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NOTES

Poliovirus Chimeras Expressing Sequences from the Principal Neutralization Domain of Human Immunodeficiency Virus Type 1

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Sequences from the principal neutralization domain of human immunodeficiency virus type 1 (HIV-1) strain LAI or RF have been expressed in antigenic site 1 of the capsid of the Sabin strain of poliovirus type 1. A number of the resulting chimeras were viable. Viable variants bearing mutations within the insertion site spontaneously arose from several nonviable chimeras. In general, these mutations result in a decrease in positive charge in the substituted antigenic site 1. Two of the chimeras were genetically stable and have been further characterized. Both chimeras were neutralized by various HIV-1 neutralizing antibodies. In rabbits, both chimeras produced high levels of antibodies which react with HIV-1 gp120/160 in immunoprecipitation and enzyme-linked immunosorbent assays. One of the chimeras (HIV-1_{LAI}) produced a significant but weak HIV-1 neutralizing response.

Several lines of evidence suggest that the induction of neutralizing antibodies is a key factor in protective immunity against human immunodeficiency virus (HIV) infection (2, 9, 13, 39, 42). The principal neutralization domain (PND) of HIV-1 lies in the third variable domain of the gp120 moiety of the external envelope glycoprotein gp160 (14, 18, 38, 43). This region forms a disulfide-bonded loop, the so-called V3 loop (amino acids 301 to 336 for strain LAI [49]; all HIV-1 numberings are according to Myers et al. [35]). Most efforts toward the development of an HIV vaccine are based on the use of gp160 or gp120 (see reference 12 for a review). However, several immunosuppressive activities mediated by the interaction of gp120 with the CD4 molecule, the receptor for HIV, have been reported in normal humans (8). Moreover, gp120 bound to CD4 could serve as a target for immune attack of noninfected lymphocytes in infected vaccinees (24). There might therefore be advantages in using only sequences from the PND to induce high levels of HIV neutralizing antibodies. We have tried to use poliovirus as a carrier for that purpose.

The ability to use poliovirus as a foreign epitope expression vector stems from studies demonstrating that the neutralization antigenic site I (N-AgI) of poliovirus type 1 (PV-1), which is located on a highly exposed loop (the B-C loop) of capsid polypeptide VP1 (16), could be substituted by the corresponding sequence from serotype 2 or 3 (4, 26, 33, 34). The resulting chimeric viruses showed dual antigenicity and immunogenicity. A variety of heterologous chimeras that express sequences from other picornaviruses (5, 21) or from unrelated pathogens such as human papillomavirus (19) or HIV-1 (10, 40) have subsequently been described.

Engineering of a VP1 B-C loop mutagenesis cartridge. To allow the replacement of N-AgI of PV-1 Sabin by virtually any foreign sequence in a single-step procedure, we engineered a mutagenesis cartridge containing unique restriction sites on either side of the VP1 B-C loop in an infectious PV-1 Sabin cDNA clone. The starting material was plasmid pVS(1)IC-O(T) (a generous gift from A. Nomoto), which contains a full-length cDNA copy of the PV-1 Sabin genome and carries simian virus 40 replication and transcription signals as well as the sequence encoding the simian virus 40 large T antigen (22). A *Pst*I-*Pst*I fragment (nucleotides 2247 to 3421 of the PV-1 Sabin cDNA) was isolated from pVS(1)IC-O(T) and cloned into the polylinker of M13mp11. Oligonucleotide-directed mutagenesis was carried out (44) to introduce restriction sites *Sal*I and *Sac*I at positions 2753 and 2790, respectively, in the viral cDNA. Engineering the *Sac*I site resulted in the change of VP1 residue 103 from Lys to Glu (Fig. 1). The mutations were then reintroduced into pVS(1)IC-O(T) by exchanging the wild-type *Nhe*I-*Sna*BI fragment (nucleotides 2470 to 2956 of the PV-1 Sabin cDNA) for the mutated one, generating cassette vector pJFC82 (Fig. 1), in which the nucleotide sequence encoding N-AgI (VP1 residues 93 to 104) is framed by unique restriction sites *Sal*I and *Sac*I.

Construction of PV-1/HIV-1 antigen chimeras. The HIV-1 PND sequences chosen included the entire V3 loop (gp120 amino acids 302 to 335) from HIV-1_{LAI} (vLAI V3) or sequences from the V3 loop of HIV-1_{LAI} (residues 310 to 321; vLAI 12 series) or from HIV-1_{RF} (residues 321 to 330; vRF 10 series) (Table 1). The latter two sequences correspond to most of the PND of the LAI and RF isolates, respectively, as judged from the ability of synthetic peptides to elicit, and to bind to, neutralizing antibodies (18, 29). A

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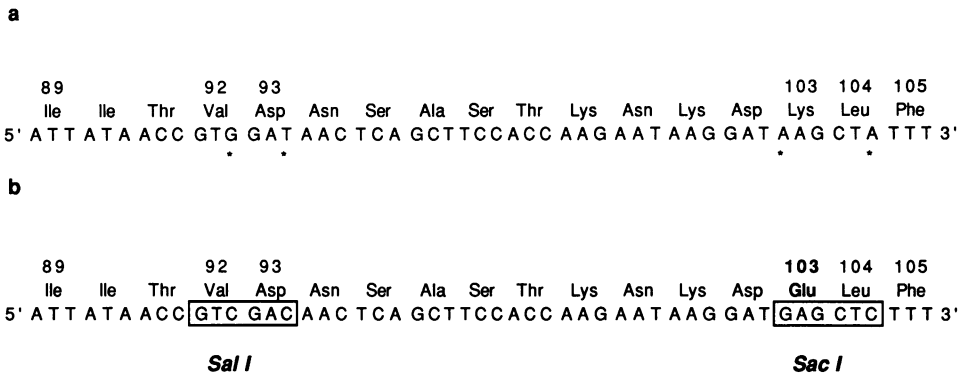

FIG. 1. Nucleotide and amino acid sequences of N-AgI in the PV-1 Sabin cDNA (a) and in the cassette vector pJFC82 (b). Nucleotides marked by an asterisk have been changed to introduce unique restriction sites *Sal*I and *Sac*I (boxed). The resulting change of VP1 residue 103 from Lys to Glu is indicated in boldface.

chimera (vLAI-RF) containing an artificial HIV-1 PND sequence consisting of amino terminal LAI-specific residues and carboxy-terminal RF-specific residues was also engineered, as a peptide of similar sequence has been shown to induce antibodies neutralizing both isolates (18). The HIV-1 PND sequences were either inserted into the B-C loop of VP1 between residues 97 and 98 (vLAI 12C) or substituted for the B-C sequence (all other chimeras) (Table 1). In the latter case, a limited and variable number of amino acids forming part of native PV-1 Sabin N-AgI were retained in the chimeras. In some constructs, additional amino acids were inserted to restore the N-terminal sequence of the VP1 B-C loop of poliovirus type 2 (vLAI V3 and vRF 10B) or type 3 (vLAI 12B and vLAI-RF).

Chimeric cDNAs were constructed by ligating annealed synthetic oligonucleotides with *Sal*I-*Sac*I-digested pJFC82. Whenever possible, the codons of the synthetic oligonucleotides were chosen according to the codon usage of PV-1 Sabin. The sequence at the *Sac*I extremity of the oligonucleotides was also designed so as to restore the original Lys-103 in the chimeras. Recombinant cDNAs were selected on the basis of restriction sites contained within the inserted fragment, and their nucleotide sequences in the hybrid region were verified by the chain termination method (46). The same DNA preparation used for sequence determination was used to transfect Vero cells by the DEAE-dextran method as described previously (26). When a complete cytopathic effect was observed, virus was recovered and its genomic RNA sequence was ascertained over ca. 200 bp spanning N-AgI as previously described (11).

Viability of PV-1/HIV-1 antigen chimeras. The construct containing the entire V3 loop of HIV-1_{LAI} (vLAI V3) failed to produce viable virus in spite of repeated transfection experiments (Table 1). This was also the case for the constructs which contained the shorter LAI or RF V3 sequences and retained the fewer poliovirus residues from N-AgI (vLAI 12A and vRF 10A). By contrast, chimeras that both retained the C-terminal amino acids (Lys-Asp-Lys-

TABLE 1. Amino acid sequence of N-AgI and viability of PV-1/HIV-1 PND chimeras

Virus	N-AgI amino acid sequence[a]	Length of the B-C loop[b]	Viability[c]
	94 105		
PV-1 Sabin	**VDNSAS** **TKNKDKLF**	10	+
vJFC 82	**VDNSAS** **TKNKDELF**	10	+
	*		
vLAI V3	**VDN** DTRPNNNTRKSIRIQRGPGRAFVTIGKIGNMRQAHKDKLF	39	−
vLAI 12A	**VDN** KSIRIQRGPGRA **KLF**	14	−
	**		
vLAI 12B	**VDN** EQKSIRIQRGPGRA **KDKLF**	18	+
vLAI 12C	**VDNSAS** KSIRIQRGPGRA **TKNKDKLF**	22	−
	(E) (E)		+[d]
vLAI 12D	**VDNSAS** KSIRIQRGPGRA **KDKLF**	19	−
	(G)(T)(E)(T) (Q)		+[d]
	(E)		+[d]
vRF 10A	**VDN** TKGPGRVIYA **KLF**	12	−
	*		
vRF 10B	**VDN** DTKGPGRVIYA **KDKLF**	15	+
	**		
vLAI-RF	**VDN** EQKSIRIQRGPGRVIYAT **KDKLF**	22	+

[a] Amino acid residues from the V3 loop of HIV-1 are shown in lightface; amino acid residues which have been retained from the PV-1 Sabin sequence are in boldface. Residues marked with an asterisk are additional residues from equivalent positions in N-AgI of type 2 (D) or type 3 (EQ) poliovirus.

[b] Number of amino acid residues between VP1 Asn-94 and Phe-105 (16).

[c] Viable (+) or nonviable (−) chimeric virus.

[d] Single amino acid changes found in the genomes of independent viable variants are shown in parentheses under the corresponding residues of the dead chimera.

TABLE 2. In vitro phenotypic characteristics of PV-1/HIV-1 PND chimeras

Virus	Plaque diam (mm)[a]	Heat lability (log PFU)[b]	*rct*[c]
PV-1 Sabin	3.14 (0.5)	1.0	4.4
vRF 10B	2.35 (0.7)	1.2	4.2
vLAI 12B	0.88 (0.3)	3.5	2.75

[a] Mean (standard deviation) of at least 40 measurements on HeLa cells infected at 34°C and stained 66 h after infection.
[b] Expressed as the loss of infectivity of the virus preparation after 10 min of incubation at 45°C.
[c] Expressed as the logarithm of the ratio between the titer at the optimal temperature (34°C) and the titer at the supraoptimal temperature (40°C), as assayed by plaque formation on HeLa cell monolayers.

Leu) of the Sabin 1 B-C loop and contained additional, negatively charged residues from the N-terminal part of the poliovirus type 2 or type 3 B-C loop (vLAI 12B, vRF 10B, and vLAI-RF) were viable. Constructs in which the native Sabin 1 B-C loop was totally (vLAI 12C) or partially (vLAI 12D) restored on both sides of the HIV sequence gave rise to viruses that all contained mutations in the N-AgI region (Table 1). These mutations affected either the HIV or the poliovirus sequence. Interestingly, their effect was to change the overall charge of the B-C loop by decreasing the number of positively charged residues and/or increasing the number of negatively charged residues.

Chimeras with a correct hybrid sequence were amplified on HeLa cell monolayers at a multiplicity of infection of 1 PFU per cell. The vLAI-RF chimera was genetically unstable upon amplification and could not be propagated as a pure population. After one passage on HeLa cells, the vLAI 12B chimera was found to carry a mutation in the poliovirus sequence, at VP1 position 90 (Ile → Leu). These mutational events indicate the existence of a strong selective pressure against the hybrid sequence of the two chimeras. The vLAI 12B virus with the Leu-90 residue was genetically stable during the following passages on HeLa cells. It was therefore used for further studies, together with the vRF 10B virus.

Phenotypic characteristics of vLAI 12B and vRF 10B chimeric viruses. The in vitro phenotypic characteristics of these chimeras were different (Table 2). Chimera vRF 10B showed a large-plaque phenotype at 34°C, and the heat lability of its capsid was not different from that of the parental PV-1 Sabin virus. By contrast, vLAI 12B showed a minute-plaque phenotype and a very heat-labile capsid. However, in standard one-step growth experiments (19), the two chimeric viruses had growth rates and reached final titers comparable to those of the parental PV-1 Sabin virus (not shown). This observation suggests that the minute-plaque phenotype of vLAI 12B may reflect the alteration of some step involved in the cell-to-cell spread of the virus. Surprisingly, whereas the *rct* marker (reproductive capacity at supraoptimal temperature) was not affected for vRF 10B, the vLAI 12B chimera was markedly less temperature sensitive than PV-1 Sabin at 40°C (Table 2). The reasons for this property are unknown; it might be related to the mutation at position 90 and/or to additional unidentified amino acid changes in the Sabin sequence, or it might be conferred by the foreign HIV-1_{LAI} sequence.

Antigenic properties of the PV-1/HIV-1 chimeras. The antigenicity of the vLAI 12B and vRF 10B chimeras was examined in a standard neutralization assay. The vLAI 12B chimera was neutralized by various HIV-1_{LAI} neutralizing sera (Table 3). These included a rabbit anti-V3 peptide serum (41) and a serum from a chimpanzee (C-499) immunized with recombinant gp160 and V3 peptides and protected against challenge with HIV-1_{IIIB} (13). Furthermore, vLAI 12B was neutralized by HIV-1 neutralizing monoclonal antibodies 110.4 (48), 0.5β (27), and F58/H3 (3), which are specific for the V3 loop of HIV-1_{LAI} (Table 4). These results indicate that the antigenicity of the HIV-1 sequence expressed on the surface of the chimeric poliovirus closely mimicked that of the PND in HIV-1. The vRF 10B chimera was neutralized by a rabbit antipeptide serum directed toward the V3 loop of HIV-1_{RF} (41) (Table 3).

Immunogenicity of the PV-1/HIV-1 chimeras. The immunogenicity of the chimeras was investigated in rabbits, using either native or heated (1 h at 56°C) virus. High-titer virus stocks were prepared on suspension-cultured HeLa cells, and viral particles were pelleted through a sucrose cushion and purified in a CsCl density gradient (23). New Zealand

TABLE 3. Neutralization of PV-1/HIV-1 PND chimeras with anti-HIV-1 sera

Serum from[a]:	Specificity[b]	Neutralization of:				
		HIV-1[c]		PV-1 chimera[d]		PV-1 Sabin[d]
		IIIB	RF	vLAI 12B	vRF 10B	
Rabbit 32						
Preimmune		>1:2	NT[e]	>1:256	NT	NT
Immune	NTRKSIRIQRGPGRAFVTIGKIGN	1:128	NT	1:32,768	NT	>1:256
Rabbit 97						
Preimmune		NT	>1:2	NT	>1:256	NT
Immune	NTRKSITKGPGRVIYATGQ	NT	>1:2	NT	1:4,096	>1:256
Chimpanzee C-499						
Preimmune		>1:2	NT	>1:128	NT	NT
Immune	NTRKSIRIQRGPGRAFVTIGKIGN	1:1,024	NT	<1:524,288	NT	>1:8

[a] Rabbits 32 and 97 were immunized with LAI and RF V3 peptides, respectively (41). C-499 serum is from a chimpanzee immunized with recombinant gp160 and V3 peptides (13).
[b] Amino acid sequence within the V3 loop reactive with the indicated immune sera. Residues present in vLAI 12B and vRF 10B chimeras are underlined.
[c] Measured by inhibition of syncytium formation in CEM-ss cells (36). Results are given as the first serum dilution that gave 50% reduction in the number of input syncytium-forming units (ca. 200).
[d] Measured in HeLa cells. Titers are expressed as the first serum dilution that neutralized 50% of one dose (50 PFU) of input virus in a standard plaque reduction assay.
[e] NT, not tested.

TABLE 4. Neutralization of the vLAI 12B chimera with anti-HIV-1 monoclonal antibodies

Neutralizing monoclonal antibody[a]	Specificity[b]	Neutralization of:		
		HIV-1_{IIIB}[c]	vLAI 12B[d]	PV-1 Sabin[d]
110.4	IQRGPGRAF	70	90	>1,450
0.5β	RGPGRAFVTIGKIG	1	0.006	>12.5
F58/H3	RKSIRIQRGPGRAFV	2	0.01	>25

[a] Monoclonal antibodies 110.4 (48), 0.5β (27), and F58/H3 (3) have been described previously.
[b] Amino acid sequence within the V3 loop reactive with the indicated monoclonal antibodies. Residues present in the vLAI 12B chimera are underlined.
[c] Measured by inhibition of syncytium formation on CEM-ss cells (36). Results are given as the lowest concentration of monoclonal antibody (micrograms per milliliter) that gave 50% reduction in the number of input syncytium-forming units (ca. 200).
[d] Measured in HeLa cells. Titers are expressed as the lowest concentration of monoclonal antibody (micrograms per milliliter) that neutralized 50% of one dose (50 PFU) of input virus in a standard plaque reduction assay.

TABLE 5. Immunogenicity of PV-1/HIV-1 PND chimeras in rabbits[a]

Immunogen	Rabbit	Neutralizing antibody titer[b]		ELISA titer[c]	
		PV-1	HIV-1	Anti-PND peptide	Anti-gp160
vLAI 12B native	1	131,072	<2	12,450	38,000
	2	16,384	<2	11,600	59,000
	3	32,768	32	310,000	360,000
vLAI 12B heated	4	<512	<2	880	150
	5	<512	<2	2,700	150
	6	<512	128	170,000	510,000
	7	<512	<2	450	250
V3 LAI peptide[d]	32	ND[e]	128	180,000	140,000
vRF 10B native	8	65,536	<2	195,000	ND
	9	131,072	<2	75,000	ND
	10	32,768	<2	73,000	ND
	11	16,384	<2	28,000	ND
vRF 10B heated	12	<512	<2	510,000	ND
	13	<512	<2	33,000	ND
	14	<512	<2	21,000	ND
	15	<512	<2	77,000	ND

[a] Only titers of the third bleeding (IS3; see text) of the animals are shown. Titers for all preimmune sera were <8 (PV-1 Sabin neutralization), <2 (HIV-1 neutralization), and <100 (ELISA).
[b] Expressed as the reciprocal of the first serum dilution that neutralized 50% of one dose (50 PFU) of PV-1 Sabin in a standard plaque reduction assay or of one dose (200 syncytium-forming units) of the homologous strain of HIV-1 in a syncytium inhibition assay (36).
[c] Expressed as the reciprocal of the highest serum dilution giving an optical density reading of 0.1 at 490 nm.
[d] Titers of a serum sample from a rabbit immunized with a V3 peptide (residues 307 to 327 of HIV-1_{LAI} gp120) conjugated to ovalbumin are given for comparison (41).
[e] ND, not done.

White rabbits were immunized by two intradermal injections of 10^8 PFU of purified virus in Freund's complete adjuvant at 3-week intervals and then given booster injections by the intramuscular route at 6-week intervals with the same dose of virus in Freund's incomplete adjuvant. Blood was withdrawn after the first (IS1), second (IS2), and third (IS3) booster injections. Anti-PND peptide titers were determined by an enzyme-linked immunosorbent assay (ELISA) (40), using PND synthetic peptides corresponding to residues 307 to 327 and 315 to 333 of HIV-1_{LAI} and HIV-1_{RF} gp120, respectively. Antibody titers against HIV-1_{LAI} gp160 were determined by an indirect ELISA (40) in which native recombinant soluble gp160 (20) was captured onto the solid phase by monoclonal antibody 160A directed against the C-terminal part of gp120 (obtained from the ANRS Reagent Project).

High titers of anti-PND peptide and anti-gp160 antibodies were generally detected after the first booster injection and increased with subsequent injections (Table 5). However, three of the four animals immunized with heated vLAI 12B showed very low ELISA titers. Only the results obtained after the third booster injection are shown in Table 5. The highest titers obtained were of the same order of magnitude as those we routinely obtain by immunizing rabbits with carrier-coupled V3 peptides in the presence of Freund's adjuvant (41). No correlation was found between the intensity of the PV-1 Sabin neutralizing response (Table 5) and that of the anti-HIV-1 response. This probably reflects individual variations, among the immunized animals, in the relative immunogenicity of the different neutralization antigenic sites of poliovirus. The reactivity of several immune sera against HIV-1 virions was also assessed in a radioimmunoprecipitation assay (31). As shown in Fig. 2, serum samples from animals immunized with native vRF 10B or vLAI 12B chimeras immunoprecipitated the envelope glycoprotein gp120 from the corresponding HIV-1 isolate. The antibodies induced by the chimeras were therefore able to recognize native HIV-1 envelope glycoprotein molecules.

HIV-1 neutralizing antibody response elicited by the PV-1/HIV-1 chimeras. HIV-1 neutralizing antibodies were measured by different infection inhibition assays. To carry out the syncytium inhibition assay (36), serial twofold dilutions of heat-inactivated serum were incubated for 1 h at room temperature with ca. 200 syncytium-forming units of HIV-1_{IIIB} or HIV-1_{RF} isolates grown on H9 cells. The virus was then incubated for 1 h at 37°C with 50,000 DEAE-dextran-treated CEM-ss cells made adherent to microplate dishes by poly-L-lysine. After 5 days of incubation at 37°C, syncytia were counted with a gridded ocular counter. The reverse transcriptase (RT) assay and the cell viability assay were performed essentially as previously described (45). Briefly, heat-inactivated serum was incubated at different dilutions with 10^3 50% tissue culture infectious doses of HIV-1_{LAI} for 1 h at 37°C and then added to 10^4 CEM-C113 cells. From day 7, and every 3 or 4 days thereafter, the culture supernatants were assayed for RT activity, and lysis of infected cells was measured by using tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) as a vital stain. Sera from animals immunized with native or heated vRF 10B virus showed no neutralizing activity against the HIV-1_{RF} isolate (Table 5). Two of the three rabbits immunized with the native vLAI 12B chimera showed low neutralizing antibody titers in the RT assay (1:20 for 50% reduction of RT activity) but were negative in the syncytium inhibition assay (Table 5). The third animal (rabbit 3), which showed the highest anti-PND peptide and anti-gp160 ELISA titers, had a neutralization titer of 1:100 (for 90% reduction of RT activity) in the RT assay. Presence of neutralizing antibodies was confirmed in the cell viability assay (not shown) and in the syncytium inhibition assay (Table 5). The same serum was devoid of neutralizing activity against the HIV-1_{RF} isolate (not shown), consistent with the fact that the PND from LAI and RF isolates are antigenically (50) and immunologically (18, 38) distinct. Among the animals immunized with the heated vLAI 12B chimera, only one (rabbit 6)

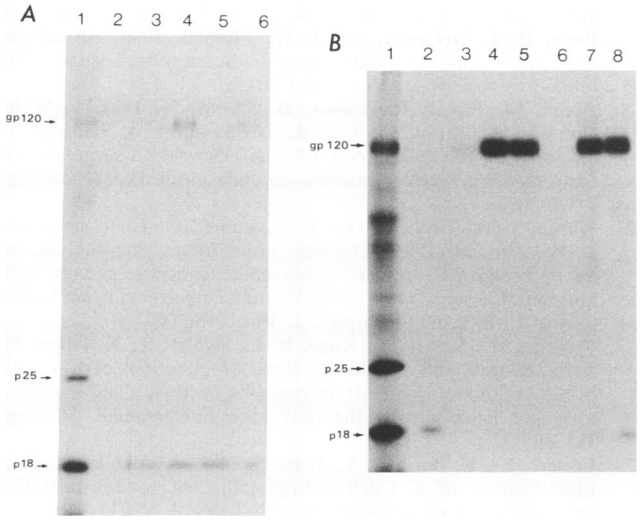

FIG. 2. Immunoprecipitation of HIV-1 antigens by antibodies induced by the vLAI 12B and vRF 10B chimeras. Serum samples from rabbits immunized with a native vRF 10B (A) or vLAI 12B (B) chimera were tested by immunoprecipitation of metabolically labelled HIV-1_{RF} (A) or HIV-1_{LAI} (B) virions from culture supernatants of chronically infected CEM cells (31). (A) Lanes: 1, positive control serum; 2, immune serum (IS1) from control rabbit 238 immunized with PV-1 Sabin; 3 and 4, preimmune and immune (IS3) sera, respectively, from animal 10; 5 and 6, preimmune and immune (IS3) sera, respectively, from animal 11. All sera were assayed at a 1:5 dilution. (B) Lanes: 1, positive control serum; 2 to 5, preimmune (lane 2) and immune (IS1 [lane 3], IS2 [lane 4], and IS3 [lane 5]) sera from rabbit 3; 6, immune serum (IS1) from control rabbit 238; 7 and 8, immune sera (IS3) from rabbits 1 and 2, respectively. All sera were assayed at a 1:12 dilution.

showed high ELISA titers and had a moderate neutralizing antibody titer in the syncytium inhibition assay (Table 5).

Peptide-size fragments of HIV-1 gp120 containing neutralization epitopes have been used as immunogens in the form of free (41) or coupled (17, 18, 38) oligopeptides or expressed by genetic recombination at the surface of bacterial (6), viral (10, 30, 40), or viruslike (15) particles. Our approach of using a vaccine strain of poliovirus as a vehicle offers several advantages: PV-1 Sabin is among the most effective live virus vaccines currently in use, it induces a strong systemic and mucosal antibody response, and as a replicating agent it could also be expected to elicit a cytotoxic cellular immune response. Evans et al. (10) have reported the construction of a PV-1 Sabin chimera expressing a neutralization epitope from the transmembrane glycoprotein gp41 of HIV-1_{LAI}. This virus induced neutralizing antibodies active against several HIV-1 isolates. The construction of poliovirus chimeras expressing sequences from the V3 loop of HIV-1 gp120 is of high interest, since this domain induces cytotoxic T lymphocytes (47) and antibody-dependent cell-mediated cytotoxicity (3) as well as neutralizing antibodies. It has been attempted unsuccessfully so far (7), despite the fact that a large variety of amino acid sequences can be naturally (see the report of Murray et al. [34] for a compilation) or artificially (5, 10, 19, 21, 25, 40) accommodated by the N-AgI of PV-1. Our observations confirm that sequences from the V3 loop of HIV-1 are highly detrimental to the viability of PV-1 Sabin. It has been suggested (7) that the volume occupied by the B-C loop is important for the viability of PV-1 Sabin chimeras. Other studies (32) have shown that the growth characteristics of PV-1 Mahoney chimeras could be improved by extending the length of the B-C loop and restoring at least part of its original sequence. We have observed that the viability of PV-1/HIV-1 PND chimeras was highly dependent on the presence of additional negatively charged amino acid residues and that mutants that spontaneously arose and were selected from nonviable chimeras all carried charge modifications in the B-C loop (Table 1). This finding suggests that the charge density and/or repartition in the B-C loop is an important determinant of the viability of PV-1 Sabin chimeric viruses modified in N-AgI. This constraint may be critical for PV-1/HIV-1 PND chimeras, given the high content in positively charged amino acids within the HIV-1 V3 loop sequences.

When used to immunize rabbits, PV-1/HIV-1 PND chimeras elicited a strong anti-HIV-1 response. Anti-PND peptide and anti-gp160 ELISA titers were in the same range as those obtained with carrier-coupled V3 peptides. It should be noted that one immunizing dose of chimeric virus (10^8 PFU) contained approximately 2 to 10 ng of the PND peptide component, compared with ca. 100 μg used for conjugated V3 peptides (poliovirus is not known to replicate in rabbits, which precludes any possibility of amplification through vector replication). This emphasizes the magnitude of the carrier effect provided by the poliovirus particle. However, the chimeras induced no (vRF 10B) or only a weak (vLAI 12B) HIV-1 neutralizing antibody response. A possible explanation is that the three-dimensional conformation of the HIV-1 sequence expressed at the surface of the chimeric poliovirus was somewhat different from that required for neutralization of HIV-1. The existence of HIV-1 variants which are resistant to V3-specific neutralizing antibodies due to amino acid changes in gp120 located outside of the V3 loop (1, 28, 37) has already illustrated the importance of the three-dimensional conformation of the PND for HIV-1 neutralization. However, the observation that the vLAI 12B chimera was neutralized by HIV-1 neutralizing monoclonal antibodies argues against this hypothesis. Alternatively, it may be that the presentation of the PND to the immune system was inappropriate in these experiments. We shall presently investigate the immunogenicity of vLAI 12B and vRF 10B chimeras in primates by the oral route, which is expected to result in amplification of the antigenic load and may allow a more efficient stimulation of the immune system, including possibly an improved T-cell response.

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