope gene from HIV-1 SF2. Various deletions encompassing part or all of a hypervariable domain are also shown (D1, D2, D3, D4 and D5). Two regions of predominantly hydrophobic amino acids are denoted by dark shading, where the transmembrane anchor has been labeled TM. The signal sequence is highlighted at the N-terminus, as well as the processing site separating gp120env (positions 1-1527) from gp41env (positions 1528-2565).

Hypervariable domains are characterized by a substantial lack of homology (e.g., as low as 10%) among independent HIV-1 isolates. Furthermore, there is a substantial variation in length among the hypervariable domains from various isolates due to the prevalence of insertion and deletion mutations. Thus, these regions cannot be characterized from one isolate to the next by having any substantial degree of amino acid sequence homology, and can only be assigned an approximate length. The primary characterization of hypervariable domains is their location within the envelope glycoprotein and their presumed tertiary structure (i.e., loops). The conserved or constant domains, as well as all 18 cysteines in the envelope, are highly conserved. Corresponding hypervariable domains are found to be located identically relative to surrounding constant domains and cysteines from one isolate to the next. Furthermore, the tertiary structure of the hypervariable domain appears to be highly conserved; e.g., two nonhomologous hypervariable domains from different HIV-1 isolates will usually both exhibit the same three-dimensional conformation, such as an exposed loop. Thus, hypervariable domains from new HIV-1 isolates can be readily identified by sequencing the new isolates and comparing the sequence to known HIV-1 sequences so that the conserved domains and cysteines are aligned. See, e.g., Modrow et al., *supra* and Figure 2 herein.

The location of the various domains, both constant and hypervariable, will be described hereinafter with reference to the sequence and numbering of the HIV-1 SF2 isolate. It is to be understood that this is for convenience only; the invention is not limited to analogs containing only HIV-1 SF2 sequences, but also encompasses analogs employing corresponding domains or sequences from other isolates. A domain from another HIV-1 isolate envelope protein can be easily identified as corresponding to an SF2 sequence by

those of ordinary skill in the art by alignment of the conserved domains and the 18 cysteines of both isolates. Such an alignment is shown in Figure 2, where the corresponding sequences of 15 HIV-1 isolates are aligned. The cysteine residues are marked with an asterisk, and potential N-linked glycosylation sites for the HXB2 isolate are indicated by "[]". The cleavage site for the signal peptide and for the mature gp120env and gp41env proteins are shown, along with the constant domains, C1-C5. Specific deletions, D1-D5, are also shown in the figure.

The constant domains of the SF2 isolate are as follows: C1, Ser29-Cys130; C2, Cys199-Leu291; C3, Ser366-Cys387; C4, Cys415-Gly456; and C5, which is Phe466-Arg509 for gp120env analogs, or Phe466-Leu855 for gp160env analogs. Since these domains are highly conserved among HIV-1 isolates, the corresponding sequences from isolates other than SF2 will be substantially homologous to these SF2 domains; i.e., a minimum of about 70-75% amino acid sequence homology, with certain highly conserved domains exhibiting a minimum of about 80%, or even 85-90% homology. The differences in amino acid sequences found in these conserved domains are generally attributable to point mutations in the nucleic acid sequence, as opposed to the deletion and insertion mutations which typify the differences in hypervariable domains.

As shown in Figures 1 and 2, the hypervariable domains lie between the conserved domains, C1-C5. Not all of the amino acids in these intervening domains, however, comprise hypervariable regions. Indeed, due to the heterogeneity among HIV isolates in the hypervariable domain, it is not feasible to generally define precise limits of the hypervariable domain. Thus, for the convenience of describing the present invention, the domains between the constant domains will be referred to as "variable" or "intervening" domains, and it will be understood that they may comprise both hypervariable regions as well as less variable, yet not highly conserved, sequences. Thus, the analogs of the present invention can be described schematically according to the following formula:

C1-V1-V2-C2-V3-C3-V4-C4-V5-C5 (I)

C1-C5 and V1-V5 in formula I are defined as described above in the Summary of the Invention. V1-V5 consist of the variable or intervening domains which contain hypervariable regions. The polypeptides of the present invention will contain a deletion in at least one of these variable domains when compared to a native gp120env or gp160env. The deletions will typically be at least about one-third of the variable domain, and oftentimes will consist of a deletion of the entire variable domain. Furthermore, it is also preferred to delete sequences from more than one of the variable domains, including the deletion of all five of the variable domains in their entirety. Thus, in accordance with the present invention, any of the variable domains, V1-V5, can be anywhere from zero to a maximum number of amino acids in length, the maximum being an approximation of the longest of such domains found in HIV-1 isolates. At least one of the V domains, however, will have deleted therefrom at least one-third of that maximum number of amino acids, or one-third of the length of the corresponding domain from the homologous HIV-1 isolate.

Since the variable or intervening domains of formula I can comprise more than the true hypervariable domains, a selected Vn domain can be expressed by the following formula:

Sn-HVn-Sn' (II)

wherein n is 1, 2, 3, 4 or 5 (as in V1, V2, etc.), HVn is a hypervariable domain of x amino acid residues in length, and Sn and Sn' are nonhypervariable sequences flanking HV in the Vn domain, the flanking sequences being y and y' residues in length, respectively. In the native protein, the sum of x, y and y' equals the maximum number of residues for Vn in a chosen isolate, and y and/or y' may be zero. As for the analogs of the present invention, the sum of x, y and y' will be anywhere from zero to the maximum number. The actual values for x, y and y' for a particular Vn can be determined by sequence comparison of a representative number of HIV-1 isolates. See, e.g., Figure 2 and Modrow et al., *supra*.

The following are the intervening domains of the SF2 isolate, which can be used for comparison purposes to determine the corresponding intervening domains in other HIV-1 isolates. See, e.g., Figure 2. V1 of SF2 is about 24 amino acids in length, encompassing Thr131 through Asn154 and, optionally, Cys155. This C-terminal cysteine is one of the 18 highly conserved cysteine residues found in gp120env. The corresponding domains from independent HIV-1 isolates sequenced to date appears to range from about 22 to about 31 amino acid residues in length. V2 of SF2 spans from Ser156 through Ser198. The corresponding domain in other HIV-1 isolates sequenced to date appears to range from about 39 to about 52 amino acid residues in length. V3 of SF2 spans Asn292 through Glu365, and the corresponding domains in other HIV-1 isolates sequenced to date appears to range from about 88 to about 90 amino acid residues in length. V4 of SF2 spans from Asn388 through Pro414, and the corresponding domains from other HIV-1 isolates appear to range from about 28 to about 33 residues in length. V5 of SF2 spans from Thr457 through Thr463, and corresponding domains from other HIV-1 isolates appear to range from about 10 to about 11 residues in length.

In selecting the areas of the intervening domains for deletions, it is preferred to either select those portions showing hypervariability, or areas known to encode epitopes which elicit a significant immune response in vivo. Examples of such epitopes in variable regions of the SF2 isolate include, without limitation, residues 137-158 (V1), residues 189-209 (V2), residues 300-327 (V3), residues 367-384 (V3), and residues 404-420 (V4). Corresponding epitopes from other isolates are known. Modrow et al., supra. Examples of preferred deletions for SF2, D1-D5, are shown in Figures 1 and 2: Thr131-Asn154 (D1), Ser156-His198 (D2), Thr300-His332 (D3), Asn388-Pro414 (D4), and Thr457-Thr463 (D5). The polypeptides of the present invention may also have deletions from more than one variable domain, for example, V1 and V2; V1, V2 and V3; V1, V2 and V5; V3, V4 and V5; or V1, V2, V3, V4, and V5.

In a preferred embodiment, the HIV-1 envelope analogs of the present invention have deleted therefrom an epitope found in the intervening domains which is bound by antibodies produced by a mammal infected or immunized with the particular HIV-1 isolate, or its native envelope protein. It is particularly preferred that the deleted epitopes are those which produce isolate-specific immunodominant responses in a mammal. While applicants do not wish to be bound by this theory, it is believed that the deletion of these variable epitopes unmasks epitopes in the conserved domains which then become visible to the immune system by virtue of the absence of the epitopes from the variable domains.

In general, the amino acid sequence according to formula I will range from about 300 to about 850 amino acid residues in length, the actual length not being critical. The sequence of formula I can be contained within a larger polypeptide, for example a fusion protein. Such fusion proteins can include, for example, a fusion between the N-terminal sequence of superoxide dismutase (human or yeast) or beta-galactosidase, where these non-HIV-1 sequences are fused to the N-terminal of the C1 domain. Alternatively, non-HIV-1 sequences could also be fused to the C-terminal of the C5 domain. The C1 domain may also be fused to a signal peptide (e.g., yeast alpha factor, or tpa signal) to provide for secretion of the HIV-1 envelope analog from a cellular host expressing the analog.

The HIV-1 envelope muteins of the present invention can be produced by any suitable method, such as direct peptide synthesis or recombinant DNA expression. The preferred method is to prepare the polypeptides by recombinant DNA techniques.

The methodology for preparing such recombinant polypeptides is within the skill of the art, and the techniques are fully explained in the literature. See, e.g., Maniatis et al., Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, Volumes I & III (D.N. Glover, ed., 1985); Oligonucleotide Synthesis: A Practical Approach (M.J. Gate, ed., 1984); Perbal, A Practical Guide to Molecular Cloning - (1984). The production of recombinant HIV-1 polypeptides is known in the art. See, e.g., Luciw et al., (1984) Nature 312:760; Sanchez-Pescador et al., (1985) Science 227:484; Hahn et al., (1984) Nature 312:167; Alizon et al., (1984) Nature 312:757; Ratner et al., (1985) Nature 313:636; Muesing et al., (1985) Nature 313:450; Wain-Hobson et al., (1985) Cell 40:9; EPO Pub. Nos. 181,150; 187,041; 227,169; 230,222; and PCT Pub. Nos. WO87/02038; WO87/02989; WO87/04459; WO87/04728. Methods of recombinant expression are also disclosed in commonly owned U.S. patent application Serial No. 138,894, filed 24 December 1987, entitled "Human Immunodeficiency Virus (HIV) Nucleotide Sequences, Recombinant Polypeptides, and Applications Thereof," the disclosure of which is incorporated herein by reference.

To prepare the polypeptides of the present invention by recombinant methods, a DNA coding sequence for the polypeptides must be provided. Such a coding sequence is a DNA sequence that can be transcribed and translated into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by and include the translation start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. DNA sequences encoding the polypeptides of the present invention can be prepared either by constructing a synthetic gene from overlapping oligonucleotides, or by site-directed mutagenesis of a sequence encoding native HIV-1 envelope. See, e.g., Zoller, & Smith (1983) Meth. Enzymol. 100:468-500.

DNA coding sequences for the polypeptides of the present invention can be maintained on a replicon, which is any genetic element (e.g., plasmid, cosmid, chromosome, virus) that functions as an autonomous unit of DNA replication in vivo; i.e., capable of replication under its own control. Vectors are replicons such as a plasmid, phage, or cosmid to which a DNA coding sequence may be attached so as to bring about the replication of the attached segment in vivo.

A host cell is then transformed with a DNA construct containing the coding sequence under the control of appropriate regulatory sequences in order to bring about the expression of the DNA coding sequence into the desired polypeptide. Cellular hosts can include, but are not limited to, bacteria (e.g., *E. coli*, *Bacillus subtilis*, *B. amyloliquefaciens*, *Salmonella typhimurium*, *Klebsiella pneumoniae* or *Erwinia amylovora*), yeast (e.g., *Saccharomyces cerevisiae*, *S. carlsbergensis*, *S. kluyveri*, *Kluyveromyces lactis*, *Pichia*, or *Schizosaccharomyces*), mammalian cells (e.g., CHO cells, COS cells, 293 cells or *Xenopus* oocytes), and insect cells

(e.g., *Drosophila* embryos or *Spodoptera frugiperda*). The transformed cellular hosts may contain the DNA constructs encoding the polypeptides of the present invention either on an extrachromosomal element, or integrated into the chromosome.

Typically, the DNA coding sequence is placed into an expression cassette which comprises the DNA coding sequence flanked by appropriate regulatory sequences that control transcription initiation and termination within the cellular host. Preferably, the expression cassette contains convenient restriction sites at either end to permit convenient cloning of the cassette into an appropriate vector for transformation of the cellular host.

Transcription initiation and termination sequences recognized by the cellular host are DNA regulatory regions which flank a coding sequence are responsible for the transcription of an mRNA homologous to the coding sequence which can be translated into the desired polypeptide. Transcription initiation sequences include host promoter sequences, which are DNA regulatory sequences capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. A coding sequence is "under the control" of transcription initiation and termination sequences when RNA polymerase binds the transcription initiation sequences and transcribes the coding sequence into mRNA terminating at the transcription termination sequence, and the mRNA is then translated into the polypeptide encoded by the coding sequence (i.e., expression).

Cellular hosts transformed with appropriate DNA constructs for the expression of polypeptides of the present invention are typically grown in a clonal population under appropriate conditions which bring about the expression of the DNA coding sequence of interest. The appropriate growth conditions will depend upon the cellular host and the transcriptional and translational regulatory sequences employed. Upon expression, the recombinant polypeptide is recovered from the culture by any appropriate method, e.g., gel chromatography, immunoabsorption, or gel electrophoresis.

Polypeptides of the present invention can be used as reagents in immunoassays for detecting the presence in a sample of either anti-HIV-1 antibodies or viral antigen. In an immunoassay for viral antigen, for example, polypeptides according to the present invention can be labeled and used as a competing antigen in a standard competitive ELISA or radioimmunoassay (RIA).

Configurations for immunoassays for antibodies to HIV-1 envelope vary widely, and the present invention contemplates the use of the polypeptides described herein in any such format. Such formats are well known in the art. See, e.g., *Immunoassay: A Practical Guide* (P.W. Chan & M.T. Perlestein, eds., 1987); McDougal et al., (1985) *J. Immunol. Meth.* 76:171-183; U.S. Pat. Nos. 4,629,783; 4,281,061; 4,520,113; 4,591,552; 4,134,792 (incorporated by reference herein). Whether the assay format is homogeneous or heterogeneous, and the measurement method is direct or indirect (e.g., competition), all such immunoassays have three common steps. First, a liquid sample suspected of containing the antibodies is provided. This sample is then contacted with polypeptides according to the present invention under conditions which will allow any antibodies having an epitope on the polypeptides to become bound thereto. Finally, there is a detecting step wherein it is determined whether or not any antibody bound to the polypeptide.

A preferred format for an anti-HIV-1 antibody assay is a heterogeneous immunoassay in which the polypeptides of the present invention are immobilized on, for example, a solid support. The selection of the appropriate solid support for immobilization of the polypeptide is conventional and within the skill of the art. In a typical assay, the sample suspected of containing the antibodies is contacted with the immobilized polypeptide and allowed to incubate under the appropriate conditions. The immobilized polypeptide is then separated from the sample and washed to remove any unbound antibody. The detecting step can constitute, for example, contacting the washed, immobilized polypeptide with an antibody that will recognize an epitope located on the anti-HIV-1 antibodies (i.e., anti-xenogenic), this second antibody being appropriately labeled for detection (e.g., radiolabeled, enzyme conjugated, avidin/biotin). After an appropriate incubation and washing step, the immobilized polypeptide is then assayed for the presence of the labeled second antibody. Alternatively, a competition immunoassay can be used where a labeled reference antibody to the immobilized polypeptide is incubated along with the sample suspected of containing the anti-HIV-1 antibodies, and the presence of such antibodies are determined by measuring a reduction or inhibition of binding of the labeled reference antibody to the immobilized polypeptide. See, e.g., PCT Pub. No. WO87/07957.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the polypeptides of the present invention and any antibodies used in the assay, in suitable containers along with remaining reagents and materials required by the assay format; e.g., incubation media, wash media, and means for measuring the presence of the analyte (e.g., enzyme-labeled antibodies and enzyme substrate). Other labels useful in the practice of immunoassays according to the present invention include radioisotopes and fluorescing compounds.

Polypeptides according to the present invention can be employed to selectively raise antibodies in a mammal to epitopes found in the constant domains of gp120env or gp160env. For example, parenterally administering polypeptides of the present invention to a mammal will cause an immune reaction in the animal, thereby producing antibodies to epitopes found in the conserved domains. Such antibodies can be recovered to make polyclonal antiserum, or antibody-producing cells recovered for fusion (or another immortalization technique) to produce monoclonal antibody-producing cell lines. See, e.g., M. Schreier et al., *Hybridoma Techniques* (1980); Hammerling et al., *Monoclonal Antibodies and T Cell Hybridomas* - (1981); Kennett et al., *Monoclonal Antibodies* (1980); U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887. Antiserum produced by the above method will be advantageous in that it will possess a high titer of antibodies which are reactive to all or most HIV-1 isolates. In a similar manner, the screening of hybridomas for non-isolate-specific antibodies will be facilitated by the elimination of some or all of the immunodominant epitopes of the hypervariable regions.

To generate such an antibody response, polypeptides of the present invention are typically formulated with a pharmaceutically acceptable carrier for parenteral administration. The formulation of such compositions, including the concentration of the polypeptide and the selection of the vehicle and other components, is within the skill of the art.

A pharmaceutically acceptable vehicle, suitable for parenteral injection, is usually nontoxic and non-therapeutic. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and Hank's solution. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Parenteral vehicles may also take the form of suspensions containing viscosity-enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. The vehicle will also usually contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and tris buffer, while examples of preservatives include thimerosal, m- or c-cresol, formalin and benzyl alcohol. The muteins of the present invention may also be formulated into liposomes for parenteral administration. Standard formulations will either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a nonliquid formulation, the vehicle may comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline could be added prior to administration.

Various adjuvants are known in the art which can also be employed in the vaccine formulations of the present invention; e.g., Freund's adjuvant, avridine, aluminum salts $[Al(OH)_3AlPO_4]$, $Al_2(SO_4)_3$, $Ca_3(PO_4)_2$, saponin, DDA, Pluronic, oil-in-water emulsions (containing, e.g., avridine, dextran sulfate or vitamin E), water-in-oil emulsions (containing, e.g., polysorbate 8), and muramyl peptides (e.g., di- and tripeptides in carriers such as oil-water emulsions or liposomes). The selection of the appropriate adjuvant and its concentration in the vaccine composition is within the skill of the art.

Many protocols for administering the vaccine composition of the present invention to animals are within the skill of the art. The preferred route of administration is parenteral, particularly intramuscular, although administration may also be intravenous. The concentration of the polypeptide antigen in the vaccine composition is selected so that an effective dose is presented to the host mammal (e.g., primate) to elicit antibodies to the polypeptide's epitopes. Within wide limits, the dosage is not believed to be critical. Typically, the vaccine composition is administered in a manner which will deliver between about 1 to about 1,000 ug of the polypeptide antigen in a convenient volume of vehicle (e.g., about 1-10 ml). Preferably, the dosage in a single immunization will deliver from about 1 to about 500 ug of polypeptide antigen, more preferably about 5-10 to about 100-200 ug (e.g., 10-100 ug). It may also be preferred, although optional, to administer a second, booster immunization to the mammal several weeks to several months after the initial immunization. It may be helpful to readminister a booster immunization to the mammal once every several years to maintain high antibody titer.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the claims in any way.

EXAMPLES

The following example describes the construction of DNA sequences encoding HIV-1 SF2 envelope muteins according to the present invention. The hypervariable regions and the corresponding deletions are shown below in Table 1.

Table 1

Hypervariable Regions and Deletion Mutants			
Hypervariable Regions		Corresponding Deletion Mutant(s)	
V1	131 through 154	D1	131 through 154
V2	156 through 198	D2	156 through 198
V3	292 through 365	D3	300 through 332
V4	388 through 414	D4	388 through 414
V5	456 through 465	D5	457 through 463

DNA encoding sequences for mutins of the present invention were prepared by mutagenesis of a gp120env SF2 gene. Mutagenesis was achieved employing the procedure described by Zoller and Smith, (1983) *Meth. Enzymol.* 100:468-500, modified as described below. Synthetic DNA mutagenesis and sequencing primers (Table 2) were prepared by automated oligonucleotide synthesis on a silica support as described by Urdea et al., (1983) *Proc. Natl. Acad. Sci. USA* 80:7461-7465, using N,N-diisopropyl phosphoramidites. Sequencing primers were designed for sequencing by the dideoxynucleotide chain termination method in bacteriophage M13. Sanger et al., (1977) *Proc. Natl. Acad. Sci. USA* 74:5483. The sequencing primers were designed to be complementary to M13mp8 recombinant templates, of 18 bases in length, and to anneal at a position at least 50 bases away from the mutation locus. Table 2 indicates the oligomers employed for mutagenesis screening and sequencing.

The mutagenesis template contained the entire plasmid pSV7dARV120tPA, which contains the SF2 gp120env gene coupled to the tPA signal sequence (described below). The plasmid was linearized at its unique *Pst*I site and cloned into the unique *Pst*I site of M13mp8.

Table 2

Mutagenesis Primers	
D1	5' CCACTCTGTGTTACTTTAAATTGCTGCTCTTTCAATATCACCACAAGC 3'
D2	5' ATAAAAGGAGAAATAAAAACTGCTGTAACAGATCAGTCATTACACAG 3'
D3	5' AATGAATCTGTAGCAATTAAGTGTGTAACATTAGTAGAGCACAATGG 3'
D4	5' GGGGAATTTTCTACTGTTGTAGAATAAAACAAATTATAAACATGTGG 3'
D5	5' CTGCTATTAACAAGAGATGGTGGTGAGGTCTTCAGACCTGGAGGAGGA 3'
D1 + D2	5' CCACTCTGTGTTACTTTAAATTGCTGCTGTAACAGATCAGTCATTACA 3'
DNA Sequencing Primers	
D1,D2, D1 + D2	5' TAATCAGTTTATGGGATC 3'
D3	5' CTGTTAAATGGCAGTCTA 3'
D4,D5	5' CAATCCTCAGGAGGGGAC 3'
Screening Probes	
D1	5' TTAAATTGCTGCTCTTTC 3'
D2	5' AAAAAGTCTGTAACAGA 3'
D3	5' AATTAAGTGTGTAACATTA 3'
D4	5' GGGGAATTTTCTACTGTTGTAGAATAAAACAAATTATAAACATGTGG 3'
D5	5' ATGGTGGTGAGGTCTT 3'
D1 + D2	5' CCACTCTGTGTTACTTTAAATTGCTGCTGTAACAGATCAGTCATTACA 3'

Mutagenesis of M13/pSV7dARV120tPA recombinants was performed using purified templates by annealing and extending the appropriate primer with the Klenow fragment of DNA polymerase I. Individual deletion mutants D1, D2, D3 and D5 were obtained utilizing the appropriate mutagenesis primer in a single round of mutagenesis (see Table 2). Deletion mutant D4 was obtained utilizing the appropriate mutagenesis primer with the annealing and elongation reactions performed at 37°C and in the presence of 125 ug/ml gene 32 protein. Combination deletion mutant D1 + D2 was obtained utilizing the appropriate mutagenesis primer using a template derived from deletion mutant D1.

Following transfection of JM101 cells (Zoller and Smith, *supra*), plaques were grown at a density of 200-1,000/plate and lifted onto filters and screened by hybridization with the appropriate mutagenesis primer or probe (see Table 2).

The DNA sequence of putative positive clones was determined using suitable primers and template preparations. Once the mutagenized locus and flanking segments (i.e., at least 50 bases) were confirmed by DNA sequence analysis, replicative form (RF) DNAs were digested by *Pst*I restriction endonuclease and the entire mutagenized mammalian expression vector pSV7dARV120tPA containing the deletion was recovered. The vector provides an SV40 early promoter and enhancer for expression of SF2 gp120env gene, SV40 polyadenylation site, and an SV40 origin of replication for use of the vector in COS cells.

Following recovery of pSV7dARV120tpa plasmids for wild-type gp120 or for a deletion mutant, the plasmid was digested with *Sall* to excise the complete gene spliced to the human tPA 5' untranslated sequences and signal sequences. The *Sall* fragment was subcloned into the unique *Sall* cloning site of the mammalian cell expression vector pCMV6a. The resulting plasmid was screened to verify the correct orientation of the gene with respect to the promoter and polyadenylation signals, and this plasmid was named pCMV6ARV120tpa for the wild-type gp120 sequences. In cases where the pSV7d vector was not recovered directly as a plasmid, the *Sall* fragment containing the gene was subcloned from the M13 mutagenesis template clone into pCMV6a.

Combination deletion mutant D4-D5 was obtained utilizing the appropriate D5 mutagenesis primer using a template derived from deletion mutant D4.

Combination deletion mutant pSV7d120D3-D4-D5 was obtained by subcloning the region containing D4-D5 by digestion of M13pSV7d120D4-D5 with *Mst*II and *Hind*III and insertion of this 334 bp fragment into *Mst*II and *Hind*III digested pSV7d120D3. The *Sall* fragment from pSV7d120D3-D4-D5 containing D3-D4-D5 was subcloned into the *Sall* site of pCMV6a to create pCMV6a120D3-D4-D5.

Combination deletion mutant D1-D2-D5 was obtained by subcloning the region containing D1-D2 by digestion of pCMV6a120D1-D2 with *Nhe*I and *Bgl*II and insertion of this 540 bp fragment into *Nhe*I and *Bgl*II digested pCMV6a120D5.

Combination deletion mutant D1-D2-D3-D4-D5 was obtained by subcloning the fragment containing the D1-D2 region by digestion of M13pSV7d120D1-D2 with *Nhe*I and *Bgl*II and insertion of this 539 bp fragment into *Nhe*I and *Bgl*II digested pCMV6a120D3-D4-D5.

Expression vector pCMV6a can be regenerated by excising the coding sequence for gp120 from pCMV6ARV120tpa with *Sall*. The mutagen coding sequences described above can all be constructed from the wild-type gp120 coding sequence in pCMV6ARV120tpa as described for pSV7dARV120tpa. Table 3 sets forth the names and deletions of the various M13-pSV7d- and pCMV6a-based vectors made according to the above protocol.

Table 3

Gene Version	M13 Vector	pSV7d Vector	pCMV6a Vector
Wild-type	M13pSV7d120	pSV7dARV120tpa	pCMV6ARV120tpa
D(1 + 2)	M13pSV7d120D1 + 2	not made	pCMV6a120D1 + 2
D1	M13pSV7d120D3	pSV7d120D1	pCMV6a120D1
D2	M13pSV7d120D2	pSV7d120D2	pCMV6a120D2
D3	M13pSV7d120D3	pSV7d120D3	pCMV6a120D3
D4	M13pSV7d120D4	not made	pCMV6a120D4
D6	M13pSV7d120D5	not made	pCMV6a120D5
D3 + D4	M13pSV7d120D3 + D4	not made	not made
D4 + D5	M13pSV7d120D4 + D	pSV7d120D4 + D5	not made
D3 + D4 + D5	not made	pSV7d120D3 + D4 + D5	pCMV6a120D3 + D4 + D5
D(1-5)	not made	not made	pCMV6a120D1-5
D(1 + 2 + 5)	not made	not made	pCMV6a120D(1 + 2 + 5)

pSV7dARV120tPA (Figure 3) was constructed as follows. An env gene was modified by in vitro mutagenesis to eliminate any potential transmembrane domains and to provide a stop codon following the processing site between the gp120 and gp41 domains of the gp160 protein. This mutagenesis was accomplished by subcloning the fragment which encodes the env gene from clone pSV7c/env (ATCC Accession No. 67593) by excising with *Hind*III and *Xho*I (SF2 clone positions 5582 and 8460) and inserting the 2.8 kb fragment into M13mp19 previously digested with *Hind*III and *Sall*. A 37 bp oligonucleotide of the

following sequence was used to alter the sequence at the gp120/gp41 processing site at position 7306 to encode 2 stop codons and two restriction endonuclease sites.

5'-GAACATAGCTGTCGACAAGCTTCATCATCTTTTTTCT-3'

The sequence of the wild-type gene and the mutant are shown below:

5

Wild-type sequence:

	Processing Site										
¹⁰ Position:	7288	7294	7300							7318	
Amino acid:	Val	Gln	Arg	Glu	Lys	Arg	Ala	Val	Gly	Ile	Val
DNA:	GTG	CAG	AGA	GAA	AAA	AGA	GCA	GTG	GGA	ATA	GTA

gp120 mutant:

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15
Position:      7288      7294      7300      7306      7317
Amino Acid:   Val Gln Arg Glu Lys Arg OP  OP
DNA:          GTG CAG AGA GAA AAA AGA TGA TGA AGC TTG TCG AC
Restriction site:                               HindIII SalI

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20

Following mutagenesis, the gene was engineered for optimal secretion into the medium. A 268 bp XbaI-NdeI fragment containing the heterologous 5' untranslated sequences and signal sequences from human tPA fused to the 5' end of env was excised from the M13 clone M13tpaS.Nhelenv described below. This fragment was ligated with a 1363 bp NdeI-Sall fragment encoding the remainder of the gp120 coding region which was isolated from the gp120 mutant (positions 5954 and 7317) described above, and both fragments were inserted into the vector pSV7d (described below) previously digested with XbaI and Sall.

Expression vector pSV7d can be generated by digesting pSV7c/env with BglII and XbaI, and then ligating the digested plasmid with the following linker:

30

BglII EcoRI SmaI XbaI BamHI SalI

5'-GATCTCGAATTCCCCGGGTCTAGAGGATCCGTCGAC
ABCTTAAGGGGCCAGATCTCCTAGGCACGTGGATC-5'

35

M13tpaS.Nhelenv was constructed as follows. The 5' end of the env coding sequence was modified to accept a heterologous signal sequence known to direct efficient secretion of both the homologous gene (human tissue plasminogen activator) and deletion variants of this gene. van Zonneveld et al., (1986) Proc. Natl. Acad. Sci. USA 83:4670.

40

A portion of the HIV-1 SF2 gene in a lambda phage (ATCC Accession No. 40143) was excised with *SacI* and *StuI* (positions 5555 and 6395) and was subcloned into the vector M13mp19 [Yanisch-Perron et al., (1985) *Gene* 33:103-109] between *SacI* and *SmaI*. Oligonucleotide-directed mutagenesis [Zoller et al., (1983) *Meth. Enzymol.* 100:468-500] was used to create an *NheI* site at the junction of the natural signal peptide and the mature envelope polypeptide using the following oligonucleotide:

45

5'-GATGCTCTGTTCAGCTAGCGAAAAATTGTGG-3'

This mutagenesis changes cytosine-5867 to guanine and adenine-5868 to cytosine, thereby creating an NheI site and altering the codon for threonine-30 to code for serine.

In parallel, the tPA gene was likewise mutagenized in M13 to place an NheI site near the carboxyl end of the tPA signal peptide. The following sequences show the 5' UT sequences and signal for wild-type tPA leader and for the NheI variant.

Wild-type sequence of the tPA signal:

5' untranslated sequences

55

AGAGCTGACATCCTACAGGAGTCCAGGGCTGGAGAGAAAACCTCTGCGAGGAAAGGGAAGGA

GCAAGCCGTGAATTTAAGGGACGCTGTGAAGCAATCATGGATGCAATGAAGAGAGGGCTC
MetAspAlaMetLysArgGlyLeu

TGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCCAGGAAATCCATGCC
CysCysValLeuLeuLeuCysGlyAlaValPheValSerProSerGlnGluIleHisAla

mature tPA
CGATTCAGAAGAGGAGCCAGA TCTTACCAAGTG
ArgPheArgArgGlyAlaArg SerTyrGlnVal

The NheI variant of the tPA signal:
5' untranslated sequences

AGAGCTGAGATCCTACAGGAGTCCAGGGCTGGAGAGAAAACCTCTGCGAGGAAAGGGAAGGA

GCAAGCCGTGAATTTAAGGGACGCTGTGAAGCAATCATGGATGCAATGAAGAGAGGGCTC
MetAspAlaMetLysArgGlyLeu

TGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCTAGC
CysCysValLeuLeuLeuCysGlyAlaValPheValSerProSerAlaSer

Following mutagenesis and sequence verification, a 174 bp fragment containing 99 bp of 5' untranslated sequence and the signal sequence from tPA was excised from the tPA-containing M13 clone using Sall and NheI and fused to the 559 bp fragment containing the 5' end of the env gene which was excised from the env-containing M13 clone with NheI and HindIII (contributed by the M13 polylinker), and these fragments were subcloned into M13mp18 between Sall and HindIII to give plasmid M13tpas.NheIenv. The DNA and amino acid sequence of the tPA signal fused to the 5' end of env gene is:

...tPA signal... 5869 (amino acid 31 of env)
Phe Val Ser Pro Ser Ala Ser Glu Lys Leu Trp Val Thr Val
TTC GTT TCG CCC AGC GCT AGC GAA AAA TTG TGG GTC ACA GTT
NheI

Example II

This example describes the expression of HIV-1 env analogs in mammalian cells.

DNA encoding the complete env gene with the substituted tPA signal sequence was excised from the plasmid pSV7dARV120tpa using the restriction endonuclease Sall and inserted into the unique Sall site of the mammalian cell expression vector pCMV6a, and the resulting plasmid DNA was screened to verify the correct orientation of the gene with respect to the promoter and polyadenylation signals (see Example I). The resulting plasmid, pCMV6ARV120tpa, and the plasmid pSV7dARV120tpa were used to transfect COS-7 cells to test expression and secretion of gp120 into the medium. pCMV6ARV120tpa was at least 50-fold more efficient in expressing gp120 compared with the pSV7dARV120tpa expression plasmid in these cells.

Permanent cell lines were isolated as follows: human kidney 293 cells were plated at a density of 50-70% confluency in DME supplemented with glutamine (292 mg/L), sodium pyruvate (110 mg/L), glucose (4.5 g/L), penicillin (1000 U/L), streptomycin (1000 U/L), 3.7 g/L sodium bicarbonate, and fetal calf serum (10% v/v). Cells were exposed to a calcium phosphate coprecipitate following standard techniques with 10 ug each of the HIV env expression plasmid pCMV6ARV120tpa (wild type or deletion mutant) and a plasmid encoding the selectable marker neomycin-resistance, pSV2neo (Ref), for six hours at 37°C in a 10% CO₂ atmosphere. Cells were washed and exposed to a 3 to 4 minute shock of 15% DMSO or glycerol in

HEPES-buffered saline, and growth medium (described above) was replaced for 48 hours. Trypsinized cells were replated at a lower density in 400 ug/ml G418 (Sigma) in DME supplemented as above. Colonies grew to the 100 cell per focus stage in one week to ten days, and these colonies were transferred individually to 96 well plates. Clones were screened for gp120 production by testing the conditioned cell medium using an ELISA described below. Positive clones were scaled up to T75 flasks, aliquots of cells frozen, and cell supernatants were collected for further characterization, e.g. CD4 binding.

Alternatively, CHO dhfr- cells plated at a density of 50-70% confluence were cotransfected by calcium phosphate coprecipitation using 10 ug each of the plasmids pCMV6ARV120tpa (or analogous deletion mutant expression vector) and the selectable marker dhfr encoded in the plasmid pAd-dhfr. Following exposure to the coprecipitate and shock solutions as described above, cells were incubated for 48 hours in Ham's F12 supplemented with glutamine (292 mg/L), sodium pyruvate (110 mg/L), sodium bicarbonate (3.7 g/L), glucose (4.5 g/L), penicillin (1000 U/L), streptomycin (1000 U/L), proline (150 mg/L), and fetal calf serum (10%). Forty-eight hours after transfection, cells were plated at a density of approximately one tenth in DME supplemented as described for F12 above, except that the fetal calf serum was replaced with dialyzed fetal calf serum (10%). Colonies were transferred individually to 96 wells after about two weeks, then screened using the ELISA assay for gp120 secreted into the medium. Positive clones were scaled up as described above.

For detection of gp120 or gp120 hypervariable region deletion mutants in the supernatants of COS, CHO, or 293 cells, conditioned medium was assayed by ELISA specific for gp120 sequences. Pooled HIV-positive human serum inactivated by treatment with psoralen was affinity purified on Staphylococcus Protein A Sepharose by standard techniques. This serum was coated on Immulon 1 96 well ELISA plates at a concentration of 5 ug/ml in PBS and plates were incubated 12 hours to two months at 4°C. Following incubation, plates were washed as described for the titration ELISA (Example IV), and samples and standards (including purified recombinant HIV-1 gp120env from yeast) were applied to the plate in two-fold dilution series using the dilution buffer described for the titration ELISA. The range of the assay is 100 ng/ml to 200 pg/ml. Samples were incubated for 12 hours at 4°C. Samples were aspirated and plates were washed as above. Samples were then incubated with an appropriate dilution of rabbit serum from rabbits immunized with recombinant SF2 gp120env analog (usually 1:100 dilution of Protein A Sepharose affinity-purified serum in dilution solution) for 1 hour at 37°C, followed by washing. Color development was with ABTS, as described for the titration ELISA. Plates were read as described, and the amount of gp120 in each sample was determined by using a standard curve derived from the standard on the same plate. The assay was verified by showing that HIV-infected HUT 78 cells (infected cell lysate) gave a positive signal, while uninfected cell lysates were negative.

Example III

This example describes the recombinant production of muteins in yeast hosts according to the present invention.

The starting plasmid used for the construction of yeast expression vectors was plasmid pJS150. This plasmid is similar to plasmid pBS24.1/SOD-SF2env4-5 (U.S. serial no. 138,894, filed 24 December 1987, supra), and has had the yeast promoter and HIV coding sequences located between the unique BamHI and Sall sites replaced with a yeast ADH2/GAPDH promoter and a portion of an Zairan HIV-1 isolate envelope coding sequence. In addition, the NheI restriction site located in the plasmid vector portion was destroyed by cutting with NheI, nuclease S1 treatment, followed by ligation.

Plasmid pJS150 was digested with restriction enzyme NcoI, which cuts just after the translation initiation ATG codon downstream from the ADH2/GAPDH promoter (as well as at other sites in the vector). The fragments were ligated to an NcoI/NheI adaptor having the sequence:

5'-CATGGCTAGCCCC
CGATCGGGG-5'

After ligation, the DNA sequences were digested with BamHI and NheI to generate a 1.2 kb BamHI-NheI fragment containing the ADH2/GAPDH promoter and an NheI sticky end immediately downstream. This DNA fragment was isolated by gel electrophoresis and is referred to as Fragment A.

A second digest was performed by cutting plasmid pJS150 with BamHI and Sall to generate a 13 kb linear DNA vector. This DNA fragment was also purified by gel electrophoresis, and is referred to as

Fragment B. A third DNA fragment containing a coding sequence for gp120 D3 mutein was isolated from pCMV6a120D3 (Table 3 of Example I) by digestion with NheI and Sall. The approximate 1.2 kb coding fragment was then gel isolated, and is referred to as Fragment C.

The three fragments (A, B and C) were then ligated together, and the resulting ligation mix was used to transform *E. coli* strain HB101 to ampicillin resistance. A plasmid containing all three fragments in the proper orientation is shown in Figure 5. This D3 yeast expression vector was designated pHL15. Expression vectors for additional muteins were also constructed by cloning the mutein-encoding sequence of the pCMV6a-based vectors (Example I, Table 3) into NheI/Sall-digested pHL15, thereby replacing the D3 coding sequence. The deletions and corresponding vectors that were made are shown in Table 4.

Table 4

Deletion	Vector
D1	pHL24
D2	pHL25
D3	pHL15
D4	pHL26
D5	pHL27
D1 + D2	pHL22
D3 + D4 + D5	pHL21
D1 through D5	pHL20

The yeast expression vectors described above were used to transform *Saccharomyces cerevisiae* strain JSC308 (ATCC accession No. 20879, deposited 5 May 1988) to uracil prototrophy. Uracil prototrophs were then streaked onto leucine selective plates to isolate leucine prototrophs (as a result of plasmid amplification *in vivo*). Expression of the deletion muteins was achieved by growing a seed culture of the leucine prototrophs in leucine selective medium and then diluting it into approximately 10 liters of a rich medium containing yeast extract, peptone, and glucose. Either 2% or 4% glucose was used as the carbon source in the media, whichever appeared optimal.

Muteins were purified as follows. Frozen yeast cells were thawed and suspended in 1 volume of lysis buffer, 0.001 M PMSF, 0.001 M EDTA, 0.15 M NaCl, 0.05 M Tris-HCl pH 8.0, and 1 volume of acid-washed glass beads added. Cells were broken in a noncontinuous system using a 300 ml glass unit of Dyno-mill at 3000 rpm for 10 min. the jacket was kept cool by a -20°C ethylene glycol solution. Glass beads are decanted by letting the mixture set for 3 min on ice. The cell extract was recovered and centrifuged at 18,000 rpm (39,200 x g) for 35 min. The supernatant was discarded and the precipitate (pellet 1) further treated as indicated below.

Pellet 1 was resuspended in 4 volumes of Tris-HCl buffer (0.01 M Tris-HCl, pH 8.0, 0.01 M NaCl, 0.001 M PMSF, 1 ug/ml pepstatin, 0.001 M EDTA, 0.1% SDS) and extracted for 1 hr at 4°C with agitation. The solution was centrifuged at 6,300 x g for 15 min. The insoluble fraction (pellet 2) was resuspended in 4 volumes (360 ml) of PBS (per liter: 0.2 g KCl, 0.2 g KH₂PO₄, 8.0 g NaCl, 2.9 g Na₂HPO₄·12H₂O), 0.1% SDS, 0.001 M EDTA, 0.001 M PMSF, 1 ug/ml pepstatin, and centrifuged at 6,300 x g for 15 min. This pellet (pellet 3), was suspended in 4 volumes of PBS, 0.2% SDS, 0.001 M EDTA, 0.001 M PMSF, 1 ug/ml pepstatin and extracted for 12 hr at 4°C with agitation on a tube rocker. The solution was then centrifuged at 6,300 x g for 15 min. The soluble fraction was recovered for further purification as indicated below. (The pellet can be reextracted by resuspending it in 4 volumes of 2.3% SDS, 5% beta-mercaptoethanol, and boiling for 5 min. After boiling, the solution is centrifuged at 6,300 x g for 15 min. The soluble fraction is recovered for further purification.)

The soluble fraction was concentrated by precipitation with 30% ammonium sulfate at 4°C. The pellet (pellet 4) was then resuspended in 2.3% SDS, 5% beta-mercaptoethanol, and chromatographed on an ACA 34 or ACA 54 (LKB Products) gel filtration column (depending on the size of the mutein). The column was equilibrated with PBS, 0.1% SDS, at room temperature. Chromatography was developed in the same solution with a flow rate of 0.3 ml/min. If needed, pooled fractions were concentrated by vacuum dialysis on Spectrapor #2 (MW cutoff 12-14K).

Example IV

This example describes an immunoassay for anti-HIV antibodies employing HIV-1 env analogs.

Immulon-1 96 well immunoassay plates are coated with gp120 antigen by dispensing 100 ul per well of a 2 ug per ml purified antigen produced in yeast (Env SF2 wild type, env HTLV wild type, env Zr6 wild type, env SF2-D3, env SF2-D1-D2, env SF2-D3-D4-D5, env SF2-D1-D2-D3-D4-D5 in 50 mM borate pH 9.2 at 4°C for at least 12 hours and less than 60 days. The coating is aspirated from the plate, and the plate is washed six times by dunking in a solution of 0.137 M NaCl (0.8%), 0.05% Triton-X 100. The plate is patted dry, and 100 ul per well of 100 mM sodium phosphate, 0.1% casein, 1 mM EDTA, 1% Triton-X 100, 0.5 M NaCl, 0.01% thimerosol pH 7.5 (dilution solution) is added.

Sera to be tested are prepared for analysis by diluting 5 ul test serum (from HIV-positive humans and normal humans, or from immunized animals) in 500 ul of the dilution solution above (1/100 dilution v/v). The solution in the top wells is aspirated off and 150 ul of diluted test serum is added. Using a multichannel pipettor set at 50 ul, dilutions down each column are carried out, taking 50 ul each time (1/3 dilution v/v). The plates are then incubated 1 hour at 37°C with the plate wrapped in plastic wrap. The samples are then aspirated off and washed 6 times as above. Then, 100 ul per well of goat anti-human IgG conjugated to horseradish peroxidase (Tago 2733 Lot 330102) diluted 1/2000 in dilution solution is added. The plates are then incubated 30 minutes at 37°C covered in plastic wrap. The solution is again aspirated off and washed 6 times as above. 100 ul per well of color developing solution is added [100 ul ABTS stock (15 mg/ml 2,2'-Azino-di-(3 ethylbenzthiazolene sulfonic acid), Sigma A-1888, in water, stored in the dark at 4°C) plus 3.3 ul 30% hydrogen peroxide in 10 ml citrate buffer (10.5 g citric acid per liter water, pH to 4.0 with 6M NaOH)]. The ABTS solution is made no more than 10 minutes prior to use, and the solution should be made with citrate buffer at room temperature. Plates in plastic wrap are incubated at 37° for 30 minutes in the dark. The reaction was stopped with 50 ul per well of 10% sodium dodecyl sulfate.

Plates were read at 415 nm with a reference wavelength at 600 nm. Titers are determined as follows from the raw data: absorbance (linear axis) is plotted vs. dilution factor (log axis) on semilog paper or computer program.

Included in the test sera are standard reference sera as positive controls. For titration of human sera, HIV-positive serum 20058 from the Interstate panel was used; for goat sera, a goat serum developed by immunization with envSF2 wild-type, reference 02GT097.2 was used; and for titration of guinea pig sera, a standard reference guinea pig serum was used, likewise obtained by immunization with envSF2 wild-type, reference +Gp sera 1935/77. From the values obtained on the ELISA reader, an absorbance value was chosen that is half-maximal (OD₅₀) (between 0.5 and 0.7) in the linear portion of the standard curve (positive control), for each plate. The average titer for the standards on the plate were determined. The average titer was divided by the "Reference Titer" for that species and antigen plate, and the resulting number is the "correction factor". The test sera titers were divided by the correction factor to yield the adjusted, normalized titers.

A panel of selected human sera was tested to determine their titers on all of the recombinant antigens, including the deletion muteins env SF2 D1-2, env SF2-D-3, env SF2-D1-2-3-4-5, and env SF2-D3-4-5. Results are shown in Figure 6 (North American serum panel) and Figure 7 (African serum panel). The graphs show that for these serum samples, the recombinant antigen env SF2-D1-2 is as efficient or more efficient in detecting sera as is env SF2 wild-type. Recombinant antigens env SF2-D3, env SF2-D3-4-5 and env SF2-D1-2-3-4-5 are as efficient as env HTLVIII wild-type or env Zr6 wild-type.

Example V

This example describes the immunization of mammals with recombinant hypervariable region muteins and the detection of immune responses in these animals in response to these injections. Recombinant env antigens purified from yeast were used to generate anti-HIV antibodies in experimental animals of very high titer. In both goats and guinea pigs, the titers of the resulting sera were at a similar level, whether the immunogen was env SF2 wild-type or an env SF2 hypervariable mutein.

Immunization of Guinea Pigs

In order to test if guinea pigs immunized with env SF2 muteins derived from yeast were capable of generating a strong immune response, these animals were immunized with several muteins and control antigens.

For each antigen, six Hartley guinea pigs were immunized in the footpad with 50 ug each antigen mixed with 50 ug adjuvant (see below) at three week intervals with a total of seven injections. Blood samples were taken at the time of each injection (prebleed at injection 1), and the serum was monitored by titration in the ELISA described above in Example IV for the production of antibodies directed against the immunizing antigen and against heterologous env antigens.

Antigens:

Env SF2-D3
Env SF2-D-1-2-3-4-5
Env SF2-D3-4-5
Env HTLVIII wild-type

Adjuvant:

Muramyl-tripeptide-phosphatidyl ethanolamine in squalene-Tween.

Vaccine:

Mix adjuvant with correct volume of 10X Squalene-Tween (carrier) to make a final concentration of 1X (4% squalene, 0.008% Tween-20), antigen, and PBS to make the correct volume and dose (50 ug each of antigen and adjuvant per animal in 100 ul injection volume per animal. Warm the mixture for 5 min at 45°C. Pass the warm mixture in and out of a 23 ga. needle six times, taking care to avoid introducing air into the mixture. Inject into the animals immediately. If the injection procedure takes more than 5 min, remix the emulsion by shaking by hand every few minutes.

Control:

Control animals receive the adjuvant in carrier.

Results:

The results of the ELISA are shown in Figure 8. As can be seen, the mutants of the present invention are as effective as wild-type envelope in generating high antibody titers. A standard virus neutralization assay was also conducted the guinea pig sera. The results are shown in Table 5. The data show that deletion mutants of the present invention can generate significant levels of neutralizing antibodies.

Table 5

Virus Neutralization by Guinea Pig Sera			
Antigen	Animal Number	ELISA Titer/env SF2	Neutralization Titer HIV-SF2
Env SF2 D3 (COOH half)	2477	280,000	50
	2476	221,000	500
	2479	274,000	250
	2480	190,000	100
	2481	115,000	<20
Env SF2 D3	2471	45,000	>500
	2473	57,000	<20
	2474	116,000	<20
	2475	26,000	<20
	2476	16,000	<500
Env SF2 D(3-5) (full length)	2489	342,000	500
	2490	88,000	30
	2491	131,000	45
	2492	83,000	25
	2494	253,000	30
Env SF2 D(1-5)	2483	131,000	<20
	2484	71,000	<20
	2485	60,000	<20
	2486	54,000	<20
	2487	100,000	<20
	2488	133,000	<20
Env HTLVIII	2523	23,000	<20
	2524	37,000	<20
	2525	52,000	<20
	2526	23,000	<20
	2527	70,000	<20
	2528	14,000	<20

Immunization of Goats

In order to test if goats immunized with env SF2 muteins derived from yeast were capable of generating a strong immune response, these animals were immunized with several muteins and control antigens.

For each antigen, two goats were immunized intramuscularly with 1 mg each primary injection (complete Freund's adjuvant) and 0.5 mg each booster injection (incomplete Freund's adjuvant) at three week intervals with a total of six injections. Blood samples were taken at the time of each injection (prebleed at injection 1), and the sera were monitored by titration in the ELISA assay described above in Example IV for the production of antibodies directed against the immunizing antigen and against heterologous env antigens.

Antigens:	Env SF2-D3	Lot 3064/1a
	Env SF2-D1-a2-3-4-5	Lot 3064/5a
	Env HTLVIII wild-type	Lot 3064/15a

Adjuvant: Complete Freund's and incomplete Freund's.

Vaccine: Mix 0.5 ml antigen (1 mg) with 0.5 ml complete Freund's. Emulsify by standard procedures and inject. For boosters, mix 0.5 ml antigen (0.5 mg) with 0.5 ml incomplete Freund's. Emulsify and inject.

Control: Control animals receive the adjuvant in carrier.

Results: The results of the ELISA are shown in Figure 9. As can be seen, the muteins of the present

invention are at least as effective as wild-type polypeptides in antibody titer levels generated.

Deposit of Biological Materials

Vectors pCMV6ARV120tpa and pHL15, both in *E. coli* HB101, were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, USA, on 13 September 1988, and will be maintained under the provisions of the Budapest Treaty on the Deposit of Microorganisms. The accession number for the pCMV6ARV120tpa deposit is 67792, and the accession number for the pHL15 deposit is 67793.

These deposits are provided for the convenience of those skilled in the art, and are neither an admission that such deposits are required to practice the present invention, nor that equivalent embodiments are beyond the skill of the art in view of the present disclosure. The availability of these deposits is not a grant of any license (e.g., to make, use or sell the deposited materials) under this or any other patent. The nucleic acid sequences of the deposited materials are incorporated in the present disclosure by reference and are controlling if in conflict with any sequence described herein.

Although the foregoing invention has been described in some detail by way of illustration and example, it will be obvious that changes and modifications may be practiced within the scope of the appended claims.

Claims

1. A human immunodeficiency virus type 1 (HIV-1) gp120env or gp160env polypeptide which comprises the domains conserved among HIV-1 isolates characterised in that at least one epitope from a hypervariable domain of the polypeptide has been deleted.

2. A polypeptide comprising epitopes that bind antibodies to HIV-1 gp120env or gp160env within an amino acid sequence according to the formula:

C1-V1-V2-C2-V3-C3-V4-C4-V5-C5

wherein:

C1 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Ser29 through Cys130 of HIV-1 SF2;

C2 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Cys199 through Leu291 of HIV-1 SF2;

C3 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Ser366 through Cys387 of HIV-1 SF2;

C4 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Cys415 through Gly456 of HIV-1 SF2;

C5 comprises an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Phe466 through Arg509 or Leu855 of HIV-1 SF2;

V1 is an amino acid sequence of 0 to a maximum of about 30 residues;

V2 is an amino acid sequence of 0 to a maximum of about 50 residues;

V3 is an amino acid sequence of 0 to a maximum of about 90 residues;

V4 is an amino acid sequence of 0 to a maximum of about 30 residues; and

V5 is an amino acid sequence of 0 to a maximum of about 10 residues;

with the proviso that at least one of the V domains selected from the group consisting of V1, V2, V3, V4 and V5 contains no more than about one-third of the said maximum number of residues for the V domain.

3. The polypeptide of claim 2 wherein:

V1, if present, comprises an HIV-1 sequence spanning from the C-termini of C1 to a Cys corresponding to the conserved Cys155 of HIV-1 SF2;

V2, if present, comprises an HIV-1 sequence spanning from said C-termini Cys of V1 to the N-termini of C2;

V3, if present, comprises an HIV-1 sequence spanning from the C-termini of C2 to the N-termini of C3;

V4, if present, comprises an HIV-1 sequence spanning from the C-termini of C3 to the N-termini of C4; and

V5, if present, comprises an HIV-1 sequence spanning from the C-termini of C4 to the N-termini of C5;



invention are at least as effective as wild-type polypeptides in antibody titer levels generated.

Deposit of Biological Materials

Vectors pCMV6ARV120tpa and pHL15, both in *E. coli* HB101, were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, USA, on 13 September 1988, and will be maintained under the provisions of the Budapest Treaty on the Deposit of Microorganisms. The accession number for the pCMV6ARV120tpa deposit is 67792, and the accession number for the pHL15 deposit is 67793.

These deposits are provided for the convenience of those skilled in the art, and are neither an admission that such deposits are required to practice the present invention, nor that equivalent embodiments are beyond the skill of the art in view of the present disclosure. The availability of these deposits is not a grant of any license (e.g., to make, use or sell the deposited materials) under this or any other patent. The nucleic acid sequences of the deposited materials are incorporated in the present disclosure by reference and are controlling if in conflict with any sequence described herein.

Although the foregoing invention has been described in some detail by way of illustration and example, it will be obvious that changes and modifications may be practiced within the scope of the appended claims.

Claims

1. A human immunodeficiency virus type 1 (HIV-1) gp120env or gp160env polypeptide which comprises the domains conserved among HIV-1 isolates characterised in that at least one epitope from a hypervariable domain of the polypeptide has been deleted.

2. A polypeptide comprising epitopes that bind antibodies to HIV-1 gp120env or gp160env within an amino acid sequence according to the formula:

C1-V1-V2-C2-V3-C3-V4-C4-V5-C5

wherein:

C1 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Ser29 through Cys130 of HIV-1 SF2;

C2 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Cys199 through Leu291 of HIV-1 SF2;

C3 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Ser366 through Cys387 of HIV-1 SF2;

C4 is an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Cys415 through Gly456 of HIV-1 SF2;

C5 comprises an amino acid sequence with a minimum of about 70-75% amino acid sequence homology to Phe466 through Arg509 or Leu855 of HIV-1 SF2;

V1 is an amino acid sequence of 0 to a maximum of about 30 residues;

V2 is an amino acid sequence of 0 to a maximum of about 50 residues;

V3 is an amino acid sequence of 0 to a maximum of about 90 residues;

V4 is an amino acid sequence of 0 to a maximum of about 30 residues; and

V5 is an amino acid sequence of 0 to a maximum of about 10 residues;

with the proviso that at least one of the V domains selected from the group consisting of V1, V2, V3, V4 and V5 contains no more than about one-third of the said maximum number of residues for the V domain.

3. The polypeptide of claim 2 wherein:

V1, if present, comprises an HIV-1 sequence spanning from the C-termini of C1 to a Cys corresponding to the conserved Cys155 of HIV-1 SF2;

V2, if present, comprises an HIV-1 sequence spanning from said C-termini Cys of V1 to the N-termini of C2;

V3, if present, comprises an HIV-1 sequence spanning from the C-termini of C2 to the N-termini of C3;

V4, if present, comprises an HIV-1 sequence spanning from the C-termini of C3 to the N-termini of C4; and

V5, if present, comprises an HIV-1 sequence spanning from the C-termini of C4 to the N-termini of C5;



with the proviso that for said selected V domain having no more than one-third the maximum residue, if said HIV-1 sequence is present, said HIV-1 sequence is missing an epitope.

4. The polypeptide of claim 2 wherein said selected V domain is V1.
5. The polypeptide of claim 2 wherein said selected V domain is V2.
6. The polypeptide of claim 2 wherein said selected V domain is V3.
7. The polypeptide of claim 2 wherein said selected V domain is V4.
8. The polypeptide of claim 2 wherein said selected V domain is V5.
9. The polypeptide of claim 2 wherein said selected V domains are V1 and V2.
10. The polypeptide of claim 2 wherein said selected V domains are V1, V2 and V3.
11. The polypeptide of claim 2 wherein said selected V domains are V1, V2 and V5.
12. The polypeptide of claim 2 wherein said selected V domains are V3, V4 and V5.
13. The polypeptide of claim 2 wherein said selected V domains are V1, V2, V3, V4 and V5.
14. A polypeptide analog of human immunodeficiency virus type 1 (HIV-1) gp120env or gp160env comprising (a) about 300 to about 850 amino acid residues in length; (b) constant domains, in the N-termini to the C-termini direction, Ser29-Cys130 of HIV-1 SF2 (C1), Cys199-Leu291 of HIV-1 SF2 (C2), Ser386-Cys387 (C3), Cys415-Gly456 (C4) of HIV-1 SF2, and Phe466-Arg509 or Phe466-Leu855 of HIV-1 SF2 (C5), or domains substantially homologous to said C1, C2, C3, C4 or C5; and (c) the intervening domains, if any, located between said constant domains comprising sequences found between said constant domains in native HIV-1 gp120env, with the proviso that at least one of said intervening domains between said constant domains is either missing or missing an epitope.
15. The polypeptide of claim 14 wherein said missing epitope is a neutralizing epitope.
16. The polypeptide of claim 14 wherein said intervening domains are HIV-1 SF2 sequences.
17. The polypeptide of claim 16 comprising an HIV-1 SF2 gp120env deletion mutain wherein C5 is Phe466-Arg509 and at least one deletion is selected from the group consisting of:
 - (a) Thr131-Asn154;
 - (b) Ser156-Ser198;
 - (c) Thr300-His332;
 - (d) Asn388-Pro414; and
 - (e) Thr457-Thr463;
 or a polypeptide with a minimum of about 70-75% amino acid sequence homology thereto.
18. The polypeptide of claim 17 wherein said deletion comprises Thr131-Asn154.
19. The polypeptide of claim 17 wherein said deletion comprises Ser156-His198.
20. The polypeptide of claim 17 wherein said deletion comprises Thr300-His332.
21. The polypeptide of claim 17 wherein said deletion comprises Asn388-Pro414.
22. The polypeptide of claim 17 wherein said deletion comprises Thr457-Thr463.
23. The polypeptide of claim 17 wherein said deletions comprise Thr131-Asn154 and Ser156-His198.



24. The polypeptide of claim 17 wherein said deletions comprise Thr131-Asn154, Ser156-His198 and Thr457-Thr463.
25. The polypeptide of claim 17 wherein said deletions comprise Thr300-His332, Asn388-Pro414 and Thr457-Thr463.
26. The polypeptide of claim 17 wherein said deletions comprise Thr131-Asn154, Ser156-His198, Thr300-His332, Asn388-Pro414 and Thr457-Thr463.
27. An immunoassay for the detection of antibodies to human immunodeficiency virus type 1 (HIV-1) comprising:
 - (a) providing a liquid sample to be tested for the presence of anti-HIV-1 antibodies;
 - (b) contacting said sample with a polypeptide according to claim 1 under conditions whereby any anti-HIV-1 antibodies present in said sample may bind to an epitope to provide sample-contacted polypeptide; and
 - (c) detecting any antibody bound to said sample-contacted polypeptide.
28. A polypeptide according to any one of claims 1-26 for use in a method of immunisation of a mammal.
29. A composition comprising a polypeptide according to claim 1 and a pharmaceutically acceptable carrier.
30. The composition of claim 29 further comprising an adjuvant.
31. A DNA sequence encoding a polypeptide according to claim 1.
32. A cellular host comprising the DNA sequence of claim 31 under the control of transcriptional and translational control sequences whereby the polypeptide encoded by said DNA sequence is expressed by said cellular host.
33. The cellular host of claim 32 wherein said cellular host is a mammalian cell.
34. The cellular host of claim 32 wherein said cellular host is a yeast cell.
35. A method of producing a recombinant analog of human immunodeficiency virus type 1 (HIV-1) envelope protein comprising growing culture of cellular hosts according to claim 32 under conditions whereby said polypeptide encoded by said DNA sequence is expressed, and recovering said polypeptide from said culture.

40 Patentansprüche

1. gp120env- oder gp160env-Polypeptid vom menschlichen Immunschwächevirus Typ 1 (HIV-1), umfassend die unter den HIV-1-Isolaten konservierten Domänen, dadurch gekennzeichnet, daß mindestens ein Epitop einer hypervariablen Domäne des Polypeptids deletiert ist.
2. Polypeptid, umfassend Epitope, die Antikörper gegen HIV-1-gp120env oder -gp160env innerhalb einer Aminosäuresequenz mit der folgenden Formel binden:
C1-V1-V2-C2-V3-C3-V4-C4-V5-C5
in der:
 - C1 eine Aminosäuresequenz ist mit einer Aminosäuresequenz-Homologie von mindestens etwa 70 bis 75 % zu Ser29 bis Cys130 von HIV-1-SF2,
 - C2 eine Aminosäuresequenz ist mit einer Aminosäuresequenz-Homologie von mindestens etwa 70 bis 75 % zu Cys199 bis Leu291 von HIV-1-SF2,
 - C3 eine Aminosäuresequenz ist mit einer Aminosäuresequenz-Homologie von mindestens etwa 70 bis 75 % zu Ser366 bis Cys387 von HIV-1-SF2,
 - C4 eine Aminosäuresequenz ist mit einer Aminosäuresequenz-Homologie von mindestens etwa 70 bis 75 % zu Cys415 bis Gly456 von HIV-1-SF2,
 - C5 eine Aminosäuresequenz umfaßt mit einer Aminosäuresequenz-Homologie von mindestens etwa 70



- bis 75 % zu Phe466 bis Arg509 oder Leu855 von HIV-1-SF2,
V1 eine Aminosäuresequenz von 0 bis maximal etwa 30 Resten ist,
V2 eine Aminosäuresequenz von 0 bis maximal etwa 50 Resten ist,
V3 eine Aminosäuresequenz von 0 bis maximal etwa 90 Resten ist,
5 V4 eine Aminosäuresequenz von 0 bis maximal etwa 30 Resten ist, und
V5 eine Aminosäuresequenz von 0 bis maximal etwa 10 Resten ist,
mit der Maßgabe, daß mindestens eine der V-Domänen, ausgewählt aus V1, V2, V3, V4 und V5, nicht
mehr als etwa ein Drittel der maximalen Zahl von Resten für die V-Domäne enthält.
- 10 3. Polypeptid nach Anspruch 2, wobei:
V1, falls vorhanden, eine HIV-1-Sequenz umfaßt, die sich von den C-Termini von C1 bis zu einem
Cystein entsprechend dem konservierten Cys 155 von HIV-1-SF2 erstreckt,
V2, falls vorhanden, eine HIV-1-Sequenz umfaßt, die sich von den C-Termini Cys von V1 bis zu den N-
Termini von C2 erstreckt,
15 V3, falls vorhanden, eine HIV-1-Sequenz umfaßt, die sich von den C-Termini von C2 bis zu den N-
Termini von C3 erstreckt,
V4, falls vorhanden, eine HIV-1-Sequenz umfaßt, die sich von den C-Termini von C3 bis zu den N-
Termini von C4 erstreckt, und
V5, falls vorhanden, eine HIV-1-Sequenz umfaßt, die sich von den C-Termini von C4 bis zu den N-
20 Termini von C5 erstreckt,
mit der Maßgabe, daß für die ausgewählte V-Domäne, die nicht mehr als ein Drittel der maximalen
Reste aufweist, wenn die HIV-1-Sequenz vorhanden ist, der HIV-1-Sequenz ein Epitop fehlt.
4. Polypeptid nach Anspruch 2, wobei die ausgewählte V-Domäne V1 ist.
- 25 5. Polypeptid nach Anspruch 2, wobei die ausgewählte V-Domäne V2 ist.
6. Polypeptid nach Anspruch 2, wobei die ausgewählte V-Domäne V3 ist.
- 30 7. Polypeptid nach Anspruch 2, wobei die ausgewählte V-Domäne V4 ist.
8. Polypeptid nach Anspruch 2, wobei die ausgewählte V-Domäne V5 ist.
9. Polypeptid nach Anspruch 2, wobei die ausgewählten V-Domänen V1 und V2 sind.
- 35 10. Polypeptid nach Anspruch 2, wobei die ausgewählten V-Domänen V1, V2 und V3 sind.
11. Polypeptid nach Anspruch 2, wobei die ausgewählten V-Domänen V1, V2 und V5 sind.
- 40 12. Polypeptid nach Anspruch 2, wobei die ausgewählten V-Domänen V3, V4 und V5 sind.
13. Polypeptid nach Anspruch 2, wobei die ausgewählten V-Domänen V1, V2, V3, V4 und V5 sind.
- 45 14. Polypeptid analog zu gp120env oder gp160env des menschlichen Immunschwächevirus Typ 1 (HIV-1),
umfassend (a) etwa 300 bis etwa 850 Aminosäurereste in der Länge, (b) konstante Domänen, in der
Richtung N-Termini zu C-Termini, Ser29-Cys130 von HIV-1-SF2 (C1), Cys199-Leu291 von HIV-1-SF2
(C2), Ser366-Cys387 (C3), Cys415-Gly456 (C4) von HIV-1-SF2 und Phe 466-Arg509 oder Phe466-
Leu855 von HIV-1-SF2 (C5) oder Domänen, die im wesentlichen homolog zu C1, C2, C3, C4 oder C5
sind, und (c) dazwischenliegende Domänen, falls vorhanden, die zwischen den konstanten Domänen
50 liegen, umfassend Sequenzen, die zwischen diesen konstanten Domänen im nativen HIV-1-gp120env
zu finden sind, mit der Maßgabe, daß mindestens eine der zwischen den konstanten Domänen
liegenden Domänen entweder fehlt oder ihr ein Epitop fehlt.
- 55 15. Polypeptid nach Anspruch 14, wobei das fehlende Epitop ein neutralisierendes Epitop ist.
16. Polypeptid nach Anspruch 14, wobei die dazwischenliegenden Domänen HIV-1-SF2-Sequenzen sind.



17. Polypeptid nach Anspruch 16, umfassend ein HIV-1-SF2-gp120env-Deletionsmucin, wobei C5 Phe466-Arg509 ist und mindestens eine Deletion ausgewählt ist aus:
- (a) Thr131-Asn154,
 - (b) Ser156-Ser198,
 - (c) Thr300-His332,
 - (d) Asn388-Pro414 und
 - (e) Thr457-Thr463;
- oder ein Polypeptid mit einer Aminosäuresequenz-Homologie von mindestens etwa 70 bis 75 % dazu.
18. Polypeptid nach Anspruch 17, wobei die Deletion Thr131-Asn154 umfaßt.
19. Polypeptid nach Anspruch 17, wobei die Deletion Ser156-His198 umfaßt.
20. Polypeptid nach Anspruch 17, wobei die Deletion Thr300-His332 umfaßt.
21. Polypeptid nach Anspruch 17, wobei die Deletion Asn388-Pro414 umfaßt.
22. Polypeptid nach Anspruch 17, wobei die Deletion Thr457-Thr463 umfaßt.
23. Polypeptid nach Anspruch 17, wobei die Deletionen Thr131-Asn154 und Ser156-His198 umfassen.
24. Polypeptid nach Anspruch 17, wobei die Deletionen Thr131-Asn154, Ser156-His198 und Thr457-Thr463 umfassen.
25. Polypeptid nach Anspruch 17, wobei die Deletionen Thr300-His332, Asn388-Pro414 und Thr457-Thr463 umfassen.
26. Polypeptid nach Anspruch 17, wobei die Deletionen Thr131-Asn154, Ser156-His198, Thr300-His332, Asn388-Pro414 und Thr457-Thr463 umfassen.
27. Immuntest zum Nachweis von Antikörpern gegen menschliches Immunschwächevirus Typ 1 (HIV-1), umfassend
- (a) Bereitstellung einer flüssigen Probe, die auf die Gegenwart von anti-HIV-1-Antikörpern getestet werden soll,
 - (b) Inkontaktbringen der Probe mit einem Polypeptid nach Anspruch 1 unter Bedingungen, unter denen anti-HIV-1-Antikörper, die in der Probe vorliegen, an ein Epitop binden können, so daß man ein mit der Probe in Kontakt befindliches Polypeptid erhält, und
 - (c) Nachweis eines Antikörpers, der an das mit der Probe in Kontakt befindliche Polypeptid gebunden ist.
28. Polypeptid nach einem der Ansprüche 1 bis 26 zur Verwendung in einem Verfahren zur Immunisierung eines Säugers.
29. Mittel, umfassend ein Polypeptid nach Anspruch 1 und einen pharmazeutisch verträglichen Träger.
30. Mittel nach Anspruch 29, das ferner ein Adjuvans umfaßt.
31. DNA-Sequenz, die ein Polypeptid nach Anspruch 1 codiert.
32. Wirtszelle, umfassend die DNA-Sequenz nach Anspruch 31 unter der Kontrolle von Transkriptions- und Translationskontrollsequenzen, wobei das von der DNA-Sequenz codierte Polypeptid von der Wirtszelle exprimiert wird.
33. Wirtszelle nach Anspruch 32, wobei die Wirtszelle eine Säugierzelle ist.
34. Wirtszelle nach Anspruch 32, wobei die Wirtszelle eine Hefezelle ist.



35. Verfahren zur Herstellung eines rekombinanten Analogons eines menschlichen Immundefizienzvirus-Typ-I (HIV-1)-Hüllproteins, wobei man eine Kultur der Wirtszelle nach Anspruch 32 unter Bedingungen züchtet, bei denen das von der DNA-Sequenz codierte Polypeptid exprimiert wird, und das Polypeptid aus der Kultur gewinnt.

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Revendications

1. Polypeptide gp120env ou gp160env du virus de l'immunodéficience humaine de type 1 (HIV-1) qui comprend les domaines conservés parmi des isolats de HIV-1, caractérisé en ce qu'au moins un épitope provenant d'un domaine hypervariable du polypeptide a été supprimé.

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2. Polypeptide comprenant des épitopes qui fixent des anticorps dirigés contre gp120env ou gp160env de HIV-1, dans une séquence d'acides aminés correspondant à la formule:

C1-V1-V2-C2-V3-C3-V4-C4-V5-C5

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dans laquelle

C1 est une séquence d'acides aminés ayant une homologie de séquence d'acides aminés d'au moins environ 70-75 % avec la séquence Ser29 à Cys130 de SF2 de HIV-1;

C2 est une séquence d'acides aminés ayant une homologie de séquence d'acides aminés d'au moins environ 70-75 % avec la séquence Cys199 à Leu291 de SF2 de HIV-1;

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C3 est une séquence d'acides aminés ayant une homologie de séquence d'acides aminés d'au moins environ 70-75 % avec la séquence Ser366 à Cys387 de SF2 de HIV-1;

C4 est une séquence d'acides aminés ayant une homologie de séquence d'acides aminés d'au moins environ 70-75 % avec la séquence Cys415 à Gly456 de SF2 de HIV-1;

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C5 comprend une séquence d'acides aminés ayant une homologie de séquence d'acides aminés d'au moins environ 70-75 % avec la séquence Phe466 à Arg509 ou Leu855 de SF2 de HIV-1;

V1 est une séquence d'acides aminés de 0 à un maximum d'environ 30 résidus;

V2 est une séquence d'acides aminés de 0 à un maximum d'environ 50 résidus;

V3 est une séquence d'acides aminés de 0 à un maximum d'environ 90 résidus;

30

V4 est une séquence d'acides aminés de 0 à un maximum d'environ 30 résidus; et

V5 est une séquence d'acides aminés de 0 à un maximum d'environ 10 résidus;

étant entendu qu'au moins l'un des domaines V choisi parmi V1, V2, V3, V4 et V5 ne contient pas plus d'environ un tiers dudit nombre maximum de résidus pour le domaine V.

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3. Polypeptide de la revendication 2, dans lequel:

V1, s'il est présent, comprend une séquence de HIV-1 s'étendant de l'extrémité C-terminale de C1 à un résidu Cys correspondant au résidu Cys155 conservé de SF2 de HIV-1;

V2, s'il est présent, comprend une séquence de HIV-1 s'étendant dudit résidu Cys C-terminal de V1 à l'extrémité N-terminale de C2;

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V3, s'il est présent, comprend une séquence de HIV-1 s'étendant de l'extrémité C-terminale de C2 à l'extrémité N-terminale de C3;

V4, s'il est présent, comprend une séquence de HIV-1 s'étendant de l'extrémité C-terminale de C3 à l'extrémité N-terminale de C4; et

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V5, s'il est présent, comprend une séquence de HIV-1 s'étendant de l'extrémité C-terminale de C4 à l'extrémité N-terminale de C5;

étant entendu que pour ledit domaine V choisi ne comportant pas plus d'un tiers du nombre maximum de résidus, si ladite séquence de HIV-1 est présente, ladite séquence de HIV-1 est dépourvue d'un épitope.

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4. Polypeptide de la revendication 2, dans lequel ledit domaine V choisi est V1.

5. Polypeptide de la revendication 2, dans lequel ledit domaine V choisi est V2.

6. Polypeptide de la revendication 2, dans lequel ledit domaine V choisi est V3.

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7. Polypeptide de la revendication 2, dans lequel ledit domaine V choisi est V4.

8. Polypeptide de la revendication 2, dans lequel ledit domaine V choisi est V5.



9. Polypeptide de la revendication 2, dans lequel lesdits domaines V choisis sont V1 et V2.
10. Polypeptide de la revendication 2, dans lequel lesdits domaines V choisis sont V1, V2 et V3.
- 5 11. Polypeptide de la revendication 2, dans lequel lesdits domaines V choisis sont V1, V2 et V5.
12. Polypeptide de la revendication 2, dans lequel lesdits domaines V choisis sont V3, V4 et V5.
- 10 13. Polypeptide de la revendication 2, dans lequel lesdits domaines V choisis sont V1, V2, V3, V4 et V5.
14. Analogue polypeptidique de gp120env ou gp160env du virus de l'immunodéficience humaine de type 1 (HIV-1) comprenant (a) une longueur d'environ 300 à environ 850 résidus aminoacide; (b) des domaines constants, dans la direction de l'extrémité N-terminale à l'extrémité C-terminale, Ser29-Cys130 de SF2 de HIV-1 (C1), Cys199-Leu291 de SF2 de HIV-1 (C2), Ser366-Cys387 (C3), Cys415-Gly456 (C4) de SF2 de HIV-1 et Phe466-Arg509 ou Phe466-Leu855 de SF2 de HIV-1 (C5), ou des domaines pratiquement homologues audit C1, C2, C3, C4 ou C5; et (c) les domaines intercalaires, s'ils existent, situés entre lesdits domaines constants, comprenant des séquences rencontrées entre lesdits domaines constants dans gp120env natif de HIV-1, étant entendu qu'au moins l'un desdits domaines intercalaires entre lesdits domaines constants est soit manquant, soit dépourvu d'un épitope.
- 15 15. Polypeptide de la revendication 14, dans lequel ledit épitope manquant est un épitope neutralisant.
16. Polypeptide de la revendication 14, dans lequel lesdits domaines intercalaires sont des séquences de SF2 de HIV-1.
- 20 17. Polypeptide de la revendication 16, comprenant une mutéine de délétion gp120env de SF2 de HIV-1, dans laquelle C5 est Phe466-Arg509 et au moins une délétion est choisie parmi le groupe constitué par:
- 30 (a) Thr131-Asn154,
(b) Ser156-Ser198,
(c) Thr300-His332,
(d) Asn388-Pro414 et
(e) Thr457-Thr463,
ou polypeptide ayant une homologie de séquence d'acides aminés d'au moins environ 70-75 % avec celui-ci.
- 35 18. Polypeptide de la revendication 17, dans lequel ladite délétion comprend Thr131-Asn154.
19. Polypeptide de la revendication 17, dans lequel ladite délétion comprend Ser156-His198.
- 40 20. Polypeptide de la revendication 17, dans lequel ladite délétion comprend Thr300-His332.
21. Polypeptide de la revendication 17, dans lequel ladite délétion comprend Asn388-Pro414.
- 45 22. Polypeptide de la revendication 17, dans lequel ladite délétion comprend Thr457-Thr463.
23. Polypeptide de la revendication 17, dans lequel lesdites délétions comprennent Thr131-Asn154 et Ser156-His198.
- 50 24. Polypeptide de la revendication 17, dans lequel lesdites délétions comprennent Thr131-Asn154, Ser156-His198 et Thr457-Thr463.
25. Polypeptide de la revendication 17, dans lequel lesdites délétions comprennent Thr300-His332, Asn388-Pro414 et Thr457-Thr463.
- 55 26. Polypeptide de la revendication 17, dans lequel lesdites délétions comprennent Thr131-Asn154, Ser156-His198, Thr300-His332, Asn388-Pro414 et Thr457-Thr463.



27. Essai immunologique pour la détection d'anticorps dirigés contre le virus de l'immunodéficience humaine de type 1 (HIV-1), comprenant:
- (a) la fourniture d'un échantillon liquide à tester pour la détection de la présence d'anticorps anti-HIV-1;
 - (b) la mise en contact dudit échantillon avec un polypeptide selon la revendication 1, dans des conditions dans lesquelles tous anticorps anti-HIV-1 présents dans ledit échantillon peuvent se lier à un épitope pour donner un polypeptide mis en contact avec l'échantillon; et
 - (c) la détection de tout anticorps lié audit polypeptide mis en contact avec l'échantillon.
28. Polypeptide selon l'une quelconque des revendications 1 à 26, pour utilisation dans un procédé d'immunisation d'un mammifère.
29. Composition comprenant un polypeptide selon la revendication 1 et un véhicule pharmaceutiquement acceptable.
30. Composition de la revendication 29, comprenant en outre un adjuvant.
31. Séquence d'ADN codant pour un polypeptide selon la revendication 1.
32. Hôte cellulaire comprenant la séquence d'ADN de la revendication 31, sous le contrôle de séquences de régulation transcriptionnelle et traductionnelle, ce par quoi le polypeptide codé par ladite séquence d'ADN est exprimé par ledit hôte cellulaire.
33. Hôte cellulaire de la revendication 32, l'hôte cellulaire étant une cellule mammalienne.
34. Hôte cellulaire de la revendication 32, l'hôte cellulaire étant une cellule de levure.
35. Procédé de production d'un analogue recombinant de la protéine d'enveloppe du virus de l'immunodéficience humaine de type 1 (HIV-1), comprenant la culture d'hôtes cellulaires selon la revendication 32, dans des conditions permettant l'expression dudit polypeptide codé par ladite séquence d'ADN, et la récupération dudit polypeptide à partir de ladite culture.