JOURNAL OF VIROLOGY, Dec. 1998, p. 10270–10274 Vol. 72, No. 12
0022-538X/98/$04.00+0

Antibody Neutralization-Resistant Primary Isolates of Human Immunodeficiency Virus Type 1

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Received 3 February 1998/Accepted 12 August 1998

Although typical primary isolates of human immunodeficiency virus type 1 (HIV-1) are relatively neutralization resistant, three human monoclonal antibodies and a small number of HIV-1$^+$ human sera that neutralize the majority of isolates have been described. The monoclonal antibodies (2G12, 2F5, and b12) represent specificities that a putative vaccine should aim to elicit, since in vitro neutralization has been correlated with protection against primary viruses in animal models. Furthermore, a neutralization escape mutant to one of the antibodies (b12) selected in vitro remains sensitive to neutralization by the other two (2G12 and 2F5) (H. Mo, L. Stamatatos, J. E. Ip, C. F. Barbas, P. W. H. I. Parren, D. R. Burton, J. P. Moore, and D. D. Ho, J. Virol. 71:6869–6874, 1997), supporting the notion that eliciting a combination of such specificities would be particularly advantageous. Here, however, we describe a small subset of viruses, mostly pediatric, which show a high level of neutralization resistance to all three human monoclonal antibodies and to two broadly neutralizing sera. Such viruses threaten antibody-based antiviral strategies, and the basis for their resistance should be explored.

There is evidence to indicate that antibody can protect or offer benefit against challenge with primary isolates of human immunodeficiency virus type 1 (HIV-1) (3). In passive-transfer experiments, the recombinant human antibody b12 completely protected against challenge with two primary isolates in the hu-PBL-SCID mouse model when it was administered pre- or shortly postexposure (11). The anti-gp41 antibody 2F5 did not protect chimpanzees against challenge with a primary virus, but seroconversion was delayed and the peak of measurable virus-specific RNA in serum was either delayed or did not reach levels comparable to those in the sera of control animals (7).

Protection in vivo appears to be directly related to neutralization in vitro. For instance, it is considerably easier to protect against challenge with readily neutralized T-cell-line-adapted (TCLA) strains of HIV-1 than with the more refractory primary isolates (11, 20). Complete protection requires serum antibody concentrations in vivo considerably in excess of the 90% neutralization titers measured in typical in vitro assays. As a rough guide, in the hu-PBL-SCID mouse model, antibody concentrations 1 to 2 orders of magnitude higher than the 90% neutralization titers are needed. For example, antibody b12 provided complete protection in the mouse model at 50 mg/kg of body weight, which corresponds to a concentration in serum of about 500 μg/ml, against two primary viruses for which the 90% neutralization titers were 15 and 5 μg/ml. A dose of 10 mg of b12 per kg offered only partial protection.

Extrapolation from the mouse model to humans is uncertain, but it seems likely that potent antibodies will be required to achieve protection. In a recent comparative study, only three human monoclonal antibodies (MAbs) were found to neutralize (90%) a range of clade B primary isolates at concentrations equal to or less than 25 μg/ml (9). These are MAb b12, which recognizes an epitope overlapping the CD4 binding site of gp120 (4, 5); MAb 2G12, which recognizes an epitope involving the base of the V3 loop and the base of the V4 loop of gp120 (2, 27); and MAb 2F5, which recognizes a linear sequence close to the transmembrane segment of gp41 (2, 8). Similar results were reported by Trkola et al. (26). In that study, a tetrameric CD4 immunoglobulin G2 (IgG2) molecule was also found to be approximately as potent as the three human MAbs. Furthermore, the antibodies and CD4 IgG2 were also highly effective against viruses from clades other than B.

Generally, comparative neutralization studies have shown that viruses resistant to one of the three antibodies described above could still be neutralized by other members of the panel. This finding is consistent with observations that neutralization escape mutants selected by growth of the primary isolate molecular clone HIV-1_{JR-CSF} in the presence of antibody b12 were still sensitive to neutralization by 2F5 and 2G12 (13). The escape mutants were shown to arise by point mutations which reduced b12 binding to mature oligomeric envelope on the virus (and gp120 monomer) but did not affect binding of the other antibodies. However, we noted previously that certain isolates with which we have worked appeared to be difficult to neutralize with several antibodies. Such isolates may be important in considering antiviral strategies, including vaccination, involving antibody. We therefore determined to investigate the neutralization properties of a number of isolates using the panel of MAbs described above and sera for which we had preliminary evidence of unusually high neutralizing titers. The viruses chosen included a panel of pediatric isolates arising from mother-child transmission. This was because interruption of mother-child transmission is a clear potential application of

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TABLE 1. Neutralization titers of sera and MAb IgG1 b12 against viruses of different clades

Serum or antibody	Dilution of serum or concn of MAb (μg/ml) that results in a 90% reduction in infectivity of virus[a]:					
	MN (clade B [TCLA], SI)	92UG029 (clade A, SI)	SF162 (clade B, NSI)	BaL (clade B, NSI)	92UG021 (clade D, SI)	93BR029 (clade F, NSI)
Sera						
RW1	1,000	64	128	64	64	64
FDA-2[b]	>512	64	32	16	32	32
FDA-2[c]	4,000	16	64	128	<16	32
RW2	>512	32	<32	<32	32	32
RW3	>512	64	32	64	64	32
RW4	>512	<16	64	32	<16	16
RW5	>512	64	128	64	<16	64
RW6	>512	<16	256	64	<16	<16
RW7	250	<16	<32	<32	<16	<16
RW8	>512	<16	32	<32	<16	16
RW9	500	<16	<32	<32	<16	32
RW10	>512	<16	<32	<32	<16	16
P	>512	<16	128	<16	<16	<16
M	>512	<16	32	32	<16	128
MAb IgG1 b12	<0.04	10	3.1	10	10	>10

[a] MN neutralization was tested with HeLa CD4 cells containing the HIV-1 long terminal repeat fused to LacZ and in H9 cells by p24 ELISA. Primary isolate neutralization was assessed in a peripheral blood mononuclear cell-based assay by determining p24 antigen production by ELISA. Only a partial DNA sequence of 92UG029 is available. Its placement in clade A has been made on the basis of its V3 loop sequence (Los Alamos National Laboratory HIV-1 sequence database [29]). SI, syncytium inducing; NSI, non-syncytium inducing.

[b] Sample obtained from the ARRRP.

[c] Sample obtained in August 1996.

prophylactic antibody (24) and because we had some evidence of more neutralization-resistant viruses in this group.

Identification of two sera showing broad neutralization of primary isolates. A panel of sera was examined at The Scripps Research Institute for neutralization of a diverse panel of isolates of different clades (Table 1). To analyze antibodies for neutralization activity against TCLA virus HIV-1_{MN}, we used an assay based on infection of HeLa cells expressing human CD4 and the HIV-1 long terminal repeat fused to *lacZ* as described previously (6, 21). Neutralization of primary isolates was performed essentially as described by Trkola et al. (26) except that we used a virus inoculum of 100 rather than 10 50% tissue culture infective doses ($TCID_{50}$). Sera from 10 individuals, designated RW1 to RW10, who had been infected for various periods from 3 to 10 years, and sera from two donors, designated P and M, from whom antibody phage libraries have been prepared (19), were used. We further included a serum from a donor, designated FDA-2, which had been shown previously to be relatively potent against a number of isolates (10, 15, 28). FDA-2 sera drawn at two different time points were tested. One sample was a pool of four blood donations drawn between April 1990 and February 1991 and was obtained from the AIDS Research and Reference Reagent Program (ARRRP), and the second sample was obtained in August of 1996. Table 1 shows that all of the sera effectively neutralized the TCLA strain HIV-1_{MN} but that neutralization of the primary isolates was much more restricted. The best sera, RW-1, RW-3, and FDA-2, neutralized all the isolates. All the other sera neutralized only some of the isolates. The two FDA-2 sera taken at different time points indicate an evolution of neutralizing antibody titers; both sera, however, are broadly neutralizing. Although FDA-2 was one of the best-neutralizing sera observed in a previous comparison of 15 different sera (15), Table 1 shows that at least two RW sera had comparable or greater potency; both these sera were from individuals who had been infected for 9 years or more. A broadening of neutralizing antibody titers in long-term-infected persons has been observed previously (14, 32). The sera RW-1 and FDA2 were chosen for detailed examination of neutralization of pediatric isolates together with RW-7, a serum that is relatively inefficient (Table 1).

Pediatric isolates resistant to neutralization by sera and MAbs. Table 2 shows a comparison of neutralization titers of the above-described sera and three potent human MAbs against 14 pediatric isolates. The panel of isolates was obtained from the ARRRP and included isolates transmitted in utero as well as isolates transmitted intra- and postpartum. The majority of isolates were neutralized by the sera RW-1 and FDA2 but not by the serum RW-7. About half of the isolates were neutralized by each of the MAbs. This is a lower figure than has been generally observed for clade B isolates (see, e.g., reference 26) and may suggest that the pediatric panel is more resistant to antibody neutralization than a more general selection of viruses (i.e., adult based). Three viruses in particular, i.e., 92US076, 92US077, and 93US143, were not neutralized by any of the sera at a 1:32 dilution or any of the three MAbs at concentrations up to 50 μg/ml. These three isolates were also resistant to a cocktail of the three MAbs, each at 17 μg/ml. It appears that they have a general enhanced resistance to antibody neutralization. Of note is that all three are syncytium-inducing viruses.

Selected resistant viruses were also tested in a second set of experiments performed at the Aaron Diamond AIDS Research Center (ADARC) (Table 3); neutralization assays were performed as described previously (26). Here also 92US077 and 93US143 were highly resistant to the three test MAbs and also to the potently neutralizing tetrameric CD4 IgG2 molecule. CD4 IgG2 has been shown to be highly effective in neutralizing primary isolates from diverse clades (26). 92US076 was sensitive to MAb 2G12, which may possibly be explained by the 10-fold lower viral inoculum used in the ADARC neutralization assay. Apart from 92US076's sensitivity to MAb 2G12, however, the results were very consistent between the two laboratories. One of the pediatric isolates (93US143) has

TABLE 2. Neutralization titers of potent neutralizing sera and MAbs against pediatric primary isolates and an adult isolate[a]

Virus isolate	Infection route	Biotype	90% virus neutralization titer						
			Serum (dilution)			MAb (μg/ml)			
			RW1	FDA-2	RW7	IgG1 b12	2F5	2G12	IgG1 b12, 2F5, and 2G12
Pediatric isolates									
90US144	Postpartum	NSI	128	512	64	>50	6.3	3.1	NT
91US056	In utero	NSI	64	256	<32	6.3	>50	25	NT
92US072	In utero	NSI	128	64	32	3.1	25	>50	50
92US076	Postpartum	SI	<32	<32	<32	>50	>50	>50	>50
92US077	Postpartum	SI	<32	<32	<32	>50	>50	>50	>50
93US073	In utero	NSI	64	32	<32	3.1	50	6.3	NT
93US140	In utero	NSI	32	32	<32	25	>50	50	NT
93US141	In utero	NSI	128	32	<32	12.5	12.5	12.5	NT
93US142	In utero	NSI	64	32	<32	>50	50	>50	NT
93US149	In utero	NSI	32	64	<32	12.5	>50	>50	NT
93US074	Intrapartum	NSI	64	32	<32	50	>50	>50	NT
93US075	Intrapartum	NSI	256	128	256	12.5	50	50	NT
93US143	Intrapartum	SI	<32	<32	<32	>50	>50	>50	>50
93US155	Intrapartum	NSI	128	512	NT	25	50	3.1	NT
Adult isolate 92US714	IVDU	NSI	32	<32	<32	50	>50	>50	NT

[a] Neutralization assays were carried out at The Scripps Research Institute with a virus inoculum of 100 $TCID_{50}$. Serum FDA-2 was the sample obtained in August 1996. NSI, non-syncytium inducing; SI, syncytium inducing; NT, not tested; IVDU, intravenous drug use.

previously been described by Mascola et al. (12) as being relatively resistant to neutralization by 2G12 and 2F5 antibodies. That study also identified another relatively resistant nonpediatric isolate, 92US714, which was obtained from a Baltimore intravenous drug user. Since another comparative study showed neutralization of 92US714 in some assay formats (9), we included this isolate in our analysis. Compared to other primary isolates, 92US714 was resistant. In contrast to the other two resistant isolates (92US077 and 93US143), however, both 92US076 and 92US714 were sensitive to CD4 IgG2 (Table 3).

To make certain that the resistant phenotypes were not a result of unusually fast growth kinetics of these viruses, we performed a kinetic study in which p24 antigen production was measured every day between day 1 and day 7 and on day 10. 92US143 replicated rapidly, peaking between days 2 and 3. 92US076 and 92US077 replicated more slowly, however, peaking between days 5 and 7, with a profile similar to that of HIV-$1_{JR\text{-}CSF}$, a virus which is sensitive to neutralization by b12 (13). There was therefore no correlation between a resistant phenotype and growth kinetics in the absence of neutralizing antibody. To control for the different growth kinetics of these isolates, we harvested virus from each individual neutralization assay over several days (day 4 to day 6 postinfection). The day on which the first peak virus production was detected was chosen to calculate neutralization titers. We consistently found a general resistance of the isolates indicated above to neutralization by potent neutralizing antibody.

Mascola et al. reported that hyperimmune anti-HIV Ig (HIVIG) was able to synergize with 2F5 and 2G12 in neutralization assays of the panel of primary isolates they examined (12). Accordingly, we assessed the ability of HIVIG or control human IgG at a high concentration (2.5 mg/ml) alone and in combination with the three MAbs (each at 17 μg/ml) to neutralize the resistant pediatric viruses. We observed some reduction in infectivity, but in no case was 90% neutralization achieved. The more neutralization-sensitive isolate, 92US072, was readily neutralized by HIVIG and HIVIG-antibody combinations (data not shown).

Finally, the ability of autologous sera to neutralize the pediatric viruses was measured (Table 4). The autologous sera also had little effect on the resistant isolates, although the autologous serum taken 6 months after virus isolation did reach 90% neutralization of 92US143 at a dilution of 1:32. The more sensitive control viruses were neutralized by these sera at dilutions comparable to those shown in Table 2.

TABLE 3. Neutralization titers of MAbs against selected isolates from Table 2[a]

Virus isolate	90% virus neutralization titer (μg/ml)			
	IgG1 b12	2F5	2G12	CD4 IgG2
92US076	>50	46	1.6	7.8
92US077	>50	>50	>50	42
93US143	>50	>50	>37	47
92US714	36	>50	>50	2.4

[a] Neutralization assays were carried out at the ADARC with a virus inoculum of 10 $TCID_{50}$.

TABLE 4. Neutralization of primary isolates by autologous sera

Virus isolate	90% neutralization titer				
	Virus in serum or plasma[a] (dilution)				MAb IgG1 b12 (μg/ml)
	92US076	92US077	93US143	93US143	
93US075	256	128	32	32	50
92US072	128	64	<64	128	6
92US076	<32	<32	<32	<32	>50
92US077	32	<32	<32	<32	>50
93US143	<32	<32	<32	32	>50

[a] Sera from the infant from whom the 93US143 virus was isolated, taken at 1 and at 6 months after infection, was provided by Merlin Robb. Serum and plasma, respectively, were obtained from the infants from whom 92US076 and 92US077 were isolated and were provided by John Sullivan. The 92US076 serum was drawn 1 month after virus isolation, and the 92US077 serum was drawn at the time of virus isolation. The first-listed 93US143 serum was obtained in March 1993, and the second-listed 93US143 serum was obtained in September 1993.

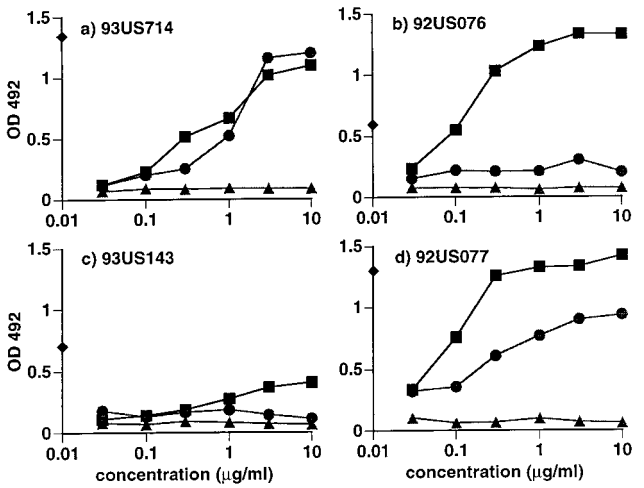

FIG. 1. Reactivity of soluble gp120 from resistant viruses with MAbs. Infectious culture supernatants were treated with detergent, and gp120 was captured in the well of an ELISA plate coated with sheep anti-gp120 antibody D7324. The reactivities of MAb IgG1 b12 (circles), MAb 2G12 (squares), and control antibody PA-53 (triangles) against HIV-2 envelope protein are shown. As a positive control for the amount of primary gp120 captured, the reactivity of a single 1:5,000 dilution of pooled seropositive human plasma (QC256; diamonds) is plotted on the *y* axis.

Reactivities of soluble gp120 and gp41 from resistant viruses with MAbs. Neutralization resistance seems to be possible through at least two mechanisms. First, local changes through point mutations which reduce the affinity of the neutralizing antibody to the virion may occur, as has been described for the V2 (31) or V3 (18) loop and in vitro escape mutants of MAb b12 (13). Second, more-global conformational changes which make viruses more refractory to neutralization by subtle alterations of the envelope antigenic makeup may occur through mutations in distal sites. One such mutation in the gp41 subunit (A-T at position 582) which made the mutant virus (a variant of TCLA virus IIIB) less sensitive to neutralization by several antisera and anti-CD4 binding site antibodies has been described (22, 23, 25, 30). By the second mechanism, certain difficult-to-neutralize viruses may acquire resistance to neutralizing antibodies to multiple antigenic sites. Resistance by the first mechanism is therefore due to a loss of the relevant epitope through primary structural variation, whereas resistance by the second mechanism is mediated through changes in tertiary and quaternary structures, such as sequestering of the epitope from the antibody-accessible surface area.

Although there is a poor correlation between the presence of an epitope on monomeric gp120 and its accessibility to antibody on oligomeric gp120 (16), the absence of an epitope on monomeric gp120, at least for the epitopes studied here, does predict its absence from the oligomer. To investigate this possibility for the resistant isolates identified, we studied the binding of MAbs IgG1 b12 and 2G12 with detergent-solubilized primary isolate gp120 captured with a sheep antibody against the gp120 C terminus immobilized on an enzyme-linked immunosorbent assay (ELISA) plate as described previously (17). The results showed that loss of binding to gp120 is not a general explanation for the resistant phenotype of these isolates. Captured solubilized gp120 from all four resistant isolates (92US076, 92US077, 93US143, and 93US714) bound MAb 2G12, whereas IgG1 b12 bound well to gp120 from 92US077 and 93US714 but not from 92US076 and 92US143 (Fig. 1). The resistant isolate 92US077 even retains the binding sites on gp120 of both MAb b12 and MAb 2G12. Similarly, there was no correlation between binding of MAb 2F5 to captured gp41 and neutralization resistance, since ELISA signals two to five times greater than background signals and comparable to those of controls were found for all viruses, except 93US143, for which binding was unclear (data not shown). From these results, it therefore seems that in some cases resistance may simply be due to the absence of epitope expression on subunits constituting the oligomeric envelope spikes but that in other cases resistance is a more complicated phenomenon, possibly reflecting a more global perturbation of the oligomeric envelope on the virion surface.

To date only three MAbs, b12, 2F5, and 2G12, that efficiently neutralize a broad range of primary isolates of HIV-1 have been identified (2, 4, 5, 8, 9, 26, 27). These antibodies are being used as molecular templates and aids in antibody-based vaccine design (3) and have potential for passive immunoprophylaxis of HIV-1 infection (11). In addition, a tetrameric CD4 IgG2 molecule which is highly effective in neutralizing diverse isolates of HIV-1 in vitro has been described (1, 26). Previous neutralization studies of panels of primary isolates indicated the majority of primary isolates to be sensitive to neutralization by at least of one the three potent MAbs (5, 9, 26), suggesting that a cocktail of these antibodies, passively transferred or elicited by a vaccine, may be effective in protecting against HIV-1 infection. In these previous studies, we identified only one (clade D) isolate which resisted all three MAbs (9, 26). Significantly, we now identify four resistant clade B primary isolates, three of which are pediatric. In addition, these four isolates resist neutralization by two potently neutralizing sera. The high frequency of neutralization-resistant viruses in the panel of pediatric primary isolates studied is noteworthy. This may result from the circumstance that perinatal HIV-1 infection is established in the presence of maternal anti-HIV-1 antibody.

In conclusion, some viruses have a general resistance to antibody neutralization. In some cases, resistance can be simply explained as loss of the relevant epitope on envelope subunits. In others, more complex phenomena involving the conformation or arrangement of oligomeric envelope spikes is probably involved. Highly resistant viruses threaten antibody-based vaccine efforts, and the basis for their resistance is being explored.

We are grateful to Robert Walker for providing a panel of sera from HIV-1$^+$ donors. We thank John Sullivan and Merlin Robb for providing sera from pediatric donors, James Robinson for providing the control MAb PA-53, and Gerald Quinnan and Harvey Alter for FDA-2 serum. HIVIG was obtained from Albert Prince via the ARRRP. Virus isolates were collected from various geographical regions of the world by the World Health Organization and the National Institute of Allergy and Infectious Diseases. All viruses were obtained via the ARRRP. Pediatric isolates were deposited by Merlin Robb, David Ho, John Sullivan, and Cecelia Hutto. Isolate 92US714 was from Kenrad Nelson.

This study was supported by NIH grants AI33292 (D.R.B.), AI40377 and AI42653 (P.W.H.I.P.), and AI36082 and HL59735 (J.P.M.) and by the Elisabeth Glaser Pediatric AIDS Foundation, of which J.P.M. is an Elisabeth Glaser Scientist and of which P.W.H.I.P. is a scholar (PFR-77348). A.T. acknowledges a fellowship from the Fonds zur Förderung der wissenschaftlicher Forschung (J01165-MED) and the Austrian Program for Advanced Research and Technology.

REFERENCES

1. **Allaway, G. P., K. L. Davis-Bruno, G. A. Beaudry, E. B. Garcia, E. L. Wong, A. M. Ryder, K. W. Hasel, M.-C. Gauduin, R. A. Koup, J. S. McDougal, and P. J. Maddon.** 1995. Expression and characterization of CD4-IgG2, a novel

heterotetramer that neutralizes primary HIV type 1 isolates. AIDS Res. Hum. Retroviruses **11:**533–539.
2. **Buchacher, A., R. Predl, K. Strutzenberger, W. Steinfellner, A. Trkola, M. Purtscher, G. Gruber, C. Tauer, F. Steidl, A. Jungbauder, and H. Katinger.** 1994. Generation of human monoclonal antibodies against HIV-1 proteins; electrofusion and Epstein-Barr virus transformation for peripheral blood lymphocyte immortalization. AIDS Res. Hum. Retroviruses **10:**359–369.
3. **Burton, D. R.** 1997. A vaccine for HIV type 1: the antibody perspective. Proc. Natl. Acad. Sci. USA **94:**10018–10023.
4. **Burton, D. R., C. F. Barbas, M. A. Persson, S. Koenig, R. M. Chanock, and R. A. Lerner.** 1991. A large array of human monoclonal antibodies to type 1 human immunodeficiency virus from combinatorial libraries of asymptomatic seropositive individuals. Proc. Natl. Acad. Sci. USA **88:**10134–10137.
5. **Burton, D. R., J. Pyati, R. Koduri, S. J. Sharp, G. B. Thornton, P. W. H. I. Parren, L. S. W. Sawyer, R. M. Hendry, N. Dunlop, P. L. Nara, M. Lamacchia, E. Garratty, E. R. Stiehm, Y. J. Bryson, Y. Cao, J. P. Moore, D. D. Ho, and C. F. Barbas.** 1994. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science **266:**1024–1027.
6. **Clavel, F., and P. Charneau.** 1994. Fusion from without directed by human immunodeficiency virus particles. J. Virol. **68:**1179–1185.
7. **Conley, A. J., J. A. Kessler II, L. J. Boots, P. M. McKenna, W. A. Schleif, E. A. Emini, G. E. Mark III, H. Katinger, E. K. Cobb, S. M. Lunceford, S. R. Rouse, and K. K. Murthy.** 1996. The consequence of passive administration of an anti-human immunodeficiency virus type 1 neutralizing monoclonal antibody before challenge of chimpanzees with a primary virus isolate. J. Virol. **70:**6751–6758.
8. **Conley, A. J., J. A. Kessler II, L. J. Boots, J.-S. Tung, B. A. Arnold, P. M. Keller, A. R. Shaw, and E. A. Emini.** 1994. Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by IAM-41-2F5, an anti-gp41 human monoclonal antibody. Proc. Natl. Acad. Sci. USA **91:**3348–3352.
9. **D'Souza, M. P., D. Livnat, J. A. Bradac, S. Bridges, The AIDS Clinical Trials Group Antibody Selection Working Group, and Collaborating Investigators.** 1997. Evaluation of monoclonal antibodies to HIV-1 primary isolates by neutralization assays: performance criteria for selecting candidate antibodies for clinical trials. J. Infect. Dis. **175:**1056–1062.
10. **Fenyö, E.-M., J. Albert, and J. McKeating.** 1996. The role of the humoral immune response in HIV infection. AIDS **10**(Suppl. A)**:**S97–S106.
11. **Gauduin, M.-C., P. W. H. I. Parren, R. Weir, C. F. Barbas, D. R. Burton, and R. A. Koup.** 1997. Passive immunization with a human monoclonal antibody protects hu-PBL-SCID mice against challenge by primary isolates of HIV-1. Nat. Med. **3:**1389–1393.
12. **Mascola, J. R., M. K. Louder, T. C. VanCott, C. V. Sapan, J. S. Lambert, L. R. Muenz, B. Bunow, D. L. Birx, and M. L. Robb.** 1997. Potent and synergistic neutralization of human immunodeficiency virus (HIV) type 1 primary isolates by hyperimmune anti-HIV immunoglobulin combined with monoclonal antibodies 2F5 and 2G12. J. Virol. **71:**7198–7206.
13. **Mo, H., L. Stamatatos, J. E. Ip, C. F. Barbas, P. W. H. I. Parren, D. R. Burton, J. P. Moore, and D. D. Ho.** 1997. Human immunodeficiency virus type 1 mutants that escape neutralization by human monoclonal antibody IgG1 b12. J. Virol. **71:**6869–6874.
14. **Montefiori, D. C., G. Pantaleo, L. M. Fink, J. T. Zhou, J. Y. Zhou, M. Bilska, G. D. Miralles, and A. S. Fauci.** 1996. Neutralizing and infection-enhancing antibody responses to human immunodeficiency virus type 1 in long term non-progressors. J. Infect. Dis. **173:**60–67.
15. **Moore, J. P., Y. Cao, J. Leu, L. Qin, B. Korber, and D. D. Ho.** 1996. Inter- and intraclade neutralization of human immunodeficiency virus type 1: genetic clades do not correspond to neutralization serotypes but partially correspond to gp120 antigenic serotypes. J. Virol. **70:**427–444.
16. **Moore, J. P., Y. Cao, L. Qing, Q. J. Sattentau, J. Pyati, R. Koduri, J. Robinson, C. F. Barbas, D. R. Burton, and D. D. Ho.** 1995. Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120. J. Virol. **69:**101–109.
17. **Moore, J. P., F. E. McCutchan, S.-W. Poon, J. Mascola, J. Liu, Y. Cao, and D. D. Ho.** 1994. Exploration of antigenic variation in gp120 from clades A through F of human immunodeficiency virus type 1 by using monoclonal antibodies. J. Virol. **68:**8350–8364.
18. **Msuda, T., S. Matsushita, M. J. Kuroda, M. Kannagi, K. Takatsuki, and S. Harada.** 1990. Generation of neutralization-resistant HIV-1 in vitro due to amino acid interchanges of third hypervariable *env* region. J. Immunol. **145:**3240–3246.
19. **Parren, P. W. H. I., and D. R. Burton.** 1997. Antibodies against HIV-1 from phage display libraries: mapping of an immune response and progress towards anti-viral immunotherapy. Chem. Immunol. **65:**18–56.
20. **Parren, P. W. H. I., M.-C. Gauduin, R. A. Koup, P. Poignard, Q. J. Sattentau, P. Fisicaro, and D. R. Burton.** 1997. Relevance of the antibody response against human immunodeficiency virus type 1 envelope to vaccine design. Immunol. Lett. **58:**125–132.
21. **Parren, P. W. H. I., D. Naniche, I. Mondor, H. J. Ditzel, P. J. Klasse, D. R. Burton, and Q. J. Sattentau.** 1998. Neutralization of HIV-1 by antibody to gp120 is determined primarily by occupancy of sites on the virion irrespective of epitope specificity. J. Virol. **72:**3512–3519.
22. **Reitz, M. S., Jr., C. Wilson, C. Naugle, R. C. Gallo, and M. Robert-Guroff.** 1988. Generation of a neutralization-resistant variant of HIV-1 is due to selection for a point mutation in the envelope gene. Cell **54:**57–63.
23. **Robert-Guroff, M., M. S. Reitz, Jr., W. G. Robey, and R. C. Gallo.** 1985. In vitro generation of an HTLV-III variant by neutralizing antibody. J. Immunol. **137:**3306–3309.
24. **Stiehm, E. R., L. Mofenson, S. Zolla-Pazner, B. Jackson, N. L. Martin, A. J. Ammann, and The Passive Antibody Workshop Participants.** 1995. Meeting report: summary of the workshop on passive immunotherapy in the prevention and treatment of HIV infection. Clin. Immunol. Immunopathol. **75:**84–93.
25. **Thali, M., M. Charles, C. Furman, L. Cavacini, M. Posner, J. Robinson, and J. Sodroski.** 1994. Resistance to neutralization by broadly reactive antibodies to human immunodeficiency virus type 1 gp120 glycoprotein conferred by a gp41 amino acid change. J. Virol. **68:**674–680.
26. **Trkola, A., A. P. Pomales, H. Yuan, B. Korber, P. J. Maddon, G. Allaway, H. Katinger, C. F. Barbas, D. R. Burton, D. D. Ho, and J. P. Moore.** 1995. Cross-clade neutralization of primary isolates of human immunodeficiency virus type 1 by human monoclonal antibodies and tetrameric CD4-IgG. J. Virol. **69:**6609–6617.
27. **Trkola, A., M. Purtscher, T. Muster, C. Ballaun, A. Buchacher, N. Sullivan, K. Srinivasan, J. Sodroski, J. P. Moore, and H. Katinger.** 1996. Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type I. J. Virol. **70:**1100–1108.
28. **Vujcic, L. K., and G. V. Quinnan, Jr.** 1995. Preparation and characterization of human HIV type 1 neutralizing reference sera. AIDS Res. Hum. Retroviruses **11:**783–787.
29. **WHO Network for HIV Isolation and Characterization.** 1994. HIV type 1 variation in World Health Organization-sponsored vaccine evaluation sites: genetic screening, sequence analysis, and preliminary biological characterization of selected viral strains. AIDS Res. Hum. Retroviruses **10:**1327–1343.
30. **Wilson, C., M. S. Reitz, Jr., K. Aldrich, P. J. Klasse, J. Blomberg, R. C. Gallo, and M. Robert-Guroff.** 1990. The site of an immune-selected point mutation in the transmembrane protein of human immunodeficiency virus type 1 does not constitute the neutralization epitope. J. Virol. **64:**3240–3248.
31. **Yoshiyama, H., H. Mo, J. P. Moore, and D. D. Ho.** 1994. Characterization of mutants of human immunodeficiency virus type 1 that have escaped neutralization by a monoclonal antibody to the gp120 V2 loop. J. Virol. **68:**974–978.
32. **Zhang, Y.-J., C. Fracasso, J. R. Fiore, A. Bjorndal, G. Angarano, A. Gringeri, and E.-M. Fenyö.** 1997. Augmented serum neutralizing activity against primary human immunodeficiency virus type 1 (HIV-1) isolates in two groups of HIV-1-infected long-term nonprogressors. J. Infect. Dis. **176:**1180–1187.