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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

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We have studied genetic variation among clades A through E of human immunodeficiency virus type 1 (HIV-1) at the levels of antibody binding to gp120 molecules and virus neutralization. We are unable to identify neutralization serotypes that correspond to the genetic clades. Instead, we observe that inter- and intraclade neutralization of primary isolates by HIV-1-positive sera is generally weak and sporadic; some sera show a reasonable degree of neutralization breadth and potency whereas others are relatively sensitive to neutralization, but no consistent pattern was found. However, a few sera were able to neutralize across clades with significant potency, an observation which may have implications for the feasibility of a broadly effective HIV-1 vaccine involving humoral immunity. Serological assays measuring anti-gp120 antibody binding also failed to identify serotypes that correspond precisely to the genetic clades, but some indications of clade-specific binding were observed, notably with sera from clades B and E. A representative protein for each clade (A through E) was selected on the basis of its specificity, defined as high seroreactivity with sera from individuals infected with virus of that clade and lower reactivity with sera from individuals infected with viruses from other clades. The seroreactivity patterns against these five proteins could be used to predict the genotype of the infecting virus with moderate success.

Although the correlates of protective immunity against human immunodeficiency virus type 1 (HIV-1) infection remain unresolved, it is reasonable to suppose that the induction of broadly neutralizing antibodies remains a desirable feature of an HIV-1 vaccine. To achieve this will, however, be no simple matter, for vaccine candidates tested to date have failed to induce antibodies able to neutralize primary HIV-1 isolates with significant potency (8, 16, 29, 50, 58). Nonetheless, should future generations of vaccines overcome this weakness, it will be important to know to what extent HIV-1 variation influences virus neutralization. Although HIV-1 isolates from different infected individuals do vary in primary sequence (19, 40, 48), a deeper level of variation is apparent between isolates from the different phylogenetically defined clades. At present, nine clades of HIV-1 (A through I), as well as the divergent O group, have been identified as a result of coordinated studies on global HIV-1 diversity (1, 4, 10–14, 22, 25–27, 30, 42, 45, 48, 52, 56). For any HIV-1 vaccine to be truly successful globally, it should be able to counter viruses from all of the clades, which may require multiple genotypes to be included in a complex vaccine formulation. It is not yet clear how well, if at all, the genetic clades correspond to neutralization serotypes, for the relationship between primary sequence and virus neutralization is complex. To address the issue of neutralization serotypes, we have performed inter- and intraclade neutralization assays using primary isolates from clades A through E and both autologous and heterologous HIV-1-positive sera. In complementary studies, we have assessed whether there are antigenic serotypes, by measuring the reactivities of HIV-1-positive sera with gp120 molecules derived from isolates of the same or different clades. We conclude that the genetic clades do not readily correspond to neutralization serotypes but that there is a relationship, albeit an imprecise one, between the genetic clades and antigenic serotypes.

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MATERIALS AND METHODS

Viruses and sera. Virus isolates were collected from various regions of the world by three organizations: the World Health Organization (WHO) Network for HIV Isolation and Characterisation (45, 56), the Henry M. Jackson Foundation for the Advancement of Military Medicine and the Military Medical Consortium for Applied Retroviral Research (HMJF/MMCARR) (25–28, 30), and the National Institute of Allergy and Infectious Diseases (NIAID) (36). Viruses designated by a code in the format exemplified by 92UG029 were provided by WHO (21) or NIAID; viruses designated by a code in the format exemplified by DJ258 were from the HMJF/MMCARR repository. A few other isolates designated AD, mostly of U.S. origin, were isolated at the Aaron Diamond AIDS Research Center. Viruses were expanded in mitogen-stimulated peripheral blood mononuclear cells (PBMC) (9, 25–28, 45), and culture supernatants containing infectious virus were stored in central repositories at −80°C. The designation of viruses into clades was made on the basis of sequence information from the *gag* gene, from gp120 or gp160, from the C2-V5 region of gp120, or, in some cases, after heteroduplex mobility analysis (3, 10, 13, 26, 27, 56).

Serum or plasma samples were also provided by the WHO, HMJF/MMCARR, and NIAID repositories. Usually, the samples were from the same individuals who provided the HIV-1 isolates or were collected from other individuals in the same geographic area. All blood samples were obtained after clearance by both in-country and institutional review boards.

Neutralization assay. Virus neutralization was assessed by using primary isolates of HIV-1 grown in activated PBMC with phytohemagglutinin-stimulated PBMC as target cells and p24 antigen output as a measurement of virus production (5, 9). Briefly, serial dilutions (1:8 to 1:256) of plasma or serum samples in culture medium were mixed with 100 50% tissue culture infective doses of each HIV-1 isolate for 30 min prior to addition to 2×10^6 activated PBMC. PBMC were prepared from Leukopacks provided by the New York Blood Center. Production of p24 antigen was measured after 5 or 7 days, when exponential viral growth is generally observed (5, 9). Care was taken to ensure that all input plasma was removed from the cultures by washing before p24 antigen measurement, to minimize assay interference by residual anti-p24 antibodies. Neutralizing titers of <1:8 were not considered significant and were scored as negative. In general, when the same serum and virus combination was tested more than once, variation in ID_{50} and ID_{90} values (highest serum dilutions that produced ≥50

and ≥90% inhibition) of 1 dilution was observed; i.e., an ID_{50} value of 32 in one experiment could be 16, 32, or 64 in a second. This is not unexpected given the irregular shapes of neutralization curves that can be influenced by variable experimental conditions such as the use of PBMC from different donors as target cells. The issue of variation in neutralization titers is addressed in more detail in the accompanying report (23).

Binding of sera to gp120. Infectious culture supernatants containing virus and free gp120 were treated with 1% Nonidet P-40 nonionic detergent to provide a source of gp120 (mostly monomeric) (36, 37). The inactivated supernatant was diluted 3- to 10-fold, as appropriate, with Tris-buffered saline containing 1% Nonidet P-40, 1% nonfat milk, and 10% fetal calf serum. A 100-μl aliquot was added for 2 h at room temperature to microplate wells (Immunlon II; Dynatech Ltd.) coated with sheep polyclonal antibody D7324. This antibody was raised to peptide APTKAKRRVVQREKR, derived from the C-terminal 15 amino acids of the clade B LAI isolate, but cross-reacts with many gp120s from other clades (36, 37). Under the assay conditions used, nonspecific absorption of gp41 and other HIV proteins to the solid phase is minimized, so almost all OD_{492} (optical density at 492 nm) values are attributable to antibody reactivity with gp120. Unbound gp120 was removed by washing with Tris-buffered saline, and a range of dilutions of serum or plasma samples diluted in TMTSS buffer was added, essentially as described previously (36, 37). Bound ligand was then detected with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) followed by AMPAK (Dako Diagnostics). Absorbance was read at OD_{492}. Background OD_{492} values derived from wells with no added gp120 were subtracted from all test OD_{492} values in both checkerboard and titration curve analyses; the background was significant usually only at a serum dilution of 1:300, sometimes at 1:1,000, and rarely at 1:3,000.

For checkerboard analyses, each serum sample was tested in duplicate against a range of gp120 molecules. The OD_{492} values were corrected for background absorption (no gp120). Aliquots of culture supernatants of between 10 and 100 μl were used in each assay, the volume depending on the amount of D7324-reactive gp120 present. To compensate for variation in the amounts of the different gp120 molecules captured onto the solid phase, each OD_{492} value was normalized by determining its ratio to the OD_{492} value obtained with a saturating concentration of CD4-IgG (1 μg/ml, in quadruplicate), essentially as described elsewhere (36). The average ratios for all gp120 molecules within each clade were then calculated. Whenever possible, the mean OD_{492} values for CD4-IgG binding to each gp120 were in the range of 0.6 to 1.2, but a few supernatants contained relatively little gp120 that could be captured by D7324, and the OD_{492} values for CD4-IgG reactivity were lower than desirable.

Seroreactivity-based clustering methodologies. Serum antibody-gp120 binding ratios (see above) were normalized by designating the highest binding ratio for each serum as equal to 1.0 and then dividing all binding ratios for that serum by the highest ratio, to create a range of reactivity values of between 0 and 1. This was done to compensate for differences in the baseline antibody levels in the different sera. A Euclidean distance was then calculated between normalized binding scores for each pair of proteins. Euclidean distance equals $(a_1 - b_1)^2 + (a_2 - b_2)^2 + (a_n - b_n)^2$, where a and b represent proteins and a_n represents the seroreactivity of protein a with serum n.

The resulting distance matrix was fed into the neighbor program of the PHYLIP package, and the proteins were organized by using the UPGMA clustering algorithm. Seroreactivity patterns for different proteins were then analyzed by using S-PLUS version 3.2 (MathSoft Inc., Seattle, Wash.).

The gp120 proteins with the most clade-specific reactivity to sera were selected by a criterion described in Results. These five proteins were then used as a basis for predicting the clade of an infecting virus by the reactivity patterns of the sera. To do this, the average normalized serum antibody/gp120 binding ratios for the set of sera from individuals infected with a virus of a given clade were calculated for each of the five proteins. A single serum was excluded from the calculation of the average reactivities and treated as an unknown. This was done iteratively for all sera. The Euclidean distances were calculated between the unknown and the average reactivities of sera from clades A through E. The clade with the minimum distance to the unknown was predicted to be the clade of the infecting virus.

RESULTS

Inter- and intraclade neutralization by HIV-1-positive sera. To determine whether HIV-1-positive sera showed clade-specific patterns of neutralization, we performed three separate checkerboard analyses using PBMC-grown primary HIV-1 isolates in a well-characterized assay, with mitogen-stimulated PBMC as target cells (5, 9). Virus replication was assessed by measuring p24 antigen production, with appropriate controls being performed to ensure that there was no interference with p24 detection by residual serum anti-p24 antibodies. Each serum sample was titrated in twofold steps over the dilution range 1:8 to 1:128 or 1:256, and the ID_{50} and ID_{90} values for each serum were determined. If 50% neutralization was not achieved at a dilution of 1:8, the result was scored as negative, for a 1:8 dilution is the least at which we generally do not observe nonspecific neutralization by normal human serum samples. Note, however, that we put little weight on ID_{50} neutralization values, as the biological significance of a twofold reduction in viral infectivity is unclear. Inferences drawn in the discussion that follows therefore relate to ID_{90} values.

The first study used virus isolates and sera collected by the WHO Network for HIV Isolation and Characterisation. The isolates were mostly derived from individuals within 18 months of seroconversion, and the sera were generally obtained contemporaneously with the isolates. Five sets of serum and virus samples from clade A, five from clade B, one from clade C, five from clade D, and five from clade E constituted this checkerboard (Tables 1 and 2). No clear pattern of neutralization could be observed on inspection of the tabulated ID_{50} (Table 1) or ID_{90} (Table 2) data. A strong, clade-specific pattern of neutralization indicative of neutralization serotypes corresponding to the genetic clades would be indicated by higher than average ID_{50} and ID_{90} titers along the diagonals of the checkerboards; no such pattern was apparent. Instead, some virus isolates were relatively sensitive to neutralization by multiple sera, and certain sera were relatively broadly neutralizing. Sensitive isolates included 92UG029 (clade A), 92UG001 and 92UG005 (clade D), and 92TH006 (clade E). Among the more broadly neutralizing sera were 92UG037 and 92RW008 (clade A). In contrast, some isolates were almost completely refractile (e.g., 92UG029, clade A) and some sera showed very little neutralizing activity (e.g., 92UG046, 92UG024, clade D). Indications of clade-specific neutralization were sometimes seen, an example being the broad neutralization of only clade E isolates by 92TH023 serum (Table 2).

In the second checkerboard, we focused on sera and isolates from clades B, C, and E, using samples collected by the NIAID Antigenic Variation Consortium. Again, the checkerboard analysis yielded no evidence for clade-specific neutralization of primary isolates (Tables 3 and 4). Some isolates in each clade (e.g., 92US716, 93MW965, and 93TH302) were relatively sensitive to neutralization; others from the same clade (e.g., 92HT594, 93MW960, and 93TH975) were highly resistant. A similar spectrum of potency was observed with the serum samples. Thus, some sera, such as 92US711, 93MW959, and 93TH966, possessed relatively broad neutralizing activity both within and across clades, whereas many other sera lacked significant neutralizing activity (Tables 3 and 4).

All sera used in checkerboards 1 and 2 were selected solely on the grounds of their availability, most having been obtained within 2 years of seroconversion. As the functional humoral immune response to HIV-1 infection can develop gradually (24, 32), we considered the possibility that at least some of the sera used had been drawn from individuals prior to their development of a fully mature antibody response to infection. We therefore screened panels of available sera for their anti-gp120 titers, using gp120 molecules derived from the same clade as each set of serum samples (Fig. 1). Sera from individuals infected with variant clade B strains circulating in Thailand and Brazil were titrated separately against gp120 from the appropriate variant isolate (Fig. 1c). Although we have found from other studies that antibody reactivity with monomeric gp120 does not correlate well with virus neutralizing titers (31, 32, 35, 37), we felt that sera with very low anti-gp120 antibody titers might have been drawn too early after infection. We therefore sought to eliminate such sera from further neutralization analyses. As expected, the serum samples from each clade displayed a wide range of anti-gp120 antibody titers, which varied by up to 1,000-fold in some cases (e.g., clade E; Fig. 1f).

TABLE 1. Reciprocals of ID_{50} values in WHO samples

Serum	Reciprocal of ID_{50} for indicated HIV-1 isolate																				
	Clade A					Clade B					Clade C	Clade D					Clade E				
	92UG029	92UG031	92UG037	92RW009	92RW008	92TH014	92TH026	92BR020	92BR021	92BR023	92BF025	92UG001	92UG005	92UG021	92UG046	92UG024	92TH006	92TH009	92TH022	92TH024	92TH023
Clade A																					
92UG029	—[a]	16	—	—	—	128	—	32	—	—	—	8	—	—	—	—	—	—	—	—	—
92UG031	>128	16	32	32	128	16	—	—	—	64	16	>128	64	—	—	—	8	16	128	16	8
92UG037	—	128	128	64	128	128	—	—	—	32	—	8	64	—	—	—	128	32	128	32	64
92RW009	—	8	8	8	128	8	—	—	—	64	—	—	64	—	—	—	128	128	128	128	128
92RW008	32	64	>128	>128	32	128	32	32	32	128	128	64	>128	8	8	32	32	16	32	16	32
Clade B																					
92TH014	>128	8	—	16	—	128	8	—	—	—	—	64	64	—	—	—	128	128	128	128	128
92TH026	>128	16	—	—	8	128	—	16	8	—	—	>128	64	—	—	—	16	32	64	—	—
92BR020	—	—	—	—	—	128	—	—	16	—	—	8	—	—	—	—	64	16	128	128	16
92BR021	—	—	—	—	—	128	—	—	8	128	—	—	—	—	—	—	—	—	—	—	—
92BR023	64	32	128	32	128	128	—	—	8	—	8	64	>128	—	—	—	—	—	—	—	—
Clade C,																					
92BR025	>128	8	64	64	32	128	—	—	—	128	8	>128	64	—	—	—	128	128	128	16	16
Clade D																					
92UG001	>128	16	—	—	128	—	8	—	—	16	—	>128	32	—	—	—	—	—	8	—	—
92UG005	>128	128	32	64	128	128	>128	128	128	128	—	>128	>128	8	128	16	32	8	128	16	8
92UG021	64	16	—	—	128	—	—	—	—	—	—	32	—	—	8	—	128	128	128	128	128
92UG046	32	—	—	—	128	—	—	—	—	—	—	8	8	—	—	—	128	64	128	32	128
92UG024	>128	—	—	—	—	8	—	—	—	—	—	>128	—	—	—	—	64	8	64	—	8
Clade E																					
92TH006	128	8	—	—	—	—	—	—	—	—	—	128	—	—	—	—	128	64	128	8	16
92TH009	—	32	64	32	—	8	—	—	8	16	—	8	128	—	—	—	32	16	32	—	8
92TH022	>128	64	64	32	8	128	8	—	—	128	8	>128	>128	—	—	—	—	16	—	16	—
92TH024	32	8	—	8	—	64	—	16	8	—	—	>128	16	>128	—	—	—	—	—	—	—
92TH023	>128	—	—	—	—	8	—	—	—	—	—	>128	—	—	—	—	128	128	128	128	128

[a] —, 50 or 90% neutralization was not achieved at a serum dilution of 1:8.

TABLE 2. Reciprocals of ID_{90} values in WHO samples

Serum	Reciprocal of ID_{90} for indicated HIV-1 isolate[a]																				
	Clade A					Clade B					Clade C	Clade D					Clade E				
	92UG029	92UG031	92UG037	92RW009	92RW008	92TH014	92TH026	92BR020	92BR021	92BR023	92BR025	92UG001	92UG005	92UG021	92UG046	92UG024	92TH006	92TH009	92TH022	92TH024	92TH023
Clade A																					
92UG029	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92UG031	>128	16	—	16	—	—	—	—	—	16	8	>128	16	—	—	—	—	—	—	—	—
92UG037	—	32	16	16	—	—	—	—	—	16	—	—	32	—	—	—	32	16	8	16	—
92RW009	—	—	—	—	64	—	—	—	—	8	—	—	16	—	—	—	128	64	128	128	—
92RW008	8	64	>128	64	—	16	32	—	—	128	64	16	>128	—	—	—	—	—	—	—	—
Clade B																					
92TH014	—	—	—	—	—	—	—	—	—	—	—	—	8	—	—	—	128	64	8	16	8
92TH026	—	—	—	—	—	—	—	—	—	—	—	64	—	—	—	—	—	—	—	—	—
92BR020	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	8	—	16	—	—
92BR021	—	—	—	—	—	—	—	—	—	128	—	—	—	—	—	—	—	—	—	—	—
92BR023	—	—	—	—	—	—	—	—	—	—	—	32	16	—	—	—	—	—	—	—	—
Clade C,																					
92BR025	—	—	—	—	—	—	—	—	—	32	8	8	32	—	—	—	16	8	8	—	—
Clade D																					
92UG001	32	—	—	—	—	—	—	—	—	—	—	64	8	—	—	—	—	—	—	—	—
92UG005	64	—	—	—	—	—	—	—	—	—	—	>128	16	—	—	—	—	—	8	—	—
92UG021	8	—	—	—	—	—	—	—	—	—	—	8	—	—	—	—	—	—	16	—	—
92UG046	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	32	32	32	8	—
92UG024	>128	—	—	—	—	—	—	—	—	—	—	16	—	—	—	—	—	—	—	—	—
Clade E																					
92TH006	—	—	—	—	—	—	—	—	—	—	—	8	—	—	—	—	16	16	—	—	—
92TH009	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92TH022	—	16	32	—	—	—	—	—	—	64	—	64	32	—	—	—	—	—	—	—	—
92TH024	—	—	—	—	—	—	—	—	8	—	—	>128	—	>128	—	—	—	—	—	—	—
92TH023	—	—	—	—	—	—	—	—	—	—	—	64	—	—	—	—	128	128	128	128	128

[a] See the footnote to Table 1. No neutralization was found for clade B strain 92BR020 and clade D strains 92UG046 and 92UG024.

TABLE 3. Reciprocals of ID_{50} values in NIAID samples

Serum	Reciprocal of ID_{50} for indicated HIV-1 isolate[a]															
	Clade B								Clade C					Clade E		
	92HT593	92HT594	92HT596	92US711	91US712	92US714	92US715	92US716	93MW959	93MW960	93MW965	93MW101	93MW102	93TH966	93TH975	93TH302
Clade B																
92HT593	>128	—	8	—	64	—	—	32	—	—	—	>128	—	8	—	8
92HT594	>128	—	—	—	—	—	—	—	—	—	—	>128	—	—	—	—
92HT596	64	—	—	—	—	—	—	—	—	—	—	>128	—	—	—	—
92US711	8	—	>128	—	—	8	8	>128	64	—	>128	>128	16	>128	—	>128
91US712	8	—	—	—	—	—	—	—	8	—	>128	>128	—	—	—	16
92US714	—	—	—	16	32	—	—	—	—	—	>128	>128	—	—	—	—
92US715	—	—	—	8	8	—	—	8	—	—	>128	64	—	—	—	—
92US716	—	—	—	—	16	—	—	8	—	—	>128	—	32	—	—	—
Clade C																
93MW959	32	—	>128	—	—	16	8	>128	>128	—	>128	>128	—	—	—	>128
93MW960	—	—	32	—	—	—	—	>128	>128	—	>128	>128	32	—	—	32
93MW965	—	—	—	—	—	—	>128	8	—	—	>128	64	—	—	—	—
93MW101	—	—	32	64	>128	—	—	>128	>128	—	—	>128	16	—	—	64
93MW102	—	—	—	—	—	—	—	—	64	—	—	—	—	16	—	32
Clade E																
93TH966	—	—	>128	—	—	8	8	>128	64	—	—	64	16	>128	—	>128
93TH975	—	—	32	—	—	—	—	>128	—	—	—	64	—	32	—	>128
93TH302	—	—	—	—	—	—	—	—	—	—	—	64	—	—	—	8

[a] See the footnote to Table 1. No neutralization was found for clade C strain 93MW960 and clade E strain 93TH975.

We then selected sera which contained relatively high anti-gp120 antibody titers for a third checkerboard neutralization analysis (Tables 5 and 6). Note that the highest-titer sera were not always available in sufficient quantity for neutralization analyses. Once again, no clade-specific pattern of neutralization was found; there was a tendency for clade C isolates to be most sensitive to neutralization by clade C sera, but these isolates were also neutralized by sera from clades A and E (Table 6).

Some discrepancies were found between the ID_{50} and ID_{90} values recorded in Tables 5 and 6 and those in Tables 1 to 4 for the same serum-virus combination. Most of the discrepant data

TABLE 4. Reciprocals of ID_{90} values in NIAID samples

Serum	Reciprocal of ID_{90} for indicated HIV-1 isolate[a]															
	Clade B								Clade C					Clade E		
	92HT593	92HT594	92HT596	92US711	91US712	92US714	92US715	92US716	93MW959	93MW960	93MW965	93MW101	93MW102	93TH966	93TH975	93TH302
Clade B																
92HT593	—	—	—	—	32	—	—	8	—	—	—	64	—	—	—	—
92HT594	—	—	—	—	—	—	—	—	—	—	—	8	—	—	—	—
92HT596	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92US711	—	—	—	—	—	—	—	>128	32	—	>128	16	—	—	—	64
91US712	—	—	—	—	—	—	—	—	—	—	>128	—	—	—	—	—
92US714	—	—	—	8	16	—	—	—	—	—	>128	—	—	—	—	—
92US715	—	—	—	—	8	—	—	—	—	—	>128	—	—	—	—	—
92US716	—	—	—	—	8	—	—	—	—	—	>128	—	16	—	—	—
Clade C																
93MW959	—	—	32	—	—	—	—	>128	64	—	>128	—	—	—	—	8
93MW960	—	—	—	—	—	—	—	>128	64	—	>128	—	8	—	—	16
93MW965	—	—	—	—	—	—	—	—	—	—	>128	—	—	—	—	—
93MW101	—	—	8	32	16	—	—	64	64	—	—	—	—	—	—	—
93MW102	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clade E																
93TH966	—	—	64	—	—	8	8	>128	16	—	—	—	—	—	—	64
93TH975	—	—	16	—	—	—	—	32	—	—	—	—	—	—	—	32
93TH302	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] See the footnote to Table 1. No neutralization was found for clade B strains 92HT593 and 92HT594, clade C strain 93MW960, and clade E strains 93TH966 and 93TH975.

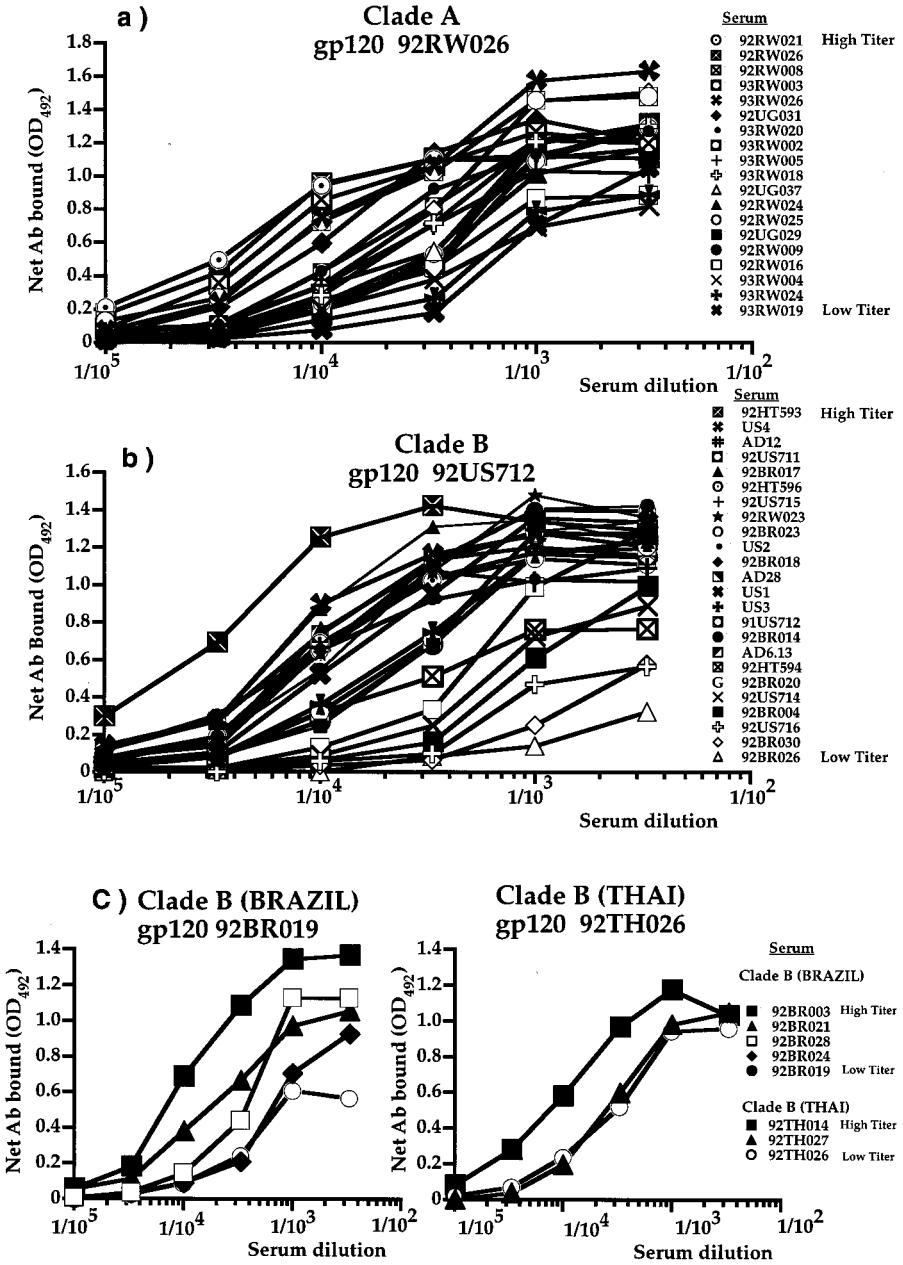

FIG. 1. Anti-gp120 antibody titers. The serum samples indicated on the right of each panel were titrated against gp120 from the same clade, and the amount of gp120-bound antibody (Ab) was determined. The rank order for anti-gp120 antibody titers in the serum samples is reflected in the order in which the sera are listed, highest titers at the top and lowest titers at the bottom. (a) Clade A; (b) clade B; (c) Thai GPGQ and Brazilian GWGR V3-loop clade B variants; (d) clade C; (e) clade D; (f) clade E. The titration curves within each clade can be compared directly with one another; interclade comparisons of titration curves may also be made but will be less precise because of variation in gp120 loading among the different sets of test plates.

are within 1 serum dilution, which is generally accepted as within the error of the assay (see Materials and Methods). However, 6 datum points in Table 2 and eight datum points in Table 4 are significantly different from the corresponding datum points in Table 6. The analyses in the accompanying report (23) show that serum neutralization curves are often irregular in shape, such that ID_{50} and ID_{90} values can easily be influenced by experimental conditions (such as different donor PBMC as target cells), resulting in >2 dilution differences in ID_{50} or ID_{90} values in some instances. A more sophisticated mathematical analysis of the neutralization data is provided in the accompanying report (23), but the conclusions drawn concerning the relationship between neutralization serotypes and the genetic clades are essentially the same as those drawn in the present report. This concordance indicates that experimental variation does not obscure the overall conclusions that we draw from these studies.

Antigenic serotyping. (i) Titration curves. The absence of any observable neutralization serotype is apparently paradoxical with our previous observations that monoclonal antibodies

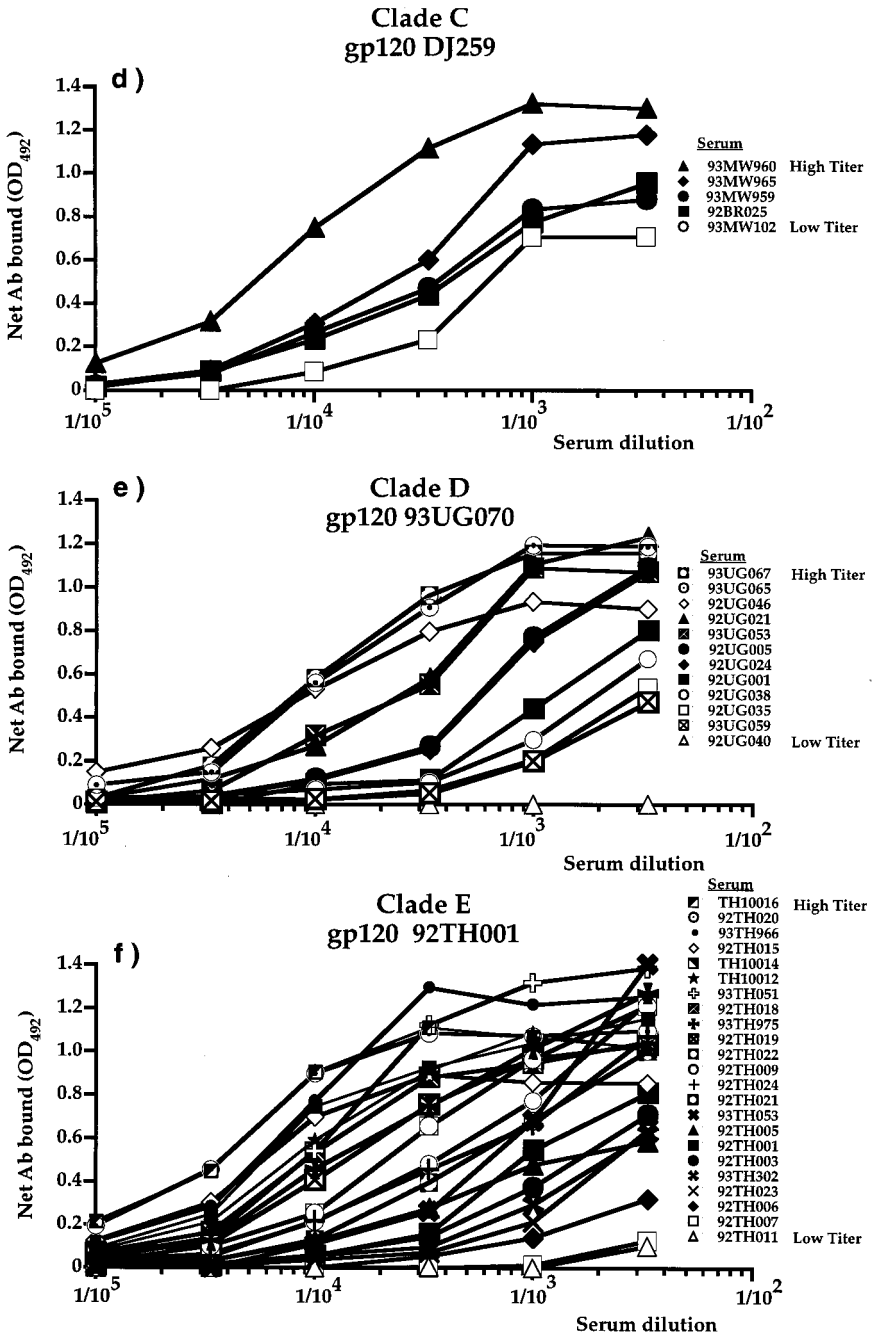

FIG. 1—*Continued.*

(MAbs) reacted with gp120 molecules from clades A through F in patterns that were at least partially clade specific (36). Furthermore, studies measuring serum antibody reactivity with V3 peptides or recombinant gp160 indicated the presence of antigenic serotypes of HIV-1 that bore some relationship to the genetic clades (6, 43, 51). To explore whether polyclonal sera from HIV-1-infected people also bound to gp120 molecules in a clade-specific manner, we carried out gp120 binding assays using virus culture supernatants treated with a nonionic detergent as antigen sources (36, 37).

In the first set of experiments, we performed serum antibody titrations against gp120s from clades A through E, as well as against gp120s from the Thai and Brazilian clade B variants (Fig. 2). The Thai clade B variant 92TH026 has the central V3 sequence IHLGPGRAWYT (21); this is a variant of the V3 sequences found in U.S. clade B viruses but also differs from the more common Thai clade B strains, which have the GPGQ motif at the crown of the V3 loop (18). The Brazilian clade B variant 92BR019 had the central V3 sequence SIHMGWG RAFYA, which is characteristic of Brazilian clade B strains (21). The sera used were among the highest titered available from each clade. To ensure that the titration curves derived from the reactions of each serum with different gp120s could be compared with one another, we attempted to ensure that

TABLE 5. Reciprocals of ID_{50} values in WHO and NIAID samples

Serum	Reciprocal of ID_{50} for indicated HIV-1 isolate														
	Clade A			Clade B			Clade C			Clade D			Clade E		
	92UG031	92UG037	92UG008	92HT593	92HT596	92TH026	93MW959	93MW960	93MW965	92UG021	92UG024	92UG046	92UG024	93TH966	93TH975
Clade A															
92UG031	8	—[a]	—	—	—	—	—	—	—	—	—	8	8	64	8
92UG037	8	—	—	—	—	32	16	—	32	—	—	—	8	8	8
92UG008	32	32	32	—	—	—	>256	—	>256	—	—	8	128	>256	—
Clade B															
92HT593	—	—	—	—	—	16	—	—	—	—	—	8	8	16	—
92HT596	—	—	—	—	—	—	—	—	—	—	—	—	8	16	—
92TH026	—	—	—	—	—	—	—	—	—	—	—	—	—	8	—
Clade C															
93MW959	32	32	—	8	—	128	>256	>256	32	—	—	—	8	>256	8
93MW960	16	8	8	—	—	—	128	16	128	—	—	—	8	>256	—
93MW965	—	—	8	—	—	—	8	—	64	—	—	—	8	16	—
Clade D															
92UG021	8	—	—	—	—	—	—	8	—	—	—	—	—	—	—
92UG024	—	—	—	—	—	—	—	8	16	—	8	—	—	8	—
92UG046	—	—	—	—	—	—	—	—	—	—	8	—	—	8	—
Clade E															
92UG024	8	—	—	—	—	—	8	—	—	—	—	—	16	128	16
93TH966	—	8	—	—	—	>256	128	32	—	—	—	—	16	>256	16
93TH975	8	8	—	—	—	128	>256	>256	—	—	—	—	8	>256	8
Clade B controls															
FDA-2 (positive)	8	64	16	8	128	128	64	8	128	16	16	64	128	128	64
Normal human serum (negative)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] See the footnote to Table 1.

TABLE 6. Reciprocals of ID_{90} values in WHO and NIAID samples

Serum	Reciprocal of ID_{90} for indicated HIV-1 isolate[a]														
	Clade A			Clade B			Clade C			Clade D			Clade E		
	92UG031	92UG037	92RW008	92HT593	92HT596	92TH026	93MW959	93MW960	93MW965	92UG021	92UG024	92UG046	92UG024	93TH966	93TH975
Clade A	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92UG037	—	—	—	—	—	—	—	—	8	—	—	—	—	—	—
92RW008	8	8	—	—	—	—	128	—	32	—	—	—	—	64	—
Clade B															
92HT593	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92HT596	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92TH026	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clade C															
93MW959	8	16	—	—	—	—	64	32	—	—	—	—	—	16	—
93MW960	8	—	—	—	—	—	64	—	64	—	—	—	—	8	—
93MW965	—	—	—	—	—	—	—	—	32	—	—	—	—	—	—
Clade D															
92UG021	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92UG024	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
92UG046	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Clade E															
92UG024	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
93TH966	—	—	—	—	—	—	32	8	—	—	—	—	—	—	—
93TH975	—	—	—	—	—	—	32	16	—	—	—	—	—	32	—
Clade B controls															
FDA-2 (positive)	—	16	—	8	—	—	—	—	8	—	—	—	—	—	—
Normal human serum (negative)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] See the footnote to Table 1. No neutralization was found for clade A strain 92RW008, clade B strains 92HT596 and 92TH026, clade D strains 92UG021, 92UG024, and 92UG046, and clade E strains 92UG024 and 93TH975.

comparable amounts of the different gp120s were attached to the solid phase. We therefore titrated each gp120 supernatant to find the amounts that gave equivalent binding to a saturating concentration of CD4-IgG (3 μg/ml) (data not shown). The appropriate amounts of each supernatant were then used in a subsequent experiment in which either CD4-IgG or seven different sera were titrated and bound antibodies were detected (Fig. 2). The pattern of CD4-IgG reactivity demonstrated that broadly comparable amounts of the seven different gp120s were captured onto the solid phase, although the clade A gp120 92RW026 and the clade E gp120 92TH001 were slightly underrepresented compared with the others (Fig. 2a); this should be noted when interpreting the serum antibody titration curves. Note also that midpoint titer estimates are imprecise because of the lack of saturation of any of the titration curves and are for guidance only.

The clade A serum 92RW021 bound to gp120 from each clade with midpoint titers that were within an approximately fivefold range of one another (Fig. 2b). The strongest reactivity was with the clade A gp120, taking into account the relatively low level of this gp120 in the assay; the weakest was with the clade D gp120. Clade B serum 92HT593 showed a strong preference for the clade B gp120 91US712, the midpoint titer against this gp120 being 5- to 30-fold greater than for the other gp120s (Fig. 2c). It was notable that serum 92HT593 reacted less strongly with the gp120s from the Thai and Brazilian clade B variants, the midpoint titers against these gp120s being approximately sixfold lower than the titer against gp120 from 91US712 (see below). The clade C serum 93MW960 and the clade D serum 93UG067 both reacted with each gp120. The midpoint titers for these sera against gp120 from each clade were within an approximately 5-fold range, except for the clade D serum against the clade A gp120, in which case the reduction in midpoint titer was about 10-fold (Fig. 2d and e). In contrast, serum 93TH252 from clade E clearly reacted most strongly with the clade E gp120 and relatively poorly with gp120s from the other clades (recall the underrepresentation of clade E gp120 in the assay, which will act to minimize the differential). Thus, the midpoint titer for the clade E serum against clade E gp120 was 10- to 30-fold greater than against the other gp120s except for the clade C gp120, in which case the titer differential was about 4-fold (Fig. 2f). The clade B variant sera 92TH014 and 92BR003 each showed preferential reactivity with gp120s from clade B rather than to gp120s from the other clades (Fig. 2g and h), the midpoint titer reductions against non-clade B gp120s being approximately 5- to 20-fold. Furthermore, the titers for the U.S. (92HT593) and Brazilian (92BR003) clade B sera against the gp120s from the same country were approximately fivefold higher than against the other clade B gp120s (Fig. 2c and h), indicating that there may be some substructure in the serological reactivity patterns within clades.

The data from the titration curves derived from a limited set of sera suggest that the genetic clades can have some relationship to antigenic serotypes. Thus, the clade B and E sera reacted preferentially with gp120s from the same clade, but they were also able to cross-react with gp120s from other clades. There is some evidence that the divergent clade B gp120s can form a distinct serotype within clade B. The sera from clades A, C, and D were more broadly reactive across clades, but some indications of clade-specific reactions can be discerned with the clade A and C sera. The clade D serum clearly did not react best with clade D gp120, and it was also notable that the clade D gp120 (93UG070) used in the titration curve experiment was often the least, or among the least, immunoreactive with sera from several clades (Fig. 2b to d and f to h).

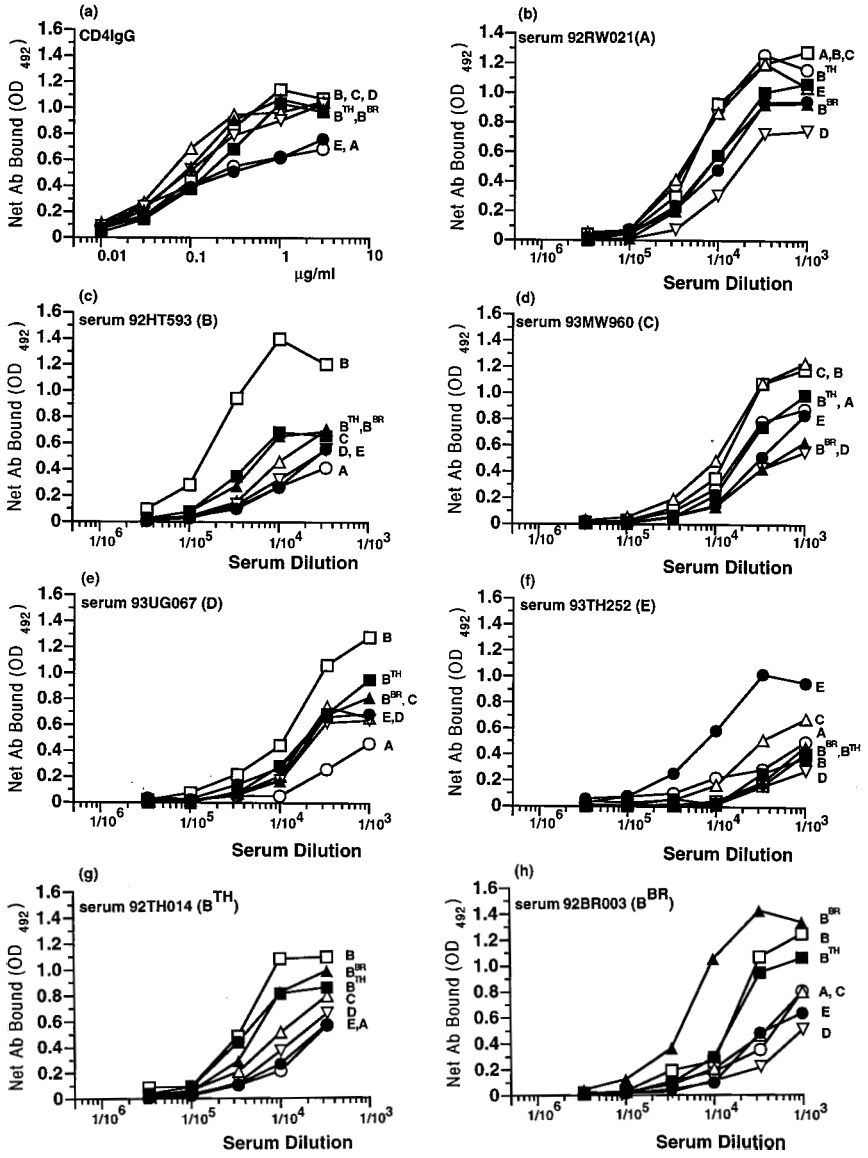

FIG. 2. Reactivities of sera from clades A through E with gp120s from clades A through E. In each experiment, gp120 from the following isolates was used: 92RW026, clade A (○); 91US712, clade B (□); DJ259, clade C (△); 93UG070, clade D (▽); 92TH001, clade E (●); 92TH026, clade B-Thai (B^{TH}; ■); 92BR019, clade B-Brazil (B^{BR}; ▲). The reagents titrated are indicated below the panel letters.

(ii) Checkerboards. To determine whether the seroreactivity patterns derived from the titration curves were more generally observed, we performed three checkerboard analyses using a wide range of sera and isolates from clades A through E (Tables 7 to 9). In each of these experiments, serum at a single dilution was reacted with a panel of gp120s from the different clades. The extent of serum binding was determined and normalized for the amount of gp120 present, as measured by the extent of CD4-IgG binding (36). The mean serum/CD4-IgG binding ratio for each serum within each clade was measured and recorded, with the highest mean value(s) among the clades in boldface in each table. A clade-specific pattern of reactivity would be indicated by boldface values along the diagonals of each checkerboard.

For the first two checkerboards, the same set of sera from individuals infected with HIV-1 strains from clades A through E was reacted with two different sets of HIV-1 gp120 molecules. Each serum was tested at a single dilution of 1:5,000. This means that low-titer sera gave lower average serum/CD4-IgG OD_{492} ratios than higher-titer sera (e.g., compare serum 92UG001 with serum TH 10016), and so binding ratios can be compared only across rows, not down columns. Virus isolates in the first two test checkerboards were selected only on the grounds of availability and the presence of sufficient capture antibody-reactive gp120 in the supernatants to provide an acceptable level of CD4-IgG and serum reactivity. The first checkerboard contained isolates from clades A through E, and the second panel contained a different set of isolates from clades A, B, D, and E, no new isolates from clade C being available. The third panel contained some isolates from clades A through E that had been used previously in the first two checkerboards, along with some new isolates. However, in this third set of experiments, the sera tested were selected on the basis of titer, only the higher-titer ones identified in the exper-

TABLE 7. Reactivities of HIV-1-positive sera with HIV-1 isolates from clades A to E, checkerboard 1

Serum		Avg serum/CD4-IgG binding ratio with gp120 from virus clade[a,b]:				
Designation	Clade	A (n = 8)	B (n = 15)	C (n = 8)	D (n = 8)	E (n = 9)
92UG029	A	0.21	**0.42**	0.31	0.22	0.26
92UG031	A	0.58	0.49	**0.75**	0.39	0.60
92RW009	A	0.33	0.39	**0.56**	0.23	0.28
92BR020	B	0.08	**0.36**	0.17	0.16	0.16
US AD6.13	B	0.31	**0.48**	0.20	0.17	0.15
US AD28	B	0.13	**0.48**	0.20	0.17	0.15
US AD12	B	0.41	**1.05**	0.59	0.45	0.41
TH 1027	B	0.22	**0.63**	0.34	0.27	0.20
92BR025	C	0.21	0.15	**0.51**	0.16	0.31
93MW959	C	0.47	0.45	**0.85**	0.25	0.47
93MW960	C	0.89	0.75	**1.23**	0.51	0.66
92UG021	D	0.06	0.09	0.13	**0.15**	0.09
92UG005	D	0.12	0.15	**0.44**	0.20	0.29
92UG021	D	0.38	0.37	0.37	**0.49**	0.37
92UG024	D	0.18	**0.34**	0.25	**0.34**	0.25
93ZR001	D	0.64	**0.76**	0.70	0.69	0.68
TH 10012	E	0.33	0.25	0.78	0.35	**1.11**
TH 10014	E	0.48	0.42	0.68	0.31	**0.96**
TH 10016	E	0.59	0.43	0.84	0.34	**1.18**
Mean		0.35	0.45	0.52	0.31	0.45

[a] The highest value(s) for each serum is in boldface. Values should be compared across rows, not down columns.

[b] The isolates from each clade used are as follows (mean OD_{492} values for CD4-IgG binding to each group of isolates are given in parentheses): clade A, DJ258, DJ263, UG273, UG276, 92UG029, 92UG031, 92RW008, and 92RW009 (0.769); clade B, US 4, 92HT596, 92HT599, 91US712, 92US715, 93US103, 93US104, 93US101, CM237, BK130, BK132, 92TH014, BZ164, BZ165, and RYCA2 (0.881); clade C, SM145, ZAM18, ZAM20, SG364, UG268, DJ259, 92BR025, and 93MW101 (0.823); clade D, UG266, UG270, UG274, SG365, 92UG001, 92UG005, 92UG021, and 92UG024 (0.976); clade E, CM235, CM238, CM240, CM244, 92TH009, 92TH021, 92TH022, 92TH023, and 93TH975 (0.948).

iment shown in Fig. 1 being used. Furthermore, each serum was tested at a dilution that gave approximately half-maximal binding to the gp120 representing the same clade (Fig. 1), to ensure a more comparable level of antibody reactivity across the entire set of sera than was achieved in the first two checkerboards.

The three checkerboard analyses yielded reasonably comparable results, in that there was a tendency for many sera to react best on average with gp120s from the same clade, leading to the highest binding ratios being on the diagonals (boldface values in Tables 7 to 9). While the differences in binding ratios are often small and their statistical robustness is uncertain, certain trends could be discerned. Thus, the preferential reactivities of serum antibodies with gp120s from the autologous clade were most frequently observed within clades B and E. Indeed, clade E sera invariably reacted best on average with clade E gp120s, and clade B sera almost always bound most strongly on average to clade B gp120s. Only one clade B serum (92US711) failed to react preferentially with clade B gp120s, and then only in one of two experiments within checkerboard 3. However, clade A and C sera did not always bind preferentially to clade A and C gp120s, and clade D sera only rarely reacted best on average with clade D gp120s.

Further examination of the distribution of reactivities for each sera with proteins from each clade revealed some clade-specific trends in the gp120 reactivities of clade A, C, and D sera. To look for patterns and trends in the data, the gp120 reactivities of each serum were normalized to range between 0 and 1, by dividing all values for a given serum by the highest reactivity for that serum. Then plots were made to show the distribution of normalized gp120 reactivities for each serum, with the proteins distinguished by clade (data not shown). Although clade D sera did not often react the best with clade D gp120s, the clade D gp120s generally had the lowest reactivities among all gp120s, and the only sera that consistently showed moderate to high reactivity with the clade D gp120s were clade D sera. Thus, while clade D sera often bound more strongly to clade B or E gp120s than to clade D gp120s, clade D sera were the only sera that reacted strongly with clade D gp120s. Furthermore, while clade A and C sera did not reveal clade-specific serotypes, they may have a shared serotype that is characterized by relatively high reactivities with clade A, C, and E gp120s and lower reactivities with clade B and D gp120s. However, clade A and C sera are clearly distinguishable from clade E sera, which show a clear preferential reactivity with clade E gp120s.

The pattern of data was not always statistically robust, in that the highest serum/CD4-IgG binding ratio was frequently only marginally above other values. In general, the same sera tested on different sets of gp120s gave similar results each time, as can be seen by comparing the results of checkerboards 1 and 2 and analyses of the Thai clade B serum TH 1027 in all three checkerboards. However, there were also some inconsistencies when the same serum was tested against more than one set of gp120s. Among inconsistent results were those for three of the

TABLE 8. Reactivities of HIV-1-positive sera with HIV-1 isolates from clades A to E, checkerboard 2

Serum		Avg serum/CD4-IgG binding ratio with gp120 from virus clade[a,b]:				
Designation	Clade	A (n = 12)	B (n = 11)	C (n = 0)	D (n = 7)	E (n = 19)
92UG029	A	0.22	**0.38**	—	0.16	0.19
92UG031	A	**0.48**	0.32	—	0.29	0.42
92RW009	A	0.42	**0.44**	—	0.27	0.41
92BR020	B	0.08	**0.29**	—	0.09	0.11
US AD6.13	B	0.25	**0.50**	—	0.31	0.30
US AD28	B	0.24	**0.54**	—	0.31	0.24
US AD12	B	0.24	**0.80**	—	0.30	0.31
TH 1027	B	0.15	**0.44**	—	0.29	0.20
92BR025	C	0.21	0.20	—	0.16	0.19
93MW959	C	0.36	0.40	—	0.29	0.48
93MW960	C	0.66	0.54	—	0.65	0.74
92UG001	D	0.06	**0.24**	—	0.13	0.11
92UG005	D	0.18	0.23	—	0.28	**0.41**
92UG021	D	0.19	0.31	—	**0.34**	0.22
92UG024	D	0.14	**0.24**	—	**0.24**	0.19
93ZR001	D	0.62	0.54	—	0.78	**0.87**
TH 10012	E	0.43	0.39	—	0.33	**1.07**
TH 10014	E	0.56	0.43	—	0.49	**1.14**
TH 10016	E	0.49	0.33	—	0.40	**1.02**
Mean		0.31	0.40	—	0.32	0.34

[a] See Table 7, footnote *a*.

[b] The isolates from each clade used are as follows (mean OD_{492} values for CD4-IgG binding to each group of isolates are given in parentheses): Clade A, 92RW016, 92RW020, 92RW021, 92RW023, 92RW024, 92RW025, 92RW026, 93RW004, 93RW005, 93RW018, 93RW020, and 93RW022 (0.664); clade B, 91HT652, 92US711, 92US716, 93US102, 92TH026, 92BR003, 92BR004, 92BR017, 92BR018, 92BR020, and 92BR030 (0.890); clade D, 92UG035, 92UG038, 93UG053, 93UG059, 93UG065, 93UG067, and 93UG070 (1.011); clade E, 92TH001, 92TH003, 92TH005, 92TH007, 92TH011, 92TH019, 92TH020, CM239, CM241, CM242, CM246, 93TH231, 93TH232, 93TH234, 93TH235, 93TH236, 93TH250, 93TH251, and 93TH253 (0.786).

TABLE 9. Reactivities of HIV-1-positive sera with HIV-1 isolates from clades A to E, checkerboard 3

Serum		Avg serum/CD4-IgG binding ratio with virus from clade[a,b]:				
Designation (dilution)	Clade	A (n = 5)	B (n = 5)	C (n = 3)	D (n = 5)	E (n = 5)
92RW021 (1/20,000)	A	**0.65**	0.22	0.38	0.22	0.37
92RW026 (1/20,000)	A	**0.60**	0.18	0.38	0.20	0.53
92RW008 (1/15,000)	A	0.97	0.32	0.59	0.28	**1.05**
92UG031 (1/5,000)	A	**0.59**	0.18	0.28	0.15	0.47
92HT593 (1/30,000)	B	0.16	**0.36**	0.12	0.16	0.14
US 4 (1/10,000)	B	0.28	**0.46**	0.10	0.11	0.13
92US711						
1/10,000	B	0.56	**0.65**	0.49	0.36	**0.84**
1/20,000		0.15	**0.19**	0.08	0.05	**0.16**
92BR003 (1/10,000)	B-Brazil	0.17	**0.26**	0.19	0.13	0.17
92BR021 (1/3,000)	B-Brazil	0.29	**0.45**	0.25	0.38	0.08
92TH014 (1/5,000)	B-Thai	0.58	**0.80**	0.46	0.42	0.55
TH 1027 (1/2,000)	B-Thai	0.40	**0.67**	0.31	0.29	0.48
93MW960 (1/10,000)	C	**0.51**	0.19	0.32	0.25	0.47
93MW965 (1/3,000)	C	**0.44**	0.20	0.40	0.10	0.22
93MW959 (1/2,000)	C	0.71	0.29	0.51	0.27	**0.83**
93UG067 (1/5,000)	D	0.47	0.56	0.46	0.53	**0.92**
93UG065 (1/5,000)	D	0.58	0.66	0.54	0.47	**0.93**
92UG046 (1/5,000)	D	**1.06**	0.92	0.85	0.95	1.05
92UG021 (1/5,000)	D	0.23	0.11	0.15	0.21	**0.26**
93TH252 (1/20,000)	E	0.20	0.02	0.04	0.07	**0.47**
92TH020 (1/20,000)	E	0.44	0.14	0.26	0.13	**0.77**
93TH236 (1/20,000)	E	0.51	0.14	0.26	0.16	**0.97**
Mean		0.48	0.36	0.34	0.27	0.54

[a] See Table 7, footnote *a*.
[b] The isolates from each clade used are as follows (mean OD_{492} values for CD4-IgG binding to each group of isolates are given in parentheses): clade A, 92UG031, 92RW023, 92RW026, DJ258, and DJ263 (0.916); clade B, 92US711, 91US712, 92US715, 92BR017, and 92TH026 (1.099); clade C, DJ259, UG268, and 94ZW106 (0.807); clade D, UG274, 92UG021, 93UG053, 93UG067, and 93UG070 (0.900); clade E, CM240, 92TH001, 92TH020, 93TH236, and 93TH251 (0.711).

five clade D sera in checkerboards 1 and 2, the clade D serum 92UG021 in all three checkerboards, and the clade C sera 93MW959 and 93MW960 in checkerboards 1 and 3. These findings reflect the lack of robustness of the patterns in the data for sera from clades A, C, and D.

An unusual degree of sequence divergence has been found in the V3 regions of a subset of clade B isolates from Thailand and Brazil. Although some Thai and Brazilian clade B isolates have V3 sequences very similar to those of strains circulating in western Europe or North America, many Thai strains B possess the GPGQ motif at the crown of the V3 loop, and a few Brazilian isolates have the GWGR motif (18, 39, 42, 44). Because of the potentially significant contribution of V3-directed antibodies to the overall anti-gp120 antibody response (31, 34), it seemed possible that the Thai and Brazilian clade B variant isolates form a distinct serotype. We therefore analyzed individual serum/CD4-IgG binding ratios for the clade B isolates and sera from checkerboards 2 and 3, as a few Thai and Brazilian clade B variant isolates and sera were used in these experiments (Tables 10 and 11). Thus, the central V3 sequences of three clade B isolates in checkerboard 2 were as follows: 92BR004, KGIPIGPGGSFYAT; 92BR003, KSIHMG WGRAFYAT; and 92TH026, KSIPLGPGQAWYTT. The Thai clade B isolate 92TH026 was also included in checkerboard 3. The remaining isolates used in these two checkerboards had V3 sequences more typical of the majority of isolates found in western Europe or North America (data not shown). Note that although 92BR003 has a GWGR motif at the tip of the V3 loop, common to many Brazilian clade B viruses and shown to be antigenically distinct in V3 peptide enzyme-linked immunosorbent assays (ELISAs) (6, 43), 92BR004 does not have this motif. However, as the latter isolate also has a highly divergent V3 sequence, it was highlighted for comparative purposes.

Although the number of variant gp120s available was small, there were indications of distinct serotypes within clade B. Thus, the clade B sera tested in checkerboard 2 tended to react relatively weakly with gp120s from the three Brazilian and Thai clade B variant isolates whose values are in boldface, in that

TABLE 10. Serum/CD4-IgG binding ratios within clade B, checkerboard 2

Serum	Serum/CD4-IgG binding ratio[a] with gp120 from[a]:										
	91HT652 (Haiti)	92US711 (United States)	92US716 (United States)	1064 (United States)	92BR020 (Brazil)	92BR030 (Brazil)	92BR017 (Brazil)	92BR018 (Brazil)	92BR004 (Brazil)	92BR003 (Brazil)	92TH026 (Thailand)
US AD6.13	0.79	0.76	0.65	0.48	0.40	0.77	0.43	0.40	**0.30**	**0.14**	**0.42**
US AD28	0.66	0.69	0.76	0.48	0.78	0.60	0.73	0.57	**0.19**	**0.27**	**0.25**
US AD12	0.80	0.77	1.06	1.03	1.00	1.00	0.99	1.08	**0.23**	**0.23**	**0.57**
92BR020	0.30	0.47	0.34	0.25	0.42	0.38	0.35	0.29	**0.19**	**0.12**	**0.13**
TH 1027	0.72	0.86	0.61	0.27	0.45	0.48	0.32	0.31	**0.20**	**0.15**	**0.50**

[a] Values should be compared across rows, not down columns. Values for Brazilian and Thai variant gp120s are in boldface. The data are derived from Table 8.

TABLE 11. Serum/CD4-IgG binding ratios within clade B, checkerboard 3

Serum	Serum/CD4-IgG binding ratio with gp120 from[a]:				
	92US711 (United States)	91US712 (United States)	92US715 (United States)	92BR017 (Brazil)	92TH026 (Thailand)
92HT593 (Haiti)	0.42	0.50	0.32	0.43	**0.16**
US 4 (United States)	0.52	0.70	0.43	0.47	**0.20**
92US711 (United States)	1.18	0.64	0.63	0.42	**0.38**
92BR003 (Brazil)	0.36	0.30	0.35	0.12	**0.16**
92BR021 (Brazl)	0.53	0.51	0.25	0.53	**0.43**
92TH014 (Thailand)	0.84	0.94	0.55	0.62	**1.03**
TH 1027 (Thailand)	0.83	0.77	0.53	0.32	**0.92**

[a] Values should be compared across rows, not down columns. Values for Thai variant gp120s are in boldface. The data are derived from Table 9.

serum/CD4-IgG binding ratios with these gp120s were lower than the average across all the clade B gp120s (Table 10). The same observation was made in checkerboard 3, as gp120 from the Thai clade B variant isolate 92TH026 was generally less reactive than average with clade B sera from the United States and Brazil but more reactive than average with clade B sera from Thailand (Table 11). Note that serum 92TH014 binds very strongly to gp120 from isolate 92TH026 (Table 11), despite the former strain having a V3 loop containing the GPGR motif and the latter one having a GPGQ motif. V3-reactive antibodies contribute to the serological response to gp120 but do not necessarily dominate it.

(iii) Cluster analysis. To look for patterns in the antigenic potential of gp120 proteins from different genetic clades, a simple method was developed to cluster proteins according to the similarity of their serological reactivity patterns. Checkerboards 1 and 2 were fused into a single matrix for this purpose, as they incorporated the same set of 19 sera tested in binding assays with 97 gp120 proteins. Checkerboard 3 incorporated a distinct set of sera and so was analyzed separately; it includes 22 sera tested in binding assays with 23 gp120 proteins. A distance matrix was generated by comparing the seroreactivity pattern of each gp120 with patterns of all others in the set (see Materials and Methods), and clusters that group proteins according to the intensities of their responses to the different sera were created (Fig. 3). A striking feature of these clustering patterns is that the proteins representing the different genetic clades can have highly similar levels of reactivity to the spectrum of sera tested.

To facilitate identification of the patterns that are driving the formation of the clusters, we created plots showing the median, quartiles, and outlier seroreactivities of all of the sera for each cluster shown in Fig. 3. As these plots were extensive, the analyses are not shown but are summarized below. Figure 3a shows four clusters of proteins that are associated on the basis of having overall low seroreactivity, ABD, AC, D, and B, although they were notable exceptions among the sera that gave rise to the four distinct clusters. Cluster ABD includes proteins from clades A, B, and D, and they do not react well with any serum except the clade D serum 93ZR001. This is a highly cross-reactive serum in that it binds well to essentially all the gp120s tested, irrespective of their clades. The gp120s from cluster AC show moderate binding to clade A, C, and E sera and a subset of clade D sera but are overall of rather low reactivity. The cluster D proteins have low reactivity with all but clade D sera, while the cluster B proteins have high to moderate reactivity with clade B sera and also with a subset of clade A and D sera. The cluster ACE gp120s are highly reactive with the clade A and E sera and with the clade D serum 93ZR001. The cluster E gp120s are strongly reactive only with clade E sera and with the clade D serum 93ZR001. Cluster B1, B2, and B3 proteins show the same patterns of reactivity with all sera, but the reactive sera score with increasing intensity. They ranged from moderately to highly reactive with clade A and B sera and with a subset of clade D sera but were poorly reactive with clade C and E sera. The cluster C proteins were highly reactive with all but clade B sera.

Similarly, four distinct groupings are apparent in Fig. 3b. Cluster BCD is dominated by clade D proteins, and they show high reactivity with clade D sera and with clade B sera from Brazil and Thailand. Cluster E is highly reactive with clade D and E sera and with a subset of sera from the other clades. Cluster AEC is dominated by clade A proteins and is highly reactive with clade A, C, and D sera but reacts less well with clade B and E sera (note that cluster AEC in Fig. 3b is distinct from cluster ACE in Fig. 3a and so is designated differently). The B cluster proteins are highly reactive with a clade B serum and with a subset of clade A and D sera but react poorly with clade C and E sera.

Some trends can be extracted from the complex summary presented above. First, the clustering patterns suggest that serum antibodies may recognize gp120 epitopes that are shared by proteins from diverse clades but that are not necessarily preserved in all proteins from the same clade. For example, some clade E gp120s are preferentially reactive with clade E sera, whereas other clade E gp120s react well with clade A and D sera as well as with clade E sera. Second, it is not easy to reconcile the observation that while clade D gp120s tend to be poorly reactive with sera from other clades and only moderately reactive with clade D sera, a subset of the clade D sera are the most potently cross-reactive sera with gp120s from other clades. Third, the broadest cross-clade antigenicity among proteins in this study was found in proteins of clade C; they served as good antigens for all but clade B sera. Similarly, the B3 cluster proteins in Fig. 3a reacted well with a broad spectrum of sera, though they showed reduced activity with clade C and E sera. Finally, the clusters in Fig. 3 showed generally consistent trends supporting the preceding observations.

Identification of the clade of an infecting virus on the basis of a serological assay. We used the normalized matrices that had served as the basis for identifying serological clustering patterns to select 5 of the 97 gp120 proteins in checkerboards 1 and 2 as potential serological typing reagents. The aim of the exercise was to determine whether we could identify reagents that allowed the identification of the genetic clades associated with HIV-1-positive sera from clades A through E. The selected proteins were those that had shown the highest genetic clade specificity in terms of their serological reactivity patterns. The specificity of the serological reactivity shown by each

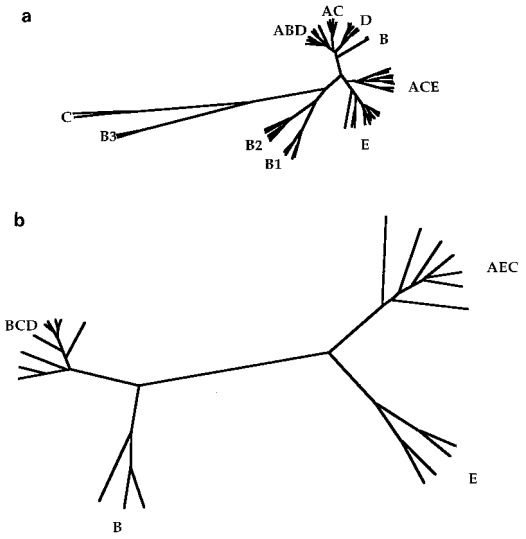

FIG. 3. Cluster analysis of protein seroreactivity patterns. Proteins are clustered on the basis of their capacities to serve as antigens for panels of sera derived from individuals infected with genetically diverse forms of HIV-1 representing the major phylogenetic clades as described in the text. The clusters are marked with letters indicating the genetic clade(s) of the proteins that predominate within that cluster. (a) Radial tree displaying the relationships in the seroreactivities of proteins included in checkerboards 1 and 2. The proteins included in each cluster are as follows: cluster ABD, A_UG276, A_92RW009, D_UG266, D_UG270, D_UG274, B_92HT599, A_92RW024, A_93RW004, B_92BR003, A_93RW020, A_93RW018, A_92RW023, A_92RW025, A_93RW005, B_92BR004, D_93UG067, D_93UG065, and B_92TH026; cluster AC, A_DJ258, C_UG268, A_92UG029, A_92RW008, C_ZAM18, C_DJ259, A_DJ263, A_92UG031, C_92BR025, and A_93RW022; cluster D, A_92RW016, D_92UG038, D_93UG070, D_92UG035, D_93UG059, D_93UG053, B_93US103, D_SG365, D_92UG024, D_92UG001, and D_92UG021; cluster B, B_93US104, B_92US715, and B_BK130; cluster ACE, A_UG273, C_93MW101, C_SG364, A_92RW021, A_92RW020, A_92RW026, E_CM241, E_92TH001, E_93TH236, E_92TH023, and E_92TH009; cluster E, E_92TH007, E_92TH011, E_92TH005, E_92TH021, E_CM238, E_93TH251, E_93TH253, E_CM235, E_92TH022, E_CM244, E_CM240, E_93TH975, E_CM242, E_93TH231, E_93TH232, E_93TH235, E_92TH019, E_92TH020, E_CM246, E_CM239, E_92TH003, E_93TH250, and E_93TH234; cluster B1, B_92BR018, B_92BR017, B_93US102, B_92BR020, B_91HT652, B_92US711, B_92BR030, and B_92US716; cluster B2, B_BK132, B_US4, B_CM237, B_RYCA2, B_BZ165, D_92UG005, B_BZ164, and B_91US712; cluster B3, B_93US101, B_92TH014, and B_92HT596; cluster C, C_SM145 and C_ZAM20. (b) Radial tree displaying the relationships in the seroreactivities of proteins included in checkerboard 3. The proteins included in each cluster are as follows: cluster AEC, A_92UG031, A_92RW026, A_92RW023, A_DJ258, C_DJ259, A_DJ263, and E_93TH251; cluster E, E_92TH001, E_93TH236, E_92TH020, and E_CM240; cluster B, B_92TH026, B_91US712, and B_92US711; cluster BCD, B_92US715, D_92UG035, D_93UG053, C_UG268, D_93UG070, D_92UG021, D_UG274, D_93UG067, and C_94ZW106. Protein names are preceded by the genetic clade with which they are associated and an underscore.

gp120 was calculated by averaging the normalized serum antibody binding ratios for each gp120, both within and outside the same clade as the gp120 under analysis; the gp120 protein that showed the greatest difference between the two average values was considered to be the protein with the most power to discriminate sera of that particular clade. Initially, we selected the best 25 gp120s (five per genetic clade), then the best 10 (two per clade), and then the best 5 (one per clade). Comparative analyses using the best 25, 10, and 5 gp120s revealed no advantage in choosing more than one gp120 per genetic clade (data not shown), and so for the sake of simplicity, we performed further analyses with one gp120 per clade only. The five gp120s deemed most discriminatory for the genetic clades A through E (A, 92RW021; B, 93US101; C, ZAM20; D, 92UG024; E, 93TH234) were then analyzed for their reactivities with sera from different clades (Fig. 4).

This analysis showed that even focusing only on those proteins that had the greatest power to discriminate between sera still allowed ambiguities that would result in a misidentification of the genetic clade associated with the infecting virus. To reduce this problem, the behaviors of the sera relative to all five proteins were considered, rather than a simple prediction of the clade of each serum based on the gp120 protein with which it had the highest reactivity. The average reactivity was calculated for each of the five gp120 proteins for each of the five groups of sera. This enabled us to identify trends: both clade A and C sera reacted strongly with the clade C gp120 ZAM20, but clade A sera tended to react better than clade C sera with the clade A gp120 92RW021. To see how well our panel of proteins served as indicators of the clade of the infecting HIV-1 strain for each given serum sample, sera were excluded one at a time from the calculation of the average behavior of the group and treated as if they were from an unknown clade. Using this method, we were able to identify correctly the genetic clades corresponding to 16 of 19 sera (all clade A, B, and E sera were correctly identified, as were 2 of 3 clade C sera and 3 of 5 clade D sera). This result compares favorably with the alternative analysis using the average binding ratios shown in Tables 7 to 9 and with predictions based on the clade of the gp120 with the highest reactivity among the 97 proteins tested; the latter would have resulted in a correct identification of only 11 of 19 sera.

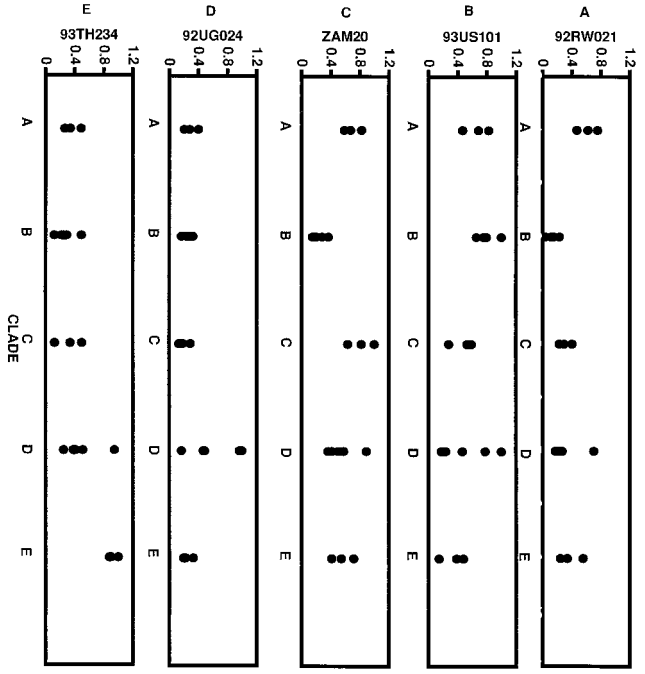

FIG. 4. Serological reactivities of the five proteins selected as those best able to distinguish the clade associated with a given serum. The ordinate shows the normalized seroreactivity scores from the ELISA checkerboards. Plot A shows the seroreactivities for the protein 92RW021, the protein best able to distinguish clade A from nonclade A sera, displayed to show the specificity and the level of cross-reactivity of 92RW021 with sera from each of the five clades tested. Similar plots are generated for the proteins best able to distinguish sera from the other four clades. The highly cross-reactive MK serum from clade D accounts for the high score among the clade D sera seen in each of plots A, B, C, and E. Only the gp120 protein best representing each clade is shown; however, similar plots were created for the top three proteins for serotyping from each clade, and the patterns were generally very similar to those presented here, although the specificity was slightly reduced in the analyses that used the second- and third-tier proteins.

It should be noted that the same test set of sera were used both to select the proteins with the most discriminating power and to test the ability of the panel of five selected gp120s to distinguish between the serotypes. To this extent, therefore, there is a bias in the analysis, and so we cannot be sure how well the selected gp120 proteins would perform if used to analyze a blinded set of sera. However, given the data currently available, the set of five proteins that we have identified would be a reasonable choice for incorporation into the design of future studies.

DISCUSSION

The data that we have obtained from three extensive checkerboard neutralization analyses have not allowed us to identify neutralization serotypes of HIV-1 that correspond to the genetic clades. An additional, more sophisticated analysis of the neutralization data reported here reinforces this conclusion and identifies factors such as infectivity enhancement that contribute to the interpretation of our present observations (23). In both studies, we find that neutralization by HIV-1-positive sera both within and across clades tends to be sporadic in nature: some isolates are fairly sensitive to neutralization, and others are resistant; some sera possess relatively broad and potent neutralizing activity, but most lack it. An additional complication is that some sera enhance rather than neutralize HIV-1 infection in vitro and that some isolates are particularly susceptible to infectivity enhancement (23).

We have previously made similar observations from studies on the neutralization of primary isolates from clade B. In one study of the development of antiviral immune responses during primary infection, autologous-virus neutralizing activity appeared in patients at different rates and to very different extents. For example, one individual, AD-6, eventually developed an autologous neutralization titer (ID_{90}) of >1:500, whereas two other individuals studied over approximately the same time course either failed to develop any autologous neutralizing antibodies (AD-13) or did so to titers of only 1:32 (AD-11) (24, 32). Furthermore, a more extensive checkerboard analysis of sera from HIV-1 (clade B)-infected individuals who progressed to AIDS revealed that neutralization of a panel of primary clade B isolates was weak and sporadic (5). In contrast, sera from long-term survivors of HIV-1 infection neutralized the same isolates much more broadly and potently, confirming that primary isolates can be neutralized by polyclonal human antibodies, provided that antibodies of an appropriate, albeit ill-defined, specificity are present (5). As the sera that we used in the present inter- and intraclade neutralization study were mostly not derived from long-term survivors, the results obtained are not inconsistent with those of our prior studies. Furthermore, others have found primary virus neutralization by HIV-1-positive sera to be, in general, fairly weak and sporadic in nature (7, 17, 28, 54, 57, 60).

Might methodological considerations affect our conclusions? We have used an assay format in which sera are titrated at a fixed virus inoculum and the ID_{50} and ID_{90} values are determined. It is not uncommon to observe only a limited degree of virus neutralization with such an assay and uncloned isolates. For example, our experience is that many HIV-1-positive sera cause 50 but not 90% neutralization, as the neutralization curves tend to plateau between the 50 and 90% marks. The residual infectious virus represents the nonneutralized fraction. Although we have presented ID_{50} and ID_{90} neutralization data, we place more emphasis on the ID_{90} values because of the questionable biological significance of 50% neutralization. However, other assay formats, such as the infectivity reduction procedure, may enable small decreases in virus infectivity to be better quantitated, allowing weak, clade-specific neutralization patterns to be discernible under certain circumstances (28, 53). It is presently uncertain whether such patterns, while statistically robust, have biological significance. We believe that our procedures would have detected strong, clade-specific patterns of neutralization had these been present, a conclusion reinforced by the analyses presented in the accompanying report (23).

The conclusions that we draw here and in the accompanying report (23) are consistent with the findings of others (7, 17, 53, 54, 57). However, one previous report indicated that clade B and E sera were best able to neutralize clade B and E isolates, respectively, which would be consistent with distinct neutralization serotypes corresponding to the genetic clades B and E (28). We were unable to discriminate between clades B and E in any of the three neutralization checkerboards that we performed. One explanation for the discrepant results between the two studies might be the use of different serum samples; it is notable that the clade E sera used by Mascola et al. (28) contained particularly high-value anti-gp120 titers (Fig. 1f). Thus, some sera or serum pools that neutralize isolates from clades B and E in a clade-specific manner can be identified (23, 28). Such sera may be of particular value as typing reagents. However, our findings suggest that most sera from individuals infected with clade B and E strains do not contain high titers of clade-specific neutralizing antibodies, a conclusion consistent with those of other studies (17, 53). The use of sera from long-term survivors of infection with HIV-1 isolates from different clades may be particularly valuable for the identification of neutralization serotypes, and of typing reagents, because of the breadth and potency of intraclade neutralization shown by long-term survivor sera against clade B strains (5). However, identifying long-term survivors of non-clade B HIV-1 infections will not be simple; for example, the HIV-1 clade E epidemic in Thailand is of such recent origin that no individual has yet been infected for sufficient time to meet the definition of a long-term survivor (5, 18, 47, 55). Furthermore, there are few studies that would help to identify long-term survivors of HIV-1 infection in Africa, where clade A, C, and D strains predominate (2, 11, 45, 56).

In contrast to our inability consistently to identify clade B- and E-specific reagents in neutralization assays, we find that clade B and E isolates and sera can be distinguished from one another serologically by the pattern of anti-gp120 antibody reactivity. This is consistent with our observations that gp120 molecules from clades B and E are highly divergent antigenically, judged by MAb reactivity profiles (36). Overall, our serological studies indicate that the clade E forms a quite distinctive serotype at the level of anti-gp120 antibodies and that clade B is also distinguishable from clades A, C, D, and E, albeit less robustly than the clade E serotype can be distinguished. Within the overall clade B serotype, Thai and Brazilian regional subserotypes may sometimes be discernible, although we have only a limited set of data pertaining to this issue. To some extent, clades A and C can be serologically distinguished from one another and from the other clades, but this is not always observed, and any distinction is weak. The observation that the gp120 molecules from clade D isolates tend to be poorly immunoreactive compared with gp120 molecules from the other clades may be a consequence of the high degree of sequence divergence found among clade D isolates (19, 20, 41). For example, clade D sera might react more poorly with heterologous clade D gp120s than with the analogous epitopes perhaps presented more prominently on gp120 molecules from other clades. Epitopes around the CD4-binding

site of gp120 are quite well conserved between clade D and B gp120s (36) and tend to stimulate serologically abundant antibodies (34); this might account for the observation that clade D sera show a tendency to react more strongly with clade D and B gp120s than with those from the other clades. It has been reported that gp120 molecules from at least some clade D isolates show unusual structural features (15, 59), that the consensus V3 loop of clade D isolates is particularly distinctive (19, 20), that some clade D isolates are abnormally cytopathic in vitro (11, 45), and that certain clade D strains appear to be exceptionally pathogenic in vivo (11, 45). Whether any or all of these observations are linked is unknown.

Our serological analyses are consistent with the prior studies of others, although our methodology is different. There is general agreement that clades B and E can be distinguished serologically by V3 peptide or gp160 binding assays and form distinctive serotypes (6, 17, 43, 51). The Brazilian clade B variant sera can also be identified by V3 peptide serology (6, 43). Sera from clades A and C are very hard to distinguish from one another by V3 peptide assays but can be distinguished from sera from clades B, D, and E (6, 43). This is a consequence of the almost identical clade A and C V3 sequences, found among a significant fraction of sequences from individuals infected with HIV-1 strains from either of those clades (20). Clade D forms a separate serotype in V3 peptide assays, although clade D sera can be fairly broadly reactive, especially with clade B V3 peptides (6, 43). As antibodies to the V3 loop contribute significantly, but probably not overwhelmingly, to the total anti-gp120 antibody response that we have measured (31, 34), it is not surprising that there are broad similarities, yet also clear differences, between our data and those derived from V3 peptide assays.

It is, however, clear that clade-specific serological and antigenic variations in gp120 molecules, or fragments thereof, do not translate into strong functional differences at the level of virus neutralization. That the genetic clades do not directly correspond to neutralization serotypes is not inherently surprising. Even if we ignore the possibility that antibody-induced enhancement of infection may to some extent counteract virus neutralization (23), there are several complexities to appreciate. Firstly, the clades of HIV-1 are defined on the basis of primary sequence, whereas virus neutralization is a much more complex process which depends on antibody interactions with epitopes that are influenced by the tertiary and quaternary structures of glycoprotein oligomers (46). Determining the relationship between primary sequence and protein conformation is difficult at best (38) and is especially so for a heterooligomer such as the HIV-1 envelope glycoprotein complex. It is obvious that certain structures, such as the CD4-binding site, in the envelope glycoproteins must be functionally conserved; to some extent, this conservation extends to the antigenic level, as some antibody epitopes on monomeric gp120 are highly preserved within and between clades (36). Unfortunately, such epitopes are not necessarily the most potent targets for primary virus neutralizing antibodies (33, 35, 37). However, certain human MAbs raised to conserved structures on clade B viruses are able to neutralize at least a subset of viruses from clades A, C, D, E, and F (49), although it should be noted that the identity of the principal neutralizing determinant on primary HIV-1 isolates is not yet known, nor is it certain that there actually is one.

In view of all of these factors, it does not seem reasonable to expect, a priori, that the meld of antibodies found in the polyclonal response of humans to HIV-1 infection should result in neutralization patterns that conform neatly to the genetic clades. Neutralization of T-cell line-adapted strains of HIV-1 is correlated with MAb binding to envelope glycoprotein oligomers on the surfaces of infected cells (46), and this is likely to be true also of primary strains. However, studies of antibody binding to the envelope glycoproteins of primary strains on the surface of infected PBMC are complicated by the presence of monomeric CD4-gp120 complexes on the surfaces of these cells (61). Alternative analytical procedures should be developed for further studies on HIV-1 neutralization serotypes.

Although there is clearly not a one-to-one correspondence between neutralization serotypes and genetic clades, the question remains: are there any serotypes at all? Rephrased, do the genetic clades have any relevance for vaccine development? The answer is complex, as protective immunity to HIV-1 is likely to involve more than just neutralizing antibodies. There is a growing understanding that an important facet of any effective HIV-1 vaccine is likely to be its ability to induce a strong cellular, particularly cytotoxic T-lymphocyte (CTL), immune response (24). The impact of genetic clades on the CTL response is poorly understood, yet CTL epitopes are continuous in nature and may be influenced significantly by clade-dependent amino acid sequence variation. As to the issue of humoral immunity, we cannot yet provide a definitive answer to the relevance of the genetic clades. Neutralization serotypes can be identified, but these do not correspond well to the genetic clades except under limited circumstances; a further discussion of this issue is presented in the accompanying report (23).

It should be noted that neutralization of primary HIV-1 isolates by most HIV-1-positive sera is generally so weak and sporadic even within a clade that any common serotype may reflect more the absence of strong cross-neutralization responses than their universal presence. This, in itself, has implications, as neutralizing antibody responses to the present generation of subunit gp120 vaccines are extremely weak or nonexistent against primary isolates from within the same clade B as the immunogen (8, 16, 29, 50, 58); responses to these vaccines do not approach even the weak neutralization response to natural infection. It is thus unreasonable to expect these immunogens to induce antibodies capable of significant cross-clade neutralization. Our results should not, therefore, be taken to support the idea of vaccine trials with the currently available clade B subunit gp120s in areas where infections with HIV-1 strains of other clades are more prevalent; we believe that such studies would not be fruitful.

More optimistically, strong cross-clade neutralization by a minority of HIV-1-positive sera, and by certain MAbs (49), can sometimes be observed. If whatever is responsible for efficient neutralization of primary HIV-1 isolates by HIV-1-positive sera can be identified and the MAb epitopes can be fully characterized, it is possible that an appropriate immunogen can be designed to induce broadly active immune responses. At least so far as humoral immunity is concerned, an immunogen with this characteristic could be effective against more than one genetic subtype of HIV-1. The binding sites on HIV-1 for antibodies able to neutralize strains from across the genetic clades are not known at present, but the V3 loop is unlikely to be a major target for them (23, 36, 37). If V3-directed antibodies were significantly involved in heterologous neutralization, neutralization serotypes with some resemblance to the patterns shown by V3 peptide- or gp120-binding antibodies might have been expected. This was not observed, and we do not expect that the V3 loop will play a significant role in any vaccine aimed at combating HIV-1 strains from multiple clades.

It is still reasonable to assume that the induction of broadly and potently neutralizing antibodies able to combat the initial

virus inoculum remains a desirable feature of an HIV-1 vaccine. The creation of an immunogen able to induce such antibodies is an important long-term goal, and the identification of the targets for such antibodies might significantly facilitate this effort. More intensive studies of the immunogenicities and antigenicities of HIV-1 proteins, combined with a greater understanding of the human immune system, should be made the highest priority for future vaccine research efforts. A fundamental research framework enabling rational vaccine design must be put in place if we are eventually to succeed in developing a globally effective HIV-1 vaccine.

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