peptides by IL-2 production were available for re-testing one to three months later, and were found to be reproducibly positive against the same antigens. One HIV^+ donor who showed no response to FLU or to any of the peptides remained negative to all of the antigens when retested one year later. PBL from 13 of the 21 HIV^+ donors who responded to FLU were typed for HLA. In this small sample, no correlation was noted between the expression of a particular HLA class I or class II antigen and ability to respond to any of the synthetic peptides (Table 2).

The individuals who responded to the synthetic peptides were two-to-three times as frequent among HIV^+/FLU^+ donors as among all HIV^+ donors (Table 3). All donors, irrespective of their HIV status, were responsive to HLA alloantigens, which is indicative of their ability to respond to some antigenic stimulus by IL-2 production. It has recently been demonstrated that T-helper cell responses by human PBL to FLU require MHC self-restricted $CD4^+$ T-helper cells whereas the response to alloantigens can use either the $CD4^+$ or $CD8^+$ pathway of T-helper cell activity[7] (C.S. Via, G. Tsokos and G.M.S., manuscript in preparation). Also, 40–50% of asymptomatic HIV^+ individuals and AIDS patients have a selective defect in CD4-mediated but not in CD8-mediated T-helper cell function (M. C. *et al.*, submitted). Therefore, almost all individuals responsive to one or more of the HIV synthetic peptides also responded to FLU, probably because this group of 21 of the 42 HIV^+ donors tested retained intact T-helper cell function to a CD4-dependent antigen. In contrast, 20 of the 21 patients unresponsive to FLU may also have failed to respond to the peptides because they had lost responsiveness to any CD4-dependent antigens. Thus, it is important when testing any HIV^+ individuals for T-helper cell responses to HIV antigens to establish whether these patients have retained an intact CD4 T-helper cell pathway.

Of the 21 FLU-responsive HIV^+ donors, 18 responded to at least one of the four T-cell epitopes. Of the 10 donors we were able to test with all four peptides, nine responded to at least one peptide. Therefore, these four helper T-cell epitopes are sufficient to elicit responses in 85–90% of an HLA-diverse group of patients. For the purposes of vaccine development, it has been feared that many T-cell epitopes would be necessary to cover most or all of the outbred human population with extensive HLA polymorphism. But the present results are much encouraging: only a few selected epitopes may be sufficient. □

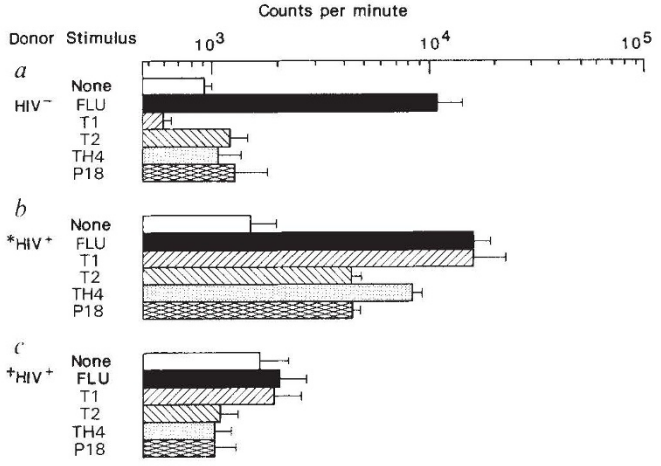

FIG. 1 IL-2 production by PBL from an HIV seronegative (*a*) and two HIV seropositive Walter Reed Stage 1 patients (*b* and *c*).
METHOD. PBL were unstimulated, or stimulated with influenza A virus (A/Hong Kong RX73, H3N2, grown in chicken eggs, final culture dilution 1:1,000) or with the HIV synthetic peptides T1, T2, TH4 or P18 at 2.5 μM. EnvT1 and T2 correspond to amino-acid residues 428–443 and 112–124 of gp120 (IIIB isolate) respectively[1]. Peptide TH4.1 corresponds to residues 834–848 of gp160 (P. Hale *et al.*, submitted). P18, corresponding to residues 315–329 of gp160 (IIIB), is a major epitope for murine anti-HIV cytolytic effector cells[11]. PBL (3×10^6) were cultured in 2 ml RPMI-1640 medium supplemented with 5% pooled AB^+ human serum for 7 d in the presence of anti-TAC (IL-2 receptor p55 chain) monoclonal antibody (to prevent IL-2 consumption). Supernatants of these cultures were collected, and five twofold dilutions of the supernatants were added to cultures of the IL-2-dependent CTLL cell line. Twenty-four hours later, the stimulated CTLL cultures were pulsed with [^{3}H]thymidine; thymidine incorporation was determined 18 h later, and is expressed in counts per minute. The data points shown in Fig. 1 and Table 1 are for a culture supernatant dilution of 1:4. We have used a stimulation index (ratio of counts per minute in stimulated cultures to that in unstimulated cultures) of greater than 2.0 as an indication of positive response. For key, see Table 1.

TABLE 3 Positive T-helper cell responses to the four HIV synthetic peptides as a function of ability to respond to influenza A virus

Donor category	IL-2 production				Proliferation			
	T1	T2	TH4.1	P18	T1	T2	TH4.1	P18
All HIV^+	14/42 (33%)	12/42 (29%)	6/29 (21%)	6/29 (21%)	4/35 (11%)	4/35 (11%)	1/22 (5%)	1/22 (5%)
HIV^+, FLU^+	14/21 (67%)	12/21 (57%)	6/10 (60%)	5/10 (50%)	4/17 (24%)	3/17 (18%)	1/6 (17%)	1/6 (17%)

Fractions and per cent (in parentheses) given for each donor category. All of the HIV seronegative control donors responded to FLU, but none of them responded to any of the four HIV synthetic peptides.

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1. Cease, K. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 4249–4253 (1987).
2. Berzofsky, J. A. *et al. Nature* **334,** 706–708 (1988).
3. DeLisi, C. & Berzofsky, J. A. *Proc. natn. Acad. Sci. U.S.A.* **82,** 7048–7052 (1985).
4. Spouge, J. L. *et al. J. Immun.* **138,** 204–212 (1987).
5. Margalit, H. *et al. J. Immun.* **138,** 2213–2229 (1987).
6. Berzofsky, J.A. *et al. Immunol. Rev.* **98,** 9–52 (1987).
7. Shearer, G. M. *et al. J. Immun.* **137,** 2514–2521 (1986).
8. Lane, H. C. *et al. New Engl. J. Med.* **313,** 79–84 (1985).
9. Redfield, R. R. *New Engl. J. Med.* **314,** 131–132 (1986).
10. Wahren, B. *et al. J. Virol.* **61,** 2017–2023 (1987).
11. Takahashi, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 3105–3109 (1988).

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An engineered poliovirus chimaera elicits broadly reactive HIV-1 neutralizing antibodies

David J. Evans*, Jane McKeating†, Janet M. Meredith*, Karen L. Burke*, Kersi Katrak‡, Ann John‡, Morag Ferguson‡, Philip D. Minor‡, Robin A. Weiss† & Jeffrey W. Almond*

* Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ, UK
† Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London SW3 6JB, UK
‡ National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QR, UK

THE Sabin type 1 vaccine strain of poliovirus is probably the safest and most successful live-attenuated vaccine virus used in humans. Its widespread use since the early 1960s has contributed significantly to the virtual eradication of poliomyelitis in developed countries. We have reported previously the construction of an intertypic antigen chimaera of poliovirus, based on the Sabin 1 strain, and proposed that this virus could be modified to express on its surface antigenic determinants from other pathogens[1]. We describe here the construction and characterization of a poliovirus

antigen chimaera containing an epitope from the transmembrane glycoprotein (gp41) of human immunodeficiency virus type 1 (HIV-1). In antibody absorption experiments, the virus chimaera inhibited neutralization of HIV-1 by antipeptide monoclonal antibodies specific for the gp41 epitope and significantly reduced the group specific neutralizing activity of HIV-1-positive human sera. Rabbit antisera raised by subcutaneous injection of the polio/HIV chimaera in adjuvant was shown to be specific for HIV-1 gp41 in peptide-binding assays and by western blotting. Moreover, the antisera neutralized a wide range of American and African HIV-1 isolates and also inhibited virus-induced cell fusion. Monoclonal antibodies against the HIV-1 derived regions of the chimaera also neutralized HIV-1. These results establish the potential of using poliovirus for the presentation of foreign antigens and suggest that Sabin 1 poliovirus/HIV chimaeras could offer an approach to the development of an HIV vaccine.

We have used recombinant DNA techniques to introduce antigenic domains from the capsid proteins of poliovirus type 3 into poliovirus type 1 to produce a virus chimaera of dual antigenicity and immunogenicity[1]. These and related observations[2,3] encouraged us to develop the Sabin type 1 vaccine strain of poliovirus as an expression vector/vaccine for the presentation of antigenic structures from unrelated pathogens. To facilitate the production of virus antigen chimaeras, we have constructed a cassette vector (pCAS1) by engineering unique restriction sites flanking the region encoding amino acids 91–102 of capsid protein VP1 (Fig. 1*a*) in a full-length complementary DNA of Sabin 1 cloned in a pBR322-derived vector. This region of VP1 is known to elicit neutralizing antibodies[4], and on the three-dimensional structure of poliovirus is seen to form a distinct surface projection at the pentameric apex of the icosahedral particle[5]. Use of pCAS1 allows the replacement of the cDNA corresponding to antigenic site 1 by synthetic oligonucleotides encoding an antigenic determinant of choice.

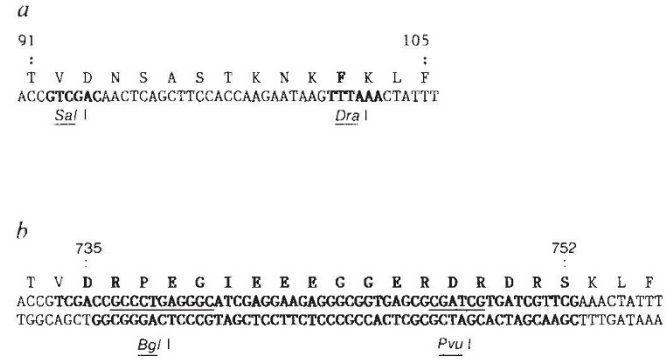

FIG. 1 Construction of the poliovirus: HIV-1 antigen chimaera S1/env/3. *a*, The nucleotide and amino-acid sequence of antigenic site 1 of the Sabin 1-derived cassette vector pCAS1. The highlighted residue represents a single amino-acid change (Asp to Phe at position 102 of VP1) generated as a result of the introduction of the *Dra*I site. *b*, The nucleotide and amino acid sequence of the corresponding region in the antigen chimaera S1/env/3. Residues in bold type indicate the 18 amino acids derived from HTLV-III$_B$ gp41 (amino acids 735–752) (ref. 25).

METHODS. *a*, Unique *Sal*I and *Dra*I restriction sites were engineered into a full-length cDNA of Sabin 1, preceded by a synthetic T7 promoter, and cloned in the pBR322-derived vector pFB1 (Pharmacia). The precise construction details of the Sabin 1-based expression vector (pCAS1) will be reported elsewhere. *b*, Complementary oligonucleotides were annealed and ligated with *Sal*I/*Dra*I digested pCAS1, and recombinants screened for *Bgl*I and *Pvu*I restriction sites (underlined). A recombinant pS1/env/3 was linearized with *Nae*I, which cuts within vector sequences of the construct, and used as a template in a T7 transcription reaction[26] prior to transfection of sub-confluent Hep2C monolayers. A cytopathic effect was observed after 4 days and the RNA sequence of ~200 base pairs spanning antigenic site 1 (2,650–2,850) of the recovered chiamaeric virus was confirmed by sequencing[27].

The envelope glycoprotein (env) of HIV-1 displays extensive sequence heterogeneity and antigenic variation[6,7]. Antigenically conserved sites within env must exist, however, as it is possible to show neutralization of a range of HIV-1 isolates with immune sera from infected individuals[8], or with antisera raised to defined regions of env[9]. Prospective vaccines should ideally contain such conserved sites so that they elicit antibodies capable of reacting with a wide range of virus isolates. We have chosen a well characterized epitope from the transmembrane glycoprotein gp41 of HIV-1 (amino acids 735–752). A synthetic peptide of this region is recognized in enzyme-linked immunosorbent assay by antisera from seropositive patients and induces antibodies which neutralize a range of HIV-1 isolates[10–12], indicating that it is a group-specific epitope. The construction of a chimaeric poliovirus containing this sequence is detailed in Fig. 1.

The recovered virus, designated S1/env/3, was neutralized and recognized in antigen-blocking tests[13] by polyclonal poliovirus type 1 antisera, and by monoclonal antibodies (mAbs) directed against antigenic sites 2 and 3 of the Sabin 1 strain, but not by antibodies specific for antigenic site 1.

The recent observation that the structure of antigenic site 1 may influence poliovirus host range[2,14] prompted us to investigate the interaction of S1/env/3 with the poliovirus receptor. S1/env/3 infection of Hep2C (human epithelial carcinoma cells) monolayers was blocked by a mAb specific for the receptor[15], thus demonstrating that the chimaera was still dependent on the normal poliovirus cellular receptor for attachement and entry into cells.

Preincubation with purified S1/env/3 but not Sabin 1 abrogated the HTLV-III$_B$ neutralizing activity of two antipeptide mAbs, ED6 and LA9, and reduced the neutralization of HTLV-III$_B$ by five of six human sera. Neutralization of HTLV-III$_B$ by a control antibody (110.3) specific for gp120 amino acids 307–321 (refs 16, 17), was not inhibited by S1/env/3 or Sabin 1.

The immunogenic potential of S1/env/3 was investigated in rabbits, and the neutralizing activity against HIV-1 was determined by infectivity inhibition and plaque reduction assays

TABLE 1 S1/env/3 inhibition of HTLV-III$_B$ neutralization

	Residual HTLV-III$_B$ neutralization titre after incubation with		
	Mock	Sabin 1	S1/env/3
Monoclonal antibodies			
ED6	640	640	0
LA9	640	640	0
110.3	1,000	1,000	1,000
Human sera			
1	160	160	20
2	40	40	10
3	40	40	10
4	80	80	20
5	320	160	40
6	80	80	80

Results are expressed as the reciprocal of the serum dilution giving >90% reduction in HIV titre[8] following pre-incubation of the mAbs or human immune sera with culture medium (Mock), Sabin 1 or S1/env/3. LA9 and ED6 are IgM mAbs specific for the gp41 epitope (HTLV-III$_B$ amino acids 735–752). 110.3 is an IgG1 mAb specific for the LAV-1 type specific neutralization loop in the second conserved domain of gp120 (amino acids 307–321) (refs 16, 17). Human immune sera numbered 1–6 represent anonymous British HIV$^+$ blood donors. Sucrose-purified S1/env/3 or Sabin 1 (5×10^4 TCID$_{50}$ (50% tissue culture infectious dose) units) were preincubated with a 1:10 dilution of ED6, LA9, a 1:100 dilution of 110.3 and a 1:1 dilution of each human serum for 1 h at 37 °C. Residual HIV-neutralizing activity was determined by incubating dilutions of the antibody/virus mixture with 10^3 infectious units (TCID$_{50}$) of HTLV-III$_B$ for 1 h at 37 °C. Aliquots (100 μl) of medium containing 2×10^4 C8166 cells were added, and the presence of syncytia recorded after 48 h as an indication of HIV infection.

(Table 2). All rabbit antisera showed neutralizing activity against HTLV-III$_B$, confirming that the chimaera has immunogenic potential. Antiserum R1, which contained the highest anti-poliovirus activity, was further tested against a range of HIV-1 isolates. This antiserum neutralized the entire test panel at various titres, including the African isolates CBL4 (Tanzania) and Z84 (Zaire). The titres observed in both neutralization assays used were in good agreement and were comparable to those of human sera (see Table 1). Antisera R7 and R8 also neutralized the HIV-1 isolates tested (HTLV-III$_{RF}$ or Z84), although the titres observed were lower against both HIV-1 and S1/env/3. The HIV-1-neutralizing activity of antiserum R1 was absorbed out by pre-incubation with S1/env/3, but not with Sabin 1, confirming that the activity was induced by the gp41 epitope and not by a chance cross-reactive poliovirus epitope (data not shown). Early syncytium formation in a mixture of HTLV-III$_B$ producing cells and uninfected C8166 cells (CD4$^+$, HTLV-I-transformed T cells)[18] was inhibited by antiserum R1, although at a lower titre than that determined for virus inhibition (data not shown).

Four mAbs specific for S1/env/3 were characterized in terms of their reaction with HIV-1. One mAb (1577) displayed neutralizing activity against all HIV-1 isolates tested, including the three African strains CBL4, Z84 and Z129 (Table 2). Monoclonal antibodies 1575 and 1583 neutralized only some of the isolates, suggesting that they recognize a defined epitope within the gp41 735–752 region, which is less well conserved. Monoclonal antibody 1578, displayed no HIV-1-neutralizing activity, suggesting that it recognized an epitope formed from both HIV-1 and poliovirus amino acids.

The specificity of the HIV-1 immune response to S1/env/3 was demonstrated by western blotting and by a peptide binding assay (Fig. 2). Antiserum R1 reacted with the envelope glycoprotein precursor gp160 and gp41 in western blots, whereas pre-immune sera were negative (Fig. 2*a*). All the rabbit antisera bound specifically to a linear synthetic peptide corresponding to the HTLV-III$_B$ epitope present on the S1/env/3 chimaera (Fig. 2*b*), whereas they failed to bind a 15-amino-acid peptide derived from the type-specific neutralization epitope on gp120 (residues 307–321). Pre-immune sera and control rabbit hyper-immune Sabin 1 antisera displayed no specific binding to either the gp41 or gp120 derived peptides (Fig. 2*c*). The three S1/env/3 monoclonal antibodies that neutralized HIV-1 (1575, 1577, 1583) also reacted with the gp41 735–752 peptide in binding assays (data not shown).

The poliovirus chimaera described here is based on the safe and effective Sabin type 1 live attenuated vaccine strain. The substitution of eight poliovirus residues by eighteen HIV-1 (HTLV-III$_B$) specified amino acids gave rise to a viable virus which possessed some of the antigenic properties of HIV-1. This substitution did not involve the alteration of amino acids believed to be important to the attenuation phenotype of Sabin 1, so we expect the virus chimaera to have retained this desirable property. The results illustrate the flexibility of the surface of the poliovirus particle and indicate that it may be possible to generate further chimaeras containing, for example, additional HIV-1, HIV-2, or composite B- and T-cell epitopes[19]. The gp41 sequence chosen here induced HIV-1 group-specific neutralizing antibodies in rabbits. The inhibition, by S1/env/3, of HTLV-III$_B$ neutralizing activity in human immune sera suggests that, in five out of six individuals, a significant proportion of the cross-reactive immune response may be directed against this epitope. The antisera also inhibited the fusion of HTLV-III$_B$ infected and uninfected C8166 cells, suggesting that fusion inhibition may be a characteristic of antibodies against this epitope.

It is possible that different regions of the envelope glycoproteins of HIV may elicit antibodies which play different roles in immunopathogenesis and protection, for example, antibody-dependent cell cytotoxicity, Fc-mediated virus enhancement, fusion inhibition or attachment inhibition. Vaccinia virus

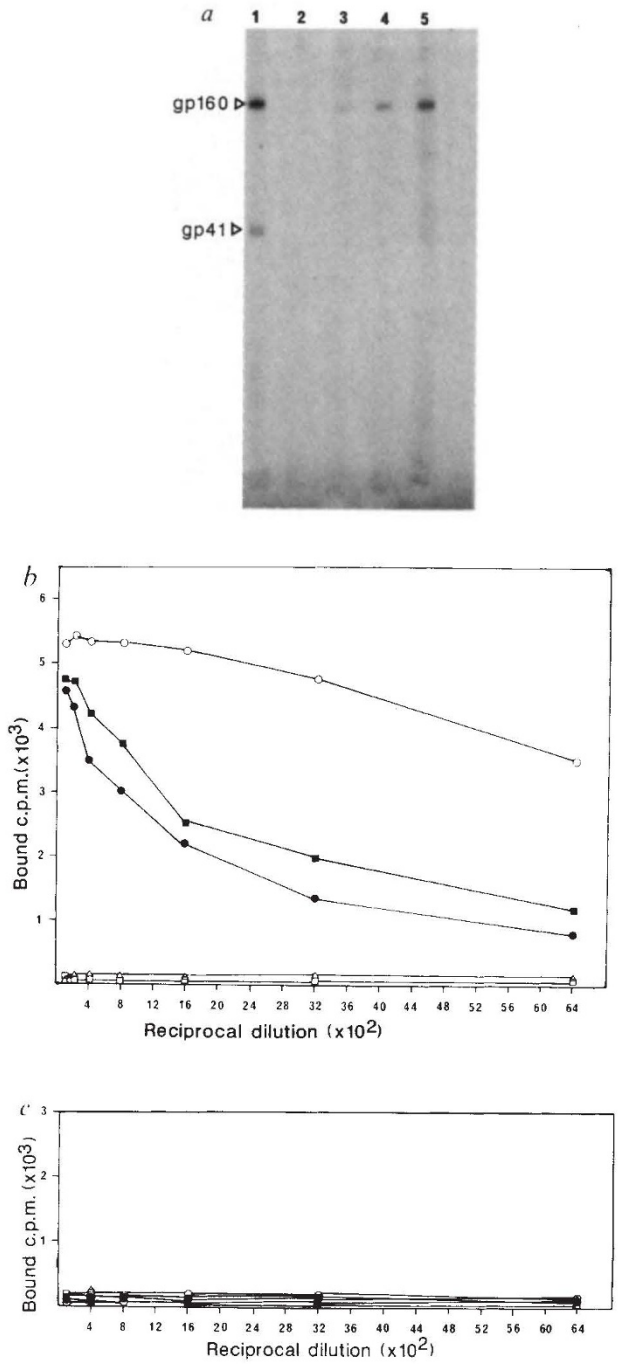

FIG. 2 Specificity of the immune response to S1/env/3. *a*, Analysis of the immune response to S1/env/3 by western blot. Reactivity of a human mAb DZ specific for HIV gp41 (lane 1), pre-immune R1 (lane 2), and S1/env/3 antiserum R1 bleed 1 (land 3), bleed 2 (lane 4) and bleed 3 (lane 5) to HIV-1 SDS-PAGE-separated viral proteins. Gp160 and gp41 are indicated. *b* and *c*, Binding of S1/env/3 antisera (R1) bleeds 1 (●), 2 (■) and 3 (○), pre-immune serum (□), and Sabin 1 antiserum (△), to (*b*) gp41 peptide amino acids 735–752 of HTLV-III$_B$ and to (*c*) gp120 peptide amino acids 307–321 of HTLV-III$_B$.

METHODS. *a*, Antisera were incubated with HIV WB strips according to the manufacturers instructions (DuPont), and bound antibodies were detected using [^{125}I]Protein A (Amersham) at 2×10^5 c.p.m. ml^{-1}. *b* and *c*, Flexible 96-well microtitre plates were coated with gp41 or gp120 peptide at 10 μg ml^{-1} (50 μl per well) in carbonate buffer, pH 9.6, for 12 h at 4 °C, washed with PBS/0.05% Tween and incubated with 1% bovine serum albumin (BSA) in PBS/0.05% Tween for 1 h at room temperature . Antiserum (50 μl) diluted in PBS/1% BSA/0.05% Tween was added and incubated for 1 h at 37 °C. After washing, bound antibodies were detected by incubation with [^{125}I]Protein A (Amersham, 10^5 c.p.m. ml^{-1}, 50 μl per well) for 1 h at 37 °C.

TABLE 2 Neutralization of HIV-1 infectivity and inhibition of syncytium formation by antisera and monoclonal antibodies

Neutralization by S1/env/3 antisera

	Reciprocal neutralization titre Virus strain						
Antiserum	HTLV-III$_B$	HTLV-III$_{RF}$	SF2	SF33	CBL4	Z84	S1/env/3
R1							
Pre-immune	<10	<10	<10	<10	<10	<10	<10
Final bleed	80	80	80	40	10	40	>28,960
	(40)	(40)	(160)	(40)	(80)	(40)	
R7							
Pre-immune	<10	<10	NT	NT	NT	<10	<10
Final bleed	20	40	NT	NT	NT	40	>2,560
R8							
Pre-immune	<10	<10	NT	NT	NT	NT	<10
Final bleed	40	20	NT	NT	NT	NT	>2,560
R9							
Pre-immune	<10	<10	NT	NT	NT	NT	<10
Final bleed	40	10	NT	NT	NT	NT	1,280
R19, R20 and R21							
Pre-immune	<10						
Post-immune	<10						

Neutralization by S1/env/3 monoclonal antibodies

	Reciprocal neutralization titre Virus strain						
mAb	HTLV-III$_B$	HTLV-III$_{RF}$	SF33	CBL4	Z84	Z129	S1/env/3
1575	40	20	40	10	10	<10	2,560
1577	40	160	80	20	20	10	640
1578	<10	<10	<10	<10	<10	<10	640
1583	40	40	20	<10	<10	<10	>28,960

Rabbit R1 was immunized intradermally with 0.1 ml ($\sim 10^8$ TCID$_{50}$ ml^{-1}) of sucrose-purified S1/env/3 in complete Freunds adjuvant, and boosted subcutaneously at two-week intervals with the same virus preparation in incomplete Freunds adjuvant. Rabbits R7–R9 (S1/env/3) and R19–R21 (Sabin 1) were immunized intramuscularly with 0.5 ml tissue culture fluid ($\sim 10^8$ TCID$_{50}$ ml^{-1}) in complete Freunds adjuvant, and boosted at two-week intervals in a similar manner. Neutralization titres were determined by incubating 10 μl of heat-inactivated antiserum with 40 μl virus supernatant containing 10^3 infectious units of HIV-1 at 37 °C for 1 h. Residual HIV-1 infectivity was measured by the infectivity inhibition assay[8] described in Table 1. Antiserum R1 was also tested for HIV-1 neutralizing activity in a plaque-reduction assay (ref. 24 and J. McK. *et al.*, in preparation) on the sensitive MT4 cell line. The results obtained by this independent assay are shown in brackets. Results are expressed as the reciprocal of the serum dilution giving >90% reduction in HIV infectivity or plaque formation. Also shown is the reciprocal neutralization titre of S1/env/3 antisera with 100 TCID$_{50}$ units of the homologous virus. NT, not tested. Murine monoclonal antibodies were raised as described previously[13], and screened against S1/env/3 and Sabin 1 in antigen-blocking tests (data not shown). The S1/env/3 and HIV-1 neutralizing activity of the ascites from four hybridomas was determined. Results are expressed as the reciprocal of the mAb ascitic fluid dilution giving >90% reduction in HIV-1 titre[8], or neutralizing 100 TCID$_{50}$ units of S1/env/3.

constructs expressing env elicit antibodies in humans that neutralize divergent HIV-1 isolates[20]. But the vaccinia-based recombinants[21] used so far express whole or slightly truncated gp160, and thus give rise to a general antibody response. The poliovirus system allows the selective expression of defined regions of HIV proteins in an immunoprominent position, and therefore enables specific immune responses to be induced and analysed. The system also facilitates the generation of monoclonal antibodies against defined HIV sequences which may provide valuable diagonostic reagents.

Poliovirus offers considerable advantages over non-replicating systems such as free peptides or hepatitis B antigen particles[22], as it induces a secretory as well as a systemic humoral immune response[23]. The presence of secretory antibodies in mucous fluids may reduce the efficiency of transmission of HIV. The immune response elicited by S1/env/3 after oral administration to primates is currently being evaluated. This should provide information on whether poliovirus chimaeras offer a realistic strategy for the development of HIV vaccines. □

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1. Burke, K. L., Dunn, G., Ferguson, M., Minor, P. D. & Almond, J. W. *Nature* **332,** 81–82 (1988).
2. Martin, A. *et al. EMBO J.* **7,** 2839–2847 (1988).
3. Murray, M. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 3203–3207 (1988).
4. Minor, P. D., Ferguson, M., Evans, D. M. A., Almond, J. W. & Icenogle, J. P. *J. gen. Virol.* **67,** 1283–1291 (1986).
5. Hogle, J. M., Chow, M. & Filman, D. J. *Science* **229,** 1358–1365 (1985).
6. Modrow, S. *et al. J. Virol.* **61,** 570–578 (1987).
7. Starcich, B. R. *et al. Cell* **45,** 637–648 (1986).
8. Weiss, R. A. *et al. Nature* **324,** 572–575 (1986).
9. Ho, D. D., Kaplan, J. C., Rackauskas, I. E. & Gurney, M. E. *Science* **239,** 1021–1023 (1988).
10. Kennedy, R. C. *et al. Science* **231,** 1556–1559 (1986).
11. Chanh, T. C. *et al. EMBO J.* **5,** 3065–3071 (1986).
12. Dalgleish, A. G. *et al. Virology* **165,** 209–215 (1988).
13. Ferguson, M. *et al. J. gen. Virol.* **65,** 197–201 (1984).
14. Murray, M. G. *et al. Science* **241,** 213–215 (1988).
15. Minor, P. D., Pipkin, P. A., Hockley, D., Schild, G. C. & Almond, J. W. *Virus Res.* **1,** 203–212 (1984).
16. Thomas, E. K. *et al. AIDS* **2,** 25–30 (1988).
17. Linsley, P. S., Ledbetter, J. A., Kinney-Thomas, E. & Shiu-Lok, H. *J. Virol.* **62,** 3695–3702 (1988).
18. Clapham, P. R. *et al. Virology* **158,** 44–51 (1987).
19. Francis, M. J. *et al. Nature* **300,** 168–170 (1987).
20. Zagury, D. *et al Nature* **332,** 728–731 (1988).
21. Chakrabarti, S., Robert-Guroff, M., Wong-Staal, F., Gallo, R. C. & Moss, B. *Nature* **320,** 535–537 (1986).
22. Michel, M-L. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 7957–7961 (1988).
23. Ogra, P. L. & Ogra, S. S. *J. Immun.* **110,** 1307–1311 (1973).
24. Harada, S. *et al. Science* **229,** 563–565 (1985).
25. Ratner, L. *et al. Nature* **313,** 277–284 (1986).
26. van der Werf, S., Bradley, J., Wimmer, E., Studier, F. W. & Dunn, J. J. *Proc. natn. Acad. Sci. U.S.A.* **80,** 5080–5084 (1983).
27. Rico-Hesse, R., Pallansch, M. A., Nottay, B. K. & Kew, O. M. *Virology* **160,** 311–322 (1987).

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