Association of HIV neutralizing antibody with lower viral load after treatment interruption in a prospective trial (A5170)

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Abstract

Objective—We investigated the impact of neutralizing antibodies (NAbs) on CD4 T-cell count and viral load in a cohort of HAART recipients who underwent extended structured treatment interruption.

Design—Substudy of NAb in the AIDS Clinical Trials Group 5170 trial.

Methods—Early plasma samples from 50 volunteers who discontinued HAART were evaluated in a peripheral blood mononuclear cell-based neutralization assay against a panel of four subtype B primary isolates.

Results—We found that high-titer (90% inhibitory dose > 500) NAb against two or more isolates was associated with reduced viral load (P=0.003 at 12-week posttreatment interruption). This effect faded with time, losing significance (P=0.161) by study conclusion. Participants possessing the highest NAb levels against individual isolates appeared more likely to have lower viral loads with the association gaining significance against the R5-tropic primary isolate US1 (P=0.005). There was no association between broader neutralization and CD4 T-cell slope over time.

Conclusion—The data suggest that high-titer NAb responses at the time of treatment interruption are associated with reduced viral load over time, but not CD4^{+} T-cell decline.

Keywords

CD4^{+} T cells; HAART; HIV; neutralizing antibodies; viral load

Introduction

HIV-infected patients on HAART are an informative population in the study of immune responses to HIV-1. Improvements in immunologic status, including increases in overall CD4^{+} T-cell counts and lymphoproliferative responses are observed in patients with suppressed viral replication [1–4]. Several studies have shown that cytotoxic T lymphocyte and neutralizing antibody (NAb) responses to HIV-1 improve, at least transiently, in most patients treated with HAART [5,6]. Although the mechanism remains unclear, HAART may slow virus evolution, allowing the immune response to catch-up [7].

Long-term administration of HAART may be associated with toxicity and drug resistance [8,9]. Treatment interruption has been proposed as a way to reduce HAART associated toxicity and boost anti-HIV immune responses [10]. To date, studies of treatment interruption have been equivocal with respect to virologic and clinical outcomes [11–14]. However, it may be important to distinguish HIV-positive individuals who initiate antiretroviral therapy (ART) shortly after infection while still possessing relatively intact immune systems from those initiating therapy after longer period of time wherein serious long-term damage has already occurred. In one recent study of HIV-1-positive individuals intiating ART within 6 months of infection [15], 40% (N=73) were able to maintain low HIV RNA concentrations (<5000 copies/ml) following treatment interruption (24 weeks) suggesting that early treatment may preserve the immune system and facilitate the development of more effective immune responses.

Cell-mediated immune responses appear to improve in breadth and magnitude during treatment interruption [12,16], whereas elevated NAb titers and broadly NAbs have been observed in some patients with poor HAART adherence and in those with chronic, untreatment infection [17–20]. In one study, overall binding and NAb development appeared to be impaired in patients who were treated with HAART shortly after seroconversion. However, some of these same patients showed strong autologous NAb upon treatment interruption suggesting that some degree of B-cell priming likely occurred prior to HAART leading to a strong secondary NAb response to the `fixed' virus population posttreatment [21].

In the present study we examined the neutralizing capacity of individual patient plasma taken at the time of treatment interruption against a panel of subtype B primary isolates. Although neutralization at the time of treatment interruption was not associated with CD4 cell count decline, there was a strong association with reduced viral load after treatment interruption. This effect waned over time, suggesting viral escape.

Methods

Clinical samples

Volunteers from 31 AIDS Clinical Trials Group (ACTG) sites were chosen to participate in an observational, prospective study (A5170) of the clinical outcome of antiretroviral treatment interruption [14]. The overall cohort consisted of 167 HIV-infected persons over the age of 18 who voluntarily agreed to discontinue ART for the duration of the study (96 weeks). Minimum requirements for study enrollment were a CD4^{+} T-cell count more than 350 cells/μl, plasma viral load less than 55 000 copies/ml and adherence to a combination of two or more predetermined antiretroviral agents for a minimum of 6 months. Immunologic and virologic markers of disease status, including CD4^{+} T-cell count and HIV-1 viral RNA copy number (viral load) were measured every 4 weeks during the period of treatment interruption. Clinical progression was defined as two, consecutive CD4^{+} T-cell counts less

than 250 cells/μl. Frozen plasma samples from 50 volunteers were obtained and heat-inactivated (568C/30 min) prior to use. Volunteers were selected by $CD4^+$ T-cell decline at 24-week posttreatment interruption and divided evenly into two groups: `progressors' and `nonprogressors' [22]. Progressors were defined by a $CD4^+$ T-cell reduction of more than 40% at week 24. Nine of the 25 progressors reinitiated ART prior to study end. Nonprogressors were defined by a $CD4^+$ T-cell decline of less than 20% over the same period. CD4 T-cell decline in this group was less than 50% of that observed in the progressor group at week 24. No one in this group re-initiated ART during the study. The two groups had similar $CD4^+$ T-cell counts and viral loads at trial entry. Additional exploratory analyses were performed by assigning volunteers to groups by number of isolates neutralized, designated as `restricted' neutralization if 0–1 isolate was neutralized with a titer (90% inhibitory dose) greater than 500 and `expanded' neutralization, if volunteer plasma neutralized two or more isolates with an 90% inhibitory dose greater than 500.

Viruses

We tested volunteer plasmas against four subtype B primary isolates [US1, US4 and 89.6 as well as a recombinant B' (CM237) isolate from Thailand], all obtained from the NIH AIDS Reagent and Research Program (Germantown, Maryland, USA). All isolates utilize CCR5 (R5) as coreceptor and represent a range of neutralization sensitivity (Weak-89.6, CM237, US4, US1-Strong) to pooled HIV-positive sera and sCD4 [6,23–26]. Of note, isolate 89.6 is also known to use CXCR4 and is considered dual-tropic (R5/X4) [27]. A single stock of each isolate (sufficient for all experiments) was generated by infection of PHA/IL-2-stimulated peripheral blood mononuclear cells (PBMCs) from a single donor (SeraCare, Inc., Milford, Massachusetts, USA) [26]. To confirm stock virus identity, a segment of the envelope region (C2–V4) was sequenced from proviral DNA.

Virus titration assay

Titration assays were performed as described [26]. Briefly, stock virus was serially diluted in cRPMI/IL-2 media cRPMI/IL-2 [RPMI 1640 (Quality Biological, Gaithersburg, Maryland, USA), 15% fetal calf serum (Gemini, Woodland, California, USA), 1% ʟ-glutamine (Quality Biological), 1% penicillin/streptomycin (Quality Biological), and 20 U/ml rhIL-2 (Roche, Indianapolis, Indiana, USA)] in a 96-well 0.5 ml plate. PHA/IL-2-stimulated PBMC (1.5×10^5 cells/well) were added to a final volume of 100 μl and incubated overnight at 37°C/5%CO_2. Cells were washed thrice in cRPMI/IL-2 and transferred to a 96-well round-bottom plate. Supernatants were harvested for measurement of p24 core antigen by sandwich ELISA on days 4, 6, and 8 and lysed according to the kit manufacturer's protocol (Coulter, Hialeah, Florida, USA). Optimal virus dilution and harvest day (>10 ng/ml of p24) were determined for use in downstream virus neutralization assays.

Virus neutralization assay

Virus neutralization assays were performed as previously described [26]. Briefly, sample plasmas and pooled HIV-1 subtype-specific plasma controls were initially diluted 1 : 5 in cRPMI/IL-2, followed by serial 1 : 4 dilutions. The final plasma dilution range including antibody, cells, and virus was 1/20–1/20 480. For baseline virus growth, plasma was replaced with cRPMI/IL-2 in a minimum of eight wells. Normal human plasma was used as a control for nonspecific neutralization (Gemini). An equal volume of appropriately diluted virus stock was added to each well. Serum and virus were incubated for 30 min at 37°C/5% CO_2 prior to the addition of PHA/IL-2 stimulated PBMC (1.5×10^5 cells/well) in a final volume of 100 μl. The plates were incubated overnight at 37°C/5% CO_2. Cells were washed thrice in cRPMI/IL-2 and transferred to a 96-well round-bottom plate. The cells were incubated according to predetermined virus growth kinetics (see above). Supernatants were

harvested for measurement of p24 core antigen by sandwich ELISA and lysed according to the kit manufacturer's protocol (Coulter). Lysed supernatants (quadruplicate wells) were pooled prior to loading on the ELISA plate. Each plasma was assayed at least twice. If the titers between experiments were more than $0.5\log_{10}$, a third experiment was performed. Titers were averaged prior to data analysis. p24 antigen in pooled experimental wells (Vn) was compared with cRMPI/IL-2 containing positive control wells (Vo). The ratio, Vn/Vo was plotted versus the reciprocal dilution of serum. Ninety percent inhibitory dose titers were calculated using linear regression analysis as previously described [28]. Titers less than 20 [no significant neutralization compared to media controls at the lowest plasma dilution (1 : 20)] were replaced with a value of 19 for statistical analyses.

Statistical analysis

Primary analyses compared NAb titers to changes in CD4 T-cell number after interruption of antiviral treatment. We calculated individual slopes for CD4 cell counts between 8 and 96 weeks after treatment interruption, to account for the two-phase decline, as previously described by Skiest *et al.*, on the entire cohort [14]. We then compared slopes by Spearman correlation coefficient with neutralization titer, as well as by neutralization category (restricted neutralization versus expanded neutralization), using a nonparametric test (Mann–Whitney *U* test). For analysis of longitudinal viral load data in the `restricted' neutralization versus `expanded' neutralization groups, we used generalized estimating equations for linear regression to account for the nonindependence of repeated viral load measurements [29,30]. We also stratified 90% inhibitory dose neutralization titer by quartile for each virus and performed a one-way analysis of variance against setpoint, log-transformed viral load, determined by taking the mean of viral load measurements up to 12 weeks after treatment interruption [31,32]. Models were compared with the lowest quartile of neutralization titer as the reference group. For all longitudinal analyses, observations after resumption of ART were censored. A two-sided level of significance of 0.05 was used for all analyses. *P* values for multiple comparisons were corrected using the Bonferroni method. Analyses were performed using Stata SE v10.0 (Stata Corp., College Station, Texas, USA).

Results

Clinical and laboratory data

The cohort consisted of 50 predominantly male (96%) volunteers (Table 1). Whites represented 67%, African Americans 19%, Hispanics 10%, and Asian/Pacific Islanders 4%. Of the 50 volunteers, 76% (38) remained on study for the full 96 weeks. Ten of the remaining 12 restarted ART prior to study conclusion, and two were lost to follow-up. The median $CD4^+$ T-cell number at study initiation was 855 (292–1402 cells/μl). HIV-1 RNA viral load at study entry ranged from less than 50 to 12 441 copies/ml. From study conclusion, the median CD4 T-cell count had fallen to 454 cells/μl. By this point, all volunteers had detectable viral loads with a mean of 4.27 $\log_{10}$ copies/ml (SD 0.80 log). To examine the impact of neutralizing activity at baseline (prior to HAART discontinuation), volunteers with similar $CD4^+$ T-cell number at study entry were separated into progressor and nonprogressor groups. Progressors were defined by a more than 40% reduction in $CD4^+$ T cells through week 24, whereas nonprogressors displayed a less than 20% decline. Mean $\log_{10}$ viral loads in the progressor and nonprogressor groups were comparable at both the beginning and end of the study. Proportions of volunteers with respect to ethnicity and sex were similar between the two groups (data not shown).

Neutralization of subtype B viruses

Table 1 summarizes the mean 90% inhibitory dose against a panel of four well characterized subtype B, PBMC-derived, low-passage primary virus isolates. When comparing geometric

mean titer between progressors and nonprogressors, we found no statistical difference. Subsequent examination of 90% inhibitory dose at the individual isolate level also failed to show statistical significance (US1, P=0.90; US4, P=0.51; CM237, P=0.83; 89.6, P=0.68).

Multi-isolate neutralization

A central question surrounding the relationship between NAb and clinical outcome is whether a high-titer heterologous response is associated with slower disease progression. To assess this in the context of the study, the number of participants with 90% inhibitory dose at least 500 for 1 virus or less (Restricted Neutralization-RN) and at least 2 virus (Expanded Neutralization-EN) were compared (Fig. 1a). Twenty of 25 participants (80%) in the Progressor group exhibited restricted neutralization. Eighteen of 25 (72%) in the nonprogressor group fell into the restricted neutralization category. Expanded neutralization was limited to nine individuals in the Progressor group and seven in the nonprogressor group. Based on the study definition, expanded neutralization was not associated with slower disease progression.

Similarly, we looked at the impact of expanded neutralization on the RNA viral load over the course of the study. At study entry both groups had a similar number with suppressed viral load on treatment, and mean $\log_{10}$ RNA viral loads were similar between the restricted neutralization and expanded neutralization groups (restricted neutralization: 2.05±0.659 versus expanded neutralization: 1.84±0.557; P0.35, Student's t test). In contrast, Fig. 1b showed that the expanded neutralization group possessed a significantly lower mean $\log_{10}$ RNA viral load posttreatment interruption (~4-week posttreatment interruption) (restricted neutralization: 4.06±1.24 versus expanded neutralization: 3.02±1.45; P=0.018, Student's t test). At study endpoint, the expanded neutralization group advantage was no longer significant (restricted neutralization: 4.33±0.909 versus expanded neutralization: 4.06±0.52; P=0.35, Student's t test), suggesting that neutralization of multiple heterologous isolates at treatment interruption was associated with lower viral load and that this effect was transient.

We next evaluated posttreatment interruption viral RNA by quartile of neutralization titer with one-way analysis of variance (Fig. 2). A statistically significant difference in mean $\log_{10}$ viral loads at 12-week posttreatment interruption by neutralization titer was observed against US1 (P=0.005) (Fig. 2a). Examination of the distribution of viral load with quartile of NAb titer appeared to be nonlinear, suggesting that there may be a discrete neutralization threshold necessary for a significant reduction in viral load. This was significantly different between the third and fourth quartiles (Bonferroni corrected P value, 0.036). A nonlinear trend test was also performed using a Wald-type test for a nonlinear hypothesis testing, which yielded an overall Bonferroni-corrected P value of 0.05 [33]. A similar pattern was also observed for the other three viral isolates. However, these differences did not reach statistical significance (Figs. 2b–d).

Duration of neutralization antibody effect

As the principal measure of progression in this study was limited to the rate of CD4^{+} T-cell decline 24 weeks after treatment interruption, we expanded the analysis to all CD4^{+} T-cell observations between restricted neutralization and expanded neutralization groups over the entire study (96 weeks or the last CD4 T-cell count prior to resumption of ART if the volunteer resumed therapy prior to 96 weeks). The expanded neutralization group (n=12) had a higher, but not statistically significant CD4 T-cell count compared to the restricted neutralization group (n=38; P=0.19). Similarly, comparison of CD4 slope between 8 and 96 weeks was not different (P=0.22). Examination of Lowess-smoothed regression plots suggested no difference over the period of study, which was confirmed by generalized estimating equation analysis comparing the two groups over time (P=0.98) (Fig. 3a). In

contrast, examination of Lowess-smoothed regression plots for $\log_{10}$ viral RNA showed a lower rate of viral replication after treatment interruption for the expanded neutralization group (Fig. 3b). Using a model that limited observations to 12, 24, 48, and 96-week intervals, we found the strongest association at 12-week posttreatment interruption ($P=0.003$), which decreased over time, losing statistical significance by week 48 ($P=0.023$ at week 24, $P=0.071$ at week 48, and $P=0.161$ at week 96).

Discussion

Although cell-mediated immunity has been associated with control of viremia, the role of NAb is less clear [34–39]. A number of studies investigating the contribution of NAbs in vertical transmission, nonhuman primate models, vaccines, long-term nonprogressors and highly exposed persistently seronegative cohorts have provided varying results with regard to acquisition and disease progression [40–45]. In this study, we compared NAb titers against a panel of heterologous subtype B HIV-1 primary isolates at time of HAART cessation to changes in viral load and CD4$^+$ T-cell number during a clinical trial of volunteers undergoing long-term structured treatment interruption [14]. There did not appear to be significant association between baseline NAb titers and either CD4 T-cell count or viral load at study conclusion. However, stratification of NAb into quartiles revealed that higher titers were associated with lower viral load, and lower NAb titers may be linked with higher viral load. In addition, a more careful analysis of high-titer multiple isolate neutralization at baseline versus viral load changes over time suggested that NAb were associated with nondurable viral load control.

Interestingly, the NAb titer relationship to viral load appeared to be nonlinear, a pattern that was consistently observed for the four viruses tested. Although the differences in mean $\log_{10}$ viral load were only significant for US1, these and other data suggest that there may be a neutralization `threshold' necessary to control viral replication [46–48]. The unexpected finding that low-to-moderate NAb titers were associated with higher viral load raised interesting questions. These were not autologous isolates nor was the study performed in the setting of transmission. Specifically, this may speak to the complex relationship between pathogen and host, where circulating, but nonneutralizing anti-HIV antibodies may have a deleterious effect on viral load [49,50].

The clinical impact of this finding is unknown, as most HIV-infected individuals do not develop broadly NAb over the course of infection [49,50]. Others have observed that antibodies coated on virions or infected cells were bound directly, or through Fc-bound complement proteins, to potential target cells (e.g., macrophages and dendritic cells) resulting in enhanced transmission [51–53]. Similarly, it has been reported that complement-mediated antibody enhancement is specifically associated with increased viral load [54]. It is unclear whether this is a function of the virus/antibody interaction, quantity of antibody present or if antibodies to specific epitopes on the viral envelope are responsible for enhancement [55–57].

When observing the effect of neutralization over time, there appeared to be a positive but nonsignificant association between expanded neutralization and less rapid decline in CD4 T cells. This was, perhaps, expected as several viral and cellular factors influence CD4$^+$ T-cell death [22,58,59]. Consistent with earlier studies, we found higher titer multi-isolate reactive NAbs were associated with reduced viral load over time and that this effect was transient [60]. We did not attempt to determine whether this was a primary effect of the antibody or a surrogate for the health of the host immune system, the latter perhaps supported by previous work describing heterologous NAb in viremic controllers [60,61]. Alternatively, persons who neutralize multiple isolates may have a greater quasispecies diversity, hence greater

epitope recognition. Previous studies have suggested that the gradual loss of an effective NAb response may be attributed to the rapidity of HIV mutation/selection that prevents the development of effective autologous neutralizing responses [62,63].

In conclusion, we found that high-titer, heterologous HIV-1 NAbs were associated with a reduction in HIV-1 viral load in individuals possessing a more extensive neutralization phenotype while there appeared to be minimal impact on CD4 T-cell decline. Of note, the time from infection to ART is unknown for these A5170 participants, and it is possible that the maturation of NAb responses seen during the natural history of infection may have been interrupted by early treatment [64]. However, the likelihood that more than a handful of these participants initiated therapy during acute or early infection is low. Similarly, the impact of pretreatment interruption ART on immune responses is complex, but in cases where undetectable viral load is achieved, antigen-driven cellular and humoral responses are ultimately diminished [65–67].

Additional studies using isolates from non-B subtypes and donors with diverse HLA haplotypes are needed to support this finding in the context of global pandemic. Further exploration of the interaction between breadth of neutralization and viral load or CD4 cell count could best be obtained from prospective cohort studies of HIV disease progression, ideally in the absence of HAART.

Acknowledgments

References

1. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, et al. Positive effects of combined antiretroviral therapy on CD4R T cell homeostasis and function in advanced HIV disease. Science. 1997; 277:112–116. [PubMed: 9204894]

2. Pontesilli O, Kerkhof-Garde S, Pakker NG, Notermans DW, Roos MT, Klein MR, et al. Antigen-specific T-lymphocyte proliferative responses during highly active antiretroviral therapy (HAART) of HIV-1 infection. Immunol Lett. 1999; 66:213–217. [PubMed: 10203057]

3. Angel JB, Kumar A, Parato K, Filion LG, Diaz-Mitoma F, Daftarian P, et al. Improvement in cell-mediated immune function during potent antihuman immunodeficiency virus therapy with ritonavir plus saquinavir. J Infect Dis. 1998; 177:898–904. [PubMed: 9534961]

4. Li TS, Tubiana R, Katlama C, Calvez V, Ait Mohand H, Autran B. Long-lasting recovery in CD4 T-cell function and viral-load reduction after highly active antiretroviral therapy in advanced HIV-1 disease. Lancet. 1998; 351:1682–1686. [PubMed: 9734884]

5. Sarmati L, d'Ettorre G, Nicastri E, Ercoli L, Uccella I, Massetti P, et al. Neutralizing antibodies against autologous human immunodeficiency virus type 1 isolates in patients with increasing CD4 cell counts despite incomplete virus suppression during antiretroviral treatment. Clin Diagn Lab Immunol. 2001; 8:822–824. [PubMed: 11427434]

6. Kim JH, Mascola JR, Ratto-Kim S, VanCott TC, Loomis-Price L, Cox JH, et al. Selective increases in HIV-specific neutralizing antibody and partial reconstitution of cellular immune responses during prolonged, successful drug therapy of HIV infection. AIDS Res Hum Retroviruses. 2001; 17:1021–1034. [PubMed: 11485619]

7. Birk M, Aleman S, Visco-Comandini U, Sonnerborg A. Proviral HIV-1 dynamics and evolution in patients receiving efficient long-term antiretroviral combination therapy. HIV Med. 2000; 1:205–211. [PubMed: 11737350]

8. Brinkman K, Smeitink JA, Romijn JA, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse-transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. Lancet. 1999; 354:1112–1115. [PubMed: 10509516]

9. Haddow LJ, Wood CW, Ainsworth JG. Discontinuation of nonnucleoside reverse transcriptase inhibitor-based highly active antiretroviral therapy due to nucleoside analogue reverse transcriptase inhibitor-related metabolic toxicity. Int J STD AIDS. 2007; 18:343–346. [PubMed: 17524198]

10. Ruiz L, Martinez-Picado J, Romeu J, Paredes R, Zayat MK, Marfil S, et al. Structured treatment interruption in chronically HIV-1 infected patients after long-term viral suppression. AIDS. 2000; 14:397–403. [PubMed: 10770542]

11. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med. 2006; 355:2283–2296. [PubMed: 17135583]

12. Rosenberg ES, Altfeld M, Poon SH, Phillips MN, Wilkes BM, Eldridge RL, et al. Immune control of HIV-1 after early treatment of acute infection. Nature. 2000; 407:523–526. [PubMed: 11029005]

13. Boschi A, Tinelli C, Ortolani P, Moscatelli G, Morigi G, Arlotti M. CD4+ cell-count-guided treatment interruptions in chronic HIV-infected patients with good response to highly active antiretroviral therapy. AIDS. 2004; 18:2381–2389. [PubMed: 15622314]

14. Skiest DJ, Su Z, Havlir DV, Robertson KR, Coombs RW, Cain P, et al. Interruption of antiretroviral treatment in HIV-infected patients with preserved immune function is associated with a low rate of clinical progression: a prospective study by AIDS Clinical Trials Group 5170. J Infect Dis. 2007; 195:1426–1436. [PubMed: 17436222]

15. Volberding P, Demeter L, Bosch RJ, Aga E, Pettinelli C, Hirsch M, et al. Antiretroviral therapy in acute and recent HIV infection: a prospective multicenter stratified trial of intentionally interrupted treatment. AIDS. 2009; 23:1987–1995. [PubMed: 19696651]

16. Lori F, Foli A, Maserati R, Seminari E, Xu J, Whitman L, et al. Control of HIV during a structured treatment interruption in chronically infected individuals with vigorous T cell responses. HIV Clin Trials. 2002; 3:115–124. [PubMed: 11976989]

17. Binley JM, Trkola A, Ketas T, Schiller D, Clas B, Little S, et al. The effect of highly active antiretroviral therapy on binding and neutralizing antibody responses to human immunodeficiency virus type 1 infection. J Infect Dis. 2000; 182:945–949. [PubMed: 10950795]

18. Ortiz GM, Nixon DF, Trkola A, Binley J, Jin X, Bonhoeffer S, et al. HIV-1-specific immune responses in subjects who temporarily contain virus replication after discontinuation of highly active antiretroviral therapy. J Clin Invest. 1999; 104:R13–R18. [PubMed: 10491418]

19. Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Finzi A, et al. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science. 2010; 329:811–817. [PubMed: 20616231]

20. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science. 2009; 326:285–289. [PubMed: 19729618]

21. Montefiori DC, Hill TS, Vo HT, Walker BD, Rosenberg ES. Neutralizing antibodies associated with viremia control in a subset of individuals after treatment of acute human immunodeficiency virus type 1 infection. J Virol. 2001; 75:10200–10207. [PubMed: 11581388]

22. Vahey MT, Wang Z, Su Z, Nau ME, Krambrink A, Skiest DJ, et al. CD4+ T-cell decline after the interruption of antiretroviral therapy in ACTG A5170 is predicted by differential expression of genes in the ras signaling pathway. AIDS Res Hum Retro-viruses. 2008; 24:1047–1066.

23. Swanson P, Devare SG, Hackett J Jr. Full-length sequence analysis of HIV-1 isolate CM237: a CRF01_AE/B intersubtype recombinant from Thailand. AIDS Res Hum Retroviruses. 2003; 19:707–712. [PubMed: 14506784]

24. Hioe CE, Xu S, Chigurupati P, Burda S, Williams C, Gorny MK, et al. Neutralization of HIV-1 primary isolates by polyclonal and monoclonal human antibodies. Int Immunol. 1997; 9:1281–1290. [PubMed: 9310831]

25. Mascola JR, Snyder SW, Weislow OS, Belay SM, Belshe RB, Schwartz DH, et al. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. The National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. J Infect Dis. 1996; 173:340–348. [PubMed: 8568294]

26. Mascola JR, Louwagie J, McCutchan FE, Fischer CL, Hegerich PA, Wagner KF, et al. Two antigenically distinct subtypes of human immunodeficiency virus type 1: viral genotype predicts neutralization serotype. J Infect Dis. 1994; 169:48–54. [PubMed: 8277197]

27. Collman R, Balliet JW, Gregory SA, Friedman H, Kolson DL, Nathanson N, et al. An infectious molecular clone of an unusual macrophage-tropic and highly cytopathic strain of human immunodeficiency virus type 1. J Virol. 1992; 66:7517–7521. [PubMed: 1433527]

28. Mascola, JR. Neutralization of HIV-1 infection of human peripheral blood mononuclear cells (PBMC): antibody dilution method. In: Michael, NL.; Kim, JH., editors. HIV protocols. Humana Press; Totowa: 1999. p. 309-315.

29. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics. 1986; 42:121–130. [PubMed: 3719049]

30. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics. 1988; 44:1049–1060. [PubMed: 3233245]

31. Jacobson JM, Pat Bucy R, Spritzler J, Saag MS, Eron JJ Jr, Coombs RW, et al. Evidence that intermittent structured treatment interruption, but not immunization with ALVAC-HIV vCP1452; promotes host control of HIV replication: the results of AIDS Clinical Trials Group 5068. J Infect Dis. 2006; 194:623–632. [PubMed: 16897661]

32. Fisher RA. The correlation between relatives on the supposition of Mendelian inheritance. Transcripts of the Royal Society of Edinburgh. 1918; 52:399–433.

33. Weisberg, S. Applied linear regression. 3rd ed.. John Wiley and Sons, Inc.; Hoboken: 2005.

34. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol. 1994; 68:6103–6110. [PubMed: 8057491]

35. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol. 1994; 68:4650–4655. [PubMed: 8207839]

36. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science. 1997; 278:1447–1450. [PubMed: 9367954]

37. Ariyoshi K, Harwood E, Chiengsong-Popov R, Weber J. Is clearance of HIV-1 viraemia at seroconversion mediated by neutralising antibodies? Lancet. 1992; 340:1257–1258. [PubMed: 1359323]

38. Pellegrin I, Legrand E, Neau D, Bonot P, Masquelier B, Pellegrin JL, et al. Kinetics of appearance of neutralizing antibodies in 12 patients with primary or recent HIV-1 infection and relationship with plasma and cellular viral loads. J Acquir Immune Defic Syndr Hum Retrovirol. 1996; 11:438–447. [PubMed: 8605588]

39. Moore JP, Ho DD. Antibodies to discontinuous or conformationally sensitive epitopes on the gp120 glycoprotein of human immunodeficiency virus type 1 are highly prevalent in sera of infected humans. J Virol. 1993; 67:863–875. [PubMed: 7678308]

40. Poignard P, Sabbe R, Picchio GR, Wang M, Gulizia RJ, Katinger H, et al. Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo. Immunity. 1999; 10:431–438. [PubMed: 10229186]

41. Carotenuto P, Looij D, Keldermans L, de Wolf F, Goudsmit J. Neutralizing antibodies are positively associated with CD4+ T-cell counts and T-cell function in long-term AIDS-free infection. AIDS. 1998; 12:1591–1600. [PubMed: 9764777]

42. Harrer T, Harrer E, Kalams SA, Elbeik T, Staprans SI, Feinberg MB, et al. Strong cytotoxic T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogres-sing HIV type 1 infection. AIDS Res Hum Retroviruses. 1996; 12:585–592. [PubMed: 8743084]

43. Lewis J, Balfe P, Arnold C, Kaye S, Tedder RS, McKeating JA. Development of a neutralizing antibody response during acute primary human immunodeficiency virus type 1 infection and the emergence of antigenic variants. J Virol. 1998; 72:8943–8951. [PubMed: 9765439]

44. Loomis-Price LD, Cox JH, Mascola JR, VanCott TC, Michael NL, Fouts TR, et al. Correlation between humoral responses to human immunodeficiency virus type 1 envelope and disease progression in early-stage infection. J Infect Dis. 1998; 178:1306–1316. [PubMed: 9780250]

45. Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, Hanson EE, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by pasive infusion of neutralizing antibodies. Nat Med. 2000; 6:207–210. [PubMed: 10655111]

46. Parren PW, Marx PA, Hessell AJ, Luckay A, Harouse J, Cheng-Mayer C, et al. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. J Virol. 2001; 75:8340–8347. [PubMed: 11483779]

47. Nishimura Y, Igarashi T, Haigwood N, Sadjadpour R, Plishka RJ, Buckler-White A, et al. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. J Virol. 2002; 76:2123–2130. [PubMed: 11836389]

48. Trkola A, Kuster H, Rusert P, von Wyl V, Leemann C, Weber R, et al. In vivo efficacy of human immunodeficiency virus neutralizing antibodies: estimates for protective titers. J Virol. 2008; 82:1591–1599. [PubMed: 18032508]

49. Simek MD, Rida W, Priddy FH, Pung P, Carrow E, Laufer DS, et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. J Virol. 2009; 83:7337–7348. [PubMed: 19439467]

50. Moore PL, Crooks ET, Porter L, Zhu P, Cayanan CS, Grise H, et al. Nature of nonfunctional envelope proteins on the surface of human immunodeficiency virus type 1. J Virol. 2006; 80:2515–2528. [PubMed: 16474158]

51. Robinson WE. Mechanism for complement-mediated, antibody-dependent enhancement of human immunodeficiency virus type 1 infection in MT2 cells is enhanced entry through CD4, CD21, and CXCR4 chemokine receptors. Viral Immunol. 2006; 19:434–447. [PubMed: 16987062]

52. Huerta L, Gomez-Icazbalceta G, Soto-Ramirez L, Viveros-Rogel M, Rodriguez R, Fuentes L, et al. Human immunodeficiency virus 1 (HIV-1) envelope-dependent cell-cell fusion modulation by HIV-positive sera is related to disease progression. J Gen Virol. 2005; 86:1961–1966. [PubMed: 15958674]

53. Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, et al. Fc receptor but not complement binding is important in antibody protection against HIV. Nature. 2007; 449:101–104. [PubMed: 17805298]

54. Szabo J, Prohaszka Z, Toth FD, Gyuris A, Segesdi J, Banhegyi D, et al. Strong correlation between the complement-mediated antibody-dependent enhancement of HIV-1 infection and plasma viral load. AIDS. 1999; 13:1841–1849. [PubMed: 10513641]

55. Takeda A, Robinson JE, Ho DD, Debouck C, Haigwood NL, Ennis FA. Distinction of human immunodeficiency virus type 1 neutralization and infection enhancement by human monoclonal antibodies to glycoprotein 120. J Clin Invest. 1992; 89:1952–1957. [PubMed: 1376330]

56. Connor RI, Dinces NB, Howell AL, Romet-Lemonne JL, Pasquali JL, Fanger MW. Fc receptors for IgG (Fc gamma Rs) on human monocytes and macrophages are not infectivity receptors for human immunodeficiency virus type 1 (HIV-1): studies using bispecific antibodies to target HIV-1 to various myeloid cell surface molecules, including the Fc gamma R. Proc Natl Acad Sci U S A. 1991; 88:9593–9597. [PubMed: 1835086]

57. Subbramanian RA, Xu J, Toma E, Morisset R, Cohen EA, Menezes J, et al. Comparison of human immunodeficiency virus (HIV)-specific infection-enhancing and -inhibiting antibodies in AIDS patients. J Clin Microbiol. 2002; 40:2141–2146. [PubMed: 12037078]

58. Stewart SA, Poon B, Jowett JB, Chen IS. Human immunodeficiency virus type 1 Vpr induces apoptosis following cell cycle arrest. J Virol. 1997; 71:5579–5592. [PubMed: 9188632]

59. Finkel TH, Tudor-Williams G, Banda NK, Cotton MF, Curiel T, Monks C, et al. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med. 1995; 1:129–134. [PubMed: 7585008]

60. Trkola A, Kuster H, Leemann C, Oxenius A, Fagard C, Furrer H, et al. Humoral immunity to HIV-1: kinetics of antibody responses in chronic infection reflects capacity of immune system to improve viral set point. Blood. 2004; 104:1784–1792. [PubMed: 15187026]

61. Pereyra F, Addo MM, Kaufmann DE, Liu Y, Miura T, Rathod A, et al. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. J Infect Dis. 2008; 197:563–571. [PubMed: 18275276]

62. Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, et al. Antibody neutralization and escape by HIV-1. Nature. 2003; 422:307–312. [PubMed: 12646921]

63. Frost SD, Wrin T, Smith DM, Kosakovsky Pond SL, Liu Y, Paxinos E, et al. Neutralizing antibody responses drive the evolution of human immunodeficiency virus type 1 envelope during recent HIV infection. Proc Natl Acad Sci U S A. 2005; 102:18514–18519. [PubMed: 16339909]

64. Tomaras GD, Haynes BF. HIV-1-specific antibody responses during acute and chronic HIV-1 infection. Curr Opin HIV AIDS. 2009; 4:373–379. [PubMed: 20048700]

65. Markowitz M, Vesanen M, Tenner-Racz K, Cao Y, Binley JM, Talal A, et al. The effect of commencing combination antiretroviral therapy soon after human immunodeficiency virus type 1 infection on viral replication and antiviral immune responses. J Infect Dis. 1999; 179:527–537. [PubMed: 9952358]

66. Morris MK, Katzenstein DA, Israelski D, Zolopa A, Hendry RM, Hanson CV. Characterization of the HIV-1 specific humoral immune response during highly active antiretroviral therapy (HAART). J Acquir Immune Defic Syndr. 2001; 28:405–415. [PubMed: 11744827]

67. Ogg GS, Jin X, Bonhoeffer S, Moss P, Nowak MA, Monard S, et al. Decay kinetics of human immunodeficiency virus-specific effector cytotoxic T lymphocytes after combination antiretroviral therapy. J Virol. 1999; 73:797–800. [PubMed: 9847391]

(a)

(b)
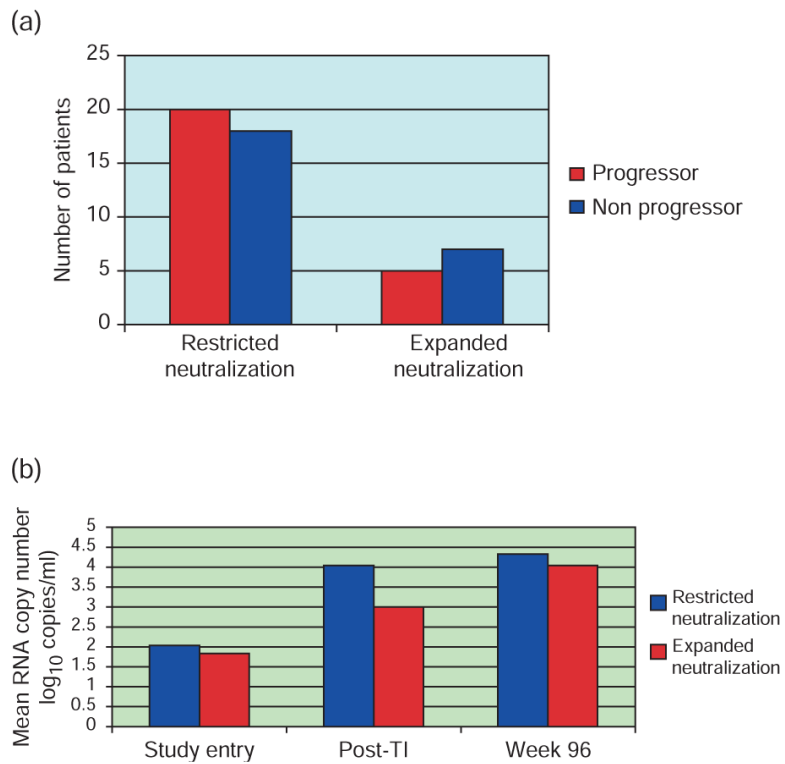
Fig. 1. Baseline neutralization and serial viral loads stratified by neutralization status
(a) Number of progressors and nonprogressors exhibiting the restricted neutralization or expanded neutralization neutralization phenotype. (b) Mean $\log_{10}$ plasma viral load in restricted neutralization versus expanded neutralization groups at study entry, 4-week posttreatment interruption and study conclusion.

Fig. 2. Distribution plots of $\log_{10}$ HIV-1 RNA setpoint by quartile of neutralizing antibody titer
Points represent average of viral load measurement up to 12 weeks for each volunteer.

(a)

(b)

Fig. 3. Lowess-smoothed regression plots
(a) CD4 and (b) viral load trajectories based on two or more 90% inhibitory dose
neutralization titers greater than 500.

Table 1

Volunteer clinical, virologic, and immunologic data.
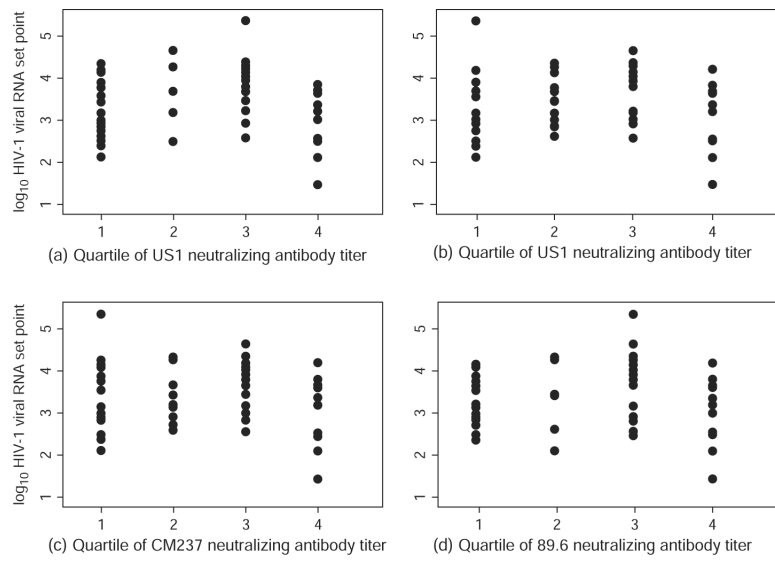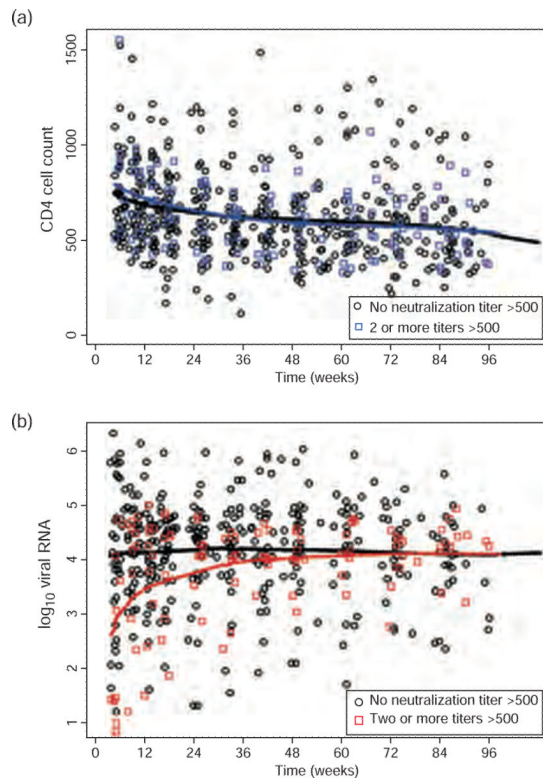
Patient no.	CD4 cell count (entry)	log$_{10}$ RNA (posttreatment interruption)	CD4 cell count (96 weeks)	log$_{10}$ RNA (96 weeks)	Geometric mean titer[a]				Restart ART
					US1	US4	89.6	CM237	
Progressors (*n*=25)									
1	887	5.76	446	4.52	176	158	<20	397	Yes
3	1009	3.80	432	4.93	36	<20	<20	61	No
5	1115	4.63	387	3.97	<20	22	19	<20	No
7	681	4.00	167	3.81	<20	<20	<20	23	Yes
8	1006	5.95	588	4.29	247	214	203	244	No
9	899	5.28	112	5.82	60	149	92	74	Yes
10	922	3.74	304	3.52	25	60	61	133	No
11	585	2.43	277	4.46	<20	<20	<20	40	No
13	1155	3.30	648	3.64	<20	35	43	70	No
18	1311	4.12	696	3.58	<20	<20	<20	<20	No
19	1118	5.78	445	4.07	180	139	373	215	No
22	671	3.91	200	4.55	62	111	54	62	Yes
23	706	5.50	539	4.52	<20	23	<20	<20	No
24	1191	2.05	454	3.80	457	1497	44	676	No
25	847	5.52	500	5.93	55	157	146	74	Yes
27	985	4.61	148	5.92	<20	<20	<20	<20	Yes
28	598	5.78	237	4.97	63	<20	126	<20	Yes
30	1141	4.16	621	3.33	<20	<20	<20	<20	No
32	707	4.73	353	4.42	1075	3917	1724	1330	No
33	649	4.70	435	5.85	<20	<20	<20	<20	Yes
41	1068	5.83	293	5.79	35	218	174	130	Yes
43	1204	5.56	722	4.40	1075	2955	859	1134	Yes
46	855	4.65	421	4.25	268	1225	592	1134	No
47	1200	1.69	384	4.34	23	<20	<20	<20	No
48	817	1.69	374	4.52	677	1900	775	1021	No
Summary[b]	922 (462)	4.37 (1.32)	421 (297)	4.53 (0.80)	65 (63–67)	96 (94–99)	64 (62–66)	91 (89–93)	10 (40%)

Patient no.	CD4 cell count (entry)	log10 RNA (posttreatment interruption)	CD4 cell count (96 weeks)	log10 RNA (96 weeks)	Geometric mean titer[a]				Restart ART
					US1	US4	89.6	CM237	
Nonprogressors (n=25)									
2	533	4.06	373	4.24	1725	5554	1530	7250	No
4	652	6.31	454	4.63	<20	26	<20	<20	No
6	856	5.18	726	4.82	<20	36	24	34	No
12	1306	2.53	1058	2.36	92	189	192	79	No
14	1117	1.69	1111	3.22	<20	<20	33	<20	No
15	810	2.32	463	3.88	57	139	117	40	No
16	989	1.69	810	4.08	446	798	2008	520	No
17	586	2.43	434	4.02	197	48	31	70	No
20	1297	3.30	897	4.06	46	75	<20	44	No
21	851	1.69	715	2.88	1983	3002	1421	2758	No
26	695	1.69	548	4.52	890	3617	845	790	No
29	1047	2.80	888	3.31	<20	48	24	50	No
31	833	4.74	493	4.45	<20	35	33	24	No
34	756	3.61	533	4.53	274	2962	1450	2059	No
35	732	4.39	75	4.69	33	24	39	<20	No
36	710	4.30	462	4.58	<20	20	<20	<20	No
37	676	3.58	346	4.49	<20	<20	<20	<20	No
38	911	2.07	903	2.93	<20	<20	<20	<20	No
39	699	3.81	894	3.15	<20	35	<20	<20	No
40	922	3.23	809	2.72	<20	<20	<20	<20	No
42	292	4.76	310	4.75	52	49	22	34	No
44	432	1.69	375	3.23	2607	5026	1252	3290	No
45	963	3.07	702	4.06	775	2015	783	1519	No
49	986	4.45	454	5.44	262	39	42	106	No
50	693	4.08	423	4.87	<20	<20	<20	<20	No
Summary[b]	810 (396)	3.34 (1.27)	533 (449)	4.00 (0.80)	85 (83–88)	123 (120–126)	84 (82–87)	96 (93–99)	0 (0)

ART, antiretroviral therapy.

[a] Geometric mean titers (GMT) based on an infectious dose for 90% neutralization (90% inhibitory dose). Titers <20 represent a titer that is below the detection limit of the assay system.

[b]Summary statistics are mean (standard deviation) for log10 viral RNA; median (interquartile range) for CD4 T-cell counts, mean (95% confidence interval) for GMT, and sum (percentage) of those re-starting ART.