Cross-reactive neutralizing humoral immunity does not protect from HIV-1 disease progression
ABSTRACT

Broadly-reactive neutralizing antibodies are the focus of HIV-1 vaccine design. However, only little is known about their role in AIDS pathogenesis and the factors associated with their development. Here we used a multi-subtype panel of 23 HIV-1 variants to determine the prevalence of cross-reactive neutralizing activity in sera obtained ~35 months post-seroconversion from 82 HIV-1 subtype B infected participants from the Amsterdam Cohort Studies. Of these patients, respectively 33%, 48%, and 20% had strong, moderate, or absent cross-reactive neutralizing activity in serum. Viral RNA load at set-point and AIDS free survival were similar for the three patient groups. However, higher cross-reactive neutralizing activity was significantly associated with lower CD4+ T cell counts before and early after infection.

Our findings underscore the importance of vaccine elicited immunity to protect from infection. The association between CD4+ T cell counts and neutralizing humoral immunity may provide new clues to achieve this.
INTRODUCTION
In HIV-1 infected individuals, neutralizing antibodies can develop against autologous HIV-1 strains within weeks of infection. In general, antibodies that can neutralize autologous virus variants are strain-specific and lack the ability to neutralize heterologous viruses. Some HIV-1 infected individuals, however, mount a potent neutralizing humoral immune response that has the \textit{in vitro} ability to neutralize HIV isolates from unrelated subjects. The exact nature of cross-reactive neutralizing activity in serum is unclear and may be the result of a single high affinity antibody directed against a highly conserved epitope in the envelope protein. Alternatively, it may reflect the activity of several neutralizing antibodies that in combination give cross-reactive neutralizing activity. Little is known about the protective properties of broadly cross-reactive neutralizing antibodies \textit{in vivo}. In non-human primate studies, passive transfer of broadly neutralizing antibodies completely blocked infection by a chimeric simian-human immodeficiency virus, while in humans, passive transfer of broadly neutralizing antibodies delayed HIV-1 rebound after cessation of antiretroviral therapy.

In our present study, we wished to determine the prevalence of cross-reactive neutralizing humoral immunity in serum among participants of the Amsterdam Cohort Studies on HIV infection and AIDS and whether the presence of HIV-1 specific cross-reactive neutralizing activity in serum was associated with delayed disease progression. In addition, we wanted to reveal factors that were associated with the development of such a potent humoral immune response.

In our cohort, 33% of participants had cross-reactive neutralizing serum activity, but no correlation between the presence of potent humoral immunity and disease course could be revealed. The mounting of a potent and cross-reactive neutralizing immune response was significantly associated with a lower CD4$^+$ T cell count at set-point but not with viral load at set-point. So although potently neutralizing humoral immunity does not seem to influence disease course, our findings may be relevant for the achievement of optimal vaccine responses.

MATERIALS AND METHODS

\textit{Study participants}

The study population consisted of 131 Caucasian, homosexual men who were HIV-1 negative at the moment of enrollment between October 1984 and March 1986 in the Amsterdam Cohort studies on the natural history of HIV-1 infection, and who seroconverted for HIV-1 antibodies between 1984 and 1996 during active follow-up. To obtain the best figure on prevalence of cross-reactive neutralizing activity in serum, which generally develops relatively late after seroconversion, we chose serum samples that were obtained at a mean of 35 months (range 30-37 months) after seroconversion. Individuals who had already reached a CD4$^+$ T cell count of less than 200 cells/µl blood, had developed AIDS, had...
initiated highly active antiviral therapy or were lost to follow-up at the time of screening were excluded, leaving 82 individuals for analysis.

For Kaplan–Meier survival analysis, individuals were censored at their first day of effective antiretroviral therapy or when lost to follow-up. When AIDS (CDC definition 1993) was used as an end point in Kaplan–Meier survival analysis, 46 individuals had an event, 13 were censored due to loss to follow-up, and 23 were censored because of initiation of highly active antiretroviral therapy (HAART). When AIDS-related death, defined as death with AIDS-related malignancy, death with AIDS-opportunistic infections, or death with AIDS-related cause not specified by the treating physician was used as an end point, 29 individuals had an event, 16 were censored due to loss to follow-up, and 37 were censored at initiation of HAART. For survival analysis after AIDS diagnosis had an event, 20 were censored due to loss to follow-up, and 37 were censored at initiation of HAART.

The Amsterdam cohort studies have been conducted in accordance with the ethical principles set out in the declaration of Helsinki and written informed consent was obtained from each cohort participant prior to data collection. The study was approved by the Amsterdam Medical Center institutional medical ethics committee.

Neutralization assay

Sera from all 82 cohort participants obtained at a mean of 35 months post-seroconversion were tested for cross-reactive neutralizing activity in a pseudovirus assay involving a single round of viral infection as developed by Monogram Biosciences. We used 2 tier 2-3 virus panels (Supplementary Table S4.1) for determining cross-neutralizing activity in serum. The first panel consisted of 20 pseudoviruses with envelope sequences from HIV-1 subtypes A, B, C, and D with 5 viruses per subtype (panel 1). Viruses were obtained recently after transmission or during the chronic phase of infection and were either moderately sensitive or neutralization resistant based on previously determined neutralization sensitivities to sera from subtype B infected individuals and monoclonal antibodies (MAbs) b12, 2G12 and 4E10. The second panel consisted of 5 pseudoviruses with envelope sequences from primary isolates of HIV-1 subtypes A, B, C and CRF01_AE (panel 2) that were resistant (n=1), moderately resistant (n=3) and moderately sensitive (n=1) based on previously determined neutralization sensitivities to sera from subtype B infected individuals and MAbs b12, 2G12 and 4E10. This 5-virus panel covered 93% of the variation in neutralization of a larger pseudovirus panel (n=15) pseudoviruses. Pseudotyped viral particles were produced by cotransfection of 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-lucPCNDO-ΔU3). 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 tested and normalized for infectivity prior to testing in the neutralization assay. Diluted pseudoviruses were incubated for 1 hour at 37 °C with
serial dilutions of the patient sera 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% (IC\textsubscript{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 IC\textsubscript{50} 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.

Viral load measurements
Viral load in plasma was routinely measured at every study visit in the cohort studies by using a quantitative HIV-1 RNA nucleic acid-based sequence amplification (Organon Teknika, Boxtel, The Netherlands) with electro-chemiluminescently labeled probes. Set point viral load data were available for all patients. Viral load data were analyzed after log\textsubscript{10} transformation.

Immunologic assays
CD4\textsuperscript{+} T cell counts in peripheral blood were first measured at the first visit after entry in the Amsterdam cohort studies, and were routinely measured at every subsequent study visit using flow cytometry. Set-point CD4\textsuperscript{+} T cell count data were available for all patients. Data on CD4\textsuperscript{+} T cell and CD8\textsuperscript{+} T cell percentages from 62 patients were available from a previous study.

Statistical analyses
For Kaplan–Meier survival analysis, left truncation was performed for time between seroconversion date and the screening date using S-PLUS 6 (Insightful Corporation, Seattle, Washington, USA). Log rank P value was used to determine differences in the clinical course of infection between groups of patients with either strong, moderate, or absent cross-reactive neutralizing activity in serum. Depending on the distribution of data as determined by the Shapiro-Wilk normality test, the analysis of variance (ANOVA) or Kruskall-Wallis test was used. The association between cross-reactive HIV-1 specific neutralizing activity in serum and viral load at set-point, CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell percentages before seroconversion, and 1 and 5 years after seroconversion (normally distributed), were tested with ANOVA. The association between cross-reactive HIV-1 specific neutralizing activity in serum and CD4\textsuperscript{+} T cell counts at set-point (not normally distributed) was tested with the Kruskall-Wallis test. Spearman’s rank correlation coefficient was used to assess the association of geometric mean titers of each patient serum that were obtained on the two viral panels. Analyses were performed in GraphPad Prism 4 (GraphPad Software, La Jolla, California, USA).
**RESULTS**

*Prevalence of cross-reactive neutralizing activity in serum in the natural course of HIV-1 infection*

We first screened sera of participants of the Amsterdam cohort studies on HIV infection and AIDS for the presence of cross-reactive neutralizing activity. As neutralizing serum activity is mounted relatively late after seroconversion, we chose to test sera obtained at 35 months (range 30-37 months) post-seroconversion, which also allowed sufficient follow-up time to perform survival analysis from the moment of screening onwards. Cohort participants who at this time-point had already progressed to disease or initiated HAART were excluded from the study. The remaining group of 82 participants had a median AIDS free follow-up time of 8.31 years (95% CI 5.95 – 10.5 years) after SC when left-truncated for time point of screening. HIV-1-specific cross-reactive neutralizing activity in the sera of these patients was measured in a cell-based infectivity assay using a panel of 20 pseudoviruses carrying a luciferase reporter gene and the envelope proteins (Env) from tier 2 HIV-1 subtype A, B, C, and D (panel 1) and a panel consisting of 6 pseudoviruses with Env from JRCSF and five tier 2 HIV-1 subtype A, B, C, and CRF_01 AE (panel 2) (Supplementary Table S4.1 and Supplementary Figures S4.1 and S4.2).

Cross-neutralizing activity of patient sera on these 2 virus panels (Supplementary Figures S4.1 and S4.2) were strongly correlated (Spearman r = 0.91, Supplementary Figure S4.3). Therefore, data from the two panels were combined for further analysis, excluding the data on CRF_01 AE as we had only 1 variant of this subtype (panel 3; Figure 4.1). The analysis of the combined data sets showed strong correlations between cross-reactive neutralizing activity in serum and geometric mean titer (Spearman r = 0.89, data not shown) and the number of viruses neutralized (Spearman r = 0.79, data not shown). Strong cross-reactive neutralizing activity in serum, defined as the ability to neutralize HIV-1 variants at an IC$_{50}$ titer of $\geq$ 100 to at least one virus from 3 or more subtypes, was observed in 27 patients (33%), similar to the prevalence in other cohorts. Sera from 39 (48%) patients neutralized HIV-1 at an IC$_{50}$ titer of $\geq$ 100 to at least one virus from 1 or 2 subtypes (moderate cross-reactive neutralizing activity) while sera from 16 patients (20%) completely lacked cross-reactive neutralizing activity (Figure 4.1). Interestingly, one patient had an average log transformed neutralizing titer of 2.9 on panel 2, with which he ranked in the top 3 of recently identified elite neutralizers (average log transformed HIV-1 neutralizing titer of $>2.5$).

*Association between cross-reactive neutralizing activity in serum and the clinical course of HIV-1 infection*

Next, we investigated the potential relationship between cross-reactive HIV-1 specific neutralizing activity in serum and the rate of HIV-1 disease progression. Kaplan-Meier and Cox Proportional Hazard analysis were performed for the period after the moment at which cross-reactive neutralizing activity in serum was measured, using clinical AIDS (definition CDC 1993) and AIDS-related death as endpoints. In addition, we analyzed the survival
Figure 4.1: Breadth and titer of HIV-1 specific neutralizing activity in serum

Shown are the IC_{50} values given as the reciprocal serum dilution of serum samples obtained at ~3 years post-SC (patient IDs, n = 82, left column). The top row shows virus panel 3 (23 viruses from subtypes A, B, C, and D, and controls on the far right: JRCSF, NL4-3 and amphotropic murine leukemia virus). IC_{50} <40 are indicated with a stripe. IC_{50} titters are color-coded as follows: white, IC_{50} <40; light gray, IC_{50} ≥ 3 times the value of aMLV; gray, IC_{50} ≥ 1:100; dark gray, IC_{50} ≥ 1:1000. Patients are ranked based on the neutralization breadth and titer in serum.
time after the moment of AIDS diagnosis, using AIDS-related death as an endpoint.

The presence of cross-reactive neutralizing activity in serum was not associated with delayed progression to AIDS according to the 1993 CDC definition (log rank \( P = 0.29 \); median AIDS free survival times [from the timepoint of screening onwards] for groups of individuals with strong, moderate, or absent cross-reactive neutralizing activity in serum at 35 months post-SC were 7.5 ± 2.2, 8.5 ± 3, and 10.5 ± 4 years, respectively; Figure 4.2A). Time from screening to AIDS-related death was also similar for the groups with strong, moderate, and absent cross-reactive neutralizing activity in serum at 35 months post-SC (log rank \( P = 0.69 \); median survival times of respectively 9.9 ± 2.5, > 7.9, and > 8.5 years; Figure 4.2B). Finally, survival time after AIDS diagnosis was also the same for the three patient groups (log rank \( P = 0.5 \); median survival time 2.3 ± 0.5 years for individuals with strong cross-reactive neutralizing activity, 2.4 ± 0.2 years for individuals with moderately cross-reactive neutralizing serum activity, and 2 ± 0.4 years for individuals who lacked cross-reactive neutralizing activity in serum; Figure 4.2C).

Factors associated with the presence of cross-reactive neutralizing activity in serum

We subsequently investigated the potential relationship between the breadth of the HIV-1 specific neutralizing activity in serum and the viral RNA load in plasma at set-point and the CD4+ T cell counts at set-point. In our cohort, cross-reactive neutralizing activity in serum at 35 months post-seroconversion was not associated with the level of plasma viremia.

**Figure 4.2: Kaplan–Meier survival analysis from seroconversion till AIDS (CDC 1993)**

(A), to AIDS-related death (B) and for time from AIDS diagnosis to AIDS-related death (C) for individuals with strong (\( n = 27 \), red bold lines), moderate (\( n = 39 \), yellow dashed lines) or absent (\( n = 16 \) green lines) cross-reactive neutralizing activity respectively. \( P \) values (log rank test) are denoted above each figure. Median survival times for groups of individuals with strong, moderate, or absent cross-reactive neutralizing activity at 35 months post-seroconversion were 7.5 ± 2.2, 8.5 ± 3, and 10.5 ± 4 years, respectively for AIDS free survival, 9.9 ± 2.5, > 7.9, and > 8.5 years respectively for median times from seroconversion to AIDS-related death, and 2.3 ± 0.5 years, 2.4 ± 0.2 years, and 2 ± 0.4 years, respectively for time from AIDS diagnosis to death.
Humoral immunity and HIV-1 control

at set-point, which was defined as the average viral load between month 18 and 24 after seroconversion (Figure 4.3A).

Interestingly, strong cross-reactive neutralizing activity in serum was significantly associated with a low median CD4+ T cell count at set-point ($P=0.011$; Figure 4.3B). To analyze whether the association between more potent humoral neutralizing activity in serum and CD4+ T cell counts may have a potential significance for vaccine efficacy, we next analyzed whether the same association could be observed between pre-seroconversion CD4+ T cell numbers and the titer of the neutralizing humoral immune response after HIV-1 infection. For this purpose, we compared the mean percentages of CD4+ and CD8+ T cells before seroconversion (at least 6 months before seroconversion) and 1 and 5 years after seroconversion within groups of individuals with strong, intermediate, or absent cross-reactive neutralizing activity in serum. Individuals with strong neutralizing activity had lower percentages of CD4+ T cells ($P=0.011$; Figure 4.3C) and higher percentages of CD8+ T

Figure 4.3: Factors associated with the presence of cross-reactive neutralizing activity in serum

Association between strong ($n=27$, ■), moderate ($n=39$, ■), and absent cross-neutralizing activity ($n=16$, □) in serum and (A) set-point log transformed viral RNA load in plasma and (B) CD4+ T cell counts at set-point. (C) Percentage CD8+ T cells or (D) CD4+ T cells before seroconversion and 1 and 5 years after seroconversion within patients with strong (■), moderate (■), and absent cross-neutralizing activity (□) in serum. In Figures C and D, the number of individuals per group ($n$) is denoted below each bar. Mean and standard deviation (A,C,D) or median with interquartile range (B) are shown. $P$-values from the ANOVA (A,C,D) or Kruskall-Wallis test (B) are denoted.
cells \( P = 0.0082; \) Figure 4.3D) before seroconversion than HIV-infected individuals who lacked cross-reactive neutralizing activity in serum. This trend was still observed 1 year after seroconversion, but was absent at year 5 of infection (Figures 4.3C and 4.3D).

**Discussion**

Previous studies have shown that autologous strain specific neutralizing activity does not contribute significantly to the control of HIV-1 infection \(^{27,29}\). Here we show that even cross-reactive neutralizing activity in serum is not associated with prolonged time to AIDS or death. This observation is in line with the finding that administration of broadly neutralizing antibody b12 before viral challenge could protect animals from infection while administration after inoculation had no effect on the control of established HIV-1 infection in vivo \(^{30}\). Moreover, it confirms recent findings in a cohort of Kenyan women in which cross-reactive neutralizing activity was not associated with time to AIDS or initiation of antiviral therapy \(^{31}\).

Cross-reactive neutralizing activity is known to accumulate with time of infection \(^{26}\). For this reason, we chose to screen for serum neutralizing activity at around 35 months post-seroconversion, when an adequate cross-reactive humoral immune response could have been developed, and excluded cohort participants who at that time-point had already developed AIDS, had initiated HAART, or had reached a CD4 count of less than 200 cells/µl blood. As a consequence, individuals with very rapid disease progression were excluded from the analysis and our study design therefore only allows for the conclusion that cross-reactive neutralizing activity has no long-term protective effect on HIV-1 disease progression.

The prevalence of strong cross-reactive neutralizing activity in serum in our study population was 33%, which is similar to observations in recent studies \(^{21,25,26}\). Simek et al. tested the neutralizing activity in sera from ~1800 individuals on different pseudovirus panels and described that screening on a panel of only 5 selected viruses (panel 2 in our study) provided similar information on the presence of cross-reactive neutralizing activity as screening on a large pseudovirus panel. Indeed, the results obtained with sera from patients in our study on either panel 1, which consisted of 20 viruses from subtypes A, B, C, and D, or panel 2 were highly concordant in geometric mean titer (Spearman \( r = 0.91 \)). This not only confirms the suitability of our large pseudovirus panel for characterization of HIV-1 neutralizing activity in patient sera, it also allows for a direct comparison of our data with previous studies.

Interestingly, Simek et al. identified 15 so-called elite neutralizers \(^{21}\), who had an average log transformed titer that was equal to or greater than 2.5 on panel 2 (including JR-CSF). In our cohort, we identified 1 elite neutralizer who reached a log transformed titer of 2.9 on this same virus panel. When compared to the elite neutralizers in the study by Simek et al. \(^{21}\), our patient ranked in the 3rd place. Since the prevalence of elite neutralizers is considered to be only 1%, the biomaterial from this Amsterdam Cohort participant is definitely interesting for the identification of potentially novel cross-reactive neutralizing antibodies.
It has been reported that the prevalence of cross-reactive neutralizing activity in serum from elite controllers was much lower as compared to LTNPs and slow progressors. A certain level of antigen is apparently required to drive the humoral immune response. Previous studies have indeed demonstrated a correlation between the breadth of neutralizing activity in serum and viral load at setpoint or at time of testing for neutralizing activity. In our present study, we did not observe a correlation between the presence of cross-reactive neutralizing activity in serum at 35 months post seroconversion and the viral load at setpoint or at the moment of screening for HIV-1 specific humoral immunity. We currently have no explanation for this apparent discrepancy. However, of the 10 patients with the lowest viral load at setpoint, 6 lacked cross-reactive neutralizing activity in serum, indicating that a certain level of antigen is indeed required to stimulate neutralizing humoral immunity. However, absent cross-reactive neutralizing activity in patients with higher viral load in plasma indicates that additional factors may be critical for the development of a cross-reactive neutralizing antibody response.

We recently demonstrated that in sera of subtype B infected patients, the neutralizing activity was stronger against the subtype B viruses in our panel than against the subtype A, C, and D viruses in our panel. We could confirm this observation in our present study as neutralization of subtype B variants was seen significantly more often than neutralization of viruses from other subtypes (Chi-square P<0.001). Indeed, in sera from 42 out of 82 patients, neutralizing activity against more than 50% of the subtype B viruses in the panel was observed, while neutralization of more than 50% of subtype A, C, or D viruses was seen in sera of only 27, 23, and 19 individuals, respectively.

Interestingly, we observed a correlation between cross-reactive neutralizing activity and a lower CD4+ T cell count at setpoint and a lower CD4+ T cell percentage prior to HIV-1 infection. In another study, this correlation was not seen, but in that study the within-subject average of CD4+ T cells from different time points was compared to neutralization breadth and can therefore not be compared to the CD4+ T cell count at setpoint or prior to HIV-1 infection.

Our data are in line with a study in a lymphocytic choriomeningitis virus (LCMV) mouse model, where either partial CD4+ T cell depletion prior to infection or exclusion of dominant CD4+ T cell epitopes from the vaccine enhanced the generation of NAb responses, due to reduced polyclonal B cell activation. In analogy, decreased CD4+ help may prevent polyclonal B cell activation and hypergammaglobulinemia in HIV infection, favoring the production of neutralizing antibodies. Although the differences in percentages or numbers of CD4+ T cells between groups may be small, it could reflect a critical threshold for proper B cell help.

The absent association between cross-reactive neutralizing immunity and the clinical course of HIV-1 infection is suggestive for rapid viral escape from humoral immune pressure, despite the fact that cross-reactive neutralizing antibodies are considered to be
directed against conserved epitopes. We have indeed observed that HIV-1 can rapidly escape from autologous humoral immunity with cross-reactive neutralizing activity (authors’ unpublished data). Apparently, these escape mutations do not come at a fitness cost to the virus, as has been described for certain escape mutations in conserved epitopes for cytotoxic T lymphocytes (CTL) 41-44. In agreement, we previously reported that the replication rates of viruses that were resistant to broadly neutralizing antibodies b12, 2G12, 2F5, and/or 4E10 were similar to the replication kinetics of the co-existing neutralization sensitive viruses from the same patient 40. 

In conclusion, cross-reactive neutralizing activity in serum does not seem to have an impact on the clinical course of HIV-1 infection. Possibly, and as observed for other viral infections, CTL rather than neutralizing antibodies may contribute to the control of already established infections while neutralizing antibodies may be essential for protection from infection 14,15. Our data suggest that a broadly neutralizing humoral immune response may be best achieved in the face of reduced CD4+ T cell numbers. Although this may be arguable unrealistic to achieve deliberately as part of a vaccination regimen, it could provide clues for achieving better efficacy of an antibody vaccine. Apart from that, the relatively large proportion of individuals with cross-reactive neutralizing humoral immunity elicited by the native HIV-1 envelope may already predict a satisfying response rate once a vaccine will be available.

ACKNOWLEDGEMENTS

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REFERENCES


Supplementary data

Supplementary Table S4.1: Neutralization profiles of pseudoviruses used in the 3 screening panels

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R, Resistant; S, Sensitive; MR, Moderately Resistant; MS, Moderately sensitive; BR, Borderline resistant; VS, Very sensitive; n.t., not tested

Supplementary Figure S4.1: Breadth and titer of HIV-1 specific neutralizing activity in patient sera on a panel of 20 HIV-1 variants from 4 different subtypes (panel 1)

Shown are the IC\textsubscript{50} values given as the reciprocal serum dilution of serum samples obtained at 3 years post-SC. In the left column, patient IDs of the 82 HIV-1 infected individuals are given. The top row shows the virus panel that was used (20 viruses from subtypes A, B, C and D and controls on the far right: JRCSF, NL4-3 and amphotropic murine leukemia virus [aMLV]). IC\textsubscript{50} titers are color-coded as follows: white (\textless 1:40), light gray, IC\textsubscript{50} \textgreater 1:100; dark gray, IC\textsubscript{50} \textgreater 1:1000. Patients are ranked based on the breadth and titer of the neutralizing activity in serum.
Supplementary Figure S4.2: Breadth and titer of HIV-1 specific neutralizing activity in patient sera on a panel of 5 HIV-1 variants from 4 different subtypes (panel 2) \(^1\)

Shown are the IC\(_{50}\) values given as the reciprocal serum dilution of serum samples obtained at 3 years post-SC tested on a reduced viral panel consisting of 5 viruses (top row) and control virus on the far right (JRCSF, NL4-3 and amphotropic murine leukemia virus [aMLV]). IC\(_{50}\) titers are color-coded as in Supplementary Figure S4.1. Patients are ranked based on the breadth and titer of the neutralizing activity in serum.
Supplementary Figure S4.3: Correlation between breadth and titer of HIV-1 specific neutralizing activity in sera on a panel of 20 (panel 1) and 5 viruses (panel 2) respectively.

The geometric mean titer was calculated for all 5 viruses plus lab strain JRSCF. The geometric mean titers of neutralization obtained against the panel of 20 viruses plus JRSCF were compared with the geometric mean titers obtained against the panel of 5 viruses. Spearman r and P-value show correlation between the different panels and are indicated in the graph. Each dot represents the geometric mean titer of one patient on both panels.

REFERENCES