28. H. Aviv and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1408 (1972).
29. H. Lehrach, D. Diamond, J. M. Wozney, H. Boedtker, *Biochemistry* **16**, 4743 (1977).
30. P. Ponte, S. Y. Ng, J. Engel, P. Gunning, L. Kedes, *Nucleic Acids Res.* **12**, 1687 (1984).
31. We thank S. H. Friend, R. A. Weinberg, and T. P. Dryja for the generous gift of the p0.9R and p3.8R probes; B. E. Johnson for the primary tumor material; W. Nash for some of the cytogenetic data; and J. F. Battey, E. A. Sausville, I. R. Kirsch, M. J. Birrer, M. Zajac-Kaye, and M. M. Nau for critical review of the manuscript. J.W.H. is a Howard Hughes Medical Institute–NIH Research Scholar.

24 March 1988; accepted 7 June 1988

Type-Restricted Neutralization of Molecular Clones of Human Immunodeficiency Virus

DAVID J. LOONEY,* AMANDA G. FISHER, SCOTT D. PUTNEY, JAMES R. RUSCHE, ROBERT R. REDFIELD, DONALD S. BURKE, ROBERT C. GALLO, F. WONG-STAAL

In a study of the immunologic significance of the genetic diversity present within single isolates of human immunodeficiency virus type 1 (HIV-1), the neutralization of viruses derived from molecular clones of the HIV-1 strain HTLV-III$_B$ by an extensive panel of sera was compared. Sera from HIV-1–infected patients and from goats immunized with polyacrylamide gel–purified HIV-1 envelope glycoprotein (gp120), native gp120, or gp120-derived recombinant peptides, showed marked heterogeneity in neutralizing activity against these closely related viruses. The change of a single amino acid residue in gp120 may account for such "clonal restriction" of neutralizing activity.

ONE STRATEGY FOR IMMUNOPROphylaxis against acquired immunodeficiency syndrome is the administration of an immunogen intended to elicit protective virus neutralizing antibodies. Neutralizing activity (NA) against HIV-1 has been documented by many investigators using a variety of techniques (*1–5*). Domains within the HIV-1 envelope protein (gp120) (*6–8*) and the transmembrane glycoprotein (gp41) (*9*) have been shown to elicit or absorb NA. Sera from infected patients generally neutralize a broad range of isolates. In contrast, antisera produced in experimental animals by immunization with gp120 or recombinant or synthetic peptides display restricted capacity to neutralize isolates other than that from which the immunogen was derived (*10–12*). Recent observations indicate that individual isolates of HIV-1 are composed of populations of genetically and biologically distinct variants (*13*). To determine the implications of such heterogeneity for vaccine development and evaluation, we compared the susceptibility of three viruses molecularly cloned from HTLV-III$_B$ (HIV-1/NIH/USA/1983/ HTLV-III$_B$) to the neutralizing effects of a number of antisera.

The molecularly cloned viruses designated HX10, HXB2, and HXB3, were obtained from a single cell line that was infected with HIV-1 (*14, 15*). They differ by less than 1% in nucleotide sequence, at 19 sites (24 amino acids) in the envelope region. Clones HXB2 and HXB3 were constructed by inserting fragments containing full-length proviral sequences into the expression vector pSP62, transfecting DH-1 bacteria, and recovering virus by protoplast fusion (*16–18*). HX10 was derived from the λBH10 phage proviral molecular clone (*14*), which lacks a 190-bp segment in the 5′-LTR, by inserting the 8.1-kb Cla I–Xho I fragment of λBH10 (which contains sequences coding for residue 14 of *gag* through residue 443 of the *nef* region) into pHXB2gpt2, and virus was recovered by protoplast fusion (*17*). The cloned viruses and the parent isolate replicate well in neoplastic T cell lines and possess similar infectivity and cytopathogenicity for CD4$^+$ cells (*17*).

We initially examined the NA of a panel of 38 human sera from 25 seropositive patients against HTLV-III$_B$, HX10, HXB2, and HXB3. The NA of some sera against the different viruses differed (Table 1). Some neutralized one or two clones in high (≥1:256) titer although they showed low (<1:256) NA against other clones (see Tables 1, HS-01 and HXB3, HS-12 and HX10, and HS-23 and HXB2); others neutralized all viruses to some degree (HS-06) or lacked appreciable NA against any virus

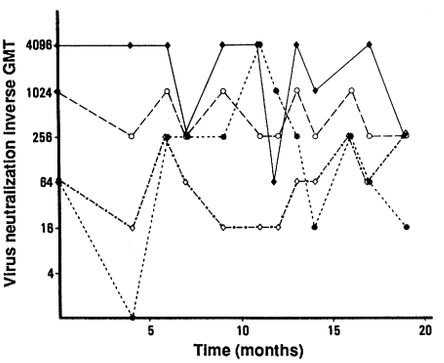

Fig. 1. Variation in neutralization of HTLV-III$_B$ and its clones by sequential patient sera. Sequential serum samples obtained from a single patient at the specified intervals were used to investigate the time course of NA against HTLV-III$_B$ (●) and its clones. Neutralization assays were performed with an inoculum of 20× TCID$_{50}$ of the respective viruses, and 50% reduction of virus expression was the end point used. The titers shown represent the inverse geometric mean titers (GMT) of duplicate determinations. ○, HX10; ◆, HXB2; ◇, HXB3.

Table 1. Restricted neutralization of viruses derived from HTLV-III$_B$ by selected patient sera. Inverse geometric mean titers (GMT) and standard errors of paired duplicate determinations on different virus preparations with inocula of four times the 50% infectious dose (4× TCID$_{50}$) are shown. Complete inhibition of viral expression at 2 weeks was used as the end point. Sera samples in parentheses share similar patterns of NA. All viruses were grown in H9 lymphoblastoid cells, and supernatants were concentrated 1000-fold by ultracentrifugation. Titrations and neutralization assays were done with an immunofluoresence technique to detect HIV-1 p24 expression (*1*). A 14- to 16-day incubation time was used for both neutralization assays and calculation of TCID$_{50}$ from eight parallel dilutions.

Sera	Virus neutralization (inverse GMT) for virus			
	HTLV-III$_B$	HX10	HXB2	HXB3
HS-06 (HS-15, -20)	360 ± 240	430 ± 290	510 ± 320	4000 ± 0
HS-12 (HS-07, -26)	180 ± 150	1600 ± 730	64 ± 0	64 ± 60
HS-23 (HS-22)	23 ± 16	130 ± 100	1000 ± 600	510 ± 390
HS-01 (HS-10, -13, -16)	1 ± 2	11 ± 8	16 ± 0	2000 ± 800
HS-08 (HS-04, -05, -18, -21)	1 ± 0	2 ± 1	4 ± 0	2 ± 1

D. J. Looney, R. R. Redfield, D. S. Burke, Department of Viral Diseases, Walter Reed Army Institute of Research, Washington, DC 20307.
A. G. Fisher, R. C. Gallo, F. Wong-Staal, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20892.
S. D. Putney and J. R. Rusche, Repligen Corporation, One Kendall Square, Building 700, Cambridge, MA 02139.

*To whom all correspondence should be addressed.

Fig. 2. Amino acid sequences of HIV-1 proviral clones and synthetic oligopeptides in the Sub2 area. Alignments shown are from the Los Alamos Laboratories Human Retroviruses 1987 envelope sequence listings (*16–18, 24*), with the addition of the oligopeptide sequences. For HX10, read also BH10.

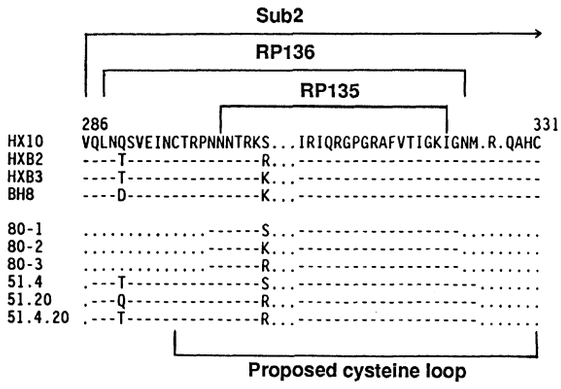

Table 2. Variable neutralizing activity of specific goat antisera against HTLV-III$_B$ and its constituent clones. Shown are inverse geometric mean titers (±SEM) for specific antisera against the specified viruses 20× TCID$_{50}$ inocula and 50% reduction of virus expression as an end point. Determinations were made in quadruplicate with concentrated virus preparations (1000-fold), or in duplicate with unconcentrated preparations. NT, not tested.

Sera*	Virus preparation	Virus neutralization (inverse GMT) for virus			
		HTLV-III$_B$	HX10	HXB2	HXB3
G505	Concentrated	2000 ± 1000	2000 ± 700	130 ± 100	180 ± 120
	Unconcentrated	NT	500 ± 0	NT	16 ± 0
G2935	Concentrated	2900 ± 1400	2000 ± 800	45 ± 37	180 ± 130
	Unconcentrated	NT	1000 ± 0	NT	32 ± 16
987	Concentrated	4100 ± 0	2900 ± 800	140 ± 120	1000 ± 600
	Unconcentrated	NT	2000 ± 1000	NT	130 ± 100
3121	Concentrated	4100 ± 0	2000 ± 1000	23 ± 14	64 ± 40
IMM-6	Unconcentrated	4 ± 0	NT	260 ± 0	NT
96-16	Unconcentrated	NT	2000 ± 1000	NT	16 ± 0

*G505 and G2935 are goat antisera against gp120 of HTLV-III$_B$, 987 against PB-1 (BH10), 3121 against sub2 (BH10), IMM-6 against gp160 (BH8); 96-16 is a mouse monoclonal antibody against gp120 (HTLV-III$_B$).

tested (HS-08). In addition, we found different patterns of NA against HTLV-III$_B$ and its clones in sequential sera from a single individual (Fig. 1). Over an 18-month period, the NA of this patient's serum against HTLV-III$_B$ rose to high levels (from <1:4 to ≥1:4096) and then declined, whereas HXB2 was neutralized at high titer (≥1:4096) from the beginning of this period, titers against HX10 were slightly lower (≥1:256 to ≥1:1024), and HXB3 was neutralized at lower titers (range ≥1:16 to ≥1:256) throughout.

To determine if sera directed against defined regions of gp120 exhibited similar restriction of NA, we selected a panel of goat antisera including G505 (antisera to immunoaffinity-purified gp120 derived from HTLV-III$_B$) (*19*), G2935 (antisera to polyacrylamide gel–purified gp120) (*19*), 987 (antisera to PB1, a recombinant peptide corresponding to the BH10 envelope region that lies between the Pvu II and second Bam HI restriction sites, residues 286–467) (*6*), and 3121 (antisera to Sub2, a peptide containing the 106 amino-terminal amino acids of PB1) (*20*). These reagents were chosen because the PB1, Sub2, and RP136 (Fig. 2) regions contain a dominant neutralizing epitope (*6*), and peptides containing this epitope are capable of absorbing type-specific NA present in the goat antisera raised against gp120 (*20*).

Table 3. Effects of preincubation with oligopeptides corresponding to the RP135 and RP136 regions of HX10, HXB2, and HXB3 upon neutralization by specific antisera. The mean percentage of HIV-1 p24-positive cells (±SEM) in the presence or absence of indicated peptides and antisera are shown. Three assays were performed in quadruplicate by adding 10 μl of purified peptide at a concentration of 1 mg/ml to 10 μl of undiluted sera in a final volume of 100 μl of phosphate-buffered saline (PBS). Sera were incubated for 24 hours at 4°C, and samples clarified by centrifugation. The supernatant (20 μl) was added to 20 μl of virus containing 20× TCID$_{50}$; the mixture was incubated for 45 minutes at 4°C; 4 × 10^4 polybrene-treated H9 cells were added; cells were incubated for 1 hour at 37°C; and 20 μl was transferred to each of two duplicate plates for incubation for 2 weeks at 37°C. Peptides 80-1, 80-2, 80-3 (301–324) were of first-cut purity (50%) only; peptides 51.4, 51.20, and 51.4.20 (287–326) were chromatographically purified. Concentrations were adjusted by absorption at 260 nm. Sequences of peptides are shown in Fig. 2.

Sera	Virus preparation	HIV-1 p24-positive cells (%) with							
		Peptide							PBS
		RP136	80-1	80-2	80-3	51.4	51.20	51.4.20	
G505	HTLV-III$_B$*	10.1 ± 1.9							4.3 ± 1.0
	HX10*		12.3 ± 1.9	6.4 ± 0.6	7.2 ± 0.5				1.6 ± 0.6
	HX10†					NT	NT	NT	NT
G2935	HTLV-III$_B$*	10.1 ± 0.5							0.0
	HX10*		7.2 ± 1.0	4.2 ± 0.7	2.9 ± 1.0				0.0
	HX10†					15.2 ± 3.9	2.1 ± 1.3	7.3 ± 1.5	0.0
987	HTLV-III$_B$*	10.8 ± 1.1							0.0
	HX10*		13.6 ± 1.1	9.0 ± 0.3	7.8 ± 0.7				0.2 ± 0.1
	HX10†					22.6 ± 2.3	7.9 ± 0.8	6.4 ± 2.2	0.0
96-16	HTLV-III$_B$*	NT							
	HX10*		NT	NT	NT				
	HX10†					14.0 ± 1.4	6.8 ± 1.8	8.0 ± 1.2	0.0
HS-25	HTLV-III$_B$*	0.0							
	HX10*		NT	NT	NT				
	HX10†					0.0	0.0	0.0	0.0
NGS‡	HTLV-III$_B$*	11.2 ± 1.2							11.1 ± 1.0
	HX10*		12.1 ± 0.8	13.5 ± 0.5	11.2 ± 1.2				12.2 ± 1.1
	HX10†					20.4 ± 2.7	20.0 ± 0.9	25.5 ± 3.2	22.8 ± 1.1

*Concentrated (1000-fold). †Unconcentrated. ‡NGS, normal goat serum.

These antisera showed high NA against HTLV-III$_B$ and HX10, but NA against HXB2 and HXB3 was substantially lower in every case (Table 2). Neutralizing activity of antiserum to Sub2 against HTLV-III$_B$ and HX10 was seen at dilutions of ≥1:2048 to ≥1:4096, but NA against comparable inocula of HXB2 and HXB3 was lost at dilutions of ≥1:23 to ≥1:281. A murine monoclonal antibody to HTLV-III$_B$–derived gp120 (96–16) displayed similar specificity. In contrast, goat antisera against recombinant gp160 derived from the biologically inactive BH8 molecular clone (IMM-6) showed NA against HXB2 that was 64 times as high as NA against HTLV-III$_B$. The amino acid sequence of HXB2 differs from that of HX10 in the Sub2 region at only two positions (289 and 305), and those of BH8 and HXB3 differ at these same locations, including the presence of arginine or lysine instead of serine (representing a single nucleotide change) within a possible cysteine loop structure (*20*) that lies in the amino-terminal portion of Sub2 (Fig. 2).

In a series of competition experiments, we used synthetic oligopeptides spanning the RP135 or RP136 regions containing residues at the 289 and 305 positions corresponding to those of HX10, HXB2, or HXB3 (Fig. 2 and Table 3). Sera G2935 and 987 completely inhibited infection by HTLV-III$_B$ in the absence of RP136 but showed no inhibition from control values when RP136 was added. In contrast, inhibition by a patient serum (HS-25) was not blocked by RP136. Peptides 51.4 and 80-1 (corresponding to HX10 at position 305) were significantly more effective than 51.20, 51.4.20, 80-2, or 80-3 (peptides containing either threonine or glutamine at position 289 but with arginine or lysine instead of serine at 305 as in HXB2, HXB3, and BH8) in blocking the neutralizing effects of G2935 and 987 on HX10 infection, supporting the conclusion that a change at the 305 position is sufficient to account for the observed restriction of NA in heterologous sera.

Although it is unlikely that differences in areas other than the *env* region are responsible for this restriction of NA, conceivably changes in other genes might alter gp120 processing, presentation, or stability (*21*). Also, differences at distant sites in gp120 or gp41 could induce conformational changes in the RP135 region. Finally, while there was no difference in the average titers of the panel of human sera against these viruses, suggesting HXB2 and HXB3 are not intrinsically more resistant to neutralization, such might not be the case for the specific goat antisera studied.

Our data suggest that a single amino acid change in the virus envelope may result in profound changes in recognition by neutralizing antisera, and extend the previous concept of type-specificity of neutralization (*20*) to differing variants of a single isolate. This suggests that the group-specific NA of some human sera could be directed against a large number of type-specific determinants, as well as conserved epitopes. This could have important implications for vaccine development. Inability to demonstrate protection by candidate vaccines after challenge with homologous virus isolates could represent the selection of minor clonal variants not well recognized by the immunized host (*22, 23*). Only the use of cloned HIV-1 variants will permit direct evaluation of the hypothesis that neutralizing antibodies are capable of protecting against HIV-1 infection.

REFERENCES AND NOTES

1. M. Robert-Guroff, M. Brown, R. C. Gallo, *Nature* **316**, 72 (1985).
2. R. A. Weiss *et al.*, *ibid.*, p. 69.
3. D. D. Ho, T. R. Rota, M. S. Hirsch, *N. Engl. J. Med.* **312**, 649 (1985).
4. S. Rasheed *et al.*, *Virology* **150**, 1 (1986).
5. S. Harada, Y. Koyanagi, N. Yamamoto, *Science* **229**, 563 (1985).
6. S. D. Putney *et al.*, *ibid.* **234**, 1392 (1986).
7. L. A. Lasky *et al.*, *ibid.* **233**, 209 (1986).
8. K. S. Steimer *et al.*, in *Vaccines '88* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, in press).
9. T. C. Chanh *et al.*, *EMBO J.* **5**, 3065 (1986).
10. T. J. Matthews *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9709 (1986).
11. S. Matsushita *et al.*, *J. Virol.* **62**, 2107 (1988).
12. R. A. Weiss *et al.*, *Nature* **324**, 572 (1986).
13. B. H. Hahn *et al.*, *Science* **232**, 1548 (1986); M. Saag *et al.*, *Nature*, in press; A. G. Fisher *et al.*, *ibid.*, in press.
14. G. M. Shaw *et al.*, *Science* **226**, 1165 (1984).
15. B. Hahn *et al.*, *Nature* **312**, 166 (1984).
16. A. G. Fisher *et al.*, *ibid.* **316**, 262 (1985).
17. L. Ratner *et al.*, *AIDS Res. Hum. Retrovir.* **3**, 57 (1987).
18. R. Crowl *et al.*, *Cell* **41**, 979 (1985).
19. W. G. Robey *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7023 (1986).
20. J. R. Rusche *et al.*, *ibid.* **85**, 3198 (1988).
21. A. G. Fisher *et al.*, *Science* **237**, 888 (1987).
22. P. L. Nara *et al.*, *J. Virol.* **61**, 3173 (1987).
23. S.-L. Hu *et al.*, *Nature* **328**, 721 (1987).
24. G. Meyers *et al.*, Eds., *Human Retroviruses and AIDS, 1987*, (Los Alamos National Laboratory, Los Alamos, NM, 1987), vol. 2, p. 38; L. Ratner *et al.*, *Nature* **313**, 277 (1984).

25. We thank S. Petteway, L. Ivanoff, D. Waselefsky (Dupont), and J. Derge (Frederick Cancer Research Facility) for synthesis of oligopeptides, F. Dorner (IMMUNO) for goat antisera to gp160, A. Bodner (Biotech Research) for monoclonal antibody 96-16, and L. Arthur and G. Robey for goat antisera to gp120. We also thank A. Rose, S. Kensek, V. Kao, L. Simek, A. Bodner, and J. Lemp for technical support and assistance.

12 January 1988; accepted 6 May 1988

Acoustical Detection of High-Density Krill Demersal Layers in the Submarine Canyons off Georges Bank

C. H. GREENE, P. H. WIEBE, J. BURCZYNSKI, M. J. YOUNGBLUTH

High-density demersal layers of krill have been detected in the submarine canyons off Georges Bank by means of a high-frequency, dual-beam bioacoustical technique. Krill densities in these demersal layers were observed to be two to three orders of magnitude greater than the highest densities observed in water-column scattering layers. Such abundances may help explain the unusually high squid and demersal fish production estimates attributed to the Georges Bank ecosystem.

EUPHAUSIIDS TYPICALLY PLAY A MAJOR role in the economy of pelagic marine ecosystems. In the productive waters of the North Atlantic, the species *Meganyctiphanes norvegica* provides an important link in the food chain between lower trophic level plankton and higher trophic level consumers, including decapod crustaceans, squids, fishes, marine mammals, and birds (*1, 2*). Early Norwegian whalers referred to *M. norvegica* as krill, a term that has now been expanded to encompass all species of euphausiids (*3*). A feature of krill ecology that makes them particularly vulnerable to successful exploitation by higher trophic level consumers is their tendency to form highly aggregated distributions (*1, 2, 4*). Krill aggregations have been categorized into four basic types: patches, shoals, swarms, and schools, with the last two types corresponding to high-density aggregations of more than 1000 animals per cubic meter (*2*). Such high-density aggregations of *M. norvegica* have been reported, but those reports have been of surface swarms often associated

C. H. Greene, Ecosystem Research Center, Corson Hall, Cornell University, Ithaca, NY 14853, and Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.
P. H. Wiebe, Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543.
J. Burczynski, BioSonics Incorporated, 4520 Union Bay Place NE, Seattle, WA 98105.
M. J. Youngbluth, Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Fort Pierce, FL 34946.