Published in final edited form as:

J Acquir Immune Defic Syndr. 2011 May 1; 57(1): 9–15. doi:10.1097/QAI.0b013e3182100c1b.

Correlation between circulating HIV-1 RNA and broad HIV-1 neutralizing antibody activity

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Abstract

Objective—To examine the relationship between HIV-1 antigenic load (plasma RNA copies/ml) and broad HIV-1 neutralizing antibody activity.

Methods—Plasma from 120 HIV-1 infected patients, including HIV-1 Natural Viral Suppressors (similar to Elite Controllers), was tested for neutralization against 15 Tier 1/Tier 2 HIV-1 pseudoviruses. Broad HIV-1 neutralizing antibody activity was confirmed with IgG and heterologous clade testing (18 pseudoviruses from Clades A, C, and CRF02_AG). Statistical analysis was performed to determine factors associated with broad HIV-1 neutralizing antibody activity.

Results—Ten individuals with broad HIV-1 neutralizing antibody activity were identified. These individuals had a median CD4 count of 589 cells/ul (range 202–927), 1,611 HIV-1 RNA copies/ml (range 110–8,964), and 13 years since HIV diagnosis (range 1–22). There was a significant correlation between the presence of broadly neutralizing antibodies in those with HIV-1 RNA between 100 and 10,000 copies/ml compared to those <100 or >10,000 copies/ml ($p=.0003$ and $p=.0245$, respectively). Individuals with HIV-1 RNA 100–10,000 copies/ml had a higher number of Tier 2 viruses neutralized compared to the <100 or >10,000 copies/ml groups ($p=<.0001$ and $p=.076$, respectively). Male sex was associated with broad HIV-1 neutralizing antibody activity ($p=.016$).

Conclusion—These results indicate that low but persistent HIV antigen expression correlates with broad HIV-1 neutralizing antibody activity. At higher levels of plasma viremia, neutralization titers were diminished. Conversely, at lower levels, there appears to be insufficient antigen stimulation to maintain high neutralization titers. These findings may have important implications in furthering the understanding of the humoral response to HIV infection.

Keywords

HIV; broadly neutralizing antibody; neutralizing activity; HIV RNA; natural viral suppressor; elite controller

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Conflict of Interests: The authors (MMS, YG, ALD, MSS, MH, GKL, RRR) have no conflict of interests to report.

Introduction

The immune system requires the presence of sufficient quantities of antigen in order to elicit an immune response. Despite numerous studies in HIV-infected patients, the relationship between HIV-1 antigenic load and HIV-1 neutralization remains unclear. Several lines of evidence suggest that the antibody response to the HIV-1 envelope lacks durability.^{1,2} Because of this, we hypothesized that a persistent but low amount of circulating HIV-1 virus would be necessary to produce and maintain a robust humoral immune response. This study was undertaken to examine the relationship between HIV-1 antigenic load (as reflected by HIV-1 plasma RNA copies/ml) and broad HIV-1 neutralizing antibody activity.

Methods

Study Patients

Characterization of HIV-1 antigenic load and neutralizing activity was performed in 120 HIV-infected patients. These included HIV-1 infected patients with a wide range of viremia, who belonged to one of the following 4 cohorts: 1) HIV-1 Natural Viral Suppressors (NVS) defined as individuals with HIV-1 infection by both Western Blot and proviral DNA, and at least a 2-year period with <400 HIV-1 RNA copies/ml in the absence of highly active antiretroviral therapy (HAART);^{3,4} 2) Low Viral Load (LVL) cohort consists of individuals with 500–20,000 HIV-1 RNA copies/ml in the absence of HAART; 3) Medium/High Viral Load (MHVL) cohort consists of individuals with >20,000 HIV-1 RNA copies/ml in the absence of HAART; and 4) HAART cohort which consists of individuals on their first HAART regimen with suppressed viral loads for at least one year. Demographic characteristics of these cohorts are given in Table 1. Forty eight NVS, 36 LVL, 18 MHVL, and 18 HAART patients were tested. This study has IRB approval, and all individuals provided informed consent.

HIV-1 neutralization testing

HIV-1 neutralization testing was performed using a luciferase-based assay in TZM.bl cells as previously described.⁵ This assay measures the reduction in luciferase expression following a single round of virus infection. For plasma, starting at a 1:20 dilution, 3-fold serial dilutions of serum were performed in duplicate. 200 TCID₅₀ of pseudovirus was added to each well (with plasma or IgG) and incubated for 1 hour at 37°C. TZM.bl cells were then added (1×10^4 /well) in 10% D-MEM medium containing DEAE-Dextran (Sigma) at a final concentration of 11 µg/ml. Following 48 hours (37°C), 150 µl of medium was added to 100 µl of Bright-Glo luciferase reagent (Promega, Madison, WI), and luminescence measured using a Victor 3 luminometer (Perkin Elmer, Waltham, MA). Stocks of Env-pseudotyped viruses were prepared by transfection of 293T/17 cells.⁵ All patient plasma was tested against 3 Tier 1 Clade B pseudoviruses (SF162.LS, BaL.26, SS1196.1), 12 Tier 2 Clade B pseudoviruses (6535.3, QH0692.42, SC422661.8, PVO.4, TRO.11, AC10.0.29, RHPA4259.7, THRO4156.18, REJO4541.67, TRJO4551.58, WITO4160.33, CAAN5342.A2), and MuLV control.

The presence of broadly neutralizing antibodies in plasma was confirmed with purified IgG tested against the above Tier 1 and 2 viruses. IgG was purified by Protein A (GE Healthcare, Piscataway, NJ), and tested in neutralization assays starting at 333 µg/ml or greater, with 3-fold serial dilutions. For patients with both plasma and IgG broadly neutralizing antibodies, heterologous clade testing was performed with the following 18 virus panel, depending on sample availability: Clade C (Du156.12, Du172.17, Du422.1, ZM53M.PB12, ZM135M.PL10a, ZM197M.PB7, ZM214M.PL15), Clade A (Q23.17, Q259.d2.17, Q461.e2,

Q168.a2, 3415.v1.c1, 0439.v5.c1, 0260.v5.c1, 3365.v2.c20), CRF02_AG (257-31, 263-8, 211-9), and MuLV control.

Statistical analysis and definitions

Inhibitory dose 80 titer (ID80) was defined as the plasma dilution causing 80% reduction in relative luminescence units (RLU) compared to controls. Broadly neutralizing was defined as plasma with ID80 neutralization titers at least twice the MuLV control against 8 or more of the 12 Tier 2 viruses.⁶ Statistical analysis was performed with Graph Pad Prism (San Francisco, CA) for the Mann-Whitney and Fisher's exact tests. Logistic regression was done with STATA statistical software (College Station, TX). P values <.05 were considered statistically significant.

Results

Identification of and characteristics of individuals with broadly neutralizing antibodies

Twelve of the 120 individuals demonstrated broadly neutralizing plasma, with the ability to neutralize at least 75% of the Tier 2 viruses. After testing of purified IgG against the 15 Tier 1 and Tier 2 viruses, 10 individuals had plasma neutralization profiles that closely matched their IgG neutralization profiles. Table 2 provides a summary of the plasma Tier 2 testing of the 10 patients with broadly neutralizing antibodies. The other 2 individuals had IgG that had no neutralizing activity against Tier 2 viruses, and in retrospect these 2 individuals had a positive MuLV control during ID50 plasma testing (but not ID80). One of these 2 individuals also had a contemporaneous serum sample tested, which did not show broadly neutralizing activity against HIV-1 (or activity against MuLV). Heterologous virus testing of IgG was done in 6 of 10 individuals with broadly neutralizing antibodies. Results demonstrated broad activity against Clade A, Clade C, and CR0F_AG viruses in all individuals tested. Table 3 provides a summary of the heterologous clade testing.

Overall, 2 of 48 NVS (4.2%), 8 of 34 LVL (22.2%), 0 of 18 MHVL (0%), and 0 of 18 HAART (0%) individuals had broadly neutralizing antibodies. These 10 carried a diagnosis of HIV-1 for a median of 13 years (range 1–22). Their median CD4 count was 589 cells/ul (range 202–927), and HIV-1 viral load of 1,611 copies/ml (range 110–8,964).

Six of these 10 individuals with broadly neutralizing plasma continue to maintain normal CD4 counts without therapy. However HAART was initiated in 4 individuals (three in the LVL group and one in the NVS group). One patient, 14 years after diagnosis, had an increase in HIV-1 RNA from a baseline of 5,000–10,000 copies/ml to close to 30,000 copies/ml with a CD4 loss from 507 to 288 cells/ul in 6 months. Two patients, while maintaining HIV-1 RNA <5,000 copies/ml, had slow but progressive CD4 loss to less than 300 cells/ul requiring HAART therapy. The final patient, although maintaining HIV-1 RNA <200 copies/ml over many years, likewise had a slow CD4 loss over and HAART therapy was initiated at a CD4 count of 489 (CD4% of 18) 19 years after diagnosis of HIV-1.

Correlation of the presence of broadly neutralizing antibodies with viral load

The presence of broadly neutralizing antibodies was analyzed according to cohort as well as HIV-1 viral load. Although all of the patients with broadly neutralizing antibodies were in the NVS and LVL groups, there was no correlation between presence of broadly neutralizing plasma and patient cohort (data not shown). However, there was a significant correlation between plasma viral load and the presence of broadly neutralizing antibodies in those with HIV-1 RNA between 10^2 and 10^4 copies/ml compared to those $<10^2$ and those with $>10^4$ HIV-1 RNA copies/ml ($p=.0003$ and $.0245$, respectively) with the two-tailed Fisher's exact test. In addition, individuals with HIV-1 RNA between 10^2 and 10^4 copies/ml

had a higher number of Tier 2 viruses neutralized compared to the $<10^2$ or $>10^4$ copies/ml groups. This reached significance when compared to the $<10^2$ group ($p<.0001$) and there was a trend toward significance in the $>10^4$ group ($p=.076$) with the two-tailed Mann Whitney test. The correlation of broadly neutralizing antibodies with viral load, and the correlation between the total number of Tier 2 viruses neutralized for the 10^2 – 10^4 compared to the $<10^2$ group was significant whether or not the HAART-treated patients were included in the analyses ($p=.0018$ and $p=.0002$, respectively). Please refer to Figure 1 for a summary of the above.

In addition, individuals with HIV-1 RNA between 10^2 and 10^4 copies/ml had a higher mean ID80 neutralization titer for each of the 15 viruses tested compared to the $<10^2$ or $>10^4$ copies/ml groups. This reached significance for 46.7% of the individual viruses tested when analyzed by single patient neutralization using the two-tailed Mann Whitney test. The above is seen in Table 4.

Using logistic regression, there was no relation between presence of broadly neutralizing antibody and age, HIV risk factor, duration of HIV infection, HCV status, or CD4 count (data not shown). There was, however an association with male sex ($p=.016$), as 10 of 11 persons with broadly neutralizing plasma were male.

Discussion

The data suggests that in HIV-1 infected individuals, the ability to produce broadly neutralizing antibodies correlates with plasma viremia. The LVL group, which was defined as having viral loads between 500 and 20,000 copies/ml, had the most individuals with HIV-1 broadly neutralizing plasma. In addition, there were two individuals in the NVS cohort who had broadly neutralizing activity. Importantly, both of these NVS individuals had low level viremia detectable by routine HIV-1 RNA testing, as opposed to the majority of NVS who consistently have undetectable viremia and do not have broadly neutralizing antibodies. Because of this observation, the data was analyzed according to HIV-1 RNA copies, and the presence of broadly neutralizing antibodies correlated with a viral load of 10^2 and 10^4 copies/ml. We did not observe any persons with broadly neutralizing antibodies who had $<10^2$ or $>10^4$ HIV-1 RNA copies/ml. This suggests that an optimum amount of antigen production is needed for this response, which is less likely to be seen in patients with higher or lower HIV-1 RNA copies.

Patients with $<10^2$ HIV-1 RNA copies/ml, whether due to natural control or HAART, demonstrated diminished ID80 neutralization values, an observation made in prior studies.^{2,7} Likewise, there were no individuals with broadly neutralizing activity in this group. Overall, the low neutralization titers in the NVS and those on HAART suggests that a decrease in antigen load led to a decrease in antibody titer, a situation that has been described in patients after starting HAART.² The lack of continual high titers in these situations may mirror the poor long-term immunogenicity of gp120 seen in the VaxGen vaccine trial.¹ Importantly, a low plasma neutralization titer in an NVS patient does not necessarily mean that they never had a robust humoral response to HIV. As we have shown in the NVS, a patient can harbor a large number of memory B cells that can be stimulated to produce epitope-specific HIV antibodies that can not be found in circulation.⁸

Several previous studies in LTNP groups noted increasing neutralization breadth and titers to heterologous viruses over time.^{9–11} Importantly, not all studies have shown this.^{12,13} LTNPs, which were defined by CD4 count only, have wide ranging viral loads, and the combined LVL and NVS groups in this study can be viewed as constituting a cohort similar to LTNPs (though not all would meet the CD4 or time requirements). More recent studies

have focused on cohorts of patients, such as ours, that have a definition of HIV suppression based on viral load and not CD4 count.^{2,7,14,15} In this study 12% of the combined NVS/LVL groups had broadly neutralizing plasma, which is comparable to the 12% reported for a combined cohort of elite and viremic controllers (similar to our definition of NVS and LVL).¹⁴ The disparate results in this study between those NVS who have greater or less than 10^2 HIV-1 RNA copies/ml may account for the discrepancies in prior LTNP studies.

At the high levels of plasma viremia, a diminished humoral response was observed. The ID80 titers in those with $>10^4$ HIV-1 RNA copies/ml for the Tier 1 and Tier 2 viruses were lower than those with 10^2 – 10^4 HIV-1 RNA copies/ml but comparable to those with those with $<10^2$ HIV-1 RNA copies/ml. Also, there were no persons in this group that demonstrated broadly neutralizing plasma. This data is consistent with the observation of low antibody titers and neutralization breadth seen in the later stages of HIV,^{10,11,17} which could be due to progressive B cell dysfunction caused by HIV-1.

Recently, some groups have noted increasing neutralization breadth with higher viral loads.^{16,18–20} These studies have had fewer patients than this study, and most of them have tested fewer viruses than ours. However, it is likely that some of these patients do develop broadly neutralizing antibodies, which are lost as HIV disease progresses. It should be noted that 1 of the 10 patients with broadly neutralizing antibodies in this study (who also eventually received HAART) experienced an increase in HIV-1 viral load to close to 30,000 copies/ml. In this individual we retested the plasma from the time point just before starting HAART, and broadly neutralizing antibody response with a similar neutralization pattern to what was seen from the time point 18 months prior. Thus, the development of broadly neutralizing antibodies in this individual was related to the 13 years his immune system was exposed to HIV at lower viral loads (rather than the six months with higher viral loads), and that without HAART these antibodies could have disappeared with further HIV-mediated immune dysfunction and destruction.

Besides the role of the optimum amount of antigen associated with broadly neutralizing plasma highlighted in this study, duration of infection has also been noted to be important.^{9,10,16} In this study, the patients with broadly neutralizing antibodies also had been infected with HIV for a relatively long time (a median of 11 years since HIV diagnosis), but we were unable to show an association with time since diagnosis. Furthermore, this can not be considered the sole determinant, as the NVS and LVL had comparable length of HIV infection, but a difference in HIV-1 RNA copies and ability to neutralize various strains of HIV-1. We were unable to find other correlations with broadly neutralizing plasma other than the viral load and male sex. Regarding the later, 90% (9 of 10) of the patients with broadly neutralizing plasma were male; however, the significance of this association remains unknown.

The study's strengths include the total number of patients tested (120 patients), the high number of Tier 1 and Tier 2 viruses tested (at least 15 viruses per patient). In addition, although we used a definition of broadly neutralizing that has been used previously,⁶ these were verified with both IgG and heterologous clade testing. Finally, this study was focused on individuals with low viral loads (82 NVS/LVL), where the majority of patients with broad neutralization were found. However, our findings need to be considered within the inherent limitations of a cross-sectional study. In terms of generalizability, it is important to note that the study subjects are predominantly African-American (>95%), Clade B infected, and have relatively high rates of IDU, thus the findings need to be confirmed in other cohorts of patients. In addition, although we have demonstrated a correlation between viral load and broadly neutralizing antibodies, this study was not designed to find causality or a putative mechanism (such as low antigen stimulation leading to low neutralization levels or

high viral loads causing B cell dysfunction). Finally, correlations about the potential protective effects of neutralizing antibodies can not be done in this type of study. More prospective studies starting in acute HIV infection, such as the one by Piantadosi et al., should be able to answer this question.¹⁸

Conclusion

In conclusion, these results indicate that low but persistent HIV antigen expression correlates with high levels of broad HIV-1 neutralizing antibody activity. The presence of broadly neutralizing activity was associated with 10^2 and 10^4 copies of HIV-1 RNA. At higher levels of plasma viremia, neutralization levels were diminished. Conversely, at lower levels, either due to antiretrovirals or natural suppression by other mechanisms, there appears to be insufficient antigen stimulation to maintain high neutralization levels. These findings may have important implications in furthering the understanding of the humoral response to HIV infection.

Acknowledgments

We would like to thank members of the NVS Study and Becky Boyce, RN, the study coordinator.

Sources of funding: Research conducted as part of the Comprehensive Antibody - Vaccine Immune Monitoring Consortium (CA-VIMC) under the Collaboration for AIDS Vaccine Discovery (CAVD) with support from the Bill & Melinda Gates Foundation. M.M.S. supported by award number NIH K12RR023250 (PI: Alan R. Shuldiner) and NIH 1K23AI084580-01A1 (PI: Mohammad M. Sajadi).

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Figure 1.

A. Correlation between plasma viral load and the presence of broadly neutralizing antibodies. There was a correlation between plasma viral load and the presence of broadly neutralizing antibodies in those with HIV-1 RNA between 10^2 and 10^4 copies/ml compared to those $<10^2$ or $>10^4$ copies/ml ($p=.0003$ and $.0245$, respectively). B. Correlation between plasma viral load and number of Tier 2 viruses neutralized. Presence of HIV-1 RNA between 10^2 and 10^4 copies/ml correlated with number of Tier 2 viruses neutralized when compared with those $<10^2$ ($p < .0001$) and trended toward significance when compared with those with $>10^4$ copies/ml ($p=.076$). VL= viral load (HIV-1 RNA copies/ml). Broadly neutralizing was defined as plasma with ID80 neutralization activity against 8 or more of the 12 Tier 2 viruses tested. P value $<.05$ was considered significant (by two-tailed Fisher's Exact test or two-tailed Mann Whitney test).

Table 1

Demographics of the 4 cohorts. HIV-1 Natural Viral Suppressors (NVS) are HIV-infected individuals with HIV-1 RNA <400 copies/ml in the absence of therapy (n=48).

Cohort	NVS	LVL	MHVL	HAART
Age	49 (range 29–60)	43 (range 24–63)	44 (range 22–58)	45 (range 23–61)
Sex	49% M 51% F	59% M 41% F	61% M 39% F	78% M 22% F
Race	100% AA	97% AA 3% Caucasian	89% AA 11% Caucasian	95% AA 5% Caucasian
Risk factor for HIV-1 infection	59% IDU 43% S	35% IDU 65% S	52% IDU 48% S	23% IDU 77% S
Median years with diagnosis of HIV-1	11 (range 2–21)	6 (range 0–20)	3 (range 1–21)	6 (range 1–14)
Median of latest HIV-1 viral load (copies/ml)	<50 (range <40–537)	1,785 (range 514–19,100)	202,000 (range 28,721–>1,000,000)	<50
Median of latest CD4 count (cells/ul)	817 (range 325–1929)	632 (range 246–1313)	77 (range 3–829)	344 (range 134–644)

Low Viral Load (LVL) are HIV-1 infected patients who are not treated by antiretrovirals whose HIV-1 RNA are 500–20,000 copies/ml (N=36). Medium-High Viral load (MHVL) are HIV-1 infected persons who are not on antiretroviral therapy whose HIV-1 RNA are >20,000 copies/ml (N=18). The HAART cohort (N=18) is comprised of individuals on their first antiretroviral regimen with suppressed HIV-1 viral loads for greater than one year. M=male, F=female, AA= African-American. IDU= injection drug use, S=sexual.

Table 2

Plasma ID80 HIV neutralization assay results.

Pt#	SF162	BaL. 26	SS 1196.1	SS 6535.3	QH0 692.42	SC 422661.8	PVO.4	TRO .11	AC10.0. 29	RHPA 4259.7	THRO 4156.18	REJO 4541.67	TRJO 4551.58	WITO 4160.33	CAAN 5342.A2	MuLV
1 (NVS)	8,163	1585	437	1353	153	294	488	712	1670	932	347	623	269	33	1676	<20
2 (NVS)	1,539	515	88	875	38	74	57	211	265	227	28	214	34	20	229	<20
3 (LVL)	5,112	271	561	97	93	64	81	148	32	81	90	115	<20	48	44	<20
4 (LVL)	1,652	344	86	124	47	45	24	59	46	54	<20	67	24	<20	96	<20
5 (LVL)	4,831	201	116	71	57	221	170	307	70	60	117	119	46	60	55	<20
6 (LVL)	3,120	394	245	140	80	131	217	514	177	358	42	128	72	620	72	<20
7 (LVL)	1,296	434	146	79	32	74	92	45	83	135	<20	102	22	<20	103	<20
8 (LVL)	4,275	812	279	147	110	318	174	429	<20	261	45	833	181	272	66	<20
9 (LVL)	2,258	544	194	44	91	128	89	76	<20	203	<20	196	66	<20	34	<20
10 (LVL)	7,771	499	146	149	44	79	114	120	54	57	25	125	23	68	93	<20
11 (Control)	2,905	120	51	57	<20	<20	<20	<20	<20	33	<20	<20	<20	93	<20	<20
12 (Control)	521	43	23	<20	<20	<20	<20	<20	29	<20	<20	<20	<20	<20	<20	<20
13 (Control)	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	55	<20	<20
14 (Control)	3,574	235	165	77	26	30	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
15 (Control)	1,697	130	88	80	46	45	22	74	<20	21	28	49	<20	<20	<20	<20
16 (Control)	1,206	164	108	38	33	44	<20	31	<20	<20	22	50	<20	28	<20	<20
17 (Control)	5,400	425	223	52	35	21	<20	42	<20	35	<20	51	<20	<20	<20	<20
18 (Control)	1,172	92	23	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
19 (Control)	236	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
20 (Control)	4,679	530	56	95	22	<20	<20	<20	<20	<20	30	22	<20	<20	<20	<20

Results for 20 of the 120 individuals tested are shown (All 10 with broadly neutralizing antibodies, and 10 patients with non-broadly neutralizing plasma chosen sequentially). The first 3 columns of viruses (SF162, BaL.26, and SSI 196.1 are Tier 1 viruses), the remaining 12 viruses are Tier 2 viruses, with the MuLV control virus in the last column. ID80 represents the highest concentration at which 80% of the infectious virus was neutralized. Broadly neutralizing plasma was defined as plasma demonstrating neutralization (ID80 titer at least twice the background shown in bold) in at least 8 of 12 Tier 2 HIV pseudoviruses.

Table 3

Plasma ID80 heterologous HIV neutralization assay results.

Pt#	Clade C	Clade C	Clade C	Clade C	Clade C	Clade A	Clade A	Clade A	Clade A	Clade A	CRF02_AG	CRF02_AG
	Du156 .12	Du172 .17	Du422 .1	ZM53M .PB12	ZM135 MPL10a	ZM197 M.PB7	Q23 .17	Q259 .d2.17	Q461 .e2	Q168 .a2	3415 .vl.el	0439 .v5.el
1 (NVS)	335	27	213	1,022	60	199	1,005	775	<20	420	344	185
2 (NVS)	236	198	226	60	<20	<20	608	32	<20	363	110	44
3 (LVL)	26	27	44	36	<20	<20	88	143	<20	68	97	36
4 (LVL)	26	<20	40	34	<20	52	120	35	<20	82	93	23
5 (LVL)	144	36	32	117	28	26	152	78	34	150	203	67
6 (LVL)	167	<20	24	31	24	49	124	395	38	545	404	139
7 (Control)	<20	<20	<20	<20	<20	<20	21	<20	<20	<20	<20	<20
8 (Control)	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	38	<20

Results for 8 individuals tested are shown (6 with broadly neutralizing plasma and 2 patients with non-broadly neutralizing plasma). Clade A, Clade C, and CRF02_AG viruses were tested. ID80 represents the highest concentration at which 80% of the infectious virus was neutralized. Values in bold show neutralization at least two-fold over the background. MuLV not shown but <20 for all individuals tested.

Table 4

Mean ID80 HIV-1 neutralization titers for Tier 1 and Tier 2 viruses.

Patient Viral Load	SF162	BaL.26	SS	6535.3	OH0	SC	PVO.4	TRO.11	AC10.0.29	RHPA	THRO	REJO	TRJO	W1TO	CAAN	MuLV
10 ² –10 ⁴	2330	270	104	99	37	45	51	81	72	74	33	81	37	51	70	<20
<10 ²	950*	109*	44*	29*	26*	25*	26*	26*	25	28*	25	35*	23	36	30	<20
>10 ⁴	1850	156*	52*	44	27*	43	26	31	24	28	29	24*	24	30	23	<20

The first 3 columns of viruses (SF162, BaL.26, and SSI 196.1) are Tier 1 viruses, the remaining 12 viruses are Tier 2 viruses. Patients were analyzed according to viral load <10², 10²–10⁴, or >10⁴ HIV-1 RNA copies/ml. Patients with HIV-1 RNA copies 10²–10⁴ had the highest mean titers for each of the 15 viruses tested. This was statistically significant for 46.7% (14 of 30) of the Tier 1 and Tier 2 viruses tested. There was no significant difference between the <10² and >10⁴ groups.

* p<.05 by the Mann Whitney test (results given in comparison to the 10²–10⁴ group only)

Inhibitory dose 80 titer (ID80) was defined as the plasma dilution causing 80% reduction in relative luminescence units (RLU) compared to controls.