Induction of antibodies to the human immunodeficiency virus type 1 by immunization of baboons with immunoglobulin molecules carrying the principal neutralizing determinant of the envelope protein

(immunoglobulin–peptide chimera/hypervariable region 3 peptides)

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ABSTRACT The hypervariable region 3 (V3) within the disulfide-bridged loop of the envelope protein of the human immunodeficiency virus type 1 (HIV-1) contains an amino acid sequence that was defined as a principal neutralizing determinant (PND). A 19-amino-acid residue consensus sequence (designated V3C) predicted from the PND sequences of 245 isolates as well as a sequence from the PND of the WMJ2 HIV-1 isolate (designated V3M) were expressed on the variable region of murine-human immunoglobulin (Ig) chimeras that were designated Ig-V3C and Ig-V3M, respectively. The HIV-1 sequences on the Ig chimeras preserved their antigenicity and interacted with antibodies specific for peptides encompassing the V3C and V3M sequences. In baboons, Ig-V3C and Ig-V3M induced antibodies that bound V3C and V3M peptides as well as the glycoprotein gp120 envelope protein of HIV-1 MN isolate. In addition, the baboons' antisera were able to prevent infection of CD4+ SupT1 susceptible T cells by HIV-1 MN. Finally, Ig-V3M chimeras were able to stimulate in vitro production of antibodies specific for the HIV-1 envelope-derived peptides by lymphocytes from HIV-1-infected human subjects.

A wealth of information on the human immunodeficiency virus (HIV) has been gathered since its discovery 12 years ago (1). To date, vaccines or therapeutic agents able to prevent the spread of HIV or to block the irreversible progress of HIV infection to fatal disease are not available. The development of a safe and efficient vaccine against this virus represents a major task to control its spreading. To this aim various vaccine-development approaches were undertaken. These include use of inactivated whole virus (2, 3), natural and recombinant envelope protein (4–6), viral and bacterial vectors expressing HIV sequences (7–11), synthetic peptides (12), anti-idiotypic antibodies (13), antigenized immunoglobulin (Ig) expressing a CD4 epitope (14), and naked DNA (15).

In previous studies we have shown that viral T-cell epitopes expressed on the heavy chain variable (VH) region of Ig molecules are highly immunogenic at the level of both CD4+ and CD8+ T cells (16–18). The advantage of this approach is that Ig molecules are safe as antigen delivery systems and have long half-lives when compared with synthetic peptides.

In this study, sequences of the principal neutralizing determinant (PND) from the hypervariable region 3 (V3) loop of HIV type 1 (HIV-1) envelope protein (19) were expressed on mouse–human Ig molecules, and the resulting Ig-V3 chimeras were tested with respect to induction of anti-HIV antibody response either in baboons or by in vitro stimulation of human lymphocytes. The results indicate that the Ig–peptide chimeras were able to induce in baboons anti-peptide antibodies that bind the envelope protein and neutralize HIV-1 virus. They also stimulate human lymphocytes for production of antibodies specific for HIV-1-derived peptides in vitro.

MATERIALS AND METHODS

Genetic Engineering of Antigenized Igs. Polymerase chain reaction (PCR) mutagenesis similar to those previously described to generate VN genes carrying influenza A virus epitopes (16, 17) was used to insert HIV-1 V3 loop peptides in Ig. Briefly, a pUC19 plasmid harboring the 5.5-kb EcoRI fragment carrying the VH gene of the murine anti-arsonate antibody, 91A3 (20), was used as template DNA in two PCRs to delete the diversity (D) segment of the complementarity-determining region 3 (CDR3) loop and instead insert nucleotide sequences encoding the selected HIV peptides. Two chimeric VH genes were generated. The first carries sequences encoding a 19-amino-acid-residue consensus peptide (designated V3C peptide; see the sequence below under Antigens) from the V3 loop of HIV-1 envelope protein (21), and the second harbors sequence also encoding a 19-residue peptide (designated V3M peptide) from the V3 loop of the WMJ2 HIV-1 isolate (21). These chimeric VH genes as well as the wild-type 91A3 VH gene were subcloned into the EcoRI site upstream of the exons encoding a human Ig γ1 heavy chain constant (C) region within the plasmid pSV2AHgptDNSVH-hCy1 (22) from which the EcoRI rursal danyl (dns)-conjugated VH gene was cut out. Thus, three constructs were generated: pSV2AHgpt91A3VH-V3C-hCy1, which carries nucleotide sequence encoding the consensus V3C peptide in place of the D segment; pSV2AHgpt91A3VH-V3M-hCy1, which carries nucleotide sequence encoding the V3M peptide; and pSV2AHgpt91A3VH-D-hCy1, which carries the D segment of parental 91A3VH gene. The sequences encoding the HIV peptide and surrounding regions were determined and confirmed their insertion in the correct frame (not shown).

To express these chimeric murine 91A3 VH–human γ1 heavy chain genes with a mouse–human chimeric κ light chain gene, we proceeded as follows. The 8-kb BamHI fragment encoding the entire murine 91A3 κ light chain gene (23) was

Abbreviations: BSA, bovine serum albumin; HIV-1, human immunodeficiency virus type 1; PBMC, peripheral blood mononuclear cells; PND, principal neutralizing determinant; V, variable; VH, heavy chain V region of immunoglobulin; V3, hypervariable region 3 of the envelope protein of HIV-1; Ig-V3 chimeras, Ig carrying amino acid residues from the V3 loop of HIV; TT, tetanus toxoid; dns, danyl; C, constant; D, diversity.

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subcloned into the BamHI site of pUC19 plasmid. Subsequently, a HindIII fragment encompassing the \( \kappa \) light chain promoter and the \( \kappa \) light chain coding sequences was cut out from this plasmid and subcloned into the HindIII site of pSV184\( \Delta \)HneoDNVS\( \kappa \)-cHc. upstream of the gene encoding a human \( \kappa \) light chain C region (C\( \kappa \)) from which the \( \kappa \)-conjugated V\( \kappa \) (dnsV\( \kappa \)) had been excised (24). This plasmid, which will encode a murine 91A3 V\( \kappa \)-human C\( \kappa \) light chain, is designated pSV184\( \Delta \)Hneo91A3V\( \kappa \)-cHc.

Gene cloning, plasmid purification, hybridization, and DNA fragment separation and elution from agarose gels were carried out as described (16). Nucleotide sequencing was carried out with a Sequenase kit (United States Biochemical) according to the manufacturer's instructions.

**Gene Transfection.** The plasmids pSV2\( \Delta \)Hgpt91A3V\( \kappa \)-V\( \gamma \)-cHc, pSV2\( \Delta \)Hgpt91A3V\( \kappa \)-V\( \gamma \)-cHc, and pSV2\( \Delta \)Hgpt91A3V\( \kappa \)-V\( \gamma \)-cHc were separately cotransfected with pSV184\( \Delta \)Hneo91A3V\( \kappa \)-cHc, into the non-lg-secreting murine myeloma B-cell line Sp2/0 by electroporation (220 V, 960 \( \mu \)F) as described (16, 17). The resulting chimeras were designated Ig-V\( \kappa \)C, Ig-V\( \kappa \)M, and Ig-W, respectively. Transfectants expressing both heavy and light chains were doubly selected by using mycoplasmonic acid and Genetin as described (16).

**Animals.** The baboons (Papio cynocephalus) used in this study were bred at the Southwest Foundation for Biomedical Research and were housed in gang cages for the duration of the study. Male baboons between the ages of 10 and 13 years were used. New Zealand White rabbits (3 kg) were used for the production of anti-peptide antibodies.

**Antigens.** The synthetic peptide V\( \gamma \)C (Arg-Lys-Ser-Ile-His-Leu-Gly-Pro-Gly-Arg-Ala-Phe-Thr-Thr-Gly-Glu-Ile-Ile) corresponds to a consensus sequence from the PND of the V\( \gamma \), disulfide-bridged loop of HIV-1 envelope (19). This sequence was predicted from the comparison of PND sequences of 24 isolates (21). V\( \gamma \)M (Arg-Arg-Ser-Leu-Ser-Ile-Gly-Pro-Gly-Arg-Ala-Phe-Thr-Thr-Glu-Ile-Ile-Gly) corresponds to amino acid residues of the PND of WM28 HIV-1 isolate at the same positions as in the consensus sequence (21). The peptide HIV-(519-535) (Ala-Val-Gly-Ile-Ala-Leu-Phe-Glu-Phe-Leu-Glu-Ala-Ala-Gly-Ser), corresponding to amino acid residues 519-535 of the envelope of HIV-1 IIIB isolate, was also used. Peptide synthesis, purification by reverse phase (RP)-HPLC, and conjugation to keyhole limpet hemocyanin and bovine serum albumin (BSA) were carried out by previously described techniques (25). Viral lysates from purified HIV-1 IIIB isolate were purchased from Organan Teknika-Cappell, and lysates from cells infected with the MN isolate were provided to us by A. Painter and B. Honen (New York University).

**Production of HIV Peptide-Specific Antibodies.** Anti-peptide antibodies were prepared in New Zealand White rabbits by immunization with peptide-keyhole limpet hemocyanin conjugates as described (25). Anti-peptide antibodies were purified from serum on peptide-BSA-conjugated Sepharose columns, and the antibodies were eluted with 0.2 M glycine hydrochloride (pH 2.8) buffer.

**Detection of Ig-\( \gamma \)C Chimeras in Transfectoma Supernatant.** This was done by a capture radioimmunoassay as follows. Microtiter plates were coated overnight at 4°C with 100 \( \mu \)l (at 2 \( \mu \)g/ml) of anti-human \( \kappa \) light chain monoclonal antibody HP6053 (American Type Culture Collection). The plates were then washed with phosphate-buffered saline (PBS), blocked with goat serum containing 5% BSA, washed, and incubated with transfectoma supernatant for 2 hr at room temperature. Captured chimeras were revealed with \( ^{125} \)I-labeled goat anti-human IgG1.

**Binding of Rabbit Anti-V\( \gamma \)C Peptide Antibodies to Ig-\( \gamma \)C Chimeras.** Microtiter plates were coated with Ig chimeras (100 \( \mu \)l at 2 \( \mu \)g/ml) for 18 hr at 4°C, washed with PBS, blocked for 1 hr with PBS containing 2% BSA, washed again with PBS, and incubated with graded concentrations of rabbit anti-peptide antibodies for 2 hr. The plates were then washed, and bound rabbit antibodies were revealed with \( ^{125} \)I-labeled goat anti-rabbit IgG.

**Detection of Anti-V\( \gamma \)C Peptide Antibodies in Baboon Serum and in Supernatant from Human Lymphocyte Culture.** Microtiter plates were coated overnight with peptide-BSA conjugate (100 \( \mu \)l at 2 \( \mu \)g of peptide per ml of PBS) or BSA (100 \( \mu \)l at 2 \( \mu \)g/ml). The plates were then washed and blocked with goat serum containing 5% BSA. After 1 hr, the plates were washed and incubated with various dilutions of test samples for 2 hr at room temperature. Bound baboon or human antibodies were revealed with \( ^{125} \)I-labeled goat anti-human IgG antibodies.

**Radioimmunoprecipitation Assay (RIPA).** The RIPA assay was performed as described (25). Briefly, mature virus lysates were labeled with \( ^{125} \)I and precleared on Pansorbin cells (Calbiochem), and \( 2 \times 10^6 \) cpm was incubated overnight at 4°C in 20 \( \mu \)l of baboon serum, 10 \( \mu \)l of rabbit serum, or 2 \( \mu \)l of human serum. Samples were then warmed for 10 min at 37°C, and complexes were removed with Pansorbin cells. After heat denaturation, the proteins were separated on a 10% acrylamide gel.

**HIV-1 Neutralization Assay.** Baboon sera were tested for HIV-1 MN and IIIB neutralizing activity by using two \textit{in vitro} neutralization assays that have been described (26, 27). The first assay utilized HIV-1 IIIB isolate and examined viral cytotoxicity. Briefly, 50 \( \mu \)l of the heat-inactivated test sera diluted 1:50 to 1:400 were preincubated with 100 TCID\textsubscript{50} (50% tissue culture infectious doses) for 1 hr at 37°C. This mixture was then added to susceptible SupT1 cells and incubated for 8 days. Subsequently, the vital dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) was added for 4 hr. The cultures were inactivated and the optical density at 570 nm was determined. Incorporation of MTT indicated viable cells, and the HIV-1 IIIB-induced cytotoxicity was inhibited.

The second assay utilized HIV-1 MN isolate and measures p24 production in the supernatant. This assay is used because the MN isolate is not cytotoxic for SupT1 cells. The assay was carried out as above except that at day 8, p24 was measured in the tissue culture supernatant. The level of HIV-1 p24 in the supernatant was determined by using a Coulter ELISA kit according to the manufacturer's specifications. The percent neutralization was calculated as described (26, 27).

**Stimulation of Human Peripheral Blood Lymphocytes with Ig-\( \gamma \)C Chimeras.** Peripheral mononuclear blood cells (PBMC) were isolated from two HIV-1-positive (CE and SLF) patients and one HIV-1-negative (K) patient on Ficol/Hypaque gradient (Pharmacia). The cells were subsequently washed with sterile cold PBS and reseeded at \( 1 \times 10^6 \) cells per ml of RPMI 1640 medium well per 24-well plates. Graded doses of Ig-\( \gamma \)C-M, Ig-\( \gamma \)M coupled to tetanus toxoid (Ig-\( \gamma \)M-TT), or Ig-W-TT were added, and the cultures were incubated at 37°C in an atmosphere containing 7% \( \text{CO}_2 \) for 7-10 days. The supernatants were then assayed for the presence of anti-V\( \gamma \)M and anti-V\( \gamma \)C antibodies as above.

**RESULTS**

**Antigenicity of HIV-1 Peptides Expressed on Ig Chimeras.** As indicated in Fig. 1 \textit{Upper}, three different Ig molecules were generated in which the heavy and light chain V regions are of the murine 91A3 anti-arsenate antibody, while the C regions are derived from human \( \gamma \) and \( \kappa \) chains. Ig-\( \gamma \)C carries V\( \gamma \)C peptide, and Ig-V\( \gamma \)M carries V\( \gamma \)M peptide in place of the D segment. Ig-\( \kappa \)-W is a control molecule encoded by unmodified genes and therefore carries the parental D segment. Chimeric IgGs were affinity-purified on an anti-human \( \kappa \) chain mAb column and were assayed for chain assembly and HIV peptide expression by radioimmunoassay. When Ig-\( \gamma \)C, Ig-\( \gamma \)M, and
Ig-W are captured by an anti-human κ chain mAb, they bind 125I-labeled goat anti-human Fcγ, antibodies, indicating that the replacement of the D segment with HIV peptides did not alter chain assembly or the folding of the molecule (Fig. 1 Lower a). The control molecule Ig-HA, which is entirely murine Ig, did not bind to anti-human antibodies. Furthermore, Ig-V3C and Ig-V3M, but not Ig-W or Ig-HA, bind rabbit antibodies specific for either V3C (Fig. 1 Lower b) or V3M (Fig. 1 Lower c) peptides. These results indicate that V3C and V3M peptides on Ig-V3C and Ig-V3M, respectively, are exposed and preserved their antigenicity.

Induction of HIV-1-Neutralizing Antibodies in Baboons Immunized with Chimeric Igs. Groups of baboons were immunized with Ig-V3C, Ig-V3M, or control Ig-W, and serum samples collected before and after immunization were tested for the presence of antibodies to V3C and V3M peptides and to an unrelated peptide corresponding to amino acid residues 519–535 of the HIV-1 IIIB isolate. The results of representative baboons are illustrated in Fig. 2. Baboons that were immunized with either Ig-V3C or Ig-V3M produced antibodies to the V3 loop peptides, while those immunized with Ig-W did not. These antibodies are specific for V3 peptides since they did not bind the HIV-(519–535) peptide. While serum from the baboon immunized with Ig-V3M bound only V3M peptide, serum from the baboon immunized with Ig-V3C bound both V3M and V3C peptides. A significant level of antibodies was detectable at day 65 (14 days after the third immunization). However, higher antibody levels were obtained at day 164 (14 days after the fifth immunization). The results depicted in Fig. 3 indicate that anti-peptide antibodies from baboons immunized with either Ig-V3C or Ig-V3M bind a 120-kDa protein when incubated with MN virus lysates, as does the control human serum from HIV-1-infected individuals. A rabbit anti-

![Fig. 1](image1.png)

**Fig. 1.** Schematic representation and immunochemical properties of Ig–HIV-1 peptide chimeras. (Upper) The heavy (H) and light (L) chain V regions (VH and VL) (filled boxes) of the murine anti-arsonate 91A3 antibody (20, 23) were used along with the CH and CL regions (hatched boxes) of human Ig κ heavy chain and κ light chain (22, 24) to generate Ig chimeras carrying HIV-1 V3 determinants (open squares). Ig-V3C carries a 19-amino acid residue consensus sequence from the PND of the V3 loop of HIV-1; Ig-V3M carries a 19-amino acid residue sequence from the PND of the V3 loop of the WM2 J2 HIV-1 isolate; and Ig-W carries the parental D segment. (Lower) The Ig-peptide chimeras were assayed for H and L chain assembly (a) by a capture assay in which 10 ng of purified chimeras were incubated on plates coated with anti-human κ chain mAb, and captured chimeras were revealed with 125I-labeled goat antibodies specific for the Fe region of human κ chain. The antigenicity of HIV-1 peptides on the chimeras was assessed by incubating Ig-chimera-coated plates with rabbit antibodies specific for either the consensus (b) or the WM12 (c) HIV-1 V3 sequences, and bound rabbit antibodies were revealed with 125I-labeled goat anti-rabbit IgG antibodies. Ig-HA is a murine Ig chimera expressing a T-helper epitope of influenza virus (17) and is used as negative control.

![Fig. 2](image2.png)

**Fig. 2.** Production of anti-PND antibodies subsequent to immunization of baboons with Ig-V3C and Ig-V3M. Sera from baboons were immunized with the Ig chimeras indicated in c, and were assayed for binding to the V3C-BSA conjugate (a), the V3M-BSA conjugate (b), the HIV-(519–535)-BSA conjugate (c), and BSA (not shown) as described in text. The immunization and bleeding schedule was as follows: baboons were bled on day 1 and were immunized with 500 μg of an Ig–peptide chimera emulsified in Freund's incomplete adjuvant on day 15, and in alum on days 29 and 43. The animals were bled on days 65, 80, and 101. They were administered a booster containing 500 μg of Ig–peptide chimeras in alum on days 122 and 150 and were bled on days 136, 164, and 178. The sera were tested at various dilutions (1:10, 1:100, and 1:1000), and the data shown were obtained with 1:100 serum dilution. Each dilution was tested in triplicate wells, and the indicated cpm represent the mean ± SD after deduction of background cpm obtained on plates coated with BSA.
isolates but not the IIIB isolate, while antiserum from a baboon immunized with Ig-W failed to neutralize either of the two isolates. Significant HIV-1 MN-neutralizing activity was detected in samples collected on days 101, 136, and 164 (14 days after the third, fourth, and fifth immunization, respectively) (not shown). The dose–effect relationship between neutralization activity and serum dilution indicates that neutralization is dependent upon the quantity of serum antibody.

In Vitro Production of Antibodies Specific for HIV-1-Derived Peptides by Human Lymphocytes Stimulated with

![Image]

**FIG. 3.** Radioimmunoprecipitation of HIV-1 gp120 by antiserum from baboons immunized with Ig-V3M and Ig-V3C. Precipitation was performed with lysates from MN **(Left)** and IIIB **(Right)** isolates as described in text. Lanes: 1–3 preimmune, sera collected on day 1 from baboons numbered 1–3; 1 a Ig-V3C, antiserum from baboon 1 immunized with Ig-V3C collected on day 164; 2 a Ig-V3M, antiserum from baboon 2 immunized with Ig-V3M collected on day 164; 3 a Ig-W, antiserum from baboon 3 immunized with Ig-W collected on day 164; Rab a 503–535, a positive control antiserum from a rabbit immunized with the HIV–(503–535) peptide [antisera in lanes 4–7 of Left and Right correspond to a conserved sequence at the carboxyl-terminal end of gp120 of HIV-1 and are known to precipitate the envelope protein (25)]; hum HIV-1 (+), serum from HIV-1-infected individual; and hum HIV-1 (−), serum from HIV-negative individual. These precipitation patterns were obtained with 20 µl of baboon serum, 10 µl of rabbit serum, and 2 µl of human serum.

**FIG. 4.** Neutralization of MN isolate by antiserum from baboons immunized with Ig-V3M and Ig-V3C. Sera from baboons immunized with Ig-V3C, Ig-V3M, and Ig-W chimeras were tested for HIV-1 MN **(Left)** and IIIB **(Right)** neutralizing activity as described in text. All bleeds from day 1 to day 178 were tested, and the data shown are those obtained with sera collected at day 164. The percent neutralization was calculated as described (26, 27).

**Ig-Peptide Chimeras.** Since Ig-peptide chimeras carrying HIV-1 envelope-derived epitopes were able to induce HIV-1-neutralizing antibodies in baboons, we carried out *in vitro* experiments to determine whether human lymphocytes could similarly produce anti-HIV-1 antibodies subsequent to *in vitro* stimulation with the chimeras. In preliminary experiments, we tested serum from 20 HIV-1-infected asymptomatic patients for presence of antibodies specific for V3M and V3C peptides. Nine subjects had antibodies that bound to V3M and V3C peptides (not shown). Two of these individuals (CE and SLF) and one healthy subject (K) were selected for this study. PBMC from these individuals were incubated with Ig-peptide chimeras alone or coupled to TT, and supernatants were harvested at days 7 and 10 and were tested for the presence of anti-HIV-1 antibodies. Fig. 5 depicts the results of a representative experiment among two performed at 2-month intervals. PBMC from patients (CE and SLF) incubated for 10 days with either Ig-V3M-TT or Ig-V3M produced antibodies that bound to both V3M and V3C peptides (Fig. 5a, b, d, and e). Lower antibody titers were obtained when the PBMC were incubated for only 7 days (not shown). No antibodies to V-161 peptide (negative control) could be detected in these supernatants (not shown). The wild-type Ig-W did not induce antibodies to either V3C or V3M peptides (Fig. 5c and f). PBMC from a healthy subject (K) did not produce any detectable anti-HIV-1 antibodies (open circles in Fig. 5), indicating that the cells producing the HIV-1-specific antibodies in asymptomatic patients are memory cells that have previously been primed. Optimal antibody production required lower amounts of antigen when the
chimeras were coupled to TT, suggesting that the mobilization of TT-specific T cells may have provided help for antibody production.

**DISCUSSION**

Engineered Igs represent attractive carriers for microbial peptides to generate reagents able to stimulate potent and specific immunity (14, 16, 17). Within we generated two Ig chimeras that carry sequences from the PND of the V2 loop of the envelope of HIV-1. Ig-V3M carries a consensus amino acid sequence predicted from the comparison of PND sequences of 245 isolates (21). Ig-V2,M carries amino acid sequences from the PND of the WMJ2 HIV-1 isolate (21). Ig-V2,C and Ig-V2,M bound affinity-purified rabbit antibodies to V2,C and V2,M peptides, suggesting that the HIV-1 sequences on the Ig chimeras are solvent phase-exposed and preserved their antigenicity. Study of the kinetics of the immune response in baboons to the Ig-HIV-1 peptide chimeras shows that antibodies specific for the HIV-1 peptides can be detected after the third immunization and that additional immunizations increase the titers. Baboons that were immunized with Ig-V2,C produced antibodies that reacted with both V2,M and V2,C peptides, while those immunized with Ig-V2,M produced antibodies that reacted with V2,M peptide only. This may be related to weaker immunogenicity of V2,M peptide or to a folding of V2,M sequence within the Ig context that preclude the production of cross-reactive antibodies. Antibodies from baboons immunized with either Ig-V2,C or Ig-V2,M bind to the envelope protein of the MN isolate and neutralize this strain in vitro. The baboon antisera did not bind to the envelope protein of the IIIB isolate and did not neutralize this strain. It is possible that the differences in amino acid sequences between the IIIB, MN, and WMJ2 isolates (see Fig. 6) play an important role in the shaping of antibody response to this PND.

More striking, Ig-peptide chimeras stimulated in vitro antibody response by human PBMC from HIV-1-infected patients. The response was higher when the PBMC were exposed to Ig chimeras that are coupled with TT. This suggests that TT-specific memory T cells induced by vaccination might provide help to V2,M-stimulated B cells. This is supported by the findings that Ig-V2,M-TT elicited a better antibody response than uncoupled Ig-V2,M in two different HIV-1-infected patients.

The results indicate that Ig molecules expressing HIV-1 V3 loop peptides are able to induce virus-neutralizing antibodies in baboons and to stimulate the production of HIV-1-specific antibodies by human lymphocytes. It is still not clear whether the V3 loop of the envelope protein represents the best target for the development of HIV vaccine. However, the ability of Ig chimeras to induce antibody response in asymptomatic patients pleads for the potential for development of a new generation of therapeutic rather than prophylactic vaccines (28). There is evidence indicating that vaccines can boost host-defense reactions in subjects infected with viruses or parasites (28).

![Fig. 6. Comparison of amino acid sequence of V2,C peptide to the corresponding sequences within the WMJ2 (V2,M), MN, and IIIB (BH10) isolates. The amino acid sequences were obtained from Larosa et al. (21). A dash indicates amino acid identity, and an asterisk indicates a gap.](image-url)

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