AIDS

DOI: 10.1097/QAD.0000000000001661

Title:

Potency of HIV-2-specific antibodies increase in direct association with loss of memory B-cells

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Abstract

Potent HIV neutralizing antibodies are critical for vaccination and viral reservoir control. High levels of neutralizing antibodies characterize HIV-2 infection, a naturally-occurring model of attenuated HIV disease with low to undectable viremia. We found that HIV-2-specific antibody potency increased in direct association with the loss of both switched and unswitched memory B-cells in untreated HIV-2 infection. Thus, HIV antibody affinity maturation is linked to memory B-cell exhaustion even in reduced viremia settings.

Research letter

The specificity and variability of antibodies (Abs) is achieved by affinity maturation through somatic hypermutation and clonal selection. This process proceeds for weeks after acute infections or vaccination. There is much debate about the mutation levels and chronic antigen exposure needed to generate broadly neutralizing antibodies (bNAbs) against HIV-1 [1]. In adults, bNAbs usually take two to four years to develop and are associated with high viremia and low CD4 T-cell counts [2-7]. In contrast to HIV-1, most HIV-2 infected patients develop potent bNAb responses [8-12]. In addition, HIV-2 infection usually courses with undetectable viral load and very slow rate of CD4 T-cell decline even in the absence of antiviral treatment [13, 14]. Therefore understanding the generation of broad and potent neutralizing antibodies in this unique model of naturally-occurring control of viremia is relevant for the vaccine field.

We aim to investigate the relationship between memory B-cells and NAb response in untreated HIV-2 infected adults. Previously we have shown that these patients featured

contraction of the memory B-cell compartment in direct association with the degree of CD4 T-cell depletion and markers of immune activation, suggesting the occurrence of lymphoid tissue disruption [15]. Here we asked how this finding relates with HIV-2-specific Abs.

We analysed the NAb response in 27 untreated HIV-2 infected patients, included in a previous report [15]. Twenty-one patients (78%) had undetectable viral load (detectable in 6 patients, ranging from 1189 to 13627 RNA copies/ml), and 20 (74%) had CD4 T-cell counts above 350 cells/µl (median 568, range 52-1511 cells/µl). Informed consent for blood collection and participation in the study was obtained from all participants, under the approval of the Ethical Board of the Faculty of Medicine, University of Lisbon. HIV-2 viremia was quantified by RT-PCR (detection threshold: 40 RNA copies/ml), as previously described [16, 17]. The neutralizing activity was analysed in plasma against a panel of four heterologous primary CCR5-tropic isolates (03PTHCC6, 03PTHCC12, 03PTHCC19, 03PTHSM2). Origin and characterization of these isolates, and their sensitivity to IgG NAbs, have been described elsewhere [10, 18, 19]. A luciferase reporter gene assay in TZM-bl cells was used, as described previously [20, 21]. Additionally, binding antibodies against HIV-2 envelope glycoproteins gp125 (C2V3C3 region) and gp36 were quantified in samples from a subset of the patients using an ELISA-HIV2 assay as previously described [15, 22]. Memory B-cell populations were assessed based on the expression of CD27 and surface IgD by flow cytometry, with class-switched memory B-cells defined as $CD19^{+}CD27^{+}IgD^{neg}$ and unswitched memory B-cells as $CD19^{+}CD27^{+}IgD^{pos}$, as described previously [15]. Serum B-cell activating factor (BAFF) levels were quantified by ELISA as previously described [15]. Statistical

analysis was performed with GraphPad Prism 5.0 (Graph Pad Software, Prism 5, version 5.04, 2010). P values were 2-tailed and significance was defined as P <0.05. Linear regression was performed and Spearman correlation coefficients were computed.

All patients produced Nabs against the panel of four primary R5 isolates, with a median reciprocal log10IC50 neutralization titer of 2.89 (interquartile ranged 2.76-3.18). NAb potency and breadth were directly associated (N=27, Spearman rank, r=0.4916, P=0.0092; Supplemental Digital Content 1, http://links.lww.com/QAD/B170). Moreover, Nab potency and breadth were not associated with CD4+ T-cell counts (N=27, Spearman rank, r=-0.1902, P=0.3419 and r=-0.0325, P=0.8723, respectively). Nab potency has generally not been associated with the number of CD4 T-cells in previous HIV-2 studies [9, 23], except when patients representing the full spectrum of disease were analysed [10].

NAb potency increased in direct association with the increase in C2V3C3-specific binding Abs (N=12, Spearman rank, r=0.7250, P=0.0098) (Supplemental Digital Content 2 – blue, http://links.lww.com/QAD/B170). However, no association was found between NAb potency and gp36-specific binding Abs (N=12, Spearman rank, r=0.4483, P=0.1446) (Supplemental Digital Content 2 – black, http://links.lww.com/QAD/B170). Our findings confirm the presence of broad and potent NAbs targeting the C2V3C3-envelope region during chronic HIV-2 infection, as described previously [10].

NAb potency was found to increase significantly with the loss of switched (N=27, Spearman rank, r=-0.6436, P=0.0003) and unswitched (N=27, Spearman rank, r=-4162, P=0.031) memory B-cell populations (Figure 1 – top and bottom, respectively). In accordance with the association between NAbs and binding Abs, the loss of both

switched and unswitched memory B-cell populations was directly correlated with the increase in C2V3C3-specific binding Abs (N=12, Spearman rank, r=-0.7622, P=0.0055 and r=-0.6434, P=0.0278, respectively; Supplemental Digital Content 3, http://links.lww.com/QAD/B170). No association was found between switched and unswitched memory B-cell populations and gp36-specific binding Abs (N=12, Spearman rank, r=-0.5455, P=0.0708 and r=-0.2517, P=0.4303, respectively; Supplemental Digital Content 3, http://links.lww.com/QAD/B170). No association was found between Nab titers and BAFF levels (N=11, Spearman rank, r=0.1455, P=0.6731; Supplemental Digital Content 4, http://links.lww.com/QAD/B170), despite the previous report of BAFF increase in parallel with B cell disturbances in HIV-2 infected individuals [15].

To our knowledge, this is the first study addressing the relationship between NAb responses in HIV-2-infected patients and memory B-cell disturbances. In spite of the limitations imposed by the number of patients and sample availability that only allowed to test four viral isolates, we were able to reveal a strong direct association between memory B-cell exhaustion and the generation of NAbs as well as C2V3C3 binding Abs. These findings suggest that, multiple rounds of HIV-2 infection are important for the generation of highly specific Abs, and that this happens at the expense of the memory B-cell pool. The lack of hypogammaglobulinemia [15] is likely due to the persistence of long-lived plasma cells has shown in other clinical settings [24]. Although the levels of plasma viral load were reduced in HIV-2 infection even in the absence of ART, we have shown that the extent of viral reservoirs is similar in HIV-2 and HIV-1 individuals [17, 25]. Moreover, HIV-2 has been shown to infect and induce cytopathicity in lymphoid tissues [26], supporting that there is contained local viral replication [27]. The prolonged

time of infection that characterizes HIV-2 pathogenesis, favours the continuous exposure to envelope antigens (either present in plasma viruses or bound to the surface of infected cells) and persistent immune activation [25]. This setting is ideal for the generation of highly specific Abs through affinity maturation, but ultimately leads to immune exhaustion.

Acknowledgements

NT and AES designed research. CR, JMM, RC and RT performed experiments. CR and JD analyzed and interpreted the data. CR and PB performed the statistical analysis. EV contributed with patients' data. CR, JD, AES and NT wrote the article. All authors revised and approved the final manuscript.

This work was supported by grants PTDC/SAU-FAR/115290/2009, PTDC/SAU-EPI/122400/2010 and PIC/IC/82712/2007 from Fundação para a Ciência e Tecnologia (FCT) (http://www.fct.pt), Portugal and by Programa Operacional Ciência e Inovação 2010 (POCI2010). CR, PB, RC and RT were supported by PhD grants from FCT. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: TZM-bl from Dr. John C. Kappes, Dr. Xiaoyun Wu and Tranzyme Inc. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors have no commercial or other association that might pose a conflict of interest.

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Figure 1 – Association between neutralizing antibodies and memory B-cells. Spearman's rank correlation coefficient was used to assess associations between neutralizing antibody titers and frequency of switched ($CD27^{+}IgD^{neg}$ – top) and unswitched ($CD27^{+}IgD^{pos}$ – bottom) memory within B-cells (N=27).