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Breadth of Neutralizing Antibody Response to Human Immunodeficiency Virus Type 1 Is Affected by Factors Early in Infection but Does Not Influence Disease Progression▽

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The determinants of a broad neutralizing antibody (NAb) response and its effect on human immunodeficiency virus type 1 (HIV-1) disease progression are not well defined, partly because most prior studies of a broad NAb response were cross-sectional. We examined correlates of NAb response breadth among 70 HIV-infected, antiretroviral-naïve Kenyan women from a longitudinal seroincident cohort. NAb response breadth was measured 5 years after infection against five subtype A viruses and one subtype B virus. Greater NAb response breadth was associated with a higher viral load set point and greater HIV-1 *env* diversity early in infection. However, greater NAb response breadth was not associated with a delayed time to a $CD4^+$ T-cell count of <200, antiretroviral therapy, or death. Thus, a broad NAb response results from a high level of antigenic stimulation early in infection, which likely accounts for prior observations that greater NAb response breadth is associated with a higher viral load later in infection.

Some human immunodeficiency virus (HIV)-infected individuals develop broad neutralizing antibody (NAb) responses, but the factors that lead to NAb response breadth remain elusive. Several cross-sectional studies have found that individuals with greater NAb response breadth have higher contemporaneous viral loads, suggesting that the presence of a greater amount of viral antigen may promote a greater NAb response breadth (9, 10, 25, 30, 32). However, because viral load and NAb response breadth were measured at the same time after HIV type 1 (HIV-1) acquisition in prior studies, it is difficult to discern cause and effect. There is also evidence that NAbs adapt in response to the evolving HIV-1 population throughout infection (11, 29, 35), which may contribute to a greater overall response breadth. Together, these studies support a model in which a greater NAb response breadth is driven by a higher level of antigenic stimulation, in terms of both the absolute level of virus and viral diversity. Confirmation of this model requires an assessment of the temporal relationship of viral load, HIV-1 diversity, and NAb response breadth.

In addition to uncertainty regarding the determinants of NAb response breadth, the consequences of a broad NAb response for HIV-1 disease progression remains controversial. Broad NAb responses have been found in long-term nonprogressors (LTNPs) in some studies, suggesting that NAbs may contribute to control of infection in these individuals (6–8, 22, 27, 37). Other studies have found no evidence for NAb control in LTNPs (1, 2, 14, 18), including studies in which NAb response breadth was lower in LTNPs (10) or elite controllers (15, 25) than in viremic individuals. A detailed analysis of NAb response breadth versus clinical outcome has not yet been conducted, particularly for individuals with typical HIV-1 disease progression.

To investigate the determinants and consequences of NAb response breadth in HIV-1 infection, we examined NAb responses in women in a seroincident cohort in Mombasa, Kenya, that began in 1993 (19–21). For each woman, the time of infection was defined by both HIV-1 serology and RNA testing (17). Women who had a banked plasma sample ~5 years after the estimated time of HIV-1 infection were included in this study. This time period was chosen to maximize the chances for the NAb response to broaden while generally testing prior to the beginning of clinical immunodeficiency. We only included samples prior to the initiation of antiretroviral therapy (ART), which in this cohort began in March 2004, according to the WHO and Kenyan National guidelines. Plasma samples meeting these criteria were identified from 70 women and came from a median of 5.0 (range, 4.5 to 6.8) years postinfection (ypi). This subset of women was representative of the entire cohort in terms of their behavioral, clinical, and demographic characteristics (data not shown).

HIV-1 subtype A accounts for most of the infections in this cohort (28), including 72% of the 53 women in this study for whom *env* subtype information was available (Fig. 1). Therefore, to test neutralization of viruses relevant to women in this population, we measured NAb response breadth against a panel of five recently transmitted subtype A viruses from other individuals in this cohort, which represented a spectrum of neutralization sensitivities (4). We also included one com-

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	env V1-V5	Viral Load				Neutralization IC50				
Plasma ID	Subtype	Set Point	~5 YPI	SF162	Q461.d1	Q168.b23	Q842.d16	Q769.	Q259.	NAb Breadth
QA255	A	4.81	4.07	649	1699	1404	524	126	271	5.3
QD359	A	4.6	5.33	2815	2581	3359	286	157	107	5.0
QB857	D	4.46	4.37	491	1500	1063	255	143	331	5.0
QD063	.	5.12	5.18	453	1217	1803	331	136	217	4.7
QC511	A	4.08	4.43	998	447	278	231	139	510	4.7
QA966	A	6.2	5.42	3300	1249	1136	289	85	3151	4.7
QA638	C	5.61	6.11	742	718	863	170	117	--	4.7
QA433	A	5.62	5.31	2253	2198	777	293	152	95	4.7
QC915	A/D	5.12	4.73	1042	610	830	449	250	110	4.3
QC152	A	5.85	5.94	442	915	1706	550	111	421	4.3
QA465	D	4.46	5.43	1218	1329	1575	293	74	--	4.0
QA261	A	5.33	5.5	547	2948	2386	840	--	--	4.0
QC036	A	5.02	4.95	496	900	511	349	80	128	3.7
QB765	A	5.39	4.54	206	2294	3194	145	145	128	3.7
QD151	.	5.19	5.38	731	1334	527	245	133	--	3.3
QA692	A	3.5	4.99	3322	1986	354	354	--	--	3.3
QA520	A	3.52	5.75	1625	3231	4000	178	--	--	3.3
QB554	.	5	4.77	209	2421	1192	322	--	--	3.0
QB374	A	.	5.86	372	1768	497	377	148	--	3.0
QD740	.	4.61	4.68	216	973	478	164	87	128	2.7
QC370	D	5.26	5.08	362	357	268	73	238	--	2.7
QA596	A/D	5.05	5.2	80	568	411	--	2683	2683	2.7
QA354	.	.	4.49	477	487	517	170	--	258	2.7
QA039	A	4.42	4.91	277	1894	1044	201	2025	147	2.7
QD416	A	4.64	4.5	1390	729	742	139	97	246	2.3
QC890	A	3.96	5.3	215	102	257	95	162	94	2.3
QB990	A	4.24	4.1	2585	630	109	189	--	70	2.3
QB247	A	5.17	4.7	492	424	228	98	--	285	2.3
QA917	.	4.64	5.81	871	93	--	228	--	69	2.3
QC869	A	2.11	3.32	1066	706	80	--	--	--	2.0
QC001	.	.	5.48	523	71	1391	2945	--	--	2.0
QB368	A	2.74	4.5	437	2425	1160	75	76	96	2.0
QA779	A	4.64	4.75	704	191	218	--	1367	--	2.0
QA584	D	4.78	4.28	180	263	446	109	141	120	2.0
QA284	A	4.67	4.45	264	327	484	102	73	--	2.0
QA106	A	4.67	3.23	2981	774	154	255	--	--	2.0
QA078	A	2.99	4.01	415	1790	1153	394	89	--	2.0
QD370	C	4.36	4.39	351	575	400	160	--	--	1.7
QD342	.	4.07	5.17	696	231	1030	200	--	114	1.7
QC914	.	.	3.74	557	368	128	173	86	--	1.7
QC805	A	5.01	6.66	301	648	187	109	--	--	1.7
QC449	A	5.89	5.13	947	291	271	--	--	--	1.7
QC048	D	5.59	4.12	204	313	212	153	116	171	1.7
QB670	A	4.9	4.11	1974	391	259	170	--	74	1.7
QA028	A	3.29	4.42	2025	2310	1454	1092	--	--	1.7
QD625	A	3.66	3.57	586	758	271	97	81	--	1.3
QD121	A	3.91	2.74	1056	605	25	129	104	87	1.3
QC322	A	4.72	5.2	148	290	225	129	111	151	1.3
QA538	A	4.05	4.92	136	130	289	157	105	89	1.3
QA210	A	4.82	5.61	2827	181	85	93	--	--	1.3
QC729	.	3.92	4.9	707	185	80	--	--	--	1.0
QB868	.	3.97	4.38	267	435	311	76	--	--	1.0
QB084	.	.	4.15	--	272	--	96	--	--	1.0
QB056	.	3.28	3.97	167	192	399	--	--	1367	1.0
QA595	A	4.71	5.53	286	488	214	90	--	--	1.0
QD385	A	5.21	3.44	542	127	186	--	--	94	0.7
QC406	C	5.28	5.23	82	270	457	--	--	--	0.7
QA874	A	3.69	4.47	164	317	172	89	82	--	0.7
QD481	.	2.59	4.44	--	109	345	--	85	--	0.3
QC344	.	3.58	4.26	193	191	313	141	--	--	0.3
QA203	A	3.89	5.61	--	265	--	--	--	--	0.3
QC100	D	2.68	1.7	--	--	--	--	--	--	0.0
QB593	.	.	2.48	83	153	226	81	82	85	0.0
QB216	D	5.35	5.71	123	69	195	--	76	69	0.0
QB212	A/C	3.24	4.3	210	106	--	--	--	--	0.0
QA523	D	5.48	4.47	89	168	186	82	--	--	0.0
QA376	.	5.54	5.15	79	222	--	--	--	--	0.0
QA281	A	3.8	5.07	--	161	--	--	--	--	0.0
QA268	A	4.3	4.02	126	--	--	--	--	--	0.0
QA252	D	3.11	4.97	177	96	--	--	--	--	0.0

FIG. 1. Summary of the IC_{50}s and NAb response breadth scores of 70 plasma samples. The first column indicates the subject identifier of each plasma sample, and the next three columns indicate the *env* V1 to V5 subtype (available for 53/70 women), the set point viral load (available for 64 women), and the viral load at ~5 ypi, when the NAb response breadth was measured. Data not available are indicated by a period. Each subsequent column shows the results with one panel virus (indicated at the top of the column). Results are the average of three experiments in which each plasma-virus pair was tested in duplicate. In the case of Q769 and Q259, two closely related viruses from the same individual were used in one (Q769.h5, Q259.d217) and two (Q769.b9, Q259.d226) of the three experiments. The IC_{50} for each plasma-virus pair is the reciprocal dilution of plasma that led to a 50% reduction in infectivity, averaged across the three experiments. IC_{50}s are shown in gray scale to represent increasing neutralization sensitivity, with white for values of <100, light gray for values of >101 and <1,000, and dark gray for values of >1,001. Plasma-virus

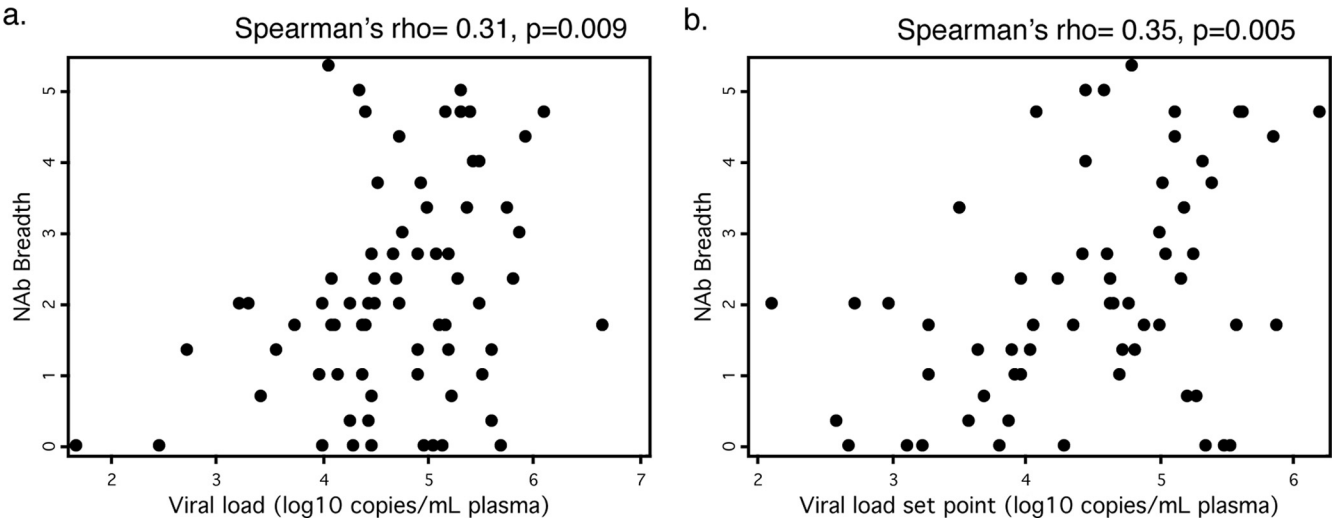

FIG. 2. Associations between NAb response breadth and viral load. In each plot, the NAb response breadth score is indicated on the *y* axis and the contemporaneous (~5 ypi) viral load (a) or viral load set point (b) is indicated on the *x* axis. Each point represents one individual. The results of Spearman correlation analysis are shown above the plots.

monly studied, easy-to-neutralize subtype B virus (SF162) for comparison to other studies. The TZM-bl neutralization assay, using pseudoviruses prepared with these six envelope variants and TZM-bl indicator cells, was performed as described previously (4, 36). The median inhibitory concentration (IC_{50}) was defined as the reciprocal dilution of plasma that resulted in 50% inhibition. Figure 1 shows the IC_{50} for each plasma-virus pair, averaged across three independent experiments that included duplicate testing of each pair.

In general, we found that the viruses that had been easily neutralized in prior screening with pooled plasma, Q461d1 and Q168b23 (4), were the most readily neutralized by individual plasma samples from women in this study (Fig. 1). Of the 70 plasma samples tested, 68 (97%) showed detectable neutralization activity (IC_{50}, >50) against Q461d1 and 60 (86%) showed activity against Q168b23. Most (76%) of the plasma samples also neutralized variant Q842d16 at detectable levels, although generally with lower IC_{50}s. By contrast, only about half of the plasma samples neutralized envelope variants Q769b9 and Q259d2.26 (51% and 46%, respectively). Almost all (93%) of the plasma samples neutralized SF162.

Given the different neutralization sensitivities of these viruses, we quantified the NAb responses in these individuals by using a previously described NAb response breadth score that takes into consideration the neutralization sensitivity of each virus (5). Briefly, the NAb response breadth score represents the number of viruses (out of six) that a given plasma sample neutralized at an IC_{50} that was higher than the median IC_{50} for that virus (across all 70 plasma samples). The response breadth score was calculated independently for each of three experiments, and the average scores are listed in Fig. 1. Among all of the individuals, the median response breadth score was 2 and the response breadth scores ranged from 0 to 5.3. A potential limitation of this approach is that response breadth was calculated by using a relatively small number of viruses. However, we found that NAb response breadth measured against this 6-virus panel was highly correlated with the NAb response breadth measured against an expanded 17-virus panel (including these 6 viruses plus an additional 11 viruses representing subtypes A, C, D, A/D, and B; J. Overbaugh et al., unpublished data), for a subset of 29 women whose plasma samples were tested against the expanded panel (Spearman's rho = 0.62, $P < 0.001$). Furthermore, the NAb response breadth score measured against this six-virus panel was highly correlated with NAb potency (Spearman's rho = 0.81, $P < 0.001$), a measure we have used in prior studies that takes into consideration the magnitude of the IC_{50} for each plasma-virus pair (5). These findings suggest that the NAb response breadth score measured against the six-virus panel is representative of the overall NAb response breadth.

We investigated whether NAb response breadth was associated with the contemporaneous plasma viral load, which was measured at the same time as NAb response breadth (4.5 to 6.8 ypi). Viral loads ranged from 1.7 to 6.7 $\log_{10}$ copies/ml among all of the individuals, with a median of 4.7 $\log_{10}$ copies/ml. As shown in Fig. 2a, individuals with higher viral loads had greater NAb response breadth (Spearman's rho = 0.31, $P = 0.009$), consistent with prior studies (9, 10, 30, 32). A similar relation-

pairs in which 50% neutralization was not detected at the highest plasma dilution (1:50) are indicated by a pair of dashes. The NAb response breadth score for each plasma sample was calculated as follows. For each experiment, the median IC_{50} for each virus (across all 70 plasma samples) was determined. Plasma samples were assigned a score of 1 for every virus against which their IC_{50} was greater than the median IC_{50}, and the score was summed across all six viruses. The NAb response breadth scores that are shown here (and which were used for analysis) were calculated by taking the average response breadth score across the three independent experiments; they were not calculated from the average IC_{50}s shown.

TABLE 1. Association between NAb response breadth and risk of HIV-1 disease progression[a]

Parameter	HR (95% CI),[b] *P* value	
	Univariate analysis	Multivariate analysis
NAb response breadth	1.27 (1.03–1.56), 0.03	1.06 (0.84–1.33), 0.6
Viral load set point	2.12 (1.38–3.25), 0.001	2.02 (1.26–3.23), 0.003

[a] HIV-1 disease progression was measured as the first occurrence of a $CD4^+$ T-cell count of <200, ART initiation, or death.
[b] CI, confidence interval.

ship was observed between viral load set point and NAb potency, a measure that takes into account the magnitude of neutralization (data not shown). There was no association between NAb response breadth and $CD4^+$ T-cell count (Spearman's rho = −0.15, $P = 0.2$) among the 64 women with contemporaneous $CD4^+$ T-cell counts available.

To further assess whether the viral load may drive NAb response breadth, we examined the relationship between the viral load set point and NAb response breadth. For each individual, the viral load set point was defined as the first available viral load measurement 4 to 24 months after infection (16), and this ranged from 2.1 to 6.2 $\log_{10}$ copies/ml (median, 4.6 $\log_{10}$ copies/ml) among the 64 individuals for whom this measurement was available. As shown in Fig. 2b, individuals with higher viral load set points had greater NAb response breadth at ~5 ypi (Spearman's rho = 0.35, $P = 0.005$). The viral load set point was also highly correlated with the viral load measured at ~5 ypi (Spearman's rho = 0.42, $P = 0.001$). Therefore, we investigated whether the relationship between NAb response breadth and the contemporaneous (~5 ypi) viral load could be explained by the viral load set point. In multivariate linear regression analysis, NAb response breadth was significantly associated with the viral load set point (coefficient of variation = 0.55, $P = 0.02$) but not with the contemporaneous viral load (coefficient of variation = 0.25, $P = 0.3$). Thus, the relationship between the contemporaneous viral load and NAb response breadth appeared to be driven by the viral load set point, with each 1-log increase in the viral load set point associated with an increase in the response breadth score of 0.55.

Given this association between the viral load set point and NAb response breadth, we wondered whether another factor in early infection—HIV-1 sequence diversity—might influence the development of NAb response breadth. Proviral HIV-1 sequences were available from 26 individuals and had been sampled a median of 87 (range, 17 to 299) days postinfection. For each individual, *gag* and *env* V1 to V5 diversity was calculated from a median of seven single-copy sequences per gene as described previously (26). Across all 26 individuals, the median *env* diversity was 0.28% (range, 0 to 4.0%) and the median *gag* diversity was 0.19% (range, 0 to 1.28%). Individuals with greater *env* diversity early in infection had greater NAb response breadth at ~5 ypi (Spearman's rho = 0.51, $P = 0.008$). However, there was no association between early *gag* diversity and NAb response breadth (Spearman's rho = 0.10, $P = 0.6$). Although both early *env* diversity and the viral load set point were associated with NAb response breadth, there was no association between these factors among the women in this study (Spearman's rho = 0.21, $P = 0.3$). However, in a larger study of 156 women in this cohort, women with greater early *env* heterogeneity (as measured by heteroduplex mobility assay) had higher viral load set points (31). Further work is needed to clarify whether early *env* diversity and the viral load set point are independent determinants of NAb response breadth or whether early *env* diversity may drive both the viral load and NAb response breadth.

Because the viral load set point and early *env* diversity have also been shown to be associated with HIV-1 disease progression in this cohort (17, 31), we explored the relationship of NAb response breadth, the viral load set point, and disease progression. We performed Cox proportional hazard analysis by using a composite survival outcome of time to the first occurrence of a $CD4^+$ T-cell count of <200, ART initiation, or death. Among all 70 women, 45 reached this composite outcome over a median of 6.8 years of follow-up after HIV-1 infection (range, 1.2 to 14.2 years). In univariate analysis, a greater NAb response breadth was associated with an increased risk of HIV-1 disease progression (Table 1, hazard ratio [HR], 1.27 per unit increase in breadth, $P = 0.03$). However, this association was attenuated, and no longer statistically significant, in a multivariate analysis adjusting for the viral load set point (HR = 1.06, $P = 0.6$). In this multivariate model, a higher viral load set point was associated with a greater risk of HIV-1 disease progression (HR = 2.02, $P = 0.003$), as expected. In a second multivariate analysis considering only those outcome events that occurred after NAb response measurement ($n = 25$ events among 50 women), there was an association between NAb response breadth and HIV-1 disease outcomes (HR = 1.39, $P = 0.03$) but again this did not persist after adjustment for the viral load (HR = 1.17, $P = 0.4$). Thus, we found no evidence that NAb response breadth affected HIV-1 disease progression independently of the viral load set point.

Based on the results of this and prior studies of the same cohort, we have begun to infer a model of the role of NAbs in natural infection (Fig. 3). Individuals with higher viral load set points and greater *env* diversity early in infection develop broader NAb responses at ~5 ypi. These findings support a

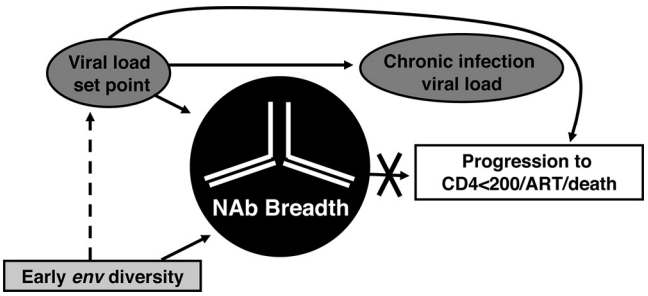

FIG. 3. Model of NAb response breadth in natural infection. Solid arrows indicate associations detected in this study, while dashed arrows indicate associations found in prior studies of the same cohort, and the crossed-out arrow indicates no association. Factors that contribute to greater NAb response breadth include the viral load set point and early *env* diversity, which have been found to be associated with one another in a prior study (31). Although NAb response breadth is associated with the chronic infection viral load in a univariate analysis, this is attributable to the viral load set point (no arrow depicted), and NAb response breadth does not affect disease progression.

model in which antigenic stimulation drives the NAb response breadth (9, 10, 30, 32). Importantly, because of the longitudinal follow-up in this study, we were able to infer a causal relationship between a higher viral load and both *env* diversity and a greater NAb response breadth. The importance of antigenic stimulation in promoting a broad NAb response is strengthened by our finding that early *env* diversity was associated with NAb response breadth while *gag* diversity was not, consistent with the fact that Gag is not considered a target for NAbs. Further evidence for this model may be derived from prior studies that demonstrated a relationship between greater time since infection and greater NAb response breadth (9, 23, 32). Taken together, these results indicate that prolonged high-level stimulation with a diverse set of antigens contributes to the development of a broad NAb response, and this process is likely to be set in motion early in HIV-1 infection.

We found no association between NAb response breadth and measures of HIV-1 disease progression (first occurrence of a $CD4^+$ T-cell count of <200, ART initiation, or death). Our results from a longitudinal study of a seroincident cohort strengthen prior evidence that NAbs do not contribute significantly to the control of HIV-1 infection (8, 12, 33). A possible explanation for the lack of association between a broad NAb response and an improved clinical outcome is that antigenic stimulation, although important for the generation of a broad NAb response, may actually impair other immune responses. Antigen persistence in chronic viral infection can lead to the loss of proliferative $CD4^+$ T cells (24), $CD8^+$ T-cell exhaustion, and loss of polyfunctional $CD4^+$ and $CD8^+$ T cells (3, 13, 34). Therefore, conditions that promote a broad NAb response may actually inhibit other protective responses in chronic HIV-1 infection. This is an important consideration for HIV-1 vaccine strategies, which may need to provide high levels and diversity of antigenic stimulation to elicit a broad NAb response while preserving other immune functions.

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