Cross-reactive neutralizing humoral immunity in HIV-1 disease: dynamics of host-pathogen interactions
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Chapter 3

Prevalence of cross-reactive HIV-1-neutralizing activity in HIV-1 infected patients with rapid or slow disease progression
Abstract

The native envelope gp160 trimer of HIV-1 is thought to shield vulnerable epitopes that could otherwise elicit effectively neutralizing antibodies. However, little is known about the prevalence of naturally occurring broadly neutralizing activity in serum of HIV-1 infected individuals.

Here we studied 35 participants of the Amsterdam Cohort Studies on HIV-1 infection (20 long-term non-progressors (LTNP) and 15 progressors) for the presence of cross-reactive neutralizing activity in their sera at 2 and 4 years after seroconversion (SC). Neutralizing activity was tested in a pseudovirus assay, against a panel of HIV-1 envelope variants from subtype A, B, C, and D.

Already at year 2 post-SC, 7 out of 35 individuals (20%) had cross-reactive neutralizing activity, which increased to 11 individuals (31%) at 4 years post-SC. There was no difference in the prevalence of cross-reactive neutralizing serum activity between LTNP and progressors. Interestingly, high plasma viral RNA load and low CD4+ T cell count at set-point were associated with early development of cross-reactive neutralizing activity. Neutralization titers in serum increased during the course of infection for 91% of individuals studied here, albeit less rapidly for those who did not develop cross-reactive neutralizing activity.

Overall, we here demonstrate a relatively high prevalence of cross-reactive neutralizing serum activity in HIV-1 infected individuals, which increased with duration of infection. These data may imply that immunogenicity of the native envelope spike of HIV-1 for eliciting cross reactive humoral immune responses may be better than previously anticipated.
INTRODUCTION

The ability to elicit potent and cross-reactive neutralizing human immunodeficiency virus type 1 (HIV-1) specific humoral immunity is one of the major goals in HIV-1 vaccine development. One of the current approaches is the characterization of the epitopes of the best broadly neutralizing antibodies (BrNAb) that are known to date and to use these epitopes as immunogens to elicit HIV-1 specific neutralizing antibodies with similar potency and breadth. However, antibodies elicited by currently available HIV-1 envelope based immunogens do not display potent and cross-reactive neutralizing activity. Interestingly, the BrNAb b12, 2G12, 2F5, and 4E10, have all been isolated from HIV-1 infected individuals suggesting that the native envelopes of the HIV-1 variants in these individuals were capable of eliciting these antibody responses.

Despite intense research efforts, the number of isolated BrNAb has remained low, which has led to the assumption that BrNAb are rare in natural HIV-1 infection. However, several recent studies have shown cross-reactive neutralizing HIV-1 specific neutralizing activity in sera from various HIV-1 infected patients. In three studies the specificity of the neutralizing activity was identified, but it is still unclear if the breadth of the neutralizing activity in serum is determined by a single high affinity antibody directed against a highly conserved epitope in the envelope protein, or if it is the combined effect of multiple co-existing neutralizing antibodies directed at multiple distinct regions of the envelope. In line with both possibilities is the observation that cross-reactive HIV-1 specific neutralizing activity in serum develops over time. Indeed, early in infection, neutralizing activity in serum is directed against autologous HIV-1 variants and rarely directed against heterologous isolates, while plasma’s collected during the chronic phase of infection display various degrees of cross neutralizing activities although a more exact prevalence of cross-reactive neutralizing activity in sera from HIV-1 infected individuals remains to be established.

To support HIV-1 vaccine development, more insight is needed into factors that are associated with the ability of the host to elicit a cross-reactive neutralizing humoral immune response, and how such a neutralizing serum response evolves over time. Here we studied the potency and breadth of HIV-1 specific neutralizing humoral immunity in serum samples that were obtained at 2 and 4 years after seroconversion from 35 participants of the Amsterdam Cohort Studies. The prevalence of cross-reactive neutralizing activity in serum in our study group was 31%. We observed a strong correlation between duration of infection and breadth of the neutralizing HIV-1 specific humoral immune response, and a high plasma viral RNA load set-point and low CD4+ T cell count set-point were both associated with the early development of cross-reactive neutralizing activity. However, the prevalence of cross-reactive neutralizing activity in serum was similar for LTNP and progressors, excluding a correlation between potent humoral immunity and the clinical course of infection.
**Materials and Methods**

**Patients**

The study group consisted of long-term non-progressors (LTNP; defined as HIV-1 infected individuals who have ≥10 years of asymptomatic follow-up with stable CD4+ cell counts that were still above 400 cells/µl in the 9th year of follow-up) and progressors (HIV-1 infected individuals who progressed to AIDS within 7 years after (imputed) seroconversion (SC)) who were all participating in the Amsterdam Cohort Studies (ACS) on HIV and AIDS in homosexual men. All individuals were infected with HIV-1 subtype B, and were either seropositive at entry in the cohort studies (seroprevalent cases with an imputed SC date on average 18 months before entry in the cohort) or seroconverted during active follow-up in the cohort studies. None of the participants received combination anti-retroviral therapy during the sampling period; samples were obtained on average at 28 months (range 24-33 months) and 51 months (range 45-83 months) after imputed or documented SC. The Amsterdam Cohort Studies are conducted in accordance with the ethical principles set out in the declaration of Helsinki and written consent was obtained prior to data collection from each participant. The study was approved by the Academic Medical Center institutional medical ethics committee.

**U87/pseudovirus assay for testing of HIV-1 neutralizing activity in serum**

Sera from all 35 patients were tested for neutralizing activity in a pseudovirus assay developed by Monogram Biosciences. The tier 2-3 virus panel that we used for determining cross-neutralizing activity in serum consisted of HIV-1 pseudoviruses from subtypes A (n=5), B (n=6), C (n=7), and D (n=5) and included recently transmitted isolates, and moderately neutralization sensitive and resistant primary HIV-1 variants, based on previously determined neutralization sensitivities to subtype B sera and MAbs b12, 2G12 and 4E10. Pseudotyped viral particles were produced by cotransfecting HEK293 cells with an expression vector carrying the HIV-1-derived gp160 gene (eETV) and an HIV-1 genomic vector carrying a luciferase reporter gene (pRTV1.F-lucPCNDP-DU3). Forty-eight hours after transfection, pseudovirus stocks were harvested and small aliquots were tested for infectivity using U87 target cells expressing CD4, CCR5, and CXCR4. Pseudovirus stocks were then diluted to result in infectivity, as measured by relative light units (RLUs), that fell within a range known to yield reproducible IC50s. A recombinant virus assay involving a single round of virus infection was used to measure cross-neutralization activity of the sera. Diluted pseudoviruses were incubated for 1 hour at 37 °C with serial dilutions of serum after which the U87 target cells were added. The ability of patient sera to neutralize viral infection was assessed by measuring luciferase activity 72 hours after viral inoculation in comparison to a control infection with a virus pseudotyped with the murine leukemia virus envelope (aMLV). Neutralization titers are expressed as the reciprocal of the plasma dilution that inhibited virus infection by 50%.
Neutralization titers were considered positive if they were 3 times greater than the negative aMLV control. 1:40 was the lowest serum dilution used in the assay. For calculation of IC₅₀ values for viruses that were not inhibited by the 1:40 serum dilution we assumed that 50% inhibition would have occurred at a 1:20 serum dilution.

**PBMC based assay for testing HIV-1 neutralizing activity in serum**

Sera from the 19 individuals with a documented SC were tested in parallel in a PBMC based neutralization assay using both resistant and sensitive tier 2-3 primary HIV-1 variants 92UG029, KNH1144 (subtype A), BX08, BK132 (GS 009) (subtype B), SM145 (GS 016) (subtype C), 92UG038, 93UG065 (subtype D) and CAM1970LE (CRF_AG) 24. PBMCs were obtained from buffy-coats from 10 healthy seronegative blood donors and pooled prior to use. Cells were isolated by Ficoll-Isopaque density gradient centrifugation and then stimulated for 3 days in Iscove’s modified Dulbecco medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 U/ml), and phytohemagglutinin (PHA; 5 µg/ml) at a cell concentration of 5 x 10⁶/ml. After inoculation, the cells (1x10⁶/ml) were grown in the absence of PHA in medium supplemented with recombinant interleukin-2 (20 U/ml; Chiron Benelux, Amsterdam, The Netherlands) and Polybrene (5 µg/ml; hexadimethrine bromide; Sigma, Zwijndrecht, The Netherlands).

To prevent possible complement-mediated antibody inhibition of virus infection, complement in human sera and fetal bovine serum, these were inactivated by incubation at 56°C for 30 minutes. From each virus isolate, an inoculum of 20 50% tissue culture infective doses in a total volume of 50 µl was incubated for 1 hour at 37°C with increasing dilutions of the serum (starting concentration 1/25) in 96-well microtiter plates. Subsequently, 10⁵ PHA-stimulated PBMCs were added to the mixtures of virus and serum. After 4 hours of incubation, PBMCs were washed once in 100 µl phosphate-buffered saline after which fresh medium was added. On day 11, virus production in culture supernatants was analyzed in an in-house p24 antigen capture enzyme-linked immunosorbent assay 25. Experiments were performed in triplicate. When possible, 50% inhibitory concentrations (IC₅₀) were determined by linear regression.

**Statistical analysis**

Statistical analyses were performed using the SPSS 16 software package. HIV-1 RNA load in plasma (copies/ml) and CD4+ T cell count in blood (number/ml) at set-point were normally distributed and compared between different groups using independent samples t-test. Titer and breadth of the neutralizing activity in serum were not normally distributed and for estimation of correlation coefficients with either viral RNA load in plasma or CD4+ T cell count in blood at set-point the non-parametric two-tailed Spearman correlation coefficient was used. Ranking for assay correlation was normally distributed and calculated with Pearson correlation. The Kruskall-Wallis test was used to compare neutralization titers
at both years 2 and 4 post-SC, per virus and between patients who had or lacked cross-reactive neutralizing activity in serum. Geometric means of serum neutralization titers were calculated for each patient per time point and the Mann-Whitney test was performed to compare neutralization titers at both years 2 and 4 post-SC.

**Results**

*Prevalence of HIV-1 specific cross-reactive neutralizing serum activity*

We studied 35 participants from the ACS for the breadth of HIV-1 specific cross-reactive neutralizing activity in sera that were obtained on average 28 months (range 24-33 months) and 51 months (range 45-83) after SC. HIV-1 specific cross-reactive neutralizing activity was measured in a cell-based infectivity assay using recombinant viruses that carry a luciferase reporter gene and that are pseudotyped with envelope proteins from tier 2-3 HIV-1 subtype A, B, C, and D. To monitor neutralizing activity not mediated by HIV-1 Env-specific antibodies, each plasma sample was also tested against a recombinant virus stock that was pseudotyped with amphotropic murine leukemia virus envelope proteins (gp70SU and p15TM). Typically, neutralization titers, expressed as the reciprocal of the plasma dilution that inhibited infection by 50% (IC$_{50}$), were <40 for amphotropic murine leukemia virus controls. At 24 months post SC, HIV-specific cross-reactive neutralizing activity, defined as an IC$_{50} \geq 100$ for at least 50% of viruses per subtype, from at least 3 different subtypes, was observed in sera from 7 individuals (20%, 3 LTNP and 4 progressors, patient IDs indicated in dark gray in Figure 3.1). At the 4 year time point, sera from these 7 individuals still had high titer cross-reactive neutralizing activity. Interestingly, at this second time point, high titer cross reactive neutralizing serum activity had developed in 4 additional individuals (3 LTNP and 1 progressor, patient IDs indicated in light gray in Figure 3.1) resulting in a prevalence of HIV-1 specific cross-neutralizing serum activity of 31% around 4 years post-SC. There was no difference in prevalence of high titer cross-reactive neutralizing activity in serum between LTNP and typical progressors at either time point of analysis (at year 2 post-SC: 15% of LTNP and 27% of progressors; at year 4 post-SC: 30% of LTNP and 33% of progressors). Moreover, there were no differences in neutralization titers between LTNP and progressors.

Since discrepancies may exist between different neutralization assays, we wanted to confirm our observations obtained with the U87/pseudovirus based neutralization assay in a PBMC based neutralization assay. As the primary viruses from which the pseudoviruses were derived were not available, we used a different panel of 8 primary HIV-1 variants from different subtypes (A, B, C, D and CRF_AG) and serum samples that were obtained 2 years post-SC from 19 ACS participants in this study with a documented SC.

The overall pattern of neutralization of the 8 viruses by the sera from the 19 patients in the BPMC assay is shown in Figures 3.2A and 3.2B, and IC$_{50}$ $\geq 1:100$ are indicated in gray. In accordance with our observations in the U87/pseudovirus based assay, the serum...
Cross-reactive HIV-1 specific humoral immunity

**Figure 3.1: Breadth and potency of HIV-1 specific neutralizing activity in sera obtained at 2 and 4 years post-SC in progressors (A) and LTNP (B)**

IC\textsubscript{50} values, given as the reciprocal serum dilution of serum samples obtained at 2 years and 4 years post-SC are shown per patient (patients IDs are in the left column). The IDs of patients with cross-reactive neutralizing activity at both 2 and 4 years post-SC are in dark gray, the IDs of patients who developed cross-reactive neutralizing activity in serum between years 2 and 4 are indicated in light gray, IDs of patients who lacked cross-reactive neutralizing activity in serum are indicated in white. In the top row a description of the virus panel is given; the tier 2-3 virus panel consisted of primary subtype A, B, C and D viruses. The references panel (right part) included strains 1196, Bal, JRCSF, NL4-3, and SF162. As a negative control (NC), the amphotropic murine leukemia virus was used. IC\textsubscript{50} titer ≥1:100 are indicated in gray. Patients are ranked based on the neutralization breadth and potency (most potent serum on the top, least potent serum on the bottom). IC\textsubscript{50}<40 are indicated with a stripe.

n.d., not done.
neutralizing activity against subtype B viruses was the strongest. Unlike the U87/pseudovirus assay, where subtype C viruses were sensitive to serum neutralization, the selected subtype C virus was resistant to neutralization by all but one of the patient sera.

Overall, neutralizing serum titers in the PBMC based assay were generally lower, reducing the sensitivity to detect the neutralization breadth of the patient sera as compared to the PV assay. Indeed, in the PBMC based assay, none of the sera were able to neutralize all HIV-1 variants from all different subtypes. However, a significant correlation between the two assays could be observed when patients were ranked based on neutralization breadth and potency (Figure 3.2C). Ranking was assigned by giving priority to serum ability to neutralize different subtypes, followed by the total amount of viruses neutralized and finally by the titers at which the viruses were neutralized.

**Figure 3.2: Inter-assay correlation of neutralization by patient sera**

Breadth and titer of HIV-1 neutralization in sera from 19 participants of the Amsterdam cohort studies who have a documented moment of seroconversion. The IC₅₀ values for the sera that were obtained 2 years post-SC from (A) progressors (n=14) and (B) LTNP (n=5). Patient IDs are shown in the top row. The sera were tested against a panel of subtype A, B, C, D and CRF_AG viruses as shown in the most left column. IC₅₀ titers ≥1:100 are color-coded gray. n.d., not done. * 1; primary virus, 2; AIDS repository virus, 3; in PBMC expanded virus. (C) Correlation between breadth and titer of neutralizing activity in patient sera as observed in a PBMC based assay and a U87/PV based assay. Sera were ranked on the basis of neutralization breadth and potency (most broad and highest titer ranked 1, least broad and lowest titer ranked 19). Each dot represents one patient serum and the solid line is the regression line.
Correlation between set-point viral load and CD4+ T cell count and breadth of HIV-1 specific cross-reactive neutralizing serum activity

Our data indicate that cross-reactive neutralizing serum activity does not develop similarly in the course of infection for each HIV-1 infected individual. To obtain some insight into factors that may influence the humoral immune response, we divided the cohort of 35 patients who participated in our study in three distinct groups: patients who had no detectable cross-reactive neutralizing activity at years 2 and 4 post-SC (group A, n=24; patient IDs in white in Figure 3.1), patients who had cross-reactive neutralizing activity already at year 2 post-SC (group B, n=7; patient IDs in dark gray in Figure 3.1), and patients who had developed cross-reactive neutralizing activity between years 2 and 4 post-SC (group C, n=4; patient IDs in light gray in Figure 3.1). These three groups were compared for plasma viral load and CD4+ T cell count at set-point (Figure 3.3A and 3.3B).

Interestingly, the presence of cross-reactive neutralizing activity at year 2 post-SC (group B) was associated with a higher plasma viral RNA load set-point, as compared to the group of patients who had not developed cross-reactive humoral immunity in the first 4 years post-SC (group A). There was no significant difference in viral RNA load set-point between groups A and C, neither between groups B and C (Figure 3.3A). These observations were reinforced by a significantly lower CD4+ T cell count at set-point in patients with cross-reactive neutralizing activity at year 2 post-SC (group B) as compared to the CD4+ T cell count at set-point in the other 2 patient groups (groups A and C; Figure 3.3B).

Interestingly, an analysis which included all 35 individual patients who participated in our study revealed a positive correlation between the plasma viral RNA load at set-point and the number of viruses that were neutralized by the serum sample obtained at year 2 post-SC (Figure 3.3C). We also observed a negative correlation between the CD4+ T cell count at set-point and the number of viruses that were neutralized by the 2 year post-SC serum sample (Figure 3.3D). No such correlations were observed for the serum neutralizing activity at year 4 post-SC (data not shown).

Viral RNA load and CD4+ T cell count at the time of sampling did not differ between the group that developed cross-neutralizing reactivity (groups B and C) and the group that did not develop cross-reactivity within 4 years post-SC (group A; data not shown).

Titers of HIV-1 specific cross-reactive neutralizing activity in serum increase with duration of infection

With increasing time since SC, we observed an increase in the geometric mean of the neutralizing titers in serum. This could not be explained by the increasing number of patients who developed cross-reactive neutralizing activity over time. Indeed, when we analyzed the 3 groups as defined above (individuals who did not develop cross-reactive neutralizing activity in the first 4 years post-SC (group A), individuals with cross-reactive neutralizing serum activity at year 2 post-SC (group B), and individuals who developed cross-reactive
neutralizing serum activity between year 2 and year 4 post-SC (group C), the increase in the geometric mean of HIV-1 neutralizing titers in serum was observed in each patient group (Figure 3.4). Even for sera from individuals who did not develop cross-reactive neutralizing activity in the first 4 years post-SC (group A), an increase of the geometric mean of the neutralizing titers was observed over time, albeit that the magnitude of the increase was less than that observed for the patients who did develop cross-reactive neutralizing activity. Finally, we observed that in 91% of patients, an increase in geometric mean of neutralizing titers in serum was observed between years 2 and 4 post-SC.

Figure 3.3: Correlation between set-point viral RNA load in plasma and CD4+ T cell count, and cross-reactive neutralizing humoral immunity
Log 10 transformed set-point viral RNA load in plasma (A) and CD4+ T cell counts at set-point (B) were compared between groups of patients who had no detectable cross-reactive neutralizing activity at years 2 and 4 post-SC (group A), patients who had cross-reactive neutralizing activity already at year 2 post-SC (group B; patients’ IDs indicated in dark gray in Figure 3.1), and patients who had developed cross-reactive neutralizing between years 2 and 4 post-SC (group C; patients’ IDs indicated in light gray in Figure 3.1). For each serum sample obtained 2 years post-SC, the number of viruses that were neutralized was determined and plotted against the log transformed viral RNA load in plasma at set-point (C) and the CD4+ T cell count at set-point (D). The solid lines are regression lines. Significant differences are indicated by their P-values.
Figure 3.4: Geometric mean of the IC₅₀ neutralizing titers in serum for sera with no cross-reactive neutralizing activity at either year 2 or 4 post-SC, with cross-reactive neutralizing activity already at year 2 post-SC, or at year 4 post-SC.

The geometric mean of IC₅₀ titers of each serum sample for all tier 2-3 viruses in the panel was determined. For each group, defined as having no cross-reactive neutralizing activity (group A), or having cross-reactive neutralizing activity at year 2 post-SC (group B), or year 4 post-SC (group C), the geometric means of IC₅₀ values of serum samples obtained at 2 and 4 years post-SC were plotted. Significant differences are indicated by their p-values.

**DISCUSSION**

We compared 20 LTNP and 15 progressors for the presence of HIV-1 specific cross-reactive neutralizing activity in serum at years 2 and 4 post-SC. Already at 2 years post-SC, 7 individuals (3 LTNP and 4 progressors; overall 20%) had potent cross-reactive neutralizing activity in their sera, defined as the ability to neutralize at least 50% of HIV-1 variants per subtype, from 3 different subtypes, with an IC₅₀ at a serum dilution of ≥1:100. Interestingly, these 7 individuals had a significantly higher set-point viral RNA load in plasma and a lower CD4+ cell count at set-point than individuals who lacked a potent cross-reactive neutralizing response. The development of potently neutralizing humoral immunity apparently requires exposure to a sufficient amount of antigen, in line with previous observations ⁴⁰. Alternatively, a better exposure of epitopes on envelope that are essential for eliciting a cross-reactive neutralizing humoral immune response may coincide with enhanced replication kinetics resulting in a higher plasma viral RNA load set-point. In a model for Lymphocytic Choriomeningitis Virus (LCMV) infection, a reduction in CD4+ T cell numbers prior to infection reduced polyclonal B cell stimulation and enhanced protective antibody responses in terms of earlier onset and higher titers without impairing protective CD8+ T cell responses ²⁷,²⁸. Although the number of patients in our study is low, our observation that early cross-reactive neutralizing activity correlated with a low CD4+ T cell count at set-point may imply that this could also be the case in HIV-1 infection.

The fact that the majority of primary HIV-1 variants are neutralized by one or more of the currently available broadly neutralizing antibodies b12, 2G12, 2F5, and 4E10, already implies that the epitopes for these broadly neutralizing antibodies are accessible on primary
viruses. It is generally assumed however that the configuration of the envelope prevents the elicitation of a neutralizing antibody response \textit{in vivo}. The relatively high prevalence of cross-reactive neutralizing serum activity, which is similar to observations in other studies \cite{8,11}, suggests however that the relevant epitopes capable of eliciting these humoral responses are accessible and immunogenic on the native gp160 spike of HIV-1, at least in a significant proportion of HIV-1 infected individuals. We may be more conclusive on this point when the exact nature of the neutralizing activity in our study group has been established. Indeed, it is unclear if the breadth of the neutralizing activity in serum is determined by a single high affinity antibody directed against a highly conserved epitope in the envelope protein, or whether cross-reactive neutralizing activity in serum can be attributed to a combination of multiple co-existing neutralizing antibodies directed at a number of distinct regions of the envelope that together give the phenotype of a cross-reactive serum neutralization. It cannot be excluded that both scenarios exist and that it may vary between individuals. Interestingly, a recent study by Scheid \textit{et al.} has demonstrated the presence of a relatively large memory B cell repertoire capable of producing different antibody specificities in HIV-1 infected individuals with cross-reactive neutralizing serum activity \cite{29}.

Irrespective of the nature of the neutralizing response, the 2 to 4 years that seem to be required to achieve a potent neutralizing immune response and then only in 31\% of patients, at least in our study population, may hamper the efficacy of vaccine induced humoral immunity. The use of optimal adjuvants may be essential to accelerate the development of broadly neutralizing antibodies after immunization. However, several studies \cite{30-32} have suggested that low levels of neutralizing titers may actually be sufficient to achieve protection from infection. These lower titers may be achieved more rapidly than the 1:100 serum dilution threshold we set for our experiments shown here.

It remains to be established how HIV-1 neutralizing activity \textit{in vitro} relates to protection from infection \textit{in vivo} \cite{26} which may be better reflected in a PBMC based neutralization assay. However, our initial data on the cross-reactive neutralizing activity in sera obtained with a pseudovirus based assay on U87 cells were confirmed in a PBMC-based assay using replication competent primary HIV-1 variants from different subtypes. Simek \textit{et al.} have demonstrated that neutralizing activity can indeed be reliably assessed using pseudovirus panels \cite{22}. The ability of our patient sera to neutralize viruses from the large virus panel that we used in our present study was strongly correlated with the ability of these same sera to neutralize viruses from the 7 virus panel used by Simek \textit{et al.} (Spearman $r = 0.77$, data not shown) not only confirming the validity of the large virus panel that we used, but also strengthening our conclusion on the broadly neutralizing ability of the patient sera tested in our study.

Although the number of patients in our study is relatively small, our data suggest that there is no correlation between the presence of cross-reactive neutralizing activity in serum and the clinical course of infection. Indeed, we observed a similar prevalence of cross-
reactive neutralizing serum activity at 2 and 4 years post SC in LTNP and progressors. Moreover, the presence of cross-reactive neutralizing antibodies in serum did not coincide with a reduction in viral load, in line with the observation that administration of broadly neutralizing antibodies to hu-PBL-SCID mice after inoculation with HIV-1 had no effect on viral load in the animals 33. Our data are supportive for the idea that it is important to achieve vaccine elicited sterilizing immunity that prevents establishment of infection, or a vaccine that can elicit potent cross-reactive neutralizing humoral immunity in combination with effective cellular immunity to delay or prevent disease progression 34. The relatively high proportion of individuals with cross-reactive neutralizing humoral immunity in our present study and other studies 8,11 suggests that the B cell repertoire in humans should indeed be sufficient to respond to a vaccine with potently neutralizing antibodies implying that a protective antibody-based vaccine against HIV-1 may be an obtainable goal.

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REFERENCES


