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Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers

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Abstract

Objective—To determine the spectrum of antiviral antibodies in HIV-1-infected individuals in whom viral replication is spontaneously undetectable, termed HIV controllers (HICs).

Design—Multicenter French trial ANRS EP36 studying the viral control in HICs.

Methods—Neutralizing Antibody (nAb) activities (neutralization assay, competition with broadly reactive monoclonal antibodies, and reactivity against the viral MPER gp41 region), Fcγ R-mediated antiviral activities, antibody-dependent cell cytotoxicity (ADCC), as well as autoantibody levels, were quantified in plasma from 22 controllers and from viremic individuals. The levels of these different antibody responses and HIV-specific CD8 T cell responses quantified by enzyme-linked immunosorbent spot (ELISPOT) IFNγ assay were compared in each controller.

Results—The levels of antibody against the gp120 CD4 binding site, gp41, as well as Env epitopes near to the sites bound by broadly nAbs 2F5 and 1b12 were not different between HICs and viremic individuals. We did not find significant autoantibody levels in HICs. The magnitude and breadth of nAbs were heterogeneous in HICs but lower than in viremic individuals. The levels of nAbs using Fcγ R-mediated assay inhibition were similar in both groups. Regardless of the type of antibody tested, there was no correlation with HIV-specific CD8 T cell responses. ADCC was detectable in all controllers tested and was significantly higher than in viremic individuals (P <0.0002).

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Author's contributions: Olivier Lambotte, Barton Haynes and Jean François Delfraissy conceived and wrote the study protocol. Georgia Tomaras and David Montefiori designed experiments and analyzed the data and helped to write the manuscript. Guido Ferrari performed the ADCC tests, Christiane Moog performed the study of Fcγ mediated inhibitory activity of antibodies on macrophages, and both also helped to write the manuscript. Alain Venet performed the ELISPOT analysis, Nicole L. Yates designed, performed and analyzed the isotype experiments. Robert Parks designed and performed the antibody binding experiments. Hua-Xin Liao analyzed the antibody binding experiments and provided reagents. Charles Hicks recruited and enrolled the chronic individuals as part of the study design. Kouros Owzar performed the statistical analysis.

Conclusion—There was no single anti-HIV-1 antibody specificity that was a clear correlate of immunity in controllers. Rather, for most antibody types, controllers had the same or lower levels of nAbs than viremic individuals, with the possible exception of ADCC antibodies.

Keywords

antibody-dependent cell cytotoxicity; FcγR; HIV controller; humoral immunity; neutralizing antibodies

Introduction

Neutralizing antibodies (nAb) provide protection against many pathogens and are detected with varying degrees of magnitude and breadth in nearly every HIV-1 chronically infected individual. HIV-1 replicates and mutates continuously in the face of neutralizing antibody responses [1,2] giving rise to escape mutants that are selected rapidly because of the high levels of viral replication. In chronically infected patients, nAb are effective against earlier viral isolates but rarely neutralize contemporaneous virus [3–5]. In addition, current HIV-1 envelope (Env)-based vaccines do not elicit an effective nAb response [2,6].

Nonetheless, some lines of evidence support a role for nAbs in HIV infection. Preexisting NAb can prevent AIDS virus infection in rhesus macaques in passive-transfer experiments [7]. In humans, Trkola *et al.* [8] showed a delay of virus rebound after cessation of antiretroviral therapy (HAART) through passive transfer of broadly neutralizing human mAb (2G12, 2F5, 4E10). Finally, a number of studies have suggested that patients defined as long-term nonprogressors (LTNP) possess strong, cross-reactive neutralizing antibodies [9–13]. These patients maintain high CD4+ T cell counts in the absence of antiretroviral therapy but have low yet detectable viral loads [9,14].

Rare HIV-1-infected patients, termed elite controllers, maintain plasma HIV RNA levels below the limit of detection for a prolonged period of time without therapy [15,16]. Several genetic and/or immune mechanisms could explain the absence of detectable HIV replication in controllers [16,17]. T cell-mediated immune control of viral replication is supported by the presence of a strong HIV-specific CD8+ T cell response and HIV-suppressive activity of CD8+ T cells [18–20]. Central memory CD4 T cells are preserved in controllers [21]. In contrast, data about the role of humoral immunity in the control of HIV replication in these patients are limited.

Prior work on LTNPs with low viral loads, did not find a strong NAb response [22]. In addition, Bailey *et al.* [23] did not find a significant difference for the NAb response between nine HIV controllers and in individuals on highly active antiretroviral therapy (HAART). Pereyra *et al.* [24] found that elite controllers had lower antibody neutralization titers than viremic controllers (viral load between 50 and 2000 RNA copies/ml) and chronic viremic patients. However, the authors noticed a marked heterogeneity among elite controllers and both studies used only neutralizing antibody assays.

Recent data in animal models, point to additional functions of potentially protective antibody types. Some antibodies can inhibit the replication of HIV-1 in macrophages and dendritic cells by mechanisms involving interactions between the Fc portion and the FcγR present on macrophages and dendritic cells [25,26]. Alternatively, the reduction of the viral load after a rectal vaccinal challenge has been linked with plasma antibody-dependent cell-cytotoxicity activity (ADCC) [27].

The French ANRS EP36 study group was established to investigate mechanisms leading to the potent control of viral replication in HIV controllers. In this setting, we undertook a large

study to describe the humoral immune response against HIV-1 in controllers compared with chronically infected viremic individuals. We quantified the levels of HIV-1 binding antibodies, including CD4 binding site and gp41 membrane proximal external region (MPER) specificities, and we measured the magnitude and breadth of neutralizing antibodies. We also looked for NAb acting by Fc-FcγR and ADCC activity as no data has been published about these 'non classical' nAb in HIV controllers.

We found similar or lower levels of all antibody types in controllers versus viremic individuals, with the exception of ADCC antibodies, which were present in greater magnitude in controllers.

Methods

Patients

Plasma was obtained from 22 HIV controllers assembled in the ANRS EP36 study group who were selected on the basis of the following characteristics: HIV-1-infected individual with a follow-up longer than 10 years, and more than 90% of the plasma HIV RNA measurements lower than 400 copies/ml (Amplicor Monitor, Roche Diagnostics, Meylan, France) during the follow-up, without any antiretroviral treatment. Controller individuals have been described in previous reports [20,28], the HIV-specific CD8 T cell immune response was available for all these individuals using enzyme-linked immunosorbent spot (ELISPOT) IFNγ assay [20]. In the 2 last years of follow up, four of these controller individuals had transient episodes of viremia (>50 but <1000 copies/ml). As a control group, 75 viremic chronically infected untreated patients with CD4 cell counts above 400 cells/mm^3 were randomly selected at the Duke University Center for AIDS Research Infectious Diseases Clinic and plasma samples collected and studied. The median ages in controllers and viremic individuals were 48 and 40 years respectively (range 39–70 and 21–77). In viremic individuals, median plasma RNA viral load was 11 950 copies (IQR 4000-27 127). The median CD4 T cell count was 819/mm^3 [IQR 732–925] in HIV controllers and 563 [IQR 445–692] in viremic individuals. All subjects gave informed consent to the study and the ethical committee of Bicêtre Hospital and the Intellectual Review Board of Duke University approved the studies performed.

Anti-HIV-1 antibody response

The anti-HIV-1 binding antibody responses against the viral proteins gp160, gp120, gp66, p55, gp41, p31, p24, and p17, were quantified using luminex using the Athena Multi-System (Zeuss Scientific, Inc., Raritan, New Jersey, USA) as previously described [29].

Competitive inhibition binding antibody assays

Competitive inhibition assays for antibodies that block the binding of sCD4, or biotylated mAbs 1b12, 2G12, 2F5, and 13H11 (a nonneutralizing Cluster II gp41 MPER antibody) to JRFL gp140 were performed as previously described [30]. Levels of antibodies directed against the HIV-1 Envelope MPER 4E10 (SLWNWFNITNWLWYIK) or 2F5 epitopes (QQEKNEQELLELDKWASLWN) and against the V3 Loop (TRPNNNTRKSIRIGPGQAFYATGDIIGDIRQAH) were quantified as previously reported by enzyme-linked immunosorbent assay (ELISA) [30].

Assay for plasma autoantibodies

Autoimmune antibody assays were performed as described for double-stranded DNA or soluble nuclear antigens SSA, SSB, Sm, RNP, Jo1, scl70, histone, and centromere using the Athena Multi-System (Zeuss Scientific, Inc.) [31]. Binding antibodies to cardiolipin and dioleoylphosphatidylethanolamine were also determined by ELISA as described [31].

Antibody-isotype assay

IgG1 and IgG2 antibodies specific for the HIV-1 P1 and immunodominant peptides were determined by ELISA as previously described [29] with minor modifications. Briefly, peptides P1 (SQNQQEKNEQELLELDKWASLWNWFNITNWLWYIK) [32] and the immunodominant region (RVLAVERYLRDQQLLGIWGCSGKLICTTAVPWNASWSNKSLNKI) were coated onto black microtiter plates. Mouse antihuman IgG1 (Cal-Biochem, San Diego, California, USA) and mouse anti-human IgG2 (Southern Biotech, Birmingham, Alabama, USA) and Attophos substrate were used for fluorescent detection (M2 plate reader, Molecular Devices, Toronto, Canada). The positive controls were HIV+ serum (HIV+ 16) and purified IgG1 and IgG2 human myeloma proteins. Standard curves to calculate μg/ml Equiv. were generated using titration curves of IgG1/IgG2 human myeloma proteins fit using four parameter logistic model curve fitting.

Neutralization assays

Neutralization assays were performed as previously described [33] using seven standard reference strains as Env-pseudotyped viruses [34] (SF162.LS, 6535.3, QH0692.42, SC422661.8, THRO4156.18, REJO 4541.67, and TRJO4551.58). These strains, all clade B, were used to infect TZM-bl cells. The 50% inhibitory dose was defined as the sample concentration that caused a 50% reduction in relative luminescence units (RLU) [34].

Study of Fcγ mediated inhibitory activity of antibodies on macrophages

Fcγ mediated inhibitory activity in plasma from HIV controllers was assessed using monocyte-derived macrophages (MDM) as target cells. This assay detects neutralizing activity as well as nonneutralizing inhibitory activity involving FcγRI mediated inhibition [26]. Briefly, monocytes purified by countercurrent centrifugal elutriation of PBMC were differentiated into macrophages in AIM lymphocytes SVF-free medium with glutamax and 1 ng/ml of GM-CSF (R&D, Minneapolis, Minnesota, USA) for 5 days. Plasma samples were heat-inactivated 30 min at 56°C. Serial dilutions of plasma were incubated for 1 h with virus HIV-1 BaL (subtype B, obtained from the NIH) or virus TV-1 (subtype C, obtained from S. Engelbrecht) at concentrations of 2–10 μg/ml of p24 (to reach 2 to 5% infected macrophages after a single round of infection according to [35]) and then the mixtures were added to MDM. Thirty-six hours after infection HIV-infected MDM were detected by intracellular staining of viral p24 Ag, and analysis by flow cytometry.

Testing for antibody-dependent cell cytotoxicity

ADCC assay was performed as previously described [36]. Briefly, CEM.NKR cells were used as targets and, according to the results of the titration, coated with recombinant gp120 representing the sequences of the MN, JRFL, and QH0692 isolates ($rgp120_{MN}$, kindly provided by Chiron, San Francisco, California, USA); $rgp120_{JRFL}$ and $rgp120_{QH0692}$ (Immune Technology Corp, Yonkers, New York, USA). The cells were simultaneously radiolabled with 100 μCi of sodium Chromate (^{51}Cr; Du Pont, Wilmington, Delaware, USA) for 90 min at 37°C. After washing, cells were plated into a round-bottom 96-well plate (Costar, Cambridge, Massachusetts, USA) at 5×10^3 cells/well. Plasma samples were heat inactivated at 56 °C for 30 min, diluted and then tested in triplicate. Cryopreserved peripheral blood lymphocytes from a seronegative human donor were used as effectors at a final effector: target ratio of 33: 1. Wells containing effectors plus target in the absence of plasma (control background), and in presence of a standard seropositive plasma were used as negative and positive controls in each assay. Wells containing target cells in presence of medium alone or plus 0.5% Triton X-100 served as control for spontaneous release and maximum release, respectively. After 6 h incubation (37°C, 5% CO_2), harvested

supernatants were counted in a gamma counter. Percentage specific lysis was calculated according to the formula [(cpm experimental release) minus (cpm spontaneous release)]/[(cpm maximum release) minus (cpm spontaneous release)] ×100. Spontaneous release did not exceed 10% of maximum release. Results were considered positive if percentage specific lysis was more than 10% after background subtraction.

Enzyme-linked immunosorbent spot assay

Interferon (IFN)-γ secretion by virus-specific $CD8^+$ T cells was quantified with an ELISPOTassay as described in detail elsewhere [37].

Statistical analysis

The levels of binding antibodies were compared in controllers versus viremic individuals using the Mann–Whitney test ($P < 0.05$). For the ADCC assays, the distributions of the group-wise (chronic versus elite) areas were compared using a two-sample Wilcoxon test.

Results

The anti-HIV-1 antibody responses against the eight viral proteins present in the western blot assays were quantified in 13 randomly selected HIV controller and 75 viremic patients. As shown in Fig. 1, there was no significant difference between the controllers and the viremic patients for the intensity of the antibody response against the main HIV proteins except for minimal differences in antibodies to p17. Competitive inhibition assays were performed with plasma from these 13 HIV controllers and 75 chronic viremic patients. The levels of antibodies that blocked the binding to JRFL gp140 of sCD4, and of the mAb 1b12, 2F5, 2G12, 13H11 were similar in the HIV controllers and viremic individuals (Fig. 2a). Moreover, HIV controllers had the same levels of direct binding antibodies to the 4E10 and 2F5 peptide epitopes, and similar levels of antibodies to a clade B V3 consensus loop peptide as did viremic HIV-1 infected individuals (Fig. 2b). As expected in both groups, there was a higher level of binding to the 2F5 peptide than the 4E10 peptide, due to the presence of nonneutralizing antibodies, targeting this region, that commonly appear in infection.

In addition, analysis of the antibody isotype (IgG1 and IgG2) responses to the MPER did not reveal significant group differences between the chronically infected patients and the 13 controllers (Fig. 2c). However, there was one controller who had a high IgG2 response to the MPER. There was a significant difference between the groups for the IgG1 antibody response to the immunodominant region. This is likely a reflection of the increased virus replication in the chronic group.

The presence of various autoantibodies was also investigated as some of these antibodies cross react with viral epitopes [31]. No significant difference was observed between 13 HIV controllers and the viremic individuals with regard to the presence of any of the autoantibodies tested or for antibodies to cardiolipin and dioleoylphosphatidylethanolamine (data not shown). None of the HIV controllers had manifestations of autoimmune disease.

Next, we assayed for the presence of plasma neutralizing antibody activity. First, we tried to amplify autologous viruses from the plasma of the HIV controllers despite the fact that all the patients had a viral load less than 50 copies/ml at the time of sampling. Despite ultracentrifugation and repeated attempts, amplification from plasma of autologous viruses was not successful. In 22 controllers and 13 viremic patients, neutralization assays were, therefore, performed against seven standard reference viruses, one defined as a tier 1 virus (SF162.LS), and six others defined as tier 2 [33,34]. As shown in Fig. 3a, the magnitude and the breadth of the neutralizing antibodies present in the plasmas of the controllers were

either similar to or lower than those in the viremic group of chronically infected patients. There was no difference in the levels of neutralizing antibodies against SF162 and 6536.3 HIV-1 strains, whereas the neutralizing antibody activities were higher in viremic individuals for the five other HIV-1 Env pseudoviruses tested. Plasmas of HIV controllers had nAb against a mean of 4.25 viruses per patient compared with 6.4 viruses for the viremic individuals. Pooling the data, the NAb response was significantly lower in controllers: the mean ID_{50} in TZM-bl cells were 962.5 RLU in controllers and 2369.3 RLU in viremic individuals ($P = 0.04$).

It should be noted that eight out of 22 controllers had strong and broad neutralizing antibody responses (Table 1). Among them, patients A11, A12, A20, A21, and B2 had some transient detectable viremias (<400 copies/ml) during a median follow-up of 18 years but A5, A10, and A17 did not have any such blips.

We hypothesized that a strong neutralizing antibody response could, in some individuals, account for the control of viral replication in those few patients in whom the HIV-specific CD8 response was low, but the presence of neutralizing antibodies was not correlated with a lower intensity of the HIV-1 specific CD8 T cell response; for example, patients A11, A12, and A21 all had strong cellular and humoral immune responses. However, taking into account the controllers with the lowest CD8 T cell response (first quartile 1798 spots), three patients of six (A5, A10, B2) had broad neutralizing antibody responses.

In parallel with neutralizing antibody activity mediated by the Fab part of the antibody, neutralizing antibodies can mediate HIV inhibitory activity via the Fc part of antibodies as demonstrated on macrophages and other cells expressing FcγR. We looked for this activity in the plasma from 22 controllers and 10 viremic individuals. As shown in Fig. 3b, the magnitude of the antibody neutralizing activity mediated via the FcγR present in the plasma of controllers was not different from those in the viremic group regardless of the virus tested (BaL or TV1, a clade C virus). But this activity was still heterogeneous in controllers and very high for some of them as shown in Table 1. The presence of Fcγ mediated inhibitory activity was not correlated with the HIV specific CD8 response. Two patients had a neutralizing antibody response only against TV1, A18 and A19, but A19 was infected with a clade A2 virus.

Overall for the six controllers with the lowest CD8 T cell responses, A22 and A23 did not have either a strong specific CD8 T cell response, nor have neutralizing antibodies either ‘classical’ or ‘nonclassical’ using Fcγ mediated inhibitory activity. Patients A5 and B2 had both strong and broad neutralizing antibodies using TZM-bl infection neutralization or Fcγ mediated inhibitory activity. Patients A10 and A13 were discordant as subject A10 had high levels of neutralizing antibodies and subject A13 had only neutralizing antibodies using the Fcγ mediated inhibitory activity against BaL in macrophages.

We also tested antibody-dependent cell cytotoxicity (ADCC) in plasma samples from 10 randomly chosen HIV controllers (marked * in Table 1) compared with those from 10 viremic individuals. The ADCC results are reported in Fig. 4 as average of the reactivities against the $rgp120_{MN}$ (similar results obtained with two other rgp120 proteins, not shown) in the two groups with the standard deviation. ADCC was detected in the plasma of all 10 controllers (100% detectable ADCC), but only in four of the viremic patients (40%). The titer of ADCC-mediating antibody responses in the HIV controllers, defined as the titer that maximum percentage of specific lysis was detected, was always at least 10 000 compared with the viremic patients who had at least 1000, but for one. The distributions of the group-wise (Viremic versus Controllers) areas, calculated from the sum of the area of three

trapezoids under each trajectory, were compared using a two-sample Wilcoxon test (*P*-value: < 0.0002).

Discussion

HIV controllers are a model of natural control of HIV infection; however, the mechanisms involved in such a control remain unclear. We, and others [19,20,24], have shown that the HIV-specific CD8 T-cell response is important to control the viral replication. However, HIV controllers are a heterogeneous group in which some patients do not have a strong HIV-specific CD8 response [24,38]. Humoral immunity can in some individuals be associated with control of viral replication as demonstrated in monkeys and in humans with an *in vivo* proof-of-concept study showing that neutralizing antibodies infused in patients are able to slow the rebound of viral replication after HAART interruption [8]. nAbs could, therefore, play a role in the control of HIV-1 in some individuals such as HIV-1 controllers.

We show here that this HIV-1 controller group had lower NAb than viremic individual controls. This result is in agreement with the two previous studies in which nAb were studied [23,24] that suggested that strong suppression of viral replication limited the stimulation and the maintenance of effective nAb responses. The finding that elite (nonviremic) controllers had lower nAb levels compared with 'viremic' controllers in a recent study [24] supports this point.

Contrasting with the lower levels of nAb, the levels of binding antibodies were similar in controllers and in viremic patients. The lack of difference in the antibody binding assays (Fig. 1) could be related to rare blips of viremia, which maintain the humoral response or could suggest that some antibody responses to HIV proteins are not maintained by chronic antigenic stimulation but rather by long-lived plasma cells that do not require continuous antigenic stimulation for long-lived antibodies. Binding assays do not distinguish between these two types of B cell response. Similarly, the results from the mAb-competition experiments, to known neutralizing epitopes, were comparable in both groups of patients. Other unknown epitopes [39] and/or antibody avidity can explain the similarities in anti-Env binding antibody levels but the differences in neutralizing antibody specificities.

The levels of nAb were heterogeneous, regardless of whether we looked at nAb with TZM-bl cells, or neutralization via macrophage Fcγ receptors. Varying levels for the CD8 T-cell responses were also observed in controllers. This heterogeneity was also documented in Pereyra's work [24] but our current study is the first one focusing on 'non classical' NAb (using FcγR also in macrophages as well as ADCC) in this subgroup of patients. To look at these different kinds of antibodies is important, as some patients seem to have high levels of nAb in macrophages acting via the FcγR and do not have nAb in the TZM-bl assay.

It should be noted that, for the first time, the NAb levels were compared with the HIV-specific CD8 T cell responses in each patient. Overall, no statistically significant correlation was found. However, individuals such as A5, A13 or B2, in whom viral replication was fully suppressed, had strong nAb levels but did not have a strong a HIV-specific CD8 T cell response. Why these patients have such a discrepancy between their cellular and humoral immune response is unclear. One hypothesis is that HIV antigens could remain trapped on follicular dendritic cells, helping the maintenance of serum antibody levels in some patients [40].

Interestingly, ADCC was detected in all controllers and was significantly higher than in viremic patients. The direct comparison of binding and ADCC-mediating antibody levels elicited by a candidate AIDS vaccine in seronegative vaccine recipients showed a direct correlation using this platform for the ADCC assay [36]. However, this was not the case in

this study of HIV infected individuals. This is potentially due to a decreased dependence of ADCC mediating antibodies on the level of circulating antigens, thus the higher in the controller, or to the sustained virus replication in progressor affecting ADCC mediating antibodies to a different extent than the binding and nAb tested here. The lysis of the target cells in our experimental model is indeed exclusively related to the presence of ADCC mediating antibody because we have previously observed that other plasma components, including complement-related factors, do not contribute to this specific lysis (Ferrari G., unpublished data). These results suggest that further and extensive investigation of the ADCC responses in HIV-1 controllers and in various states of HIV infection is required.

In summary, this study supports and extends previous results published about humoral immunity in HIV controllers. These patients have globally lower nAb and other antiviral antibodies compared with viremic patients. However, within the controller group, antiviral antibody levels were heterogeneous. Antiviral antibodies comprise multiple types of antibodies and effector mechanisms that could play a role in the control of viral replication in some HIV controllers, especially in those who do not have a strong CD8 T cell response.

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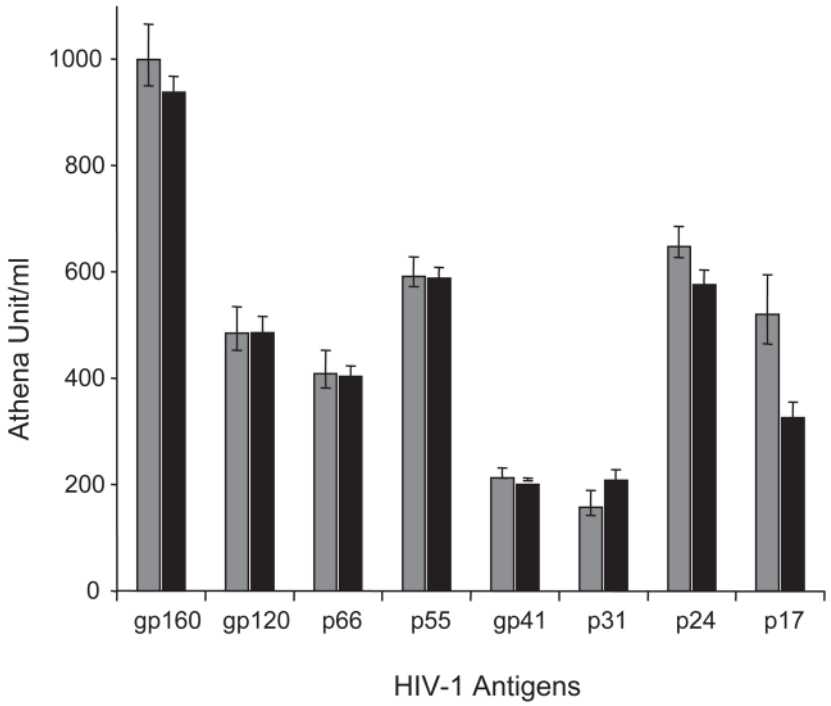


Fig. 1.

Anti-HIV-1 antibody responses in HIV controllers (grey) and chronically viremic patients (black) as measured in a quantitative luminex bead antibody-binding assay.

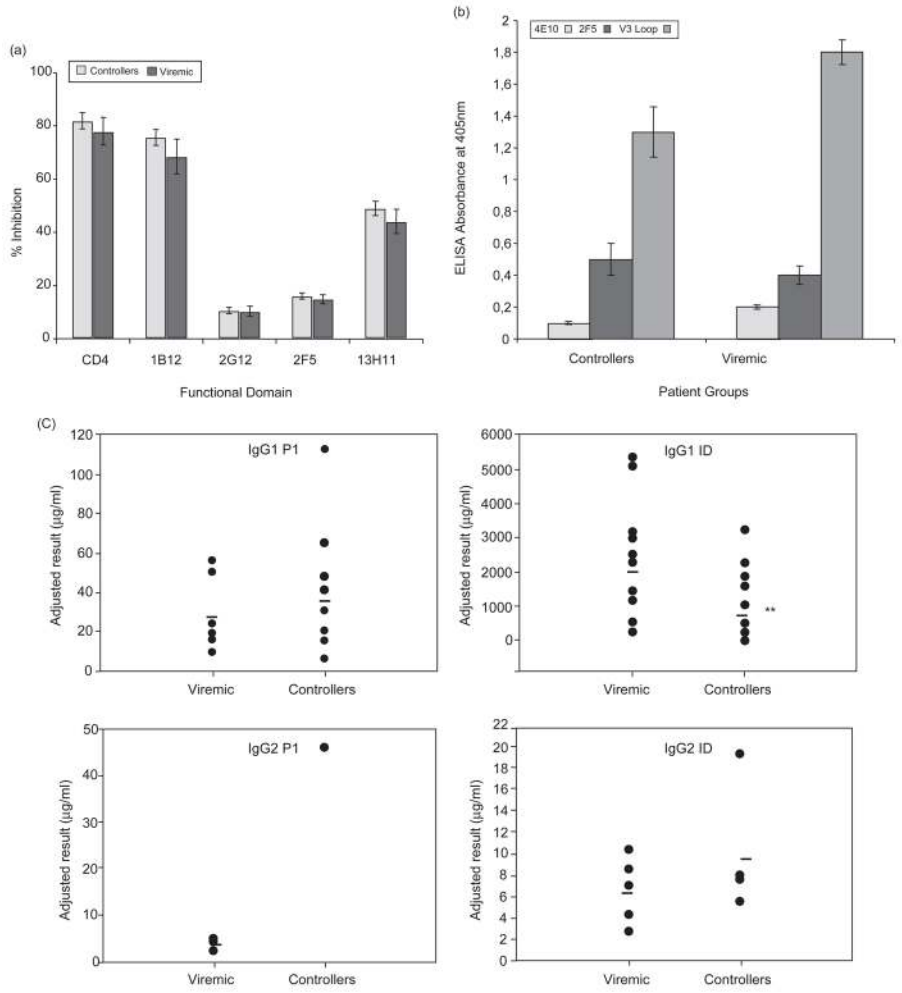
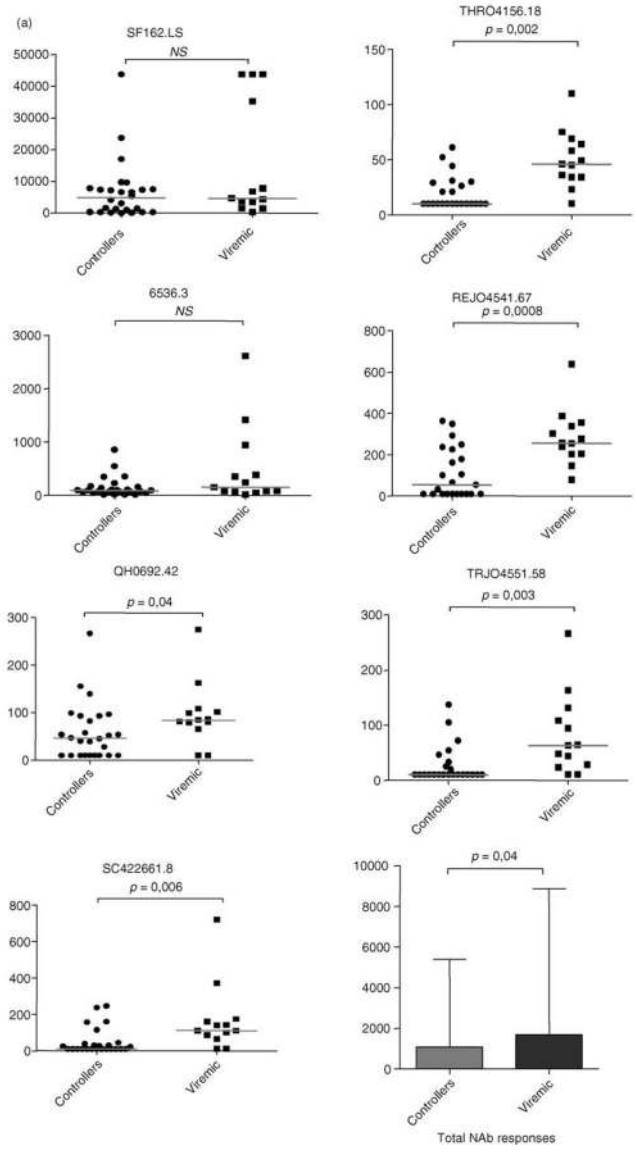


Fig. 2. Graphical representation of competitive inhibition assays in HIV controllers (grey) and chronically viremic patients (black)

(a) Competitive inhibition assays of patient sera for the ability to block the binding of soluble CD4, mAbs 1b12, 2G12, 2F5 and the MPER nonneutralizing mAb, 13H11 to Env JRFL gp140 oligomers; (b) Binding antibodies to HIV-1 Envelope MPER 4E10, 2F5 and V3 Loop peptide epitopes in HIV-1 patients; (c) IgG1 and IgG2 binding antibodies to HIV-1 envelope MPER (P1) and the immunodominant peptide (ID). $^{**}P < 0.01$.



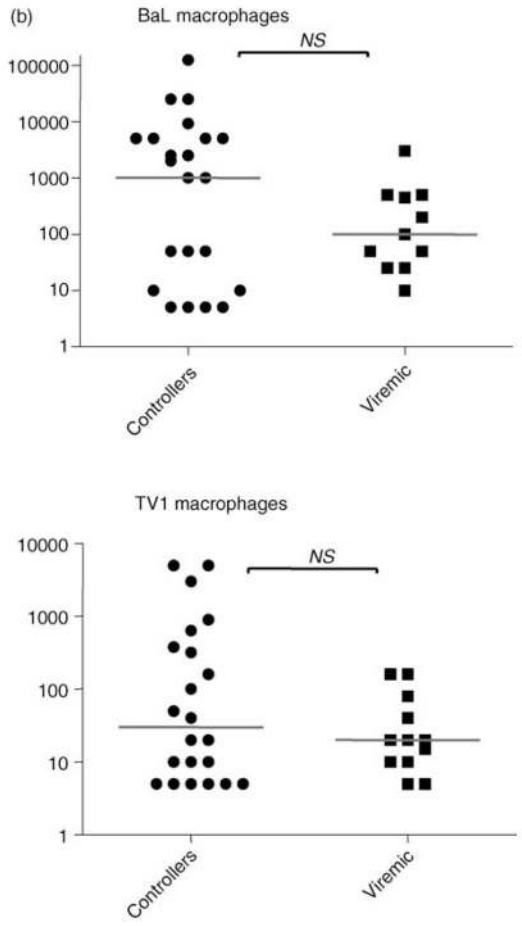


Fig. 3. Neutralizing antibodies reactivity in controllers and viremic patients

Neutralizing antibody reactivity against seven HIV strains (a) and Fcγ mediated inhibitory activity of antibodies on macrophages (b).

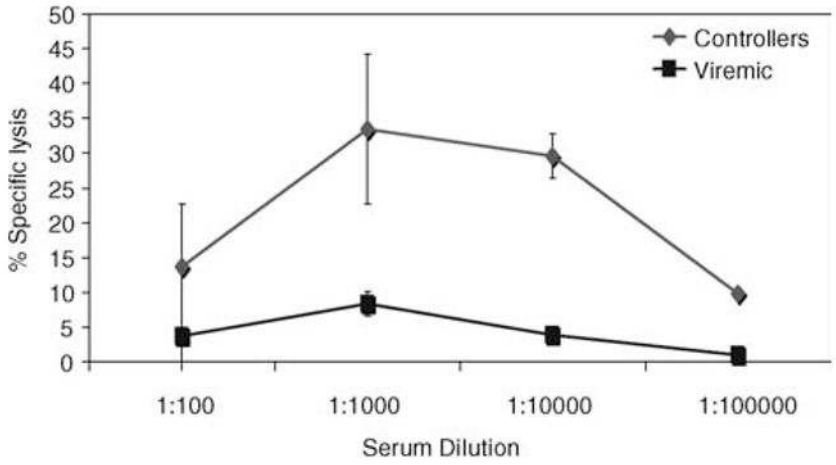


Fig. 4.
Antibody-dependent cell cytotoxicity in ten HIV controllers and in ten viremic patients.

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Table 1

Neutralizing antibody responses mediated either by the Fab portion on TZM bl cells using seven HIV strains or by FcγR tested on macrophages using BaL and TV1 viral strains compared with the HIV specific CD8 T cell responses quantified by ELISPOT INFγ.

Patients	ELISPOT CD8 INFγ	SF162.LS	6535.3	QH0692.42	SC422661.8	THRO4156.18	REJO4541.67	TRJO4551.58	BaL Mφ	TV1 Mφ	Subtype
A2*	15 087	7336	42	<20	<20	<20	55	<20	50	<10	B
A4*	13 394	7509	67	45	28	<20	163	<20	5000	40	B
A6*	13 239	7811	75	<20	<20	<20	101	<20	50	10	B
A12	12 495	6604	158	99	38	30	32	<20	1000	100	B
A21	11 075	5397	348	139	157	61	249	54	25000	>5000	B
A11	10 760	1587	106	96	44	29	105	72	2000	640	B
A7*	10 125	6586	45	<20	28	<20	292	<20	5000	20	B
B5*	7836	270	<20	<20	<20	nt	nt	nt	10	<10	B
A1*	6755	7374	<20	<20	<20	<20	<20	<20	<10	<10	B
A3*	5517	289	<20	<20	nt	nt	nt	nt	<0	<10	B
A18	5055	151	73	39	<20	<20	<20	20	<10	160	nt
A15	3405	3062	90	54	<20	<20	<20	<20	5000	320	B
A20	2472	23 695	855	266	246	21	237	105	125000	5000	B
A17	2382	9724	355	93	237	52	179	137	25000	380	B
A19	2060	314	69	40	<20	<20	<20	<20	<10	3000	A2
A16	2000	26	69	52	<20	<20	<20	<20	1000	<10	B
A5*	1730	17 028	141	155	160	26	363	33	5000	20	B
A10*	1531	>43 740	546	<20	<20	21	348	<20	<10	10	B
A23	0645	111	107	82	<20	<20	<20	<20	10	10	nt
A13	0460	1310	95	54	27	<20	<20	<20	2500	50	B
A22	0171	340	228	93	<20	<20	<20	25	50	<10	B
B2	0131	7192	171	47	115	44	227	46	2500	900	B

*Patients in whom ADCC activity was tested. nt, not tested.