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Longitudinal Antibody Responses in People Who Inject Drugs Infected With Similar Human Immunodeficiency Virus Strains

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Background: Multiple factors influence the human immunodeficiency virus (HIV) antibody response produced during natural infection, leading to responses that can vary in specificity, strength, and breadth.

Methods: People who inject drugs identified as recently infected with HIV (n = 23) were analyzed for clustering of their viral sequences (genetic distance, <2%). Longitudinal antibody responses were identified for neutralizing antibody (Nab) potential, and differences in antibody subclass, specificity, and Fc receptor ligation using pseudovirus entry and multiplexed Fc array assays, respectively. Responses were analyzed for differences between subject groups, defined by similarity in the sequence of the infecting virus.

Results: Viral sequences from infected individuals were grouped into 3 distinct clusters with 7 unclustered individuals. Subjects in cluster 1 generally had lower antibody response magnitudes, except for antibodies targeting the V1/V2 region. Subjects in clusters 2 and 3 typically had higher antibody response magnitudes, with the Fv specificity of cluster 2 favoring gp140 recognition. NAb responses differed significantly between clusters for 3 of 18 pseudoviruses examined ($P < .05$), but there were no differences in overall NAb breadth ($P = .62$).

Discussion: These data demonstrate that individuals infected with similar viral strains can generate partially similar antibody responses, but these do not drastically differ from those in individuals infected with relatively unrelated strains.

Keywords. HIV; neutralizing antibody; antibody development; people who inject drugs; cluster linkage.

A successful protective human immunodeficiency virus (HIV) vaccine will most likely need to generate a broad and diverse antibody response that can directly neutralize the virus or direct virions to other cytotoxic mechanisms. An important underlying element of any vaccine construct is the ability to generate a similar immune response in a majority of vaccinees. Previous research examining the development of the natural humoral response to HIV infection has generally focused on individuals infected with naturally diverse viral strains and has found a variable response that is influenced by early events in infection, as well as the ongoing evolution and changes of the viral population [1–3]. Currently, it is not fully understood to what extent individuals initially infected with similar viral strains generate analogous humoral immune responses when exposed for a similar time frame.

The majority of research examining the development of HIV humoral immunity has focused on the neutralizing antibody (NAb) response, and in particular the development of broadly NAbs [4–6]. In this context, it was observed that broadly NAb responses from individuals infected with HIV subtype B viruses target the CD4 binding site more potently than non-B subtype viruses, which conversely were superior at targeting the V2 loop [7]. Work in nonhuman primates comparing animals infected with identical inocula suggests that the antibody response is influenced by initial viral genotype. For example, SIVmac239 is poor at eliciting NAb, which SHIVAD8 is able to do [8]. In addition, simian-human immunodeficiency virus–based NAb response can develop to be specifically targeted to certain areas of the viral envelope, such as the V1/V2 region, similar to the targeting of the CAP256-VRC26 family of NAb [5, 9]. These

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data suggest that the underlying infecting viral strain may influence the path of NAb maturation.

HIV vaccine trials, patient cohorts, and nonhuman primate studies have highlighted the importance of antibody effector functions in the protection against infection [10–14]. In the successful RV144 HIV vaccine trial, levels of nonneutralizing antibodies targeting the V1/V2 loop that induced antibody-dependent cellular cytotoxicity function were found to be elevated in individuals protected from infection [14]. In addition, experimentally eliminating Fc receptor interactions has been shown to limit the protective effect of passive antibody protection in animal models [15, 16].

An additional indication that infection with highly similar viruses may lead to similar immune responses was reported in a case of adult monozygotic twins infected through injection drug use with identical HIV strains [17]. It was observed that the twins' immune responses and the natural evolution of their viral populations were highly similar throughout their disease [17]. However, this may not be the outcome for all matched twin infections, particularly in twins infected at birth [18].

A large study of putative transmission pairs identified in the Swiss HIV Cohort attempted to determine what the antibody imprinting capacity is for highly similar viral strains [19]. That study found that several components of the NAb and binding antibody responses were significantly associated with members of transmission pairs or clusters. The authors estimated that the infecting viral genetic make-up may contribute up to 13% and 19% of the antibody imprinting found in NAb and immunoglobulin (Ig) G binding responses, respectively [19].

In the late 1980s and early 1990s, the HIV epidemic in Baltimore, Maryland, was growing exponentially, with prevalence in emergency departments almost doubling from 1988 to 1992 (from 6% to 11%) [20]. Many of these new infections occurred because of needle sharing among people who inject drugs, which, given the early stage of the epidemic and the limited barrier for infection due to direct viral transmission, may have led to infection clusters with similar viral strains. The current study aimed to determine whether people who injected drugs early during the HIV epidemic were infected with genetically similar viral strains, and whether this viral genetic similarity influenced the resulting anti-HIV humoral response in these individuals.

METHODS

Study Population

Individuals who enrolled from 1988–1989 in the AIDS Linked to the IntraVenous Experience (ALIVE) study and who were found to be HIV positive on enrollment were screened using a validated multiserological assay algorithm for recent infection (<6 months) [21]. Briefly, patient serum or plasma samples that had a positive viral load (second-generation branched DNA assay; Chiron) were screened for lower antibody titer using the BED HIV titer assay, as well as for low antibody affinity using a modified Bio-Rad HIV enzyme-linked immunosorbent assay [22]. If a sample was found to be below established cutoffs for both values, the patient were determined to be recently infected, and of these patients, those who remained in the study for 3–6 years of follow-up without starting highly active antiroretrovial therapy had their initial plasma viral populations sequenced with a validated site-directed next-generation sequencing (NGS) assay for 2 genetic regions (gp41 and pol) [23].

Individuals whose viral populations were successfully sequenced in both regions at baseline and ≥1 subsequent time point were included for subsequent antibody analyses. In addition, the initial diagnosis sample or the closest available sample with adequate volume was sequenced with single-genome amplification (SGA) for either the entire gp160 gene or the C1-V5 region of gp120 if gp160 amplification was not successful. Heparinized plasma or serum samples for 3 time points (0.75–1.5, 2–3, and 5–6 years after diagnosis, or the closest time point available before these windows) were used for subsequent antibody assays. All participants provided informed consent for testing and sample storage for further analysis.

Viral Linkage

NGS-derived consensus sequences for pol and gp41 from each individual at their initial diagnosis sample were combined into a single consensus sequence for each region and concatenated. The concatenated viral sequences were aligned with HIV subtype B reference sequences and examined for linkage. Sequences that were genetically linked (<2% total genetic distance between the whole group) were classified into clusters or determined to be unclustered (HIV-TRACE) [24]. Clusters were phylogenetically confirmed using SGA-derived sequences from either the initial diagnosis sample or the closest time point available.

Antibody Binding, Immunoglobulin Class, and Fc Receptor Screen

A subset of plasma and serum samples from all 16 of the subjects that were clustered and 7 of the 9 unclustered subjects were analyzed for antigen binding and Fc interactions, as reported elsewhere [25, 26]. Briefly, antibodies specific for 40 antigens derived from the HIV envelope and other structural and accessory genes were probed with 15 Fc receptor and other detection reagents to profile the antibody response [27]. Samples were analyzed at a dilution of 1:5000 for FcgRs, anti-human (aHu) IgG, and aHu IgG1 detection reagents, whereas the dilution used for analysis with aHu IgA, IgD, IgM, IgG2–4, and C1q detection reagents was 1:1000. Detection with aHu IgG was also performed using 1:1000 and 1:25 000 dilutions. The median fluorescence intensity was reported for each measurement.

NAb Responses

Neutralization was measured using single-round-of-infection HIV-1 Env pseudoviruses and TZM-bl target cells, as described

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elsewhere [28, 29]. Neutralization curves were fit by nonlinear regression using a 5-parameter hill slope equation. The 50% and 80% inhibitory dilutions (ID50 and ID80) were reported as the reciprocal plasma dilutions required to inhibit infection by 50% and 80% respectively. Plasma from all available time points for clustered and unclustered individuals were screened for neutralization activity using a standard 20 pseudovirus panel, as reported elsewhere [27]. Results from 2 of the pseudoviruses (231965.c1 and 242-14) were removed from analysis because they were sensitive to residual heparin in the plasma, rendering the data unreliable. Samples that demonstrated >30% neutralization breadth were analyzed for their monoclonal NAb fingerprint, and these were compared for correlations between samples as reported elsewhere with a modification for using the 18 virus panel [28].

Statistical Analyses

Fold changes in mean values per each group and *t* tests were used to compare antigen binding, immunoglobulin class, and Fc receptor binding measurement. Excel software was used to calculate fold change and perform *t* tests. Fc array heat map and Volcano plots were generated in R using the "gplots" package. Longitudinal NAb potency for individual pseudoviruses tested and overall breadth (median inhibitory dilution, >40:1 dilution) for each individual were analyzed using a linear mixed-effects model for differences between the clusters overall, as well as the clusters versus the unclustered group.

Sequence data for the study are available through GenBank (accession nos. MN412134–MN412403). The full neutralizing antibody and binding antibody data sets are available on request (aredd2@jhmi.edu).

RESULTS

Of the individuals identified as being recently infected on enrollment into the original ALIVE cohort (n = 80), 23 had their viruses successfully sequenced in both pol and gp41 genetic regions at multiple time points (Figure 1). These individuals were all African American, mostly men (19 of 23), and they were grouped according to the genetic similarity of their NGS-derived viral sequences at their initial study time point (Figure 1). These clusters were confirmed by means of SGA of the viral envelope (Supplementary Figure 1). The demographics and HIV disease status for the individuals in the clustered groups (clusters 1, 2, and 3; n = 5, 5, and 6 respectively) did not differ significantly from the unclustered individuals (n = 7) (Table 1). It should be noted that the baseline viral loads for these patients were determined using a second-generation branched DNA assay, which has been shown to report viral loads at 2-fold lower levels than reverse-transcription polymerase chain reaction–based assays [30].

Using a high-throughput antibody binding assay that examines epitope targets (n = 40), as well as antibody Fc characteristics (n = 15), "snapshots" of the humoral response for each subject were captured. Visualizing individual feature measurements relative to one another at the earliest time point tested (mean, 1.06 years after diagnosis; interquartile range, 1.02–1.14 years) for each subject, and sorting the features by Fc and Fv specificity revealed that some antibody attributes were shared by subjects belonging to the same viral cluster (Figure 2). It was also apparent from this analysis that at a biophysical level, there were large differences in both the level of responses and the specificity of those responses between subjects, regardless of cluster. These differences may be related to different environmental factors or the distinct genetic profiles of each individual that participated in this study, among others. As anticipated, subjects typically had a wide range of responses, both to the envelope glycoprotein as well as to the other accessory, regulatory, and structural proteins of the HIV proteome; these responses were typically observed across time points.

To examine how the humoral responses of individuals differed between subjects infected with similar strains and those infected by disparate strains, the Fc array data were compared between subjects in viral clusters. Subjects in cluster 1 typically exhibited lower-intensity gp140-specific responses. In some cases, IgM antibodies that were specific for gp120 were elevated in cluster 1, particularly in comparison with cluster 2 (Figure 3A). Although the magnitude of these differences tended to decrease over time, they were still present at the later time points tested (data not shown). Similar to those in cluster 2, cluster 3 subjects had higher responses against many of the Env antigens, although there was a subset of responses to 1 specific sequence of the V1/V2 region of the viral envelope, as defined by a V1V2 epitope probe (gp70 from strain 62357) that was higher in cluster 1 than in cluster 3 (Figure 3B). These differences also held over time, with subjects in cluster 1 increasing in V1/V2 response magnitude longitudinally (data not shown). Antibodies with high C1q ligating capacity often appeared elevated among subjects in cluster 3 when compared with cluster 2, suggesting an elevated antibody-associated complement response in this cluster. Consistent with distinctions in the genetic sequence of the infecting virus, antibody profiles from nonclustered subjects did not seem to strongly support their inclusion in clusters 1, 2, or 3 (data not shown).

NAb responses were measured using an 18-psuedovirus panel, and the longitudinal ID50 values were examined for differences between viral groups as compared with the unclustered control group (Figure 4). NAb responses increased over time, culminating in a relatively broad response by 5–6 years after infection; however, the potency of the NAb response to specific pseudovirus strains differed dramatically between subjects. In addition, some NAb responses waned later in disease compared with the second time point tested.

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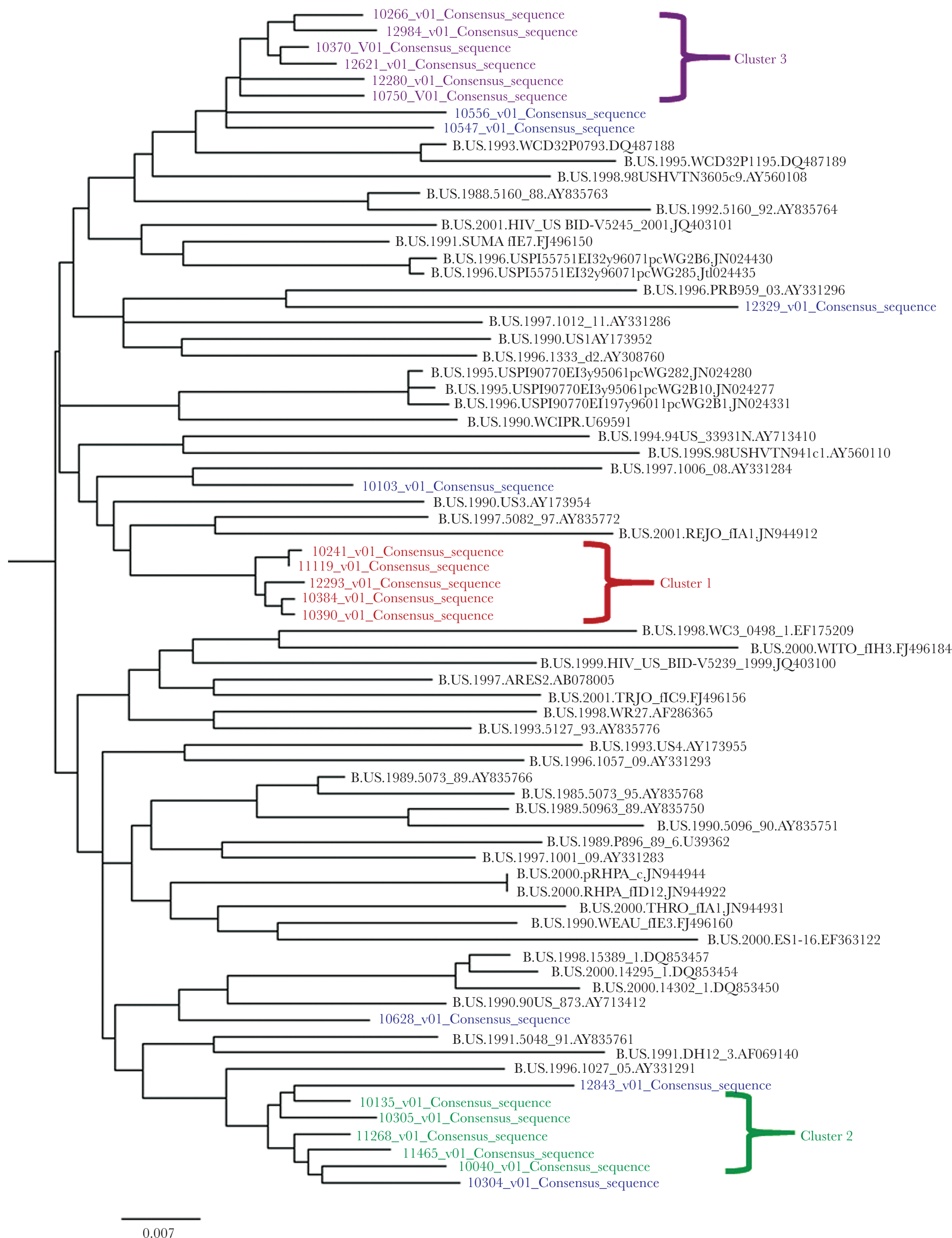

Figure 1. Maximum likelihood phylogenetic tree of concatenated consensus sequences derived from next-generation sequencing of pol and gp41. Clusters of sequences with <2% genetic distance are indicated by color (cluster 1 [*red*], cluster 2 [*green*], and cluster 3 [*purple*]), along with a group of unclustered individuals (*blue*). Subtype B reference sequences are shown in black. The scale of the phylogenetic tree is 0.007.

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Table 1. Patient Demographics by Cluster

Cluster	Patients, No.	Age, Median (IQR), y	Male Sex, %	Current IDU, %	Baseline CD4 Cell Count, Median (IQR), Cells/μL	HIV Level, Median (IQR), Copies/mL[a]
1	5	27.7 (24.2–36.4)	80	100	753 (563–1019)	1236 (305–29 712)[b]
2	5	30.4 (28.3–37.3)	100	80	821 (506–1000)	5098 (678–6312)
3	6	29.5 (27.0–35.4)	83.3	100	765 (365–1048)[b]	1725 (966–9543)[b]
Unclustered	7	30.7 (25.7–39.8)	71.4	100	858 (474–1074)	1626 (384–11 720)

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range.

[a]Circulating HIV levels were measured using a second-generation branched DNA assay (Chiron).

[b]One value missing for measurement.

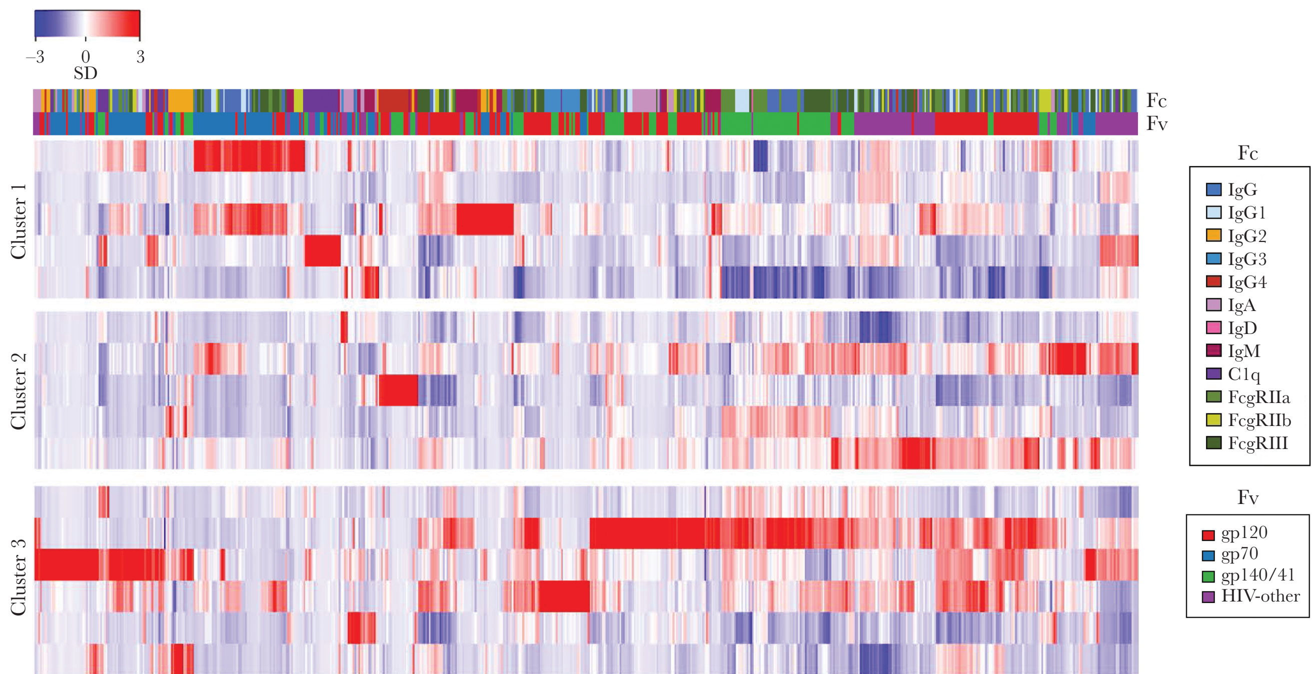

Figure 2. Heat map of antibody response features for all subjects at their first tested time point (centered, scaled, and truncated at ±3 standard deviations [SDs]). Subjects are sorted by viral sequence cluster, and features are clustered using hierarchical clustering. The color bar indicates the Fc characteristic (*top*) and Fv specificity (*bottom*) for each antibody measurement. Abbreviations: gp, glycoprotein; HIV, human immunodeficiency virus; Ig, immunoglobulin.

The longitudinal breadth of the NAb response did not differ between the 4 groups (P = .62) (Figure 5A). However, significant differences were observed between the 4 groups for 3 individual pseudoviruses: KER2008.12 (P = .02), X26191.2.48 (P = .02), and X0013095.2.11 (P = .002) (Figure 5B–5D). For these 3 pseudoviruses, the NAb responses for each group were compared alone with the unclustered group. For X0013095.2.11, each of the 3 clustered groups differed significantly from the unclustered group ($P < .02$), but only group 3 differed significantly from the unclustered group for KER2008.12 (P = .04). No individual clustered group was significantly different from the unclustered group for X26191.2.48.

A serum neutralization fingerprinting analysis with the 18-pseudovirus panel was performed for all sample time points that demonstrated >30% breadth (Supplementary Figure 2). Despite the observed viral sequence similarities within each cluster, the neutralization fingerprints of individuals within a cluster were varied and were not found to be more similar than between clusters, suggesting a diversity of antibody-specificities in the different clusters (Supplementary Figure 3).

DISCUSSION

Genetic analysis of viral strains in individuals who entered the ALIVE cohort study after being recently infected with HIV identified 3 groups of individuals who were infected with highly similar viruses, suggesting they may have been part of transmission clusters. In several other subjects, the sequence similarities of the pol and gp41 regions of their infecting strains were not similar enough to each other or the 3 groups to be classified as members of a cluster. It should be noted that genetic analysis of multiple HIV isolates reveal that the highest rate of mutation is in the gp120 region, with reduced mutation rates in gp41 and

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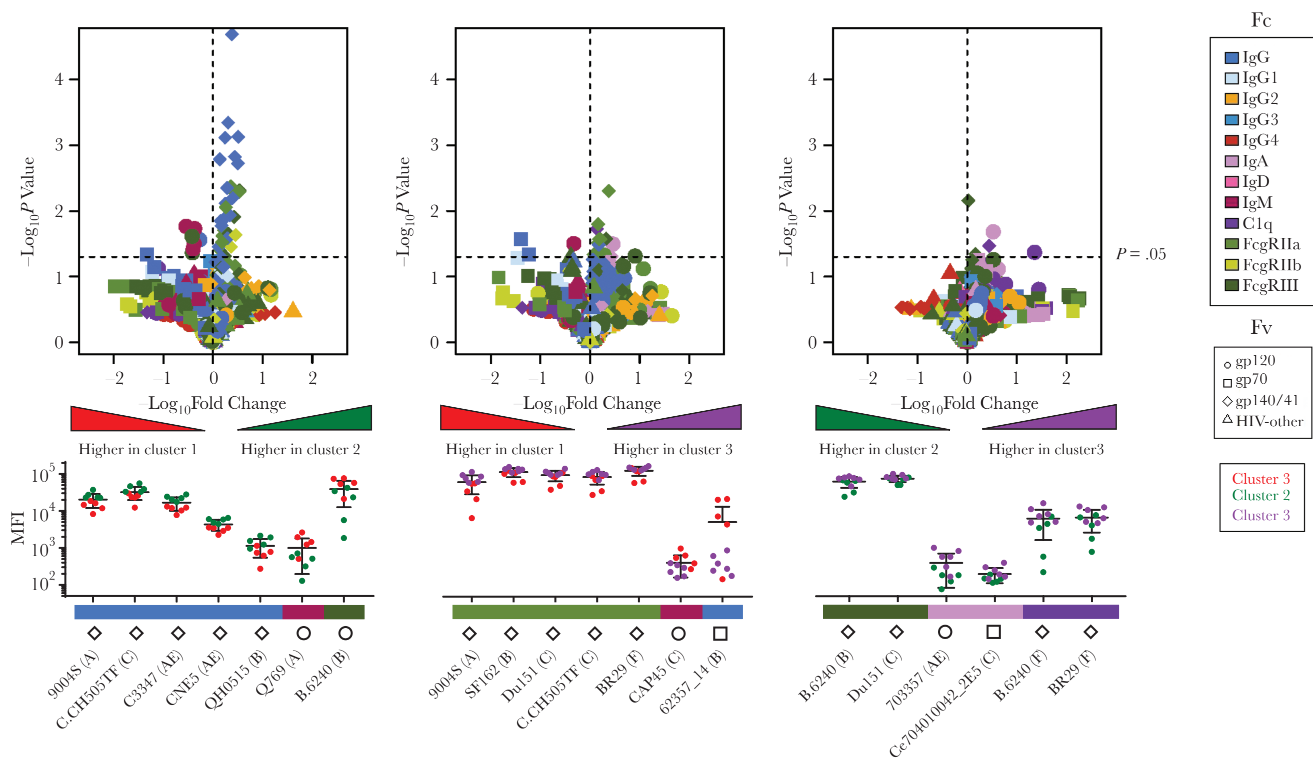

Figure 3. *A,* Volcano plots of fold change and *P* values indicating differences in binding antibody responses defined between clusters by the Fc array. Antigen specificity is indicated by shape, and Fc characteristics are indicated by colors; the dotted lines represent an unadjusted *P* value of .05 and a fold change of 0. Responses that are significantly more related to 1 cluster are shown above the dotted line, and are skewed to the left or right according their level of fold change. *B,* Dot plots of select features by group. Antigen specificity is indicated by shape and Fc characteristics are indicated in the color bar, and human immunodeficiency virus (HIV) strain/clad is labeled below the x-axis. Lines and whiskers represent means and standard deviations. Viral clusters are indicated by colored dots, including clusters 1 (*red*), 2 (*green*), and 3 (*purple*). Abbreviations: gp, glycoprotein; Ig, immunoglobulin.

pol [31]. In addition, the NGS protocol used here does not allow for determining whether the 2 regions examined are collinear on the same viral isolate. Therefore, gp160 or partial gp120 SGA-derived sequences were analyzed as well, and they found to agree with the cluster groupings.

Interestingly, the SGA and NGS data showed that the subjects were infected with relatively uniform viral inocula, in contrast to previous findings in acutely infected people who inject drugs [32]. This may be because these patients were infected early in the epidemic in Baltimore, when there were limited numbers of viral strains circulating in the community. Alternatively, these patients were identified early in infection but not during the acute stage, and the viral populations that we examined may therefore represent the strain that grew out after initial infection with multiple transmitted strains. It should also be noted that the multiassay algorithm used in our study to identify recently infected individuals may have biased our findings by excluding those who rapidly developed a potent anti-HIV antibody response [33]. This bias, together with the lower viral loads seen with the branched DNA assay, may have contributed to the lower viral loads observed in this cohort, because it has been shown that individuals with low viral loads develop mature antibody responses at slower rates [33]. However, the initial viral loads did not differ significantly between groups.

These subjects' participation in the ALIVE cohort study allowed for longitudinal analysis of the natural development of their anti-HIV humoral response before initiation of highly active antiretroviral therapy. To clarify the relationship between the infecting strain of a virus and the humoral responses it induces, tools to characterize the antibody repertoire were implemented and provided insights into the mechanisms of these differences. The Fc array data revealed distinct patterns between subject clusters for the binding antibody responses, such as stronger V1/V2 binding and weaker gp140 binding in cluster 1 subjects. Responses among subjects in clusters 2 and 3 typically had similar magnitude responses toward gp140s, whereas cluster 2 seemed to have lower-magnitude gp120-specific responses. Not only did Fv specificities diverge, but the Fc characteristics of antibodies also differed between the subject clusters, with complement binding and IgM antibodies being some of the most noticeable points of distinction between subject groups.

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Subject	Years since diagnosis	Cluster	YU2.DG	001428-2.42	Q259.17	3168_V4_C10	CH038.12	7165.18	0013095-2.11	247-23	CH070.1	CNE5	CNE55	KER2008.12	M02138	26191-2.48	Q461.e2	TH976.17	620345.c1	ZM135.10a
10241	1.14	1	<25	<25	<25	<25	<25	41	183	56	<25	<25	<25	46	132	<25	<25	<25	<25	57
10241	5.40	1	47	<25	<25	225	160	85	356	56	44	32	74	50	96	41	42	57	60	105
10384	1.23	1	104	36	56	254	<25	<25	219	<25	<25	<25	<25	<25	52	<25	<25	<25	<25	<25
10384	2.75	1	128	203	208	327	<25	27	676	49	<25	<25	<25	43	102	<25	<25	<25	<25	71
10384	5.73	1	338	120	81	109	159	253	737	155	64	47	56	89	265	59	35	283	98	269
10390	1.28	1	35	<25	694	352	<25	44	364	<25	<25	<25	<25	37	<25	<25	<25	114	<25	<25
10390	2.34	1	69	<25	222	44	<25	56	886	51	<25	<25	<25	<25	92	<25	<25	26	<25	<25
10390	4.98	1	84	<25	204	151	236	133	551	72	68	47	60	74	348	67	38	715	117	155
11119	0.97	1	<25	<25	<25	<25	<25	26	277	<25	<25	<25	<25	33	<25	<25	<25	<25	<25	45
11119	2.98	1	83	41	34	140	<25	48	403	39	<25	<25	<25	34	102	<25	<25	64	<25	54
11119	5.46	1	<25	<25	<25	<25	825	<25	199	65	60	53	46	85	259	68	45	81	85	81
12293	0.99	1	<25	135	26	184	<25	<25	45	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
12293	1.99	1	<25	<25	38	88	<25	<25	55	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
12293	5.78	1	<25	<25	<25	<25	506	<25	175	58	53	43	38	74	76	50	26	88	53	74
10040	0.59	2	<25	<25	<25	33	<25	<25	31	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
10040	2.68	2	<25	<25	<25	<25	<25	60	375	57	<25	<25	<25	<25	<25	<25	<25	127	<25	<25
10040	5.77	2	194	73	<25	62	280	176	373	100	44	60	<25	58	156	57	45	99	73	85
10135	1.55	2	45	64	<25	<25	<25	29	461	61	<25	<25	<25	<25	214	<25	<25	<25	<25	93
10135	2.03	2	110	53	28	46	25	113	967	63	<25	<25	<25	75	493	<25	<25	90	<25	265
10135	5.61	2	369	56	479	69	231	137	314	86	54	55	75	58	173	64	40	580	74	117
10305	3.05	2	227	<25	378	346	187	84	126	<25	53	<25	<25	43	123	<25	<25	154	<25	<25
10305	5.44	2	81	27	118	<25	311	166	167	62	68	89	101	76	251	49	54	265	66	58
11268	1.02	2	<25	<25	<25	128	25	173	93	<25	<25	<25	<25	53	86	<25	<25	78	<25	32
11268	2.11	2	63	37	<25	45	99	274	54	<25	<25	<25	<25	57	77	<25	<25	<25	<25	<25
11268	5.01	2	63	<25	99	97	699	449	121	68	39	50	28	59	25	52	25	59	47	48
12465	1.04	2	<25	<25	<25	<25	<25	<25	116	<25	<25	<25	61	<25	97	<25	<25	<25	<25	<25
12465	5.50	2	53	<25	<25	212	175	367	565	86	41	36	2,402	80	526	66	44	153	105	144
10266	1.09	3	<25	<25	<25	<25	<25	29	62	25	<25	<25	<25	40	<25	<25	<25	<25	<25	<25
10266	3.07	3	50	<25	1,359	<25	<25	51	42	<25	<25	<25	<25	<25	26	<25	<25	<25	<25	<25
10266	5.62	3	72	<25	325	110	188	100	187	96	65	60	34	94	97	79	41	123	108	142
10370	1.42	3	267	309	3,253	727	189	329	576	83	92	34	71	166	177	54	<25	312	178	118
10370	3.10	3	336	234	2,575	478	102	247	753	91	71	<25	<25	157	275	<25	<25	283	98	139
10370	5.10	3	60	<25	549	<25	168	258	331	92	76	45	104	119	248	57	31	976	122	99
10750	1.78	3	107	161	<25	195	49	62	185	75	44	50	56	104	342	58	31	233	32	178
10750	2.78	3	259	187	221	1,074	89	105	801	125	50	<25	167	145	504	75	<25	593	116	638
10750	3.77	3	137	42	<25	194	955	1,079	615	133	74	45	144	106	416	76	49	199	83	128
12280	1.06	3	43	415	123	140	<25	<25	139	<25	<25	<25	<25	115	135	<25	<25	54	<25	<25
12280	2.84	3	410	1,334	998	341	<25	79	252	63	<25	119	<25	70	113	240	<25	163	129	<25
12280	5.72	3	402	389	977	223	542	650	601	205	66	148	36	149	137	247	60	581	188	187
12621	1.08	3	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	27	<25	<25	<25	<25	<25	<25
12621	5.55	3	40	<25	<25	28	272	70	175	54	54	25	<25	47	89	55	<25	<25	33	39
12984	1.05	3	82	225	<25	89	28	87	353	48	<25	<25	<25	194	103	<25	<25	36	<25	107
12984	2.88	3	281	439	118	273	94	231	867	70	<25	<25	<25	360	233	143	<25	97	<25	254
12984	5.27	3	119	271	30	129	248	422	345	87	63	81	60	322	107	93	34	307	97	109
10103	1.13	99	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	99	<25	<25	<25	<25	<25
10103	1.63	99	<25	42	<25	<25	378	432	66	66	49	35	37	83	163	86	37	228	76	113
10304	1.08	99	107	<25	<25	129	<25	<25	<25	37	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
10304	2.59	99	<25	<25	<25	<25	<25	36	45	65	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
10304	3.14	99	33	<25	<25	<25	<25	<25	37	28	39	31	29	44	26	41	26	36	42	40
10547	1.05	99	37	<25	55	98	153	1,338	117	60	45	56	32	104	79	83	35	135	73	82
10556	1.04	99	<25	<25	113	41	<25	<25	<25	41	<25	<25	<25	48	55	<25	<25	128	<25	86
10556	2.57	99	28	98	118	121	<25	70	42	<25	<25	<25	<25	59	84	<25	<25	58	<25	<25
10556	3.58	99	59	92	<25	<25	316	344	40	54	37	55	45	86	113	60	46	64	71	84
10628	0.97	99	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
10628	2.47	99	<25	<25	<25	<25	<25	35	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
12329	0.99	99	<25	<25	138	1,874	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25
12329	3.01	99	30	49	66	36	<25	<25	<25	<25	<25	<25	<25	<25	74	<25	<25	<25	<25	<25
12329	3.99	99	<25	<25	44	<25	36	<25	80	67	67	52	<25	65	116	79	<25	<25	<25	80
12843	1.04	99	58	<25	<25	54	<25	<25	<25	<25	<25	<25	<25	<25	93	<25	<25	<25	<25	<25
12843	3.02	99	482	244	<25	242	<25	<25	132	<25	<25	<25	<25	92	212	<25	<25	<25	<25	<25
12843	5.46	99	248	99	515	110	290	306	650	55	46	67	30	64	489	43	<25	129	121	110

Figure 4. Heat map of longitudinal serum and plasma median inhibitory dilution (ID50) measurements of neutralizing antibody potential for all patient samples tested, grouped by cluster and time since diagnosis. The potency of neutralization for each pseudovirus tested (labeled above) is color coded, showing no neutralization (*white*) and low (*green*), moderate (*yellow*), and high (*red*) neutralization.

In most cases, these differences persisted throughout later time points. Interestingly, the binding responses of individuals with unclustered viral sequences did not present with the same patterns as the members of the cluster with which they were most closely genetically related. The neutralization patterns on a heterologous virus panel showed higher similarity within all the clusters than the unclustered viruses in only 1 instance (X0013095.2.11). Neutralization patterns are derived from the set of epitopes targeted by NAbs, a subset of those targeted by binding antibodies; these data suggest that the infecting viruses in this cohort did not trigger highly similar antibodies in different individuals.

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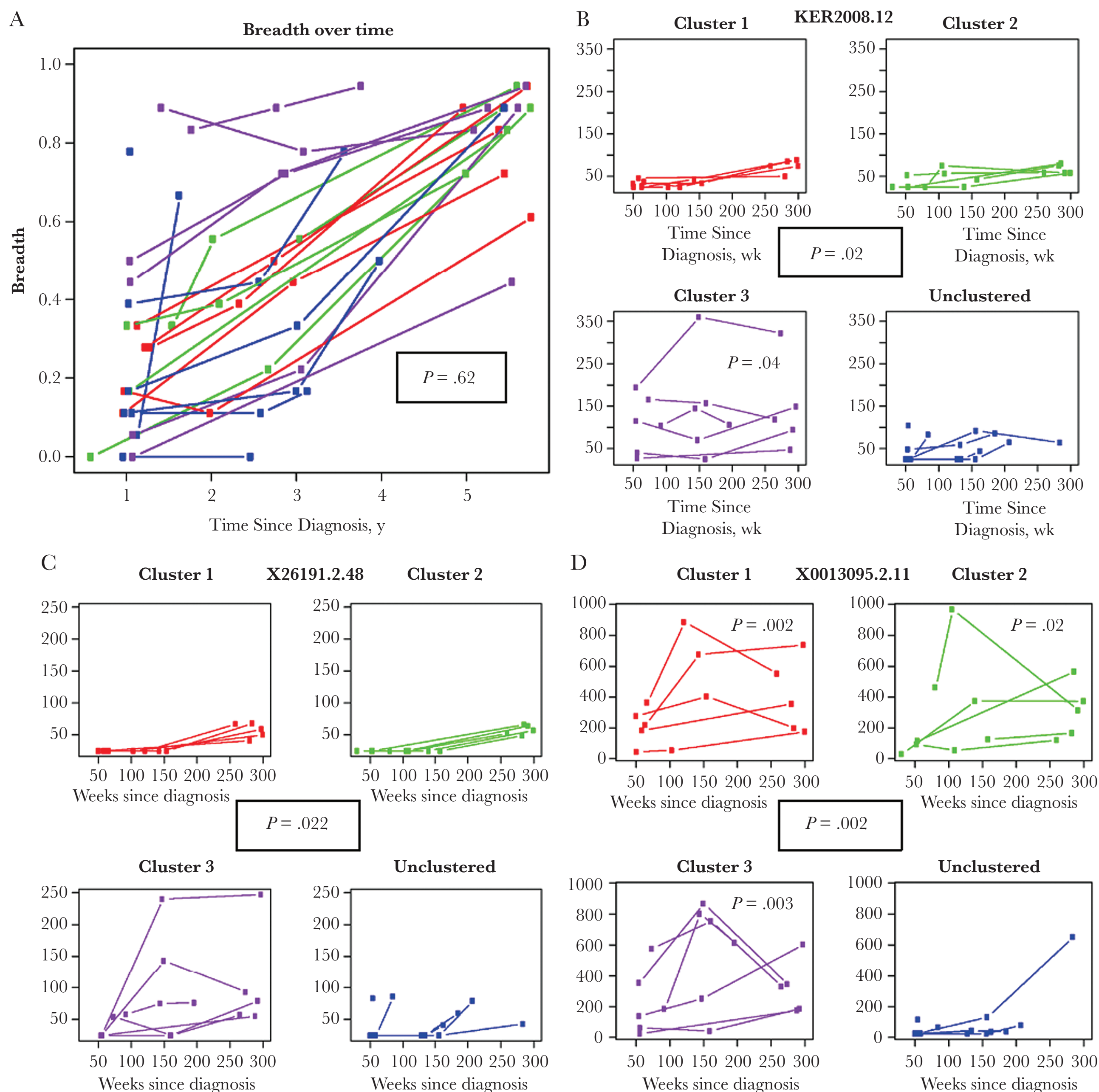

Figure 5. Longitudinal plots of the median inhibitory dilution (ID50) neutralizing antibody (Nab) potential for all samples tested. *A,* Overall breadth did not differ between groups (cluster 1 [*red*], cluster 2 [*green*], cluster 3 [*purple*], and unclustered individuals [*blue*]). *B–D,* The NAb responses to 3 pseudo typed viral strains differed between all clusters and the unclustered group (overall *P* values shown; linear mixed effects). In addition, individual clusters that differed significantly from the unclustered group are indicated.

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These data support the concept that antibody characteristics and repertoire are mildly affected by the infecting strain, an observation also made as part of an analysis of the Swiss HIV cohort study, in which it was estimated that up to 19% of the IgG binding response may be due to antibody imprinting of the infecting strain [19]. There are some critical differences, however, between these 2 studies. First, the Swiss study was significantly larger, providing it with statistical power that was not available in the current analysis. Second, several unclustered patients in our study were infected with viral sequences similar to those in some of the clustered patients, although they were greater than the 2% cutoff used. This could have diluted the effect seen between the unclustered subjects and the clustered groups.

The ALIVE cohort had several advantages compared with the Swiss cohort, particularly when we tried to investigate the effects of antibody imprinting from the infecting strain. In the ALIVE study, subjects were followed up longitudinally, whereas

the Swiss analysis was cross-sectional. More importantly, the patients whose findings we analyzed are people who inject drugs and were most likely infected intravenously, whereas the Swiss cohort comprised patients infected through a variety of modes of transmission, which may affect the types of humoral responses seen in these patients [19]. One limitation of both studies is that humoral response attributes are affected by host genetics, among other factors, which were not controlled for or investigated in our study but are expected to affect observations independent of infection strain

The results presented here further support the findings in the Swiss cohort that a portion of the NAb response may be attributable to antibody imprinting by the infecting strain [19]. The NAb responses in the clustered groups differed significantly from those in the unclustered in 3 of the 18 pseudoviruses tested, and they also trended toward differences for 3 other viruses ($P < .10$; data not shown). The Swiss cohort study found that the NAb responses to 50% of the pseudoviruses tested (7 of 14) differed significantly in putative transmission clusters [19]. The associations between NAb response, binding antibody profiles, and viral genetic similarity presented here provide evidence that the infecting viral strain may have a small but significant effect on the resulting anti-HIV NAb response.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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