Characterization of broadly neutralizing antibody responses to HIV-1 in a cohort of long term non-progressors

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Abstract

Background

Only a small fraction of HIV-1-infected patients develop broadly neutralizing antibodies (bNAb), a process generally associated to chronic antigen stimulation. It has been described that rare aviremic HIV-1-infected patients can generate bNAb but this issue remains controversial. To address this matter we have assessed bNAb responses in a large cohort of long-term non-progressors (LTNPs) with low or undetectable viremia.

Methods

Samples from the LTNP cohort of the Spanish AIDS Research Network (87 elite and 42 viremic controllers) and a control population of 176 viremic typical-progressors (TPs) were screened for bNAb using Env-recombinant viruses. bNAb specificities were studied by ELISA using mutated gp120, neutralization assays with mutated viruses, and peptide competition. Epitope specificities were also elucidated from the serum pattern of neutralization against a panel of diverse HIV-1 isolates.

Results

Broadly neutralizing sera were found among 9.3% LTNPs, both elite (7%) and viremic controllers (14%). Within the broadly neutralizing sera, CD4 binding site antibodies were detected by ELISA in 4/12 LTNPs (33%), and 16/33 of TPs (48%). Anti-MPER antibodies were detected in 6/12 LTNPs (50%) and 14/33 TPs (42%) whereas glycan-dependent HIV-1 bNAb were more frequent in LTNPs (11/12, 92%) as compared to TPs (12/33, 36%). A good concordance between standard serum mapping and neutralization-based mapping was observed.
Conclusion

LTNPs, both viremic and elite controllers, showed broad humoral immune responses against HIV-1, including activity against many major epitopes involved in bNAbs-mediated protection.

Introduction

Production of broadly neutralizing antibodies (bNAbs) against HIV represents a relatively infrequent event in HIV-infected patients [1,2]. One major issue to induce such antibodies resides in the high variability of the viral envelope and structural mechanisms hiding crucial epitopes for neutralization. Besides, maturation leading to high affinity antibodies represents a major challenge for the immune system that can be impaired by the immunodeficiency associated with HIV infection. Affinity maturation of antibodies is critical to confer effective neutralization against HIV and this maturation capacity becomes altered along infection [3–5].

Despite the complexity of such mechanisms of viral escape, some antibodies are able to overcome these barriers and display a broad neutralizing activity. These bNAbs are mainly directed to four vulnerable Env regions: the gp120 CD4-binding site (CD4bs) [6–10], the gp41 membrane proximal external region (MPER) [11–13], glycan-dependent epitopes in the second hypervariable loop (V2) [14–16] and glycan dependent epitopes around the third hypervariable loop (V3) [11,15]. In addition these four well-established sites, new epitopes at the gp120-gp41 interface recognized by some more recently discovered bNAbs have been identified [17–20].

The study of the mechanism of action of bNAbs is essential to understand the mechanisms of antibody neutralization and escape by HIV-1. Several studies have suggested that the development of neutralizing antibodies is a consequence of viral replication [1,21]. On the other hand, it is generally accepted that bNAbs are not able to contribute the control of viremia due to continuous escape by HIV from immune pressure through mutation or glycosylation. However, it has been recently described a role of bNAbs in HIV control in one patient with EC phenotype raising the possibility of an active role of bNAbs in the control of autologous viruses [22].

We have shown that patients receiving antiretroviral treatment are capable of inducing a broad and potent humoral immune response against HIV despite having undetectable levels of viremia [23]. According to these results, it is possible that long-term nonprogressors (LTNPs), individuals with low levels of viremia who maintain stable CD4 T cell counts over 10 years of infection, develop neutralizing antibodies with a high affinity profile. In fact, isolated LTNP patients the presence of bNAbs has been described [24–26]. We have explored the hypothesis that preserved B cell function in LTNPs could result in the production of a broad humoral response. To get a better understanding of this issue, we have assessed the presence of bNAbs in a large cohort of LTNP, including both viremic and elite controllers. Furthermore we have characterized the epitopes targeted by bNAbs found in LTNPs in comparison with those in HIV typical progressors (TPs).

Material and methods

HIV-1 infected subjects

This study has been approved by Research Ethics and Animal Welfare Committee of Instituto de Salud Carlos III (CEI PI 42_2011-v2).
Samples (129) from the cohort of LTNPs from the RIS (median RNA copies/ml: 104, median CD4+: 734 cells/μl and asymptomatic HIV infection over 10 year after seroconversion) were kindly provided by the HIV BioBank integrated in the Spanish AIDS Research Network (RIS) [27]. The HIV BioBank, integrated in the Spanish AIDS Research Network, is partially funded by the RD12/0017/0037 project as part of the Plan Nacional R + D + I and cofinanced by ISCIII- Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER) and Fundación para la investigación y prevención del SIDA en España (FIPSE). Samples were processed following current procedures and frozen immediately after their reception. All patients participating in the study gave their informed consent and protocols were approved by institutional ethical committees. A population of 176 untreated TPs (median RNA copies/ml: 10,241, median CD4+: 567 cells/μl) from Hospital Clinic, Barcelona, was analyzed as control [23]. The overall rate of CD4 cell decline in TPs was 50–100 cells/μL per year. Patients in the present study signed informed consent. All subjects on this study were antiretroviral naïve at the time of sampling.

LTNPs were classified as elite and viremic controllers (Table 1). Among LTNPs, 87 of them were elite controllers with persistent viral load below 50 RNA copies/ml and a median CD4+ T cell count of 773 cells/μl and 42 were viremic controllers. Viremic controllers had a median number of viral RNA copies/ml in plasma of 3,450, a median CD4+ T cell count of 655 cells/μl and viral load was always below 10000 RNA copies/ml.

### HIV-1 neutralization assays

To evaluate neutralizing antibody titers against HIV-1, Env-reporter viruses carrying a Renilla luciferase gene in nef were generated by cloning the full-length envelope in the pNL–lacZ/env–Ren vector, as previously described [28]. VI191 (A), NL4-3 (B), 92BR025 (C), 92UG024 (D), CM244 (AE) and NP1525 (CRF01_AE) envelopes were amplified from culture supernatants kindly provided by Dr. H. Holmes (NIBSC, UK) through the NeutNet consortium (Dr. G. Scarlatti) [29]. Envelopes from strains X-845-4 (F1), X-1628-2 (G), P-1261 (CRF02_AG) and 2105 (CRF14BG/B) were amplified from culture supernatants kindly provided by Dr. Lucía Pérez Álvarez, Instituto de Salud Carlos III, Spain [30]. Samples for the amplification of subtype B chronic and acute envelopes (14382, 37343, 325 and 29) were provided by Dr. José María Miró from Hospital Clinic, Barcelona. The viruses chosen represent different HIV-1 subtypes, varying neutralization sensitivity and coreceptor usage (Table 2). An amphotropic vesicular stomatitis virus (VSV) Env pseudotyped HIV-1 was added to the panel as a specificity control virus in neutralization testing.

Infectious supernatants were generated by calcium phosphate transfection in HEK 293 T cells with 5 μg of the plasmids [31]. Titrated recombinant viruses were preincubated with the dilutions of sera (1/200-1/2000) for 30 minutes at 37°C before the infection of the U87.CD4.

### Table 1. Clinical data and neutralization screening results for the patient groups.

|                           | Elite controllers | Viremic controllers | Typical progressors |
|---------------------------|-------------------|---------------------|---------------------|
| No. of sera               | 87                | 42                  | 176                 |
| Viral RNA copies/ml plasma (median) | <50*              | 3,450               | 10,241              |
| No. of CD4+ T cells/μl (median) | 773               | 655                 | 567                 |
| Years since diagnosis (median/range) | 17.3 (10–25)     | 14.8 (10–26)        | 5 (0–24)            |
| Broadly neutralizing sera | 6/87 (7%)         | 6/42 (14%)          | 33/176 (19%)        |

* In some old samples (n = 34) the threshold of detection was <500 RNA copies/ml
CCR5 or U87.CD4.CXCR4 cells (2x10^4 per well) [32]. Virus infectivity was determined 48 h postinoculation by measuring luciferase activity in cell lysates using a 96-well plate luminometer (Orion, Berthold). Sigmoid curves were generated and ID50 neutralization titers were calculated by non-linear regression using GraphPad Prism version 7.02 software. In a first screening, serum samples were tested with a minipanel of four recombinant viruses with envelopes from different subtypes and tropisms and a VSV-pseudotyped virus. Selected serum samples neutralizing all the viruses in the minipanel with an ID50 ≥ 200 were screened against a panel of 10 more viruses.

**Epitope mapping**

**i. Neutralization assays.** For the characterization of neutralizing antibodies, neutralization assays were performed using single-round infection HIV-1 Env pseudoviruses and TZM-bl target cells as previously described [33,34]. To determine the serum concentration producing 50% reduction in RLU value, serial dilutions were made and the neutralization dose-response curves were fitted by non-linear regression using a four-parameter hill slope equation.

For the assessment of CD4bs-directed neutralization, antibody-mediated neutralization was blocked with specific protein probes in a competition assay [7]. Briefly, 25 μg/ml of RSC3 (Resurfaced Stabilized Core 3) or 25 μg/ml of the mutant RSC3 Δ371I/P363N was incubated with sera serially diluted 4-fold starting from 1:10 for 30 min. JR-FL (subtype B), RW020 (subtype A) or ZA012 (subtype C) pseudovirus was added 30 min before the addition of TZM-bl cells and the infection proceeded for 48 h. CD4bs-directed activity was calculated as a 30% reduction in the ID50 values of the sera in the presence of RSC3 compared to RSC3 Δ371I/P363N [35].

For the mapping of bNAbbs directed to glycan structures in the variable region (V1V2 and V3), neutralization assays in TZM-bl were performed using JR-CSF virus with the N160K mutation and the N332A mutation respectively [36,37]. The mutation of N160 to lysine, an N-glycosylation site in the V2 loop, abolishes the neutralization mediated by PG9 and PG16 and N332A mutation removes a glycosylation site at the base of V3 loop essential for the formation of Viruses used in the neutralization assays.

| SUBTYPE | VIRUS CODE | CORECEPTOR USAGE | INFECTION STAGE | TIER |
|---------|------------|------------------|-----------------|------|
| A       | VI191*     | R5               | Chronic         | 2    |
| B       | NL4-3*     | X4               | Chronic         | 1A   |
| B       | 14382      | R5               | Acute           | n.d. |
| B       | 37313      | R5               | Acute           | n.d. |
| B       | 325        | X4               | Chronic         | n.d. |
| B       | 29         | R5X4             | Chronic         | n.d. |
| C       | 92BR025*   | R5               | Chronic         | 1B   |
| D       | 92UG024    | X4               | Chronic         | 2    |
| AE      | CM244*     | R5               | Chronic         | 2    |
| F1      | 845_4      | R5               | Chronic         | n.d. |
| G       | 1628_2     | R5               | Chronic         | n.d. |
| CRF02_AG| 1261       | R5X4             | Chronic         | n.d. |
| CRF14_BG/B| 2105     | R5               | Chronic         | n.d. |
| CRF01_AE| NP1525     | X4               | Chronic         | n.d. |

* Recombinant viruses included in the mini-panel

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of epitopes recognized by 2G12 and PGT bNAbs. A sample is considered positive if there is a decrease in ID50 greater than or equal to 50% for the mutant compared to the wild-type virus [38].

For the mapping of bNAbs to the membrane-proximal external region (MPER) of gp41, sera were tested for neutralizing activity against a chimeric HIV-2 virus containing the HIV-1 MPER region of gp41 (71312-C1) and the parenteral HIV-2 7312A clone [39].

The serum neutralizing antibodies were also mapped to the MPER region by a method of selective peptide inhibition of neutralization [40]. gp41-specific overlapping peptides (MPR.03: KKKNEQELLELDKWASLWNWFDITNRWYIRKKK, 2F5.01: NEQELLELDKWASLWNWFDITNWLWYIRKKK, 4E10.22: CNWFDITNWLWYIRKKK and Z13e1.01: WASLWNWFDITNKKK) were added to the serum for 30 min at a concentration of 25 \( \mu \text{g/ml} \) prior to the addition of 7312A-C1 virus (HIV-2/YU2 MPER chimera). A scrambled MPER region peptide (MPR.Scr.02: KKKRIYWLWNTDFWNLWASKDLELLEQENKKK) was included as negative control. In these assays the reduction of the neutralization activity caused by MPER derived peptides is compared to that due to the mock peptide (defined as an equivalent volume of DMEM medium).

ii. ELISA analyses. ELISA assays were performed as previously described [7]. Plates were coated with the antigen in PBS at 2 \( \mu \text{g/ml} \) and incubated overnight at 4˚C. The ELISA plates were coated with the following probes:

- YU2 gp120wt and YU2 gp120 D368R protein. The mutation at position 368 reduces or knocks out binding of most CD4bs Ab.

- The antigenically resurfaced glycoprotein RSC3 containing the CD4bs and RSC3 Δ371I/P363N mutant, which affects the CD4 binding loop and reduces b12 and VRC01 binding.

- The RSC3 G367R probe [7,35] that creates a steric clash for mAb b12 binding but it causes little interference with VRC01 binding.

Serum that showed a loss of reactivity to the CD4bs mutants (YU2 D368R and RSC3 Δ371I/P363N) and reacted to YU2, RSC3 and RSC3 G367R were classified as containing CD4bs antibodies as described previously [35,41]. In those samples with low reactivity to YU2 gp120wt or and RSC3 (endpoint titer below or equal to 2500) ELISA with RSC3 G367R probe was not performed.

iii. Neutralization-based serum delineation analysis. Serum specificities were delineated using a neutralization fingerprint algorithm, as described previously [42]. Briefly, a reference set of monoclonal antibody-virus neutralization data was obtained for a set of 21 diverse HIV-1 strains (subtypes A, B and C) against a set of representative monoclonal antibodies, divided into epitope-specific antibody clusters (VRC01, b12, CD4, HJ16, 8ANC195, PG9, PGT128, 2G12, 2F5 and 10E8-like). For each serum, the pattern of neutralization of the same set of 21 strains was compared to the neutralization patterns (fingerprints) of the set of reference antibodies, and the relative contribution of antibodies from each cluster to the neutralization by a given serum was estimated.

Results

In order to better understand the breadth and the spectrum of neutralization against HIV-1 in LTNP patients, we evaluated the neutralizing capacity of sera samples from the cohort of LTNP of the Spanish AIDS Research Network (RIS). The neutralizing activity in LTNPs was compared with that in the control group of TPs. In these neutralization assays, neutralizing activity of these sera were tested against a mini-panel of recombinant viruses with different subtypes (clades and tier categorizations are given in parentheses): VI191 (A, tier 2), NL4-3 (B,
tier 1A), 92BR025 (C, tier 1B) and CM244 (AE, tier 2) (S1 Fig) [10,43–45]. A VSV-pseudo-
typed HIV-1 was included as a control for nonspecific neutralizing activity and none of the
sera showed neutralizing activity against VSV-pseudotyped HIV-1. We considered that a
serum sample displayed broadly neutralizing activity (bNA) when it was capable of neutraliz-
ing all the recombinant viruses tested across with a titre ≥200, with no neutralization of the
VSV-pseudotyped control.

LTNP serum samples capable to neutralize all the viruses in the mini-panel with an
ID50 ≥200 were selected and these samples were screened against an extended panel of 10
viruses more with various subtypes (Fig 1).

The percentage of individuals with broad neutralizing responses found among LTNP's was
9.3% (12/129) and in TPs was 18.8% (33/176).

Although the number of broad neutralizer individuals in the viremic population was higher,
broad neutralizer patients were also found among the elite controllers. Six of the LTNP's with
bNA had viral loads under 50 copies/ml indicating that patients with undetectable viral loads
are capable to develop a broadly neutralizing response. These six individuals represent 7% of
the elite controllers’ population (6/87) while in the case of the viremic LTNP's the ratio of indi-
viduals with bNA was 14% (6/42) (Table 1).

The epitopes for the binding of the neutralizing antibodies contained in LTNP sera and in
TPs sera from patients with a broad neutralization profile were mapped.

NAbs directed to the CD4bs were identified with ELISA techniques. A serum was consid-
ered positive if there was a 5-fold or greater difference in serum binding to the wild type
compared to the corresponding CD4bs knockout mutant probe and if it showed a good reactivity
to YU2 gp120wt or/and RSC3 (endpoint titer above 2500). Sera with NAbs to the CD4bs were
detected in several samples of LTNP's (597473, 3227057, 2090945 and 449326) and also in TPs
(670–002, 521–006, 282–046, 363–014, 651–003, 363–014, 651–003, 308–040, 661–002, 328–017, 380–
017, 530–013, 390–012, 706–000, 738–000, 97–031, 53–036, 642–007) (Table 3).

To determine whether the CD4bs antibodies contained in sera were responsible for broad
neutralization, neutralization competition assays using RSC3 glycoprotein containing the
CD4bs were performed (Fig 2). RSC3 addition inhibited neutralization mediated by two sam-
ple of sera from LTNP's (449326 and 597473). For sample 449326 RSC3 addition inhibited

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### Table 1

| Subject | Viral load copies/ml | A | B | C | D | AE | F1 | G | CRF02_AG | CRF14_BG/B | CRF01_AE | Control Neutralized |
|---------|---------------------|---|---|---|---|----|----|---|---|----------|-----------|----------|-------------------|
| 2197004 | <50 | 2000 | 2000 | 151 | <200 | 588 | 322 | 785 | <200 | 208 | <200 | <200 | 208 | <200 | <200 | <200 | 208 | <200 | 208 | <200 |
| 582150 | <50 | 2000 | 2000 | 347 | 368 | 805 | 516 | <200 | <200 | <200 | <200 | 369 | 573 | 859 | 372 | <200 | <200 | <200 | <200 | <200 | <200 |
| 597473 | <50 | 2000 | 2000 | 925 | 2000 | <200 | <200 | >200 | >200 | <200 | <200 | 346 | 258 | 470 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
| 125966 | <50 | 2000 | 2000 | 1136 | >200 | 380 | 242 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
| 2060579 | <50 | 2000 | 2000 | 496 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
| 2017239 | <50 | 2000 | 2000 | <200 | <200 | <200 | <200 | 454 | 1608 | 1027 | >2000 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
| 440326 | 1136 | 723 | 2000 | 2000 | 1416 | 1473 | >200 | 875 | <200 | 216 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
| 3227057 | 1259 | 224 | 2000 | 824 | <200 | 277 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
| 2062015 | 2304 | <200 | <200 | <200 | <200 | <200 | <200 | 536 | >200 | >200 | >200 | 622 | 266 | >200 | >200 | >200 | >200 | >200 | >200 | >200 | >200 |
| 121875 | 3095 | <200 | <200 | <200 | <200 | <200 | <200 | 780 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
| 2090945 | 3546 | 1473 | 2000 | 321 | 203 | 264 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
| 6302555 | 3729 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 | <200 |
Table 3. Serum endpoint titers in ELISAs to determine the presence of CD4bs antibodies.

| Subject | YU2 core | YU2 D368R | Ratio | RSC3 | RSC3 Δ371I/P363N | Ratio | RSC3 G367R | RSC3 Δ371I/P363N | Ratio |
|---------|----------|-----------|-------|------|-----------------|-------|-------------|-----------------|-------|
| LTNPs   |          |           |       |      |                 |       |             |                 |       |
| ECs     |          |           |       |      |                 |       |             |                 |       |
| 2197004 | 62500    | 2500      | 25    | 2500 | 2500            | 1     | 2500        | 2500            | 1     |
| 582150  | 2500     | 1        | 2500  | 100  | 25              |        | nd          | 100             | nd    |
| 597473  | 312500   | 12500     | 25    | 312500 | 500             | 625   | 1562500     | 500             | 3125  |
| 125966  | 312500   | 62500     | 5     | 12500 | 2500            | 5     | 2500        | 2500            | 1     |
| 2060579 | 62500    | 12500     | 5     | 100   | 1               |        | nd          | 100             | nd    |
| 20172839| 12500    | 2500      | 5     | 2500  | 100             | 25    | 100         | 100             | 1     |
| VCs     |          |           |       |      |                 |       |             |                 |       |
| 3227057 | 312500   | 12500     | 25    | 62500 | 500             | 125   | 2500        | 500             | 5     |
| 2062035 | 1562500  | 62500     | 25    | 312500 | 12500          | 25    | 12500       | 12500           | 1     |
| 2090945 | 62500    | 12500     | 5     | 12500 | 500             | 25    | 2500        | 500             | 5     |
| 449326  | 312500   | 12500     | 25    | 1562500 | 12500          | 125   | 62500       | 12500           | 5     |
| 121875  | 62500    | 12500     | 5     | 62500 | 12500           | 5     | 12500       | 12500           | 1     |
| 6302555 | 62500    | 12500     | 5     | 100   | 500             | 0.2   | nd          | 500             | nd    |
| TPs     |          |           |       |      |                 |       |             |                 |       |
| 359–016 | nd       | nd        |       | 2500  | 100             | 25    | 100         | 100             | 1     |
| 734–000 | 62500    | 12500     | 5     | 2500  | 2500            | 1     | nd          | 2500            | nd    |
| 670–002 | nd       | nd        |       | 12500 | 2500            | 5     | 12500       | 2500            | 5     |
| 521–006 | 312500   | 12500     | 25    | 62500 | 2500            | 25    | 12500       | 2500            | 5     |
| 600–003 | 312500   | 312500    | 1     | 312500 | 62500          | 5     | 312500      | 62500           | 5     |
| 344–017 | nd       | nd        |       | 2500  | 500             | 5     | 2500        | 500             | 5     |
| 72–071  | nd       | nd        |       | 2500  | 100             | 25    | 2500        | 2500            | 1     |
| 339–017 | nd       | nd        |       | 2500  | 2500            | 1     | 2500        | 2500            | 1     |
| 269–049 | nd       | nd        |       | 12500 | 500             | 25    | 500         | 500             | 1     |
| 378–017 | nd       | nd        |       | 500   | 100             | 5     | 500         | 100             | 5     |
| 282–046 | nd       | nd        |       | 12500 | 100             | 125   | 12500       | 100             | 125   |
| 322–012 | nd       | nd        |       | 12500 | 500             | 25    | 500         | 500             | 1     |
| 363–014 | 312500   | 62500     | 5     | 12500 | 2500            | 5     | 62500       | 2500            | 25    |
| 651–003 | nd       | nd        |       | 12500 | 500             | 25    | 2500        | 500             | 5     |
| 701–000 | nd       | nd        |       | 2500  | 2500            | 1     | 2500        | 2500            | 1     |
| 308–040 | 312500   | 12500     | 25    | 1562500 | 500          | 3125   | 2500        | 500             | 5     |
| 661–002 | nd       | nd        |       | 12500 | 100             | 125   | 500         | 100             | 5     |
| 56–024  | nd       | nd        |       | 2500  | 500             | 5     | 500         | 500             | 1     |
| 139–020 | nd       | nd        |       | 500   | 100             | 5     | 100         | 100             | 1     |
| 328–017 | nd       | nd        |       | 2500  | 500             | 5     | 2500        | 500             | 5     |
| 380–017 | nd       | nd        |       | 12500 | 2500            | 5     | 12500       | 2500            | 5     |
| 629–005 | nd       | nd        |       | 500   | 500             | 1     | 500         | 500             | 1     |
| 530–013 | nd       | nd        |       | 312500 | 500           | 625    | 12500       | 500             | 25    |
| 390–012 | nd       | nd        |       | 12500 | 500             | 25    | 2500        | 500             | 5     |
| 706–000 | nd       | nd        |       | 62500 | 100             | 625    | 12500       | 100             | 125   |
| 528–006 | 312500   | 62500     | 5     | 62500 | 2500            | 25    | 2500        | 2500            | 1     |
| 622–005 | nd       | nd        |       | 500   | 100             | 5     | 500         | 100             | 5     |
| 738–000 | nd       | nd        |       | 12500 | 500             | 25    | 12500       | 500             | 25    |
| 97–031  | nd       | nd        |       | 62500 | 12500           | 5     | 62500       | 12500           | 5     |
| 708–002 | nd       | nd        |       | 2500  | 100             | 25    | 500         | 100             | 5     |
| 376–036 | nd       | nd        |       | 2500  | 500             | 5     | 2500        | 500             | 5     |

(Continued)
neutralization of RW020 (35.2%) and ZA012 (35.1%). For serum 597473 there was a 56% reduction in neutralization of ZA012 strain attributed to RSC3.

For the mapping of V1V2 and V3 glycan-dependent HIV-1 NAbs (Fig 3 and S2 Fig), neutralization assays in TZM-bl using JRCSF.N160K and JRCSF.N332A respectively were performed. A decrease of 2 fold or more in serum neutralization against JRCSF.N160K or JRCSF.N332A compared to the neutralization of wild-type JRCSF indicates the presence of neutralizing antibodies directed to glycans in V1V2 or V3, respectively. From the results obtained it can be deduced that glycan-dependent HIV-1 NAbs are significantly more abundant in the samples from LTNPs (11/12) than in those from TPs (12/33) (p value = 0.0017, Fisher’s exact test).

Sera were analyzed to detect antibodies directed against MPER region of gp41 (Fig 4 and S3 Fig) assessing neutralizing activity against 7312A and 7312A-C1 viruses and anti-MPER antibodies were detected in both groups of patients. To confirm the presence of MPER neutralizing antibodies in the sera, the specificity of the antibodies against MPER was inhibited with soluble peptides containing different fragments of this region (Table 4).

Table 3. (Continued)

| Subject | YU2 core | YU2 D368R | Ratio* | RSC3 | RSC3 Δ371I/P363N | Ratio* | RSC3 G367R | RSC3 Δ371I/P363N | Ratio* |
|---------|----------|-----------|--------|------|------------------|--------|------------|------------------|--------|
| 53–036  | nd       | nd        | 125    | 12500 | 500              | 25     | 2500       | 500              | 5      |
| 642–007 | 312500   | 2500      | 125    | 1562500 | 2500            | 625    | 62500      | 2500            | 25     |

nd: not determined; ECs: Elite controllers; VCs: Viremic controllers; TPs: Typical progressors

*Ratio of previous two endpoint titers. Sera with a ratio RSC3 Δ371I/P363N and a loss of activity on the cognate CD4bs mutant greater than or equal to 5-fold are considered reactive and bolded.

neutralization of RW020 (35.2%) and ZA012 (35.1%). For serum 597473 there was a 56% reduction in neutralization of ZA012 strain attributed to RSC3.

For the mapping of V1V2 and V3 glycan-dependent HIV-1 NAbs (Fig 3 and S2 Fig), neutralization assays in TZM-bl using JRCSF.N160K and JRCSF.N332A respectively were performed. A decrease of 2 fold or more in serum neutralization against JRCSF.N160K or JRCSF.N332A compared to the neutralization of wild-type JRCSF indicates the presence of neutralizing antibodies directed to glycans in V1V2 or V3, respectively. From the results obtained it can be deduced that glycan-dependent HIV-1 NAbs are significantly more abundant in the samples from LTNPs (11/12) than in those from TPs (12/33) (p value = 0.0017, Fisher’s exact test).

Sera were analyzed to detect antibodies directed against MPER region of gp41 (Fig 4 and S3 Fig) assessing neutralizing activity against 7312A and 7312A-C1 viruses and anti-MPER antibodies were detected in both groups of patients. To confirm the presence of MPER neutralizing antibodies in the sera, the specificity of the antibodies against MPER was inhibited with soluble peptides containing different fragments of this region (Table 4). Four serum samples...
from LTNP and eight serum samples from TP competed with the MPR.03 peptide that covers the complete MPER domain. One of the LTNP samples (2090945) contained antibodies similar to 4E10. One serum sample from TP (53–036) contained bNAbs directed against the same epitope than Z13 and five TP samples (600–003, 701–000, 390–012, 622–005 and 642–007) contained bNAbs directed against the same epitope than 4E10. The epitope of one TP sample (56–024) overlapped that of both 4E10 and 2F5 and the epitope of other TP sample (661–002) overlapped that of 4E10 and Z13e1.

In summary, we found that most of the bNAbs from LTNP map known neutralization epitopes and that in some subjects the neutralization breadth is mediated by antibodies with different specificities (Figs 5 and 6). A summary of serum neutralization specificities found with standard mapping is shown (Fig 5).

It has been shown that similarity in neutralization fingerprint correlates with similarity in epitope [42]. Therefore, epitope specificities of HIV-1–neutralizing antibodies in serum were
elucidated from the serum pattern of neutralization against a panel of 21 HIV-1 isolates. The patterns of neutralization of the sera were compared with a reference set of 10 epitope-specific neutralization fingerprints (one for each epitope-specific antibody cluster). The predicted prevalence of the different clusters is shown as a heat map for each serum (Fig 6A). These data exhibited a high concordance with the ones obtained using the experimental assays (Fig 5), with at least one of the top two neutralization-based specificities identified by standard mapping in 92% of the sera (Fig 6B). Epitopes involved in bNAbs-mediated protection have been characterized by these different approaches and activity against all the analyzed epitopes was displayed in LTNPs and TPs (Fig 6C).

Discussion

It has been suggested that in HIV-infected individuals high levels of viral replication and the time since infection correlates with the induction of bNAbs [1,21,46]. However, in this work we have detected broadly neutralizing antibodies against HIV-1 in a cohort of LTNPs with low

Fig 4. Detection of antibodies specific for the membrane-proximal region in the sera from LTNPs and typical progressors. For the mapping of anti-MPER neutralizing antibodies, the serum samples were tested against the parental HIV-2 isolate 7312A and the 7312A chimera containing HIV-1 MPER fragments (7312A-C1). Monoclonal antibodies 2F5 and 4E10 have been used as controls and are indicated by a black box. In this figure only samples with anti-MPER neutralizing antibodies are shown.

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or undetectable levels of viremia. Actually, LTNP were classified in two sub-groups according to viremia levels: elite controllers (persistent undetectable viremia) and viremic controllers (VL < 10,000 copies/ml). Although we have found a higher percentage of individuals with bNAbs in viremic controllers (14% vs 7%), bNAbs were also found in long-term elite controllers, suggesting that other factors besides persistently detected viremia could drive the development of bNAbs. However, we cannot exclude that this broad humoral immune response could be due to hidden viral replication in other tissues such as gut-associated lymphoid tissue (GALT) or tonsils.

LTNPs and TPs developed antibodies against all kinds of epitopes analyzed. Therefore, bNAbs in LTNPs are mapped to specific known neutralization epitopes. These data have been obtained using two different approaches, neutralization-based and standard serum mapping. The concordance between these two methods was high. These two approaches have been previously compared in a population of 21 sera [42]. The data obtained in the present study of 19 additional sera validate the previous reports of concordance between neutralization fingerprinting and standard serum mapping, further underlining the utility of the neutralization-based serum-epitope predictions.

Only two of the sera with binding antibodies against the CD4bs detected by ELISA had detectable neutralization activity confirmed by RSC3 competition neutralization assays. One possible explanation is that if the virus is neutralized by antibodies directed against other epitope, there will not be an effect on neutralization reduction mediated by RSC3. The sera samples with no inhibition in neutralization by this glycoprotein could have few antibodies against CD4bs or antibodies with low affinity incapable of mediating neutralization, only detectable

Table 4. ID50 titers in the absence and the presence of MPER-derived peptides.

| Serum | ID50 | % Neutralization inhibited by peptide |
|-------|------|-------------------------------------|
|       | Mock | MPR.Scr.02 | MPR.03 | 2F5.01 | 4E10.22 | z13e1.01 | MPR.Scr.02 | MPR.03 | 2F5.01 | 4E10.22 | z13e1.01 |
| LTNPs |      |           |       |       |         |          |           |       |       |         |          |
| ECs   | 597473 | 580 | 541 | 365 | 533 | 711 | 423 | 7 | 37 | 8 | 0 | 27 |
|       | 125966 | 85 | 100 | 92 | 89 | 117 | 103 | 0 | 0 | 0 | 0 | 0 |
|       | 20172839 | 241 | 126 | 37 | 210 | 128 | 111 | 48 | 84 | 13 | 47 | 54 |
| VCx   | 3227057 | 129 | 123 | 61 | 98 | 113 | 91 | 5 | 52 | 24 | 12 | 29 |
|       | 2062035 | 357 | 425 | 162 | 511 | 380 | 470 | 0 | 55 | 0 | 0 | 0 |
|       | 2090945 | 111 | 134 | 27 | 90 | 38 | 118 | 0 | 75 | 19 | 66 | 0 |
| TPs   | 600–003 | 4047 | 5444 | 12 | 5823 | 10 | 2563 | 0 | 100 | 0 | 100 | 37 |
|       | 651–003 | 797 | 371 | 345 | 548 | 647 | 327 | 53 | 57 | 31 | 19 | 59 |
|       | 701–000 | 2779 | 1521 | 227 | 1284 | 677 | 1447 | 45 | 92 | 54 | 76 | 48 |
|       | 661–002 | 2244 | 2236 | 771 | 1730 | 973 | 853 | 0 | 66 | 23 | 57 | 62 |
|       | 56–024 | 3279 | 3216 | 94 | 1336 | 146 | 7461 | 2 | 97 | 59 | 96 | 0 |
|       | 390–012 | 1494 | 1306 | 712 | 1806 | 591 | 856 | 13 | 52 | 0 | 60 | 43 |
|       | 706–000 | 1539 | 896 | 2313 | 876 | 993 | 514 | 42 | 0 | 43 | 35 | 47 |
|       | 622–005 | 773 | 1014 | 170 | 407 | 82 | 390 | 0 | 78 | 47 | 89 | 50 |
|       | 53–036 | 164 | 121 | 46 | 119 | 135 | 46 | 26 | 72 | 27 | 18 | 72 |
|       | 642–007 | 5932 | 8449 | 588 | 8646 | 375 | 4900 | 0 | 90 | 0 | 94 | 17 |

1 Indicator of peptide-specific neutralizing antibody response, calculated as (1 – ID50 with peptide/ID50 with mock peptide) x 100. Values higher than 50% are shown in bold

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Fig 5. Summary of experimental serum mapping of HIV-1 sera from LTNPs and TPs with a broad neutralization profile. Data obtained from the assays used to map CD4bs, V1V2 glycans, V3 glycans and MPER regions are shown. Epitope groups are marked with a plus sign (+) if predicted by the mapping assays to be present in a given serum.

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by ELISA techniques. Then, in these sera the neutralization may be due mainly to the presence of high-affinity antibodies against other domains.

When we analyzed the sera samples with the standard serum mapping, we observed that V3 glycan-dependent HIV-1 NAbS were more abundant in LTNPs (11/12) than in TPs (12/33). This prevalence of the V3 glycan-dependent NAbS in LTNPs was also detected with the neutralization-based analysis. A previous study has also found high levels of 2G12-like antibodies in broadly neutralizing samples from LTNPs [47]. A question that arises from these results is whether these antibodies are contributing to the control of viremia in LTNP. The V3 region is known to be highly immunogenic and individuals develop antibodies directed against the C3-V4 region early in infection [48,49]. HIV overcomes the response through mutation but this variability is decreased in the low viral rates of replication. It could be possible that in some LTNPs bNAbs contribute to viral control. Actually it has been recently described that Abs from one EC patient can exhibit autologous neutralization and these antibodies are contributing to elite control in this individual [22]. To address this hypothesis autologous neutralization should be detected in LTNP patients but due to extremely low viral loads, viral isolation and cloning of the envelope is unfeasible in the majority of patients.
In some subjects neutralization breadth was mediated by more than one antibody epitope specificity which is in agreement with previous observations showing that broadly neutralizing activity of some HIV-1 infected individuals is due to antibodies that target more than one epitope [38,50–52].

bNAbs require a large number of somatic mutations [7] that are related with preservation of T follicular helper cells (TFH). HIV infected individuals have several defects in the humoral immune system, including B cell abnormalities associated with HIV replication-induced immune cell activation and TFH priming [53–55]. Elite controllers with viral load consistently below 50 copies/ml could develop a robust response mediated by their well-preserved B cells generating high affinity antibodies. Therefore control of the viral load could be associated to an improved maturation of antibodies in the affinity for the antigen. TFH are involved in the development of these antibodies as B cell memory maturation and generation of high-affinity neutralizing antibodies is dependent on extensive signaling from TFH cells [56]. However, in productive HIV infection, high levels of HIV viremia drive the expansion of TFH cells which is associated with perturbation of the B cell compartment, resulting in deregulated antibody production [57,58]. One potential hypothesis could point to a better preserved B cell function in LTNPs including appropriate regulation of TFH resulting in a generation of bNAbs with high levels of somatic hypermutation despite lower levels of antigen.

**Supporting information**

S1 Fig. Neutralizing activity of sera from LTNPs and TPs. Percentages of neutralization at a 1/200 serum dilution against the mini-panel of viruses (NL4-3, VI191, 92BR025 and CM244). A white box indicates <50% neutralization, a yellow box indicates ≥50% and <70% neutralization, an orange box indicates ≥70% and <90% and a red box indicates ≥90% neutralization.

(PPTX)

S2 Fig. Detection of glycan-dependent HIV-1 neutralizing antibodies in sera from LTNPs and typical progressors (negative samples). In this figure only samples with no neutralizing antibodies directed to glycans in V1V2 and/or V3 are shown. SEMs of two independent assays are shown.

(PPTX)

S3 Fig. Detection of antibodies specific for the membrane-proximal region in the sera from LTNPs and typical progressors (negative samples). In this figure only samples with no neutralizing antibodies specific for the membrane-proximal region are shown. SEMs of two independent assays are shown.

(PPTX)

S4 Fig. Serum neutralization data (ID50s) for LTNPs and TPs against the panel of 21 HIV-1 isolates used in the antibody-sera delineation analysis. Reciprocal serum ID50 values ≥40 and <500 are highlighted in yellow, ≥500 and <5000 in orange and ≥5000 in red. For VRC01 IC50 values ≥1 and <10 are highlighted in yellow, ≥0.100 and <1 in orange and <0.100 in red.

(PPTX)

S1 Table. Serum neutralization activity (ID50) against JRFL, RW020 and ZA012 viruses used in RSC3 neutralization competition assays.

(DOCX)
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References

1. Doria-Rose NA, Klein RM, Daniels MG, O’Dell S, Nason M, Lapedes A et al. (2010) Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. J Virol 84: 1631–1636. https://doi.org/10.1128/JVI.01482-09 PMID: 19923174

2. Hraber P, Seaman MS, Bailer RT, Mascola JR, Montefiori DC, Korber BT (2014) Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. AIDS 28: 163–169. PMID: 24361678

3. Gustchina E, Louis JM, Frisch C, Ylera F, Lechner A, Bewley CA et al. (2009) Affinity maturation by targeted diversification of the CDR-H2 loop of a monoclonal Fab derived from a synthetic naive human antibody library and directed against the internal trimeric coiled-coil of gp41 yields a set of Fabs with improved HIV-1 neutralization potency and breadth. Virology 393: 112–119. https://doi.org/10.1016/j.viroi.2009.07.019 PMID: 19695655

4. Pancera M, McLellan JS, Wu X, Zhu J, Changela A, Schmidt SD et al. (2010) Crystal structure of PG16 and chimeric dissection with somatically related PG9: structure-function analysis of two quaternary-specific antibodies that effectively neutralize HIV-1. J Virol 84: 8098–8110. https://doi.org/10.1128/JVI.00966-10 PMID: 20538861

5. Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Finzi A et al. (2010) Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science 329: 811–817. https://doi.org/10.1126/science.1192819 PMID: 2092819

6. Burton DR, Barbas CF III, Persson MA, Koenig S, Chanock RM, Lerner RA (1991) A large array of human monoclonal antibodies to type 1 human immunodeficiency virus from combinatorial libraries of asymptomatic seropositive individuals. Proc Natl Acad Sci U S A 88: 10134–10137. PMID: 1719545

7. Wu X, Yang ZY, Li Y, Hoger korp CM, Schief WR, Seaman MS et al. (2010) Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science 329: 856–861. https://doi.org/10.1126/science.1187659 PMID: 20616233

8. Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C et al. (2011) Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science 333: 1593–1602. https://doi.org/10.1126/science.1207532 PMID: 21835983
9. Scheid JF, Mouquet H, Ueberheide B, Diskin R, Klein F, Oliveira TY et al. (2011) Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science 333: 1633–1637. https://doi.org/10.1126/science.1207227 PMID: 21764753

10. Corti D, Langedijk JP, Hinz A, Seaman MS, Vanzetta F, Fernandez-Rodriguez BM et al. (2010) Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals. PLoS One 5: e8805. https://doi.org/10.1371/journal.pone.0008805 PMID: 20998712

11. Buchacher A, Pred R, Strutzengerber K, Steinfellner W, Trkola A, Purtscher M et al. (1994) Generation of human monoclonal antibodies against HIV-1 proteins; electrofusion and Epstein-Barr virus transformation for peripheral blood lymphocyte immortalization. AIDS Res Hum Retroviruses 10: 359–369. https://doi.org/10.1089/aid.1994.10.359 PMID: 7520721

12. Zwick MB, Labrijn AF, Wang M, Spenlehauer C, Sapirie EO, Binley JM et al. (2001) Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. J Virol 75: 10892–10903. https://doi.org/10.1128/JVI.75.22.10892-10905.2001 PMID: 11602729

13. Huang J, Ofek G, Laub L, Louder MK, Doria-Rose NA, Longo NS et al. (2011) Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. Nature 491: 406–412. https://doi.org/10.1038/nature11544 PMID: 23151583

14. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL et al. (2009) Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science 326: 285–289. https://doi.org/10.1126/science.1178746 PMID: 19729618

15. Walker LM, Huber M, Doorey KJ, Falkowska E, Pejchal R, Julien JP et al. (2011) Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature 477: 466–470. https://doi.org/10.1038/nature10373 PMID: 21849777

16. Bonsignori M, Hwang KK, Chen X, Tsao CY, Morris L, Gray E et al. (2011) Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. J Virol 85: 9998–10009. https://doi.org/10.1128/JVI.05045-11 PMID: 21795340

17. Blattner C, Lee JH, Slepken K, Derking R, Falkowska E, de la Pena AT et al. (2014) Structural delineation of a quaternary, cleavage-dependent epitope at the gp41-gp120 interface on intact HIV-1 Env trimers. Immunity 40: 669–680. https://doi.org/10.1016/j.immuni.2013.01.019 PMID: 24768348

18. Falkowska E, Le KM, Ramos A, Doorey KJ, Lee JH, Blattner C et al. (2014) Broadly neutralizing HIV antibodies define a glycan-dependent epitope on the prefusion conformation of gp41 on cleaved envelope trimers. Immunity 40: 657–668. PMID: 24768347

19. Huang J, Kang BH, Pancera M, Lee JH, Tong T, Feng Y et al. (2014) Broad and potent HIV-1 neutralization by a human antibody that binds the gp41-gp12012 interface. Nature 515: 138–142. https://doi.org/10.1038/nature13601 PMID: 25186731

20. Scharf L, Scheid JF, Lee JH, West AP Jr., Chen C, Gao H et al. (2014) Antibody 8ANC195 reveals a site of broad vulnerability on the HIV-1 envelope spike. Cell Rep 7: 785–795. PMID: 24767986

21. Sather DN, Armann J, Ching LK, Mavranton i A, Sellhorn G, Caldwell Z et al. (2009) Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J Virol 83: 757–769. https://doi.org/10.1128/JVI.02036-08 PMID: 18997148

22. Freund NT, Wang H, Scharf L, Nogueira L, Horwitz JA, Bar-On Y et al. (2017) Coexistence of potent HIV-1 broadly neutralizing antibodies and antibody-sensitive viruses in a viremic controller. Sci Transl Med 9. https://doi.org/10.1126/scitranslmed.aal2144 PMID: 28100831

23. Medina-Ramirez M, Sanchez-Merino V, Sanchez-Palomino S, Merino-Mansilla A, Ferreira CB, Perez I et al. (2011) Broadly cross-neutralizing antibodies in HIV-1 patients with undetectable viremia. J Virol 85: 5804–5813. https://doi.org/10.1128/JVI.02482-10 PMID: 21471239

24. Carotenuto P, Logi D, Keldermans L, de WF, Goudsmit J (1998) Neutralizing antibodies are positively associated with CD4+ T-cell counts and T-cell function in long-term AIDS-free infection. AIDS 12: 1591–1600. PMID: 9764777

25. Cecilia D, Kleeberger C, Munoz A, Giorgi JV, Zolla-Pazner S (1999) A longitudinal study of neutralizing antibodies and disease progression in HIV-1-infected subjects. J Infect Dis 179: 1365–1374. https://doi.org/10.1086/314773 PMID: 1038/nat ure11544 PMID: 21849777

26. Pilgrim AK, Pantaleo G, Cohen OJ, Fink LM, Zhou JY, Zhou JT et al. (1997) Neutralizing antibody responses to human immunodeficiency virus type 1 in primary infection and long-term-nonprogressive infection. J Infect Dis 176: 924–932. PMID: 9331150

27. Garcia-Merino I, de Las CN, Jimenez JL, Gallego J, Gomez C, Prieto C et al. (2009) The Spanish HIV BioBank: a model of cooperative HIV research. Retrovirology 6: 27. https://doi.org/10.1186/1742-4690-6-27 PMID: 19272145
Characterization of bNAbs to HIV-1 in LTNPs

28. Gonzalez N, Perez-Olmeda M, Mateos E, Casajero A, Alvarez A, Spijkers S et al. (2010) A sensitive phenotypic assay for the determination of human immunodeficiency virus type 1 tropism. J Antimicrob Chemother 65: 2493–2501. https://doi.org/10.1093/jac/dkq379 PMID: 20947622

29. Fenyo EM, Heath A, Dispensieri S, Holmes H, Lusso P, Zolla-Pazner S et al. (2009) International network for comparison of HIV neutralization assays: the NeuNet report. PLoS One 4: e4505. https://doi.org/10.1371/journal.pone.0004505 PMID: 19229336

30. Cuevas MT, Fernandez-Garcia A, Pinilla M, Garcia-Alvarez V, Thomson M, Delgado E et al. (2010) Short communication: Biological and genetic characterization of HIV type 1 subtype B and nonsubtype B transmitted viruses: usefulness for vaccine candidate assessment. AIDS Res Hum Retroviruses 26: 1019–1025. https://doi.org/10.1016/j.aids.2010.00.018 PMID: 20707647

31. Pear WS, Nolan GP, Scott ML, Baltimore D (1993) Production of high-titer helper-free retroviruses by transient transfection. Proc Natl Acad Sci U S A 90: 8392–8396. PMID: 7690960

32. Bjornadal A, Deng H, Janssens B, Heyndrickx L, Heyndrickx L, Vereecken K, Janssens W et al. (2010) Characterization of neutralizing profiles in HIV-1 infected patients from whom the HJ16, HGN194 and HK20 mAbs were obtained. PLoS One 6: e25488. https://doi.org/10.1371/journal.pone.0025488 PMID: 20700449

33. Shu Y, Winfrey S, Yang ZY, Xu L, Rao SS, Srivastava I et al. (2007) Efficient protein boosting after plasmid DNA or recombinant adenosine immunization with HIV-1 vaccine constructs. Vaccine 25: 1398–1408. https://doi.org/10.1016/j.vaccine.2006.10.046 PMID: 17113201

34. Wei X, Decker JM, Liu H, Zhang Z, Arani RB, Kilby JM et al. (2002) Emergence of resistant human immunodeficiency virus type 1 isolates varies according to biological phenotype. J Virol 76: 7478–7487. PMID: 9311827

35. Lynch RM, Tran L, Louder MK, Cohen M, Dersimonian R et al. (2012) The development of CD4 binding site antibodies during HIV-1 infection, J Virol 86: 7588–7595. https://doi.org/10.1128/JVI.00734-12 PMID: 22573869

36. Scanlan CN, Pantophlet R, Wormald SE, Stanfield R, Wilson IA et al. (2002) The broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2G12 recognizes a cluster of alpha1—>2 mannose residues on the outer face of gp120. J Virol 76: 7306–7321. https://doi.org/10.1128/JVI.76.14.7306-7321.2002 PMID: 12072529

37. Walker LM, Simek MD, Pridfy D, Gach JS, Wagner D, Zwick MB et al. (2010) A limited number of antibody specificities mediate broad and potent serum neutralization in selected HIV-1 infected individuals. J Virol Pathog 6: e1001028. https://doi.org/10.1371/journal.ppat.1001028 PMID: 20700449

38. Tomaras GD, Binley JM, Gray ES, Crooks ET, Osawa K, Moore PL et al. (2011) Polyclonal B cell responses to conserved neutralization epitopes in a subset of HIV-1 infected individuals. J Virol 85: 11502–11519. https://doi.org/10.1128/JVI.00049-11 PMID: 21849452

39. Gray ES, Moore PL, Choge IA, Decker JM, Bibollet-Ruche F, Li H et al. (2007) Neutralizing antibody responses in acute human immunodeficiency virus type 1 subtype C infection. J Virol 81: 6187–6196. https://doi.org/10.1128/JVI.01992-07 PMID: 17409164

40. Li Y, Svehla K, Louder MK, Wycuff D, Phogat S, Tang M et al. (2009) Analysis of neutralization specificities in polyclonal sera derived from human immunodeficiency virus type 1-infected individuals. J Virol 83: 1045–1059. https://doi.org/10.1128/JVI.01992-08 PMID: 19004942

41. Sanchez-Merino V, Fabra-Garcia A, Gonzalez N, Nicolas D, Merino-Mansilla A, Manzardo C et al. (2016) Detection of Broadly Neutralizing Activity within the First Months of HIV-1 Infection. J Virol 90: 5231–5245. https://doi.org/10.1128/JVI.00461-16 PMID: 26984721

42. Georgiev IS, Doria-Rose NA, Zhou T, Kwon YD, Staup E, Moquin S et al. (2013) Delineating antibody recognition in polyclonal sera from patterns of HIV-1 isolate neutralization. Science 340: 751–756. https://doi.org/10.1126/science.1233989 PMID: 23661761

43. Balla-Jhaghoorsingh SS, Willems B, Heyndrickx L, Heyndrickx L, Vereecken K, Janssens W et al. (2011) Characterization of neutralizing profiles in HIV-1 infected patients from whom the HJ16, HGN194 and HK20 mAbs were obtained. PLoS One 6: e25488. https://doi.org/10.1371/journal.pone.0025488 PMID: 22016789

44. Matz J, Kessler P, Bouchet J, Combé O, Ramos OH, Barin F et al. (2013) Straightforward selection of broadly neutralizing single-domain antibodies targeting the conserved CD4 and coreceptor binding sites of HIV-1 gp120. J Virol 87: 1137–1149. https://doi.org/10.1128/JVI.00461-12 PMID: 23152508

45. Montefiori DC, Kamasa C, Huang Y, Ahmed H, Gilbert P, de Souza MS et al. (2012) Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. J Infect Dis 206: 431–441. https://doi.org/10.1093/infdis/jis367 PMID: 22634875

46. Piantadosi A, Panteleeff D, Blish CA, Baeten JM, Joaoko W, McCllelland R et al. (2009) Breadth of neutralizing antibody response to human immunodeficiency virus type 1 is affected by factors early in
infection but does not influence disease progression. J Virol 83: 10269–10274. doi:10.1128/JVI.01149-09 PMID: 19640996

47. Braibant M, Brunet S, Costagliola D, Rouzioux C, Agut H, Katinger H et al. (2006) Antibodies to conserved epitopes of the HIV-1 envelope in sera from long-term non-progressors: prevalence and association with neutralizing activity. AIDS 20: 1923–1930. doi:10.1097/01.aids.0000247113.43714.5e PMID: 16988513

48. Moore JP, Ho DD (1993) 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 67: 863–875. PMID: 7678308

49. Moore PL, Gray ES, Choge IA, Ranchobe N, Mlisana K, Abdool Karim SS et al. (2008) The c3-v4 region is a major target of autologous neutralizing antibodies in human immunodeficiency virus type 1 subtype C infection. J Virol 82: 1860–1869. doi:10.1128/JVI.02187-07 PMID: 18057243

50. Bonsignori M, Montefiori DC, Wu X, Chen X, Hwang KK, Tsao CY et al. (2012) Two distinct broadly neutralizing antibody specificities of different clonal lineages in a single HIV-1-infected donor: implications for vaccine design. J Virol 86: 4688–4692. doi:10.1128/JVI.02187-07 PMID: 22301150

51. Klein F, Gaebler C, Mouquet H, Sather DN, Lehmann C, Scheid JF et al. (2012) Broad neutralization by a combination of antibodies recognizing the CD4 binding site and a new conformational epitope on the HIV-1 envelope protein. J Exp Med 209: 1469–1479. doi:10.1084/jem.20120423 PMID: 22826297

52. Mikell I, Stamatatos L (2012) Evolution of cross-neutralizing antibody specificities to the CD4-BS and the carbohydrate cloak of the HIV Env in an HIV-1-infected subject. PLoS One 7: e49610. doi:10.1371/journal.pone.0049610 PMID: 23152926

53. Ammann AJ, Schiffman G, Abrams D, Volberding P, Ziegler J, Conant M (1984) B-cell immunodeficiency in acquired immune deficiency syndrome. JAMA 251: 1447–1449. PMID: 6608011

54. Lane HC, Masur H, Edgar LC, Whalen G, Rook AH, Fauci AS (1983) Abnormalities of B-cell activation and immunoregulation in patients with the acquired immunodeficiency syndrome. N Engl J Med 309: 453–458. doi:10.1056/NEJM198308253090803 PMID: 6224088

55. Moir S, Fauci AS (2009) B cells in HIV infection and disease. Nat Rev Immunol 9: 235–245. doi:10.1038/nri2524 PMID: 19319142

56. Crotty S (2011) Follicular helper CD4 T cells (TFH). Annu Rev Immunol 29: 621–663. doi:10.1146/annurev-immunol-031210-101400 PMID: 21314428

57. Lindqvist M, van LJ, Soghoian DZ, Kuhl BD, Ranasinghe S, Krantas G et al. (2012) Expansion of HIV-specific T follicular helper cells in chronic HIV infection. J Clin Invest 122: 3271–3280. doi:10.1172/JCI64314 PMID: 22922259

58. Perreau M, Savoye AL, De CE, Corthiaux JM, Cubas R, Haddad EK et al. (2013) Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. J Exp Med 210: 143–156. doi:10.1084/jem.20121932 PMID: 23254284