CONCISE COMMUNICATION

Identification of early-induced broadly neutralizing activities against transmitted founder HIV strains

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Objectives: Broadly neutralizing antibodies have been proposed as key actors for HIV vaccine development. However, they display features of highly matured antibodies, hampering their induction by vaccination. As protective broadly neutralizing antibodies should be induced rapidly after vaccination and should neutralize the early-transmitted founder (T/F) viruses, we searched whether such antibodies may be induced following HIV infection.

Design: Sera were collected during acute infection (Day 0) and at viral set point (Month 6/12) and the neutralizing activity against T/F strains was investigated. Neutralizing activity in sera collected from chronic progressor was analyzed in parallel.

Methods: We compared neutralizing activity against T/F strains with neutralizing activity against non-T/F strains using the conventional TZM-bL neutralizing assay.

Results: We found neutralizing antibodies (nAbs) preferentially directed against T/F viruses in sera collected shortly after infection. This humoral response evolved by shifting to nAbs directed against non-T/F strains.

Conclusion: Although features associated with nAbs directed against T/F viruses need further investigations, these early-induced nAbs may display lesser maturation characteristics; therefore, this might increase their interest for future vaccine designs.

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Introduction

Treatment with anti-HIV broadly neutralizing antibodies (bnAbs) has shown that the predominant mechanism of antiviral activity of these antibodies is through neutralization of virus entry, their Fc-mediated functions contributing only to 21% of plasma-virus decay slopes [1]. BnAbs are therefore considered to be the Grail for the development of an effective HIV vaccine. Kinetics of neutralizing antibody (nAb) induction have been largely studied. Following HIV infection, autologous nAbs are first detected [2], followed about 1 year by heterologous nAbs [3,4]. Neutralization breadth against Tier 2 strains is only detected in a subset of individuals during the chronic phase and following numerous rounds of viral replication and neutralization escape [4]. This delay of bnAbs response led to the postulate that the presence of viral quasispecies is needed to drive the humoral response for the selection of B cells directed to conserved epitopes [5]. Vaccine efforts have therefore focused on targeting conserved epitopes under evolutionary constraints thereby limiting the virus's adaptive space. However, so far, these approaches have failed *in vivo*. Indeed, antibodies displaying the characteristics of bnAbs have all the same features with high levels of somatic hypermutation, insertions and/or deletions (indels) and, often, unusually long CDRH3 (heavy chain complementarity-determining regions) [4,6]. These specific Ab characteristics have strong disadvantages in the setting of vaccine strategies, as they require long-lasting maturation and the path to bnAbs induction will therefore be difficult.

Another vaccine approach would be to focus on transmitted founder (T/F) viruses that have established the actual infection, given the hypothesis that transmissible phenotypes are limited or antigenically conserved [7]. This is indeed the case during sexual HIV transmission as only a limited number of HIV variants are transmitted. The unique traits of these T/F variants and their evolutionary trajectories with antibody response remain an unresolved question [8].

Different studies have been performed to identify common features for T/F viruses. Shorter V1V2 regions [9], lower glycosylation sites [3] and less efficient binding to $CD4^+$ [10] have been identified. These characteristics may increase infectivity and affinity to mucosal receptors or reduce sensitivity to neutralization [9,11]. However, these special features could not be confirmed by others describing increased neutralizing sensitivity [12] and less infectivity for viruses isolated early after infection [11], further suggesting that the pattern of Env evolution observed may be specific to the host and infecting strains [11]. The explanation of the viral bottleneck selecting T/F strains remains elusive. However, vaccine should induce antibodies able to neutralize these selected transmitted variants.

As only a limited number of HIV variants are acquired during transmission, we hypothesized that less potent nAbs, with limited breadth, but nonetheless able to neutralize T/F strains may be sufficient for protection. Noteworthy, previous studies have analyzed the neutralization of T/F vs. chronic viruses but did not specifically focus on serum samples from very early infection. We searched for such antibodies in sera collected from infected individuals grouped according to the time following infection. Remarkably, we detected nAbs against T/F in some samples collected during early acute infection. Neutralization was waived over time in favor of nAbs against non-T/F viruses. These results demonstrate that nAbs against T/F strains can be induced rapidly the following infection. As they were induced rapidly, they may not display the highly maturated and difficult-to-induce profile of the nAbs detected after several years of infection. They may therefore be of high interest in future vaccine designs.

Methods

Patient cohorts

Sera from 24 patients from the ANRS PRIMO cohort were obtained at two time points: Day 0 (D0) corresponding to the day of inclusion in the cohort (day of detection, <3 months after infection) and 6 or 12 months later (M6/M12). As this is a historical cohort, the patients selected were not treated at the time of sample collection. We used a historical non-treated, HIV-infected chronic progressors cohort ($n = 16$) of individuals infected with a median of 8 years. These chronic progressors had normal CD4T cell levels (median of 696/mm^3 at the time of blood sampling) but high viral load and went on therapy shortly after sample collection because of disease progression. IgG were purified by protein G columns (Sepharose 4 Fast Flow, Amersham Biosciences, Amersham, UK) according to manufacturer instructions.

All patients provided their written informed consent to give their blood sample for research purposes. The PRIMO cohort was funded and sponsored by ANRS and approved by the Ile de France III Ethics Committee, 2 July 1996, with amendment No. 15 approved 8 June. The study was conducted according to the principles expressed in the Helsinki Declaration.

TZM-bl neutralization assays

The conventional TZM-bl neutralization assay was used [13]. Serial dilution of sera (beginning at 1 : 20 dilution) was tested for its ability to neutralize various HIV-1 strains (Supplemental Table S1, http://links.lww.com/QAD/C626), and the inhibitory reciprocal serum dilution 50% (IRD50), the inhibitory reciprocal serum dilution 70% (IRD70) or the inhibitory antibody concentration 50%

(IC50) were calculated [13]. The capacity of individuals' sera to neutralize murine leukemia virus (MuLV) was assessed as a control. Two sera (M6/12) with IRD more than 50 for MuLV pseudovirus were excluded from the study. Samples with values at least 50 IRD50 and samples with values of less than 1 μg/ml IC50 were recorded as positive for neutralizing activity.

HIV-specific Ig detection

HIV-specific antibodies directed against gp41S30, gp160 HIV MN LAI (kind gift from R. El Habib, Sanofi Pasteur) or trimer folded JRFL NFL TD (kind gift from R. Wyatt, Scripps Research, San Diego, CA, USA) were detected by ELISA as previously described [14].

Statistical analysis

The statistical significance (*P* values) was evaluated using a two-tailed paired (T/F versus non-T/F) Wilcoxon test of the median neutralizing activity of sera using the Prism software (GraphPad Software Inc., San Diego, California, USA).

Results

Distinct patterns of neutralizing antibodies against T/F variants compared with non-T/F strains over time

We searched for nAb in sera collected early after infection (D0), 6–12 months after infection (M6/12) or in sera from chronic progressors. In opposition to what has been previously described, antibodies able to neutralize some HIV strains were already detected at D0 of infection (Fig. 1a and Supplementary S2, http://links.lww.com/QAD/C625). In sera from patients 350 115, 440 138, 680 115, 750 518 and 751 206, IRD50 titers with values of at least 50 against T/F viruses were detected. At that time point, the median IRD50 was significantly higher for T/F viruses compared with non- T/F viruses ($P = 0.016$). 426c, CH077, CH058 and RHPA- T/F viruses were the best neutralized (Fig. 1d). Moreover, we analyzed the neutralizing activity after IgG purification. We found a similar neutralizing profile (Supplemental Fig. S1, http://links.lww.com/QAD/C625) indicating that the neutralization detected was not due to non-specific factors present in the sera. These results show that nAbs against some T/F strains can be induced very rapidly during acute infection.

At M6/12, Samples with IRD50 of at least 50 increased with the highest neutralizing activity in patients 330 209 and 940 106. At that time point neutralization was similar for T/F and non-T/F strains ($P = 0.14$) (Fig. 1b) and was recovered in the IgG fraction (Supplement Fig. S1, http://links.lww.com/QAD/C625).

In chronic progressors, on contrary, we detected significantly more neutralizing activities against non-T/F strains compared with T/F viruses ($P > 0.0005$), suggesting that the neutralizing activity is redirected against non-T/F viruses after several years of infection (Fig. 1c).

Indeed, by following the neutralizing profile over time, neutralizing activity against T/F viruses did not increases at M6/12 and in chronic progressors compared with a gradually increased neutralization for non-T/F Tier 2 and Tier 1 strains (Fig. 1d). The general profile of neutralizing responses depicted by the radar plot showed that the mean neutralizing activity against T/F viruses was similar for the three time points (Fig. 2a). Therefore, T/F strains may display special features supporting early nAb development by the humoral immune response.

Detection of cross-neutralizing activity against T/F viruses at D0

For the vaccine design, induction of cross-neutralization against different strains is essential for a large coverage of HIV protection. We hence analyzed the capacity of the sera to cross neutralize several strains (Fig. 2b). Significantly, 17% of sera collected at D0 already neutralized four to five of the seven T/F viruses analyzed with an IRD value of at least 50, whereas none of them cross-neutralized four to five non-T/F Tier 2 strains. Cross-neutralization of non-T/F viruses appeared later with 29 and 56% of sera neutralizing more than four non-T/F s Tier 2 strains at M6/12 and for chronic progressors, respectively. This cross-neutralization only slightly evolved for T/F viruses from 17 to 29% and 31% cross-neutralization of more than four T/F viruses at M6/12 and for chronic progressors, respectively. Similar profile with neutralization detected against T/F viruses at D0 was observed by using an IRD70 read out (Supplemental Fig. S2, http://links.lww.com/QAD/C625). Again, this point to a different neutralizing activity against T/F viruses that switch over time to nAbs against non-T/F strains. Whether this cross-neutralizing activity refers to bNAbs or to polyclonal responses with induction of various nAbs needs further investigation. Moreover, to identify specific env-binding features associated with this cross-neutralizing activity, we analyzed antibody-binding capacity to various envelop proteins (Supplemental Fig. S3, http://links.lww.com/QAD/C625). Binding to gp41 protein, gp160 monomer or JRFL-folded trimer did not allow to discriminate cross-neutralizing activity (colored dots) from the other samples (black dots) as the color dots were distributed all over the panel. More precise immunomapping should be performed to identify epitopes involved in cross-neutralize activity in sera collected at D0.

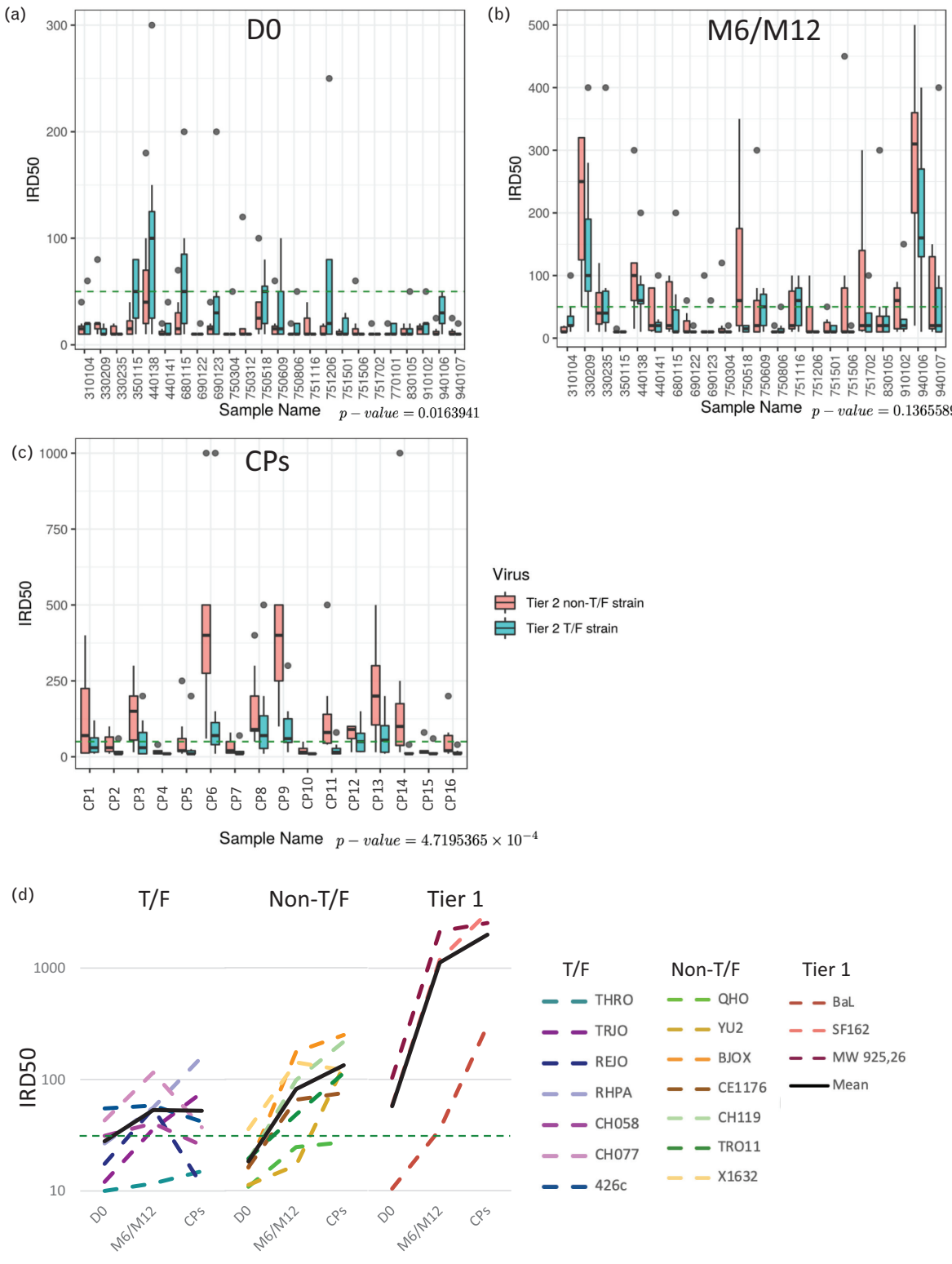

Fig. 1. Distinct pattern of neutralizing activity against tramsmitted founder (T/F) variants over time in HIV-1-infected patients. Neutralization for each serum collected at PRIMO D0 (a), month M6/12 (M6/12) (b) and chronic progressors (c) against T/F (red) and non- T/F (green) strains. Box plots indicate median inhibitory reciprocal dilution 50% (IRD50) values. Mean neutralizing activity for each virus: T/F, non- T/F and Tier 1 strains at different time points (d). Seven T/F s Tier 2 viruses (CH058, CH077, RHPA, THRO4156.18, REJO4541.67, 426c and TRJO4551.58), seven non- T/F Tier 2 viruses (QH0692.42, YU2, X1632_S2_B10, TRO.11, CH119.10, BJOX2000.03.2 and CE1176_A3) and three Tier-1 reference strains (SF162.LS, MW 925.26 and BaL) were used. Two-tailed paired (T/F versus non- T/F) Wilcoxon test of the median neutralizing activity was performed for each serum.

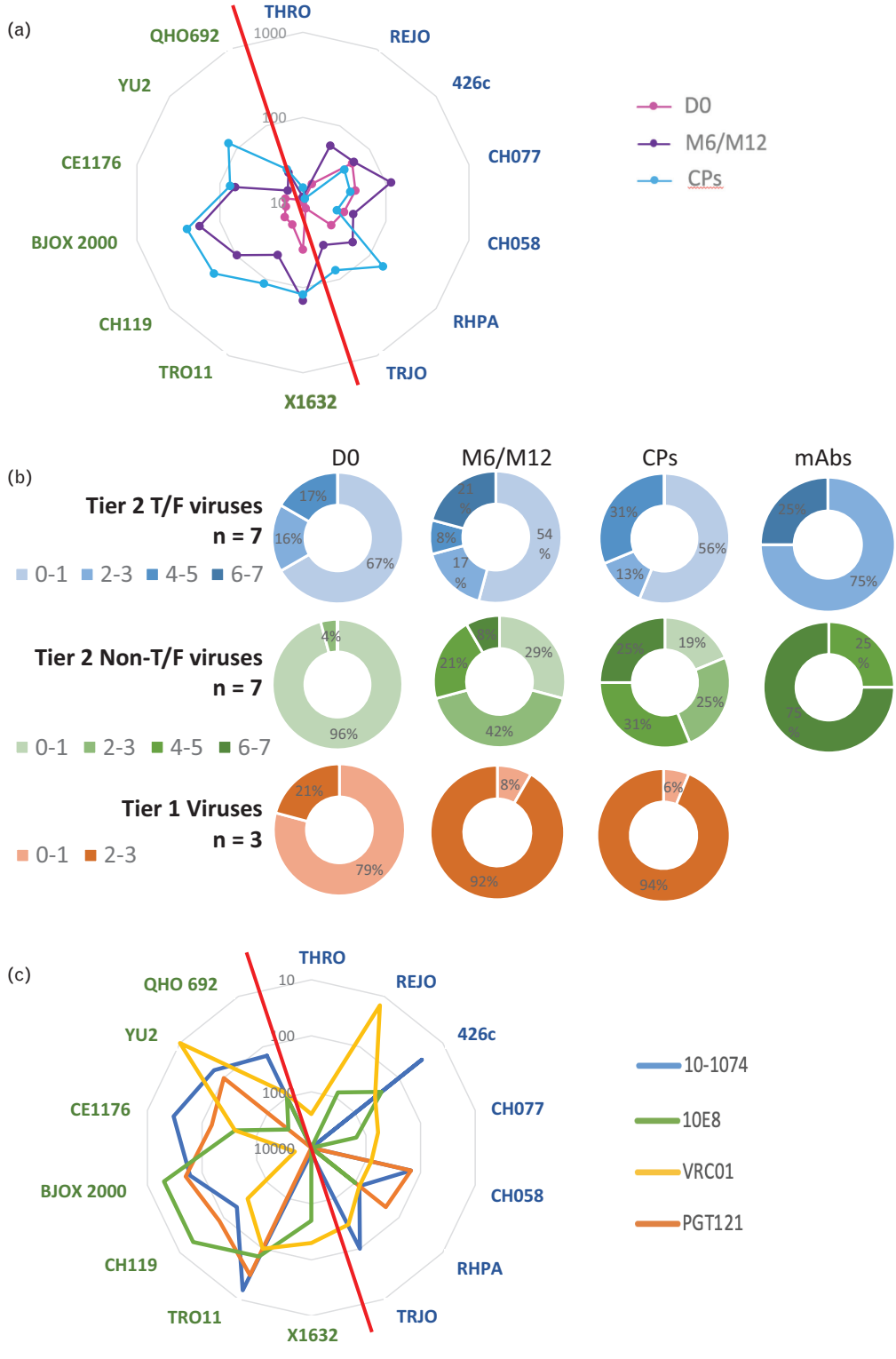

Fig. 2. Detection of cross-neutralizing activity against transmitted founder (T/F) viruses at D0. Radar chart against T/F viruses (in blue) and non- T/F (in green) for mean inhibitory reciprocal dilution 50% (IRD50) neutralizing activities of sera collected at three time points (a) or inhibitory concentration 50 (IC50) of four bmNAbs: 10–1074 and PGT121 targeting V3 glycan-dependent epitopes, 10E8 targeting the remarkably conserved gp41 membrane-proximal external region and VRC01 targeting the conformational $CD4^{+}$-binding site. (c). Pie charts representing the percentage of viruses neutralized with an IRD50 value of at least 50 for sera collected at D0, M6/M12 and chronic progressors or IC50 less than 1 μg/ml for bmNAbs 10–1074, PGT121, 10E8 and VRC01 for Tier 2 T/F (blue), non- T/F (green) and Tier 1 (orange) strains (b).

Effects of neutralizing antibodies against T/F strains on viral load

We further searched for an association between nAbs detected at D0 and viral load evolution. We did not detect any correlation between nAbs responses directed against T/F or non- T/F strains and their corresponding viral load (Supplemental Table S2, http://links.lww.com/QAD/C626 and Fig. S4, http://links.lww.com/QAD/C625). We also analyzed whether nAbs detected at D0 may associate with lower viral load at D0 or at set point at M12. Although patients displaying cross-neutralizing activity at D0 had often low post-acute viral load, no significant association could be detected between nAbs and viral load at the set point (Supplemental Fig. S4, http://links.lww.com/QAD/C625). This shows that the unexpected cross-neutralizing response detected at D0 did not influence post-acute viral load evolution at the set point.

Neutralizing sensitivity of T/F strains

Next, we determined if differences of cross-neutralizing activity between the T/F and non- T/F could be attributed to differences of virus-neutralizing sensitivity. We therefore analyzed the neutralizing capacity of four well-known monoclonal bnAbs (mbnAbs) against our T/F and non- T/F viruses (Fig. 2c). We found that these mbnAbs poorly neutralized (Fig. 2c) and cross-neutralized (Fig. 2b and Supplemental Table S3, http://links.lww.com/QAD/C626) T/F viruses (with only 25% neutralization against four or five T/F), whereas 100% of them neutralized more than four non-T/F strains tested (Fig. 2b). This low neutralization sensitivity corroborated the resistant phenotype described for transmitted founder viruses. Of note T/F viruses were significantly less inhibited by bnAbs PGT121 and 10E8 (Fig. 2c and Supplemental Table S3, http://links.lww.com/QAD/C626) further suggesting that V3 glycan-dependent epitopes and the conserved MPER may be less exposed on these viruses.

Discussion

In this study, we assessed the neutralization sensitivity against T/F strains and compared them with other Tier 2 variants. We found a modest cross-neutralizing activity against T/F strains in sera collected during acute infection (D0). This unexpected neutralizing activity barely increased over time. On the contrary, nAb response shifted to the development of neutralization against non-T/F Tier 2 strains suggesting that the early humoral immune response developed against T/F viruses was discarded in favor of other non- T/F strains. Moreover, the highly potent monoclonal bnAbs that display more than 60% coverage at IC50 less than 1 μg/ml using a large panel of selected Tier 2 isolates [4], were found to poorly neutralize the T/F viruses. This further indicates that these T/F viruses display a neutralization-resistant phenotype. Although the number of T/F strain analyzed was relatively small, this unexpected cross-neutralizing activity detected at D0 against our 'neutralization resistant' T/F viruses deserve further investigation.

Whether this unexpected cross-neutralizing activity has potential, protective effects need to be further assessed on larger cohorts of acute patient and against multiple types of T/F and non- T/F viruses. Moreover, it is unknown whether vaccine targeting more specifically T/F viruses with unambiguous identification of transmissibility signatures will give promising protection. Our data clearly demonstrate that nAbs against T/F viruses can be induced during acute infection. As these nAbs were induced very early after infection, they may display specific features (epitope recognized, less constrained maturation phenotypes) distinct from the currently identified mbnAbs. nAbs directed against T/F strains therefore open new perspectives for the development of such antibodies in future vaccine designs.

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Conflicts of interest

The authors declare no conflict of interests. This work was not presented before.

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