

Rapid Communication

Lack of neutralizing antibody response to HIV-1 predisposes to superinfection

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Abstract

Occurrences of HIV-1 superinfection offer a unique opportunity to investigate the correlates of immune protection. Here we describe the neutralizing antibody responses of a cohort of recently infected individuals who were screened for HIV superinfection. Three individuals identified with HIV superinfection had less cross-protective and autologous neutralizing antibody response than their non-superinfected case–controls. Neutralizing antibody may be crucial in the protection against superinfection and may explain why superinfection has only been documented following recent infection or treatment interruption. These data have considerable implications for vaccine development. Published by Elsevier Inc.

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Introduction

Most reports of intraclade HIV-1 superinfection have been described during primary infection (Jost et al., 2002; Koelsch et al., 2003; Yang et al., 2005; Smith et al., 2005a,b) or during a structured treatment interruption when the patient initiated antiretroviral therapy during primary infection (Altfeld et al., 2002). Both situations are associated with a relatively weaker cell-mediated and humoral immunologic response to autologous and heterologous viruses than is chronic untreated infection (Binley et al., 2004; Richman et al., 2003; Altfeld et al., 2002; Ortiz et al., 2001). Superinfection in humans, however, has been reported to occur even when there are broad CD8⁺ T-cell

responses to the primary virus, but the superinfecting virus in these cases did not share apparently important epitopes with the initial strain (Altfeld et al., 2002; Yang et al., 2005; Jost et al., 2002). Animal models suggest a role for neutralizing antibody (NAb) in the protection against superinfection (Shibata et al., 1996) and even against primary infection (Nishimura et al., 2002; Parren et al., 2001). To investigate this we performed a case–control study within a cohort previously screened for superinfection to examine NAb as a correlate of immune protection from superinfection during primary infection.

Results and discussion

All superinfected patients lacked detectable NAb to heterologous laboratory strains at baseline, which was significantly different than the detection of NAb in the non-superinfected control group (Fisher's exact: NL4-3 $p=.02$; JRCFSF $p=.05$). A weak response to autologous virus could be detected in only one

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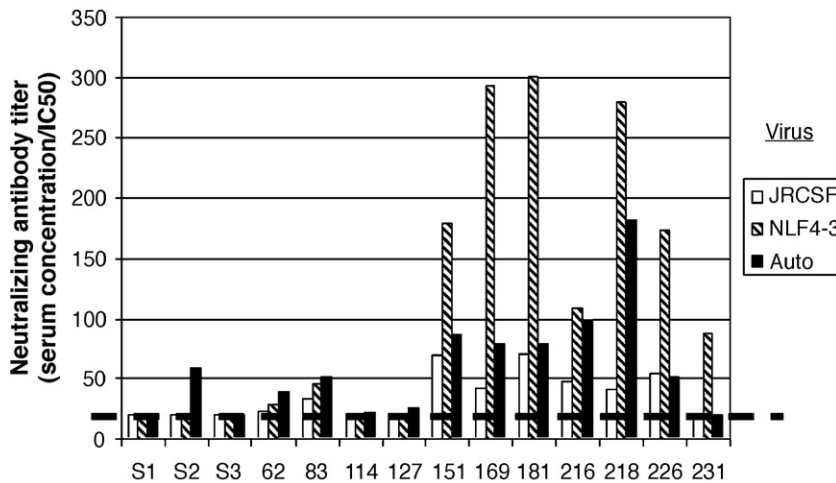
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of the three superinfected patients, suggesting a weaker response to autologous HIV-1 in these patients than in the 11 control patients (Fisher's exact; $p=.09$) (Fig. 1A). Two control patients (114 and 127) also had very little detectable NAb to NL4-3, which might mean that they were susceptible to superinfection but other factors may have prevented superinfection like degree of risk exposure.

The clinical significance of *in vitro* neutralization of laboratory strains remains unclear since NL4-3 is quite sensitive to antibody neutralization in the assay used (Richman et al., 2003). A trend (Mann-Whitney; $p=.09$) toward a longer delay between the estimated date of infection and study enrollment was observed among the superinfected group (mean 98 days) compared to the control group (mean 72 days) (Table 1). Since

the NAb responses increase throughout natural infection, this trend would theoretically bias the NAb titers to be higher in the superinfected group than the controls, in contrast to what was observed, which further underscores the observation that the superinfected group had less neutralizing antibody than the control group. We then investigated if the strength of NAb response correlated within individuals across all viruses tested at both baseline and six-month timepoints (i.e. did individuals with weaker NAb responses to one virus have weaker responses to other viruses?). We found a significant direct correlation between NAb responses to all three viruses at baseline. Measurements at 6 months, however, revealed that only the responses to the six-month autologous and JRCSF viruses remained significantly correlated (Supplementary Table 1).

A. Neutralizing antibody at baseline



B. Neutralizing antibody responses at six months

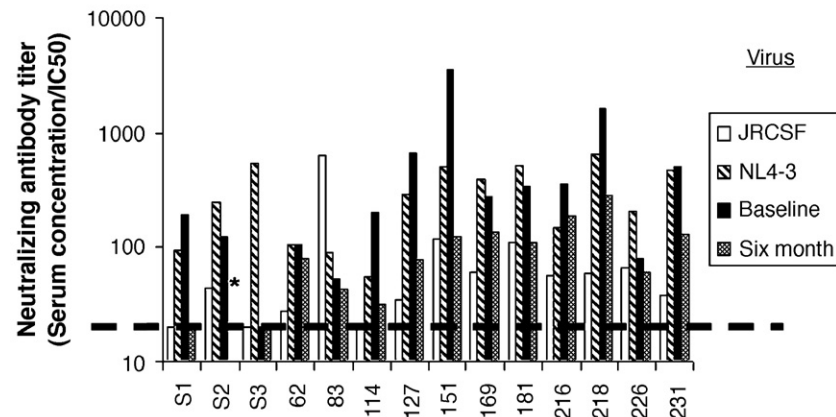


Fig. 1. Neutralizing antibody responses. (A) Neutralizing antibody responses at baseline. Baseline neutralizing antibody could be detected at the threshold 20:1 titer (dashed black line) in only 1/9 samples from superinfected patients vs. 28/33 samples from patients without evidence of superinfection ($p<0.001$). At time of baseline screening for superinfection, all superinfected patients lacked detectable neutralizing antibody to heterologous clones NL4-3 ($p=0.02$) and JR-CSF ($p=0.05$). Responses to autologous virus (Auto) could be detected in only one of the three patients ($p=0.09$), suggesting a weaker response to autologous HIV-1 in these patients than in the 11 control patients. (B) Neutralizing antibody responses at 6 months, representing after superinfection in the superinfected group. The six-month virus from S2 was unable to be reconstructed in the recombinant assay (*). Therefore, the neutralization sensitivity for this virus at this timepoint is unknown. However, when comparing NAb response to simultaneous autologous virus at 6 months, there was significantly less response in the superinfected group (S1 and S3) ($p=0.02$). There was also a trend for less NAb response to JRCSF ($p=0.08$) and baseline ($p=0.12$) viruses at 6 months, while there was no difference in responses to NL4-3.

Table 1
Patient summary

Patient	Age	HIV RNA (Log c/ml)	ΔHIV RNA	CD4	ΔCD4	Estimated days of infection at time of NAb measurement
S1	36	3.08	1.76*	571	-197	85
S2	33	3.38	2.80*	711	-385	84
S3	33	2.91	1.96*	616	-85	126
62	44	5.70	-2.01	552	-83	82
83	64	2.75	.28	1119	-383	67
114	63	3.24	-.70	492	-30	83
127	38	6.26	-.59	492	-199	43
151	35	3.89	-.10	557	-172	107
169	48	5.42	-.09	692	-165	182
181	36	5.27	.00	689	-207	45
216	37	3.07	.80	391	-95	73
218	47	5.46	-.20	311	-9	24
226	34	4.88	-.53	362	-68	55
231	31	4.28	-.58	557	-132	32

There were no significant differences in age or baseline CD4 count between the superinfected patients (bold) (S1, S2 and S3) and the controls. There were no significant differences between the two groups in the changes in CD4 counts (ΔCD4) over the 6 months after the first antibody measurement, which represented before and after superinfection in the superinfected group. However, there was a trend for baseline viral loads (Mann–Whitney; $p=0.12$) to be lower in the superinfected group. Viral loads increased significantly (ΔHIV RNA) in the superinfected group over the study period, indicating significantly poorer control of plasma viremia than in control patients ($p=0.02^*$). There was a trend (Mann–Whitney; $p=0.09$) toward a longer delay between the estimated date of infection and study enrollment among superinfected patients (mean 98 days) than non-superinfected patients (mean 72 days). This suggests a bias toward more mature antibody responses in the superinfected group, further underscoring the relative weakness of antibody responses in the superinfected patients.

Additionally, a recent report has demonstrated a positive correlation between heterologous NAb response and viral load (Deeks et al., 2006), which is in contrast to our superinfected group that demonstrated low viral loads despite low heterologous NAb responses (Supplementary Fig. 1). Perhaps, this discrepancy may be explained by vigorous cell-mediated responses, which are associated with lower HIV viral loads (Ogg et al., 1998), among these individuals prior to their superinfection, which has also been described previously (Yang et al., 2005; Altfeld et al., 2002). Taken together, these data may lend further evidence that a preventative HIV vaccine based solely on boosting the cell-mediated response may be inadequate.

In the superinfected patients, NAb responses to the baseline autologous and laboratory strain viruses were stronger after superinfection (Fig. 1B), especially the responses to NL4-3, which were no longer different between the two groups 6 months later. However, there still remained a trend for lower NAb levels in the superinfected group to baseline autologous virus (Mann–Whitney; $p=.12$) and JRCSF (Mann–Whitney; $p=.08$) (Fig. 1B). There was also a weak trend (Mann–Whitney; $p=.12$) for the superinfected group to have lower baseline viral loads, which could represent either initial infection with a less virulent virus or that the superinfected group, who was farther along in their infection, was closer to their viral setpoint than the control group whose viral loads were

elevated secondary to a more recent infection (Table 1). This is further highlighted by the significant increases in viral loads over the study period that occurred in the superinfected group (Mann–Whitney; $p=.02$) (Supplementary Fig. 1). It remains unclear how superinfection may effect HIV disease progression since the reductions in CD4 count were not statistically significant between the two groups (Mann–Whitney; $p=.29$) (Table 1). No significant differences in *env* glycosylation (data not shown), which have been shown to influence Nab activity (Wei et al., 2003), or genetic distances between viruses were observed between the two groups and either laboratory strain NL4-3 ($R^2=.012$) or JRCSF ($R^2=.10$) (Supplementary Fig. 2). Similarly, an inferred maximum likelihood phylogenetic reconstruction of the V3 coding region of the *env* sequences showed no differences between baseline viruses of the superinfected and non-superinfected individuals as compared to JRCSF and NL4-3 (Supplementary Fig. 3). From the superinfected individuals, the baseline (initial strain) and six-month (superinfecting strain) viruses were significantly different from each other confirming the incidences of superinfection, as previously described (Smith et al., 2005a,b). Furthermore, all baseline and superinfecting viruses used the CCR5 coreceptor as would be expected in primary infection (data not shown) (Connor et al., 1997).

Intraclade superinfection has been difficult to identify, and this report includes three of the reported seven cases (Smith et al., 2005a,b). Despite the small number of superinfected cases investigated, this study demonstrates that individual immune responses to primary HIV infection vary in breadth and magnitude, but that the absence of heterologous neutralizing activity probably predisposes to superinfection. The level and cross-reactivity of NAb required to protect an individual from superinfection will require characterization of additional cases. Current efforts to develop candidate HIV vaccines must consider these ‘natural’ immunization failures in the hopes of better understanding the role of virus-specific host immune responses (Smith et al., 2005a,b). These provocative data suggest the role of cross-protective neutralizing antibody in the prevention of superinfection and elucidate important goals for protective vaccine design.

Methods

We performed a case–control study, matched for primary HIV infection (<6 months) and acknowledged continuing male homosexual HIV risk exposures (4 to >100 reported exposures during the first 6 months of their infection), within a cohort previously screened for superinfection (Smith et al., 2004). Neutralizing antibody, CD4 and viral load measurements and HIV *env* sequences (gp120) (Monogram Biosciences Inc., South San Francisco) were obtained for the first eleven matched individuals within the San Diego AIEDRP cohort who screened negative for intraclade superinfection (controls) and the three individuals who became intraclade superinfected (cases). Based on previous investigations and internal validation of the assay, a threshold of 20:1 was used to identify significant NAb responses

(Richman et al., 2003). Responses were measured against autologous and clade B laboratory virus strains (NL4-3 and JRCSF) with the first available blood sample (baseline) and 6 months later (Table 1). For the superinfected individuals, these timepoint samples represent before and after superinfection. Estimated date of first HIV infection was based upon the standardized AIEDRP algorithm (<http://www.aiedrp.org/>). Briefly, the rules for estimated date of infection are listed in order of application: (1) if HIV enzyme immunoassay (EIA) is negative and HIV viral load is >5000 copies/ml, use the date 21 days prior; (2) if HIV EIA is positive and Western Blot is indeterminate, use the date 28 days prior to indeterminate Western Blot; (3) If HIV EIA is positive and Western Blot is positive, but ≤ 5 bands, then use the date 45 days prior; (4) if HIV EIA is positive and Western Blot is positive (i.e. ≥ 5 bands positive) and a detuned EIA (Vironostika) value is ≤ 1.0 and the CD4 cell percentage is greater than 14%, then use the date 85 days prior; (5) if none of the previous criteria is met, then use the midpoint between the date of the first documented positive EIA and the last historically documented negative EIA, which must be less than 365 days prior to enrollment.

The significance of comparisons between groups was assessed by non-parametric methods; a Fisher's exact test was used for categorical comparisons, Wilcoxon paired test for comparisons between antibody responses and the two-tailed Mann-Whitney test in all others. Logarithmic viral load and CD4 count changes were computed as a least-squares linear fit to all available data from the first year on study. Glycosylation pattern (Marshall, 1974), coreceptor usage (Pillai et al., 2003) and genetic distances were computed from the gp120 *env* nucleotide sequences, and phylogenetic reconstruction was performed using Phylogeny Inference Package software of the C2-V3 coding region of *env* nucleotide sequences (Felsenstein, 1993).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.virol.2006.08.009.

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