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HIV neutralizing antibodies: clinical correlates and implications for vaccines

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Neutralizing humoral immunity has been the subject of intense investigation since early in HIV research. While virtually all HIV-positive patients have antibodies capable of binding to HIV Env protein, only a subset of these antibodies, termed neutralizing antibodies (NAb), are able to block viral entry into target cells. NAb develop later than other immune responses – at least 12 weeks after infection [1,2] – and initially target only the infecting strain. Over time, in many patients this response broadens to allow recognition of heterologous strains [3,4]. It is generally thought that NAb will be a critical component of a successful vaccine-elicited immune response.

Given the immense diversity of HIV strains worldwide, vaccine-elicited NAb would ideally be broadly cross-reactive. To date, the antibodies elicited by candidate vaccines have had no or weak neutralizing activity, mainly against laboratory-adapted strains and with very limited breadth [5–7]. However, many HIV-infected individuals make NAb, and a small fraction make extremely potent NAb with activity against diverse clinical (primary) isolates [3,4,8–11]. Understanding how broad NAb develop naturally in some HIV-1 infected patients should provide guidance for vaccine design. The prevalence of, and clinical parameters associated with, broadly reactive NAb in serum have been the subject of several recent studies [10,12–14]. Euler and colleagues, in an article appearing in this issue of *the Journal* [15], examine these issues in a European cohort.

Euler et al [15] evaluated samples from 82 patients participating in the Amsterdam Cohort Studies. Because this cohort has been followed from seroconversion onward, the authors were able to look for associations between NAb and clinical outcomes, as well as immunological parameters. The authors chose samples from three years post-infection, allowing time for broad NAb to have developed. Neutralizing activity in serum was measured using the well-accepted TZMbl assay and pseudoviruses derived from primary isolates [2,16]. They found that NAb breadth varies widely among chronically infected patients. Consistent with studies of other cohorts in multiple geographic areas [10–14], the authors observed that 33% of their patients with chronic HIV infection had broad NAb. An important finding was the lack of association between breadth of NAb and the time from seroconversion to diagnosis with AIDS or AIDS-related death, or survival time after AIDS diagnosis. This agrees with findings in a Kenyan cohort [13]. The authors also noted a positive association of NAb breadth with viral load, although the association did not reach statistical significance, as it did in other cohorts [10,13,14].

Unexpectedly, Euler et al observed that broad NAb were associated with lower CD4⁺ T cell levels prior to and one year after seroconversion. This finding appears counterintuitive, as one

would expect broad NAb to be produced by highly functional B cells that have undergone class switching and multiple rounds of somatic hypermutation [17], events that require intact CD4 help. The authors speculate that lower levels of CD4+ T cells result in less HIV-induced polyclonal B cell activation, with a concomitant boost to virus-specific Ab, as seen in the LCMV mouse model [18]. However, in HIV infection, polyclonal activation and other B cell abnormalities are more pronounced in patients with depleted CD4+ T cells, and can be partially reversed by ARV treatment [19]. A second possibility is that lower initial CD4 counts lead to less effective control of viremia, and since higher viremia and extended exposure to antigen are associated with the development of broad NAb [10,14], the relationship between low CD4 and broad NAb could be a side effect of viremia rather than causal. Nonetheless, the observation by Euler et al is intriguing and needs to be validated and explored in larger cohorts.

Why are neutralizing antibodies the focus of such interest for vaccine research?

Based on the data from patients with chronic infection, NAb may not seem advantageous. Broad NAb are not associated with better clinical outcomes among chronically infected patients, as shown in Euler et al and [13], and they correlate with higher viral load [10,13,14]. Patients that control viremia to <50 copies/ml without antiretrovirals have very low autologous [20] and heterologous [9,14,21,22] NAb. Furthermore, a study of superinfection in women did not find a protective effect of broad NAb [23]. Thus, NAb may not be of value when present in chronic infection.

However, several lines of evidence strongly suggest that vaccine-elicited NAb could be useful in preventing infection. The most convincing data come from the non-human primate model of HIV using SHIVs -- chimeric viruses bearing an HIV *env* gene on an SIV backbone. In a large number of studies, macaques were administered HIV-neutralizing antibodies intravenously and subsequently challenged with SHIV. NAb could completely prevent SHIV infection by intravenous, intravaginal, or oral routes. In some cases, animals became infected, but with delayed disease kinetics and controlled viremia [24]. These studies demonstrate the potential for antibodies to prevent infection or disease if they are present at the time of exposure -- as they would be if elicited by a vaccine.

Data from other areas of research speak to the prophylactic potential of NAb. Vertical transmission of HIV may be influenced by NAb: some (although not all) studies find less frequent HIV transmission to babies by mothers with higher NAb titers. Antibodies can be passively transferred from mother to child transplacentally or in breastmilk. When transmission does occur, the transmitted variants are often those that are resistant to the mother's NAb [25]. Additionally, many licensed vaccines for other pathogens protect via neutralizing antibodies [26]. Finally, it is likely that vaccine-induced T cells would be unable to prevent infection in the absence of antibodies: in clinical trials of an Ad5-based HIV vaccine [27] and experimental adoptive transfer of CD8+ T cells in the macaque model [28], pre-existing virus-specific T cells showed no efficacy in preventing infection or lowering viral load. Thus, despite the ineffectiveness of NAb at mitigating chronic infection, the *in vivo* production of broad NAb is still a major goal for prophylactic vaccines.

The recent announcement of results from a Phase III vaccine trial in Thailand has focused much attention on vaccine-induced humoral responses. The RV144 trial tested a canarypox prime, recombinant gp120 boost regimen vs placebo in 16,000 volunteers. The modified-intent-to-treat analysis showed 31.2% vaccine efficacy (p=0.04) [29]. This modest, but positive, result surprised many in the field [30,31]. Immunogenicity studies showed a lack of CD8+ T cell responses and very low levels of CD4+ T cell responses to the vaccine; thus, cellular immunity was unlikely to have contributed to efficacy. In contrast, humoral immunity in the form of gp120-binding antibodies was noted in nearly all vaccinees. Furthermore, 71% of sera had

NABs against the laboratory-adapted HIV-MN strain. MN neutralization was also measured in the VAX004 Phase III trial of recombinant gp120 (AIDSVAX), but the effect of MN NABs on HIV acquisition was unclear [32,33]. Neutralization of primary isolates is widely assumed to be a more relevant antibody function [34]. Primary-isolate neutralization was not measured, and indeed was not expected, in RV144 samples based on over a decade of experience with gp120 vaccines [6]. However, the vaccine regimen tested in RV144 was previously shown in a Phase I/II trial to elicit a different Ab function, antibody-dependent cell mediated cytotoxicity (ADCC), in most vaccinees [35]. ADCC is the destruction of antibody-coated HIV-infected cells by natural killer cells. Measurement of ADCC responses in serum from RV144 may allow determination of their contributions to vaccine efficacy. The vaccine development field can build upon the results of this trial, and future trials of vaccines that elicit additional responses that are expected to be useful – including NABs – may have better outcomes.

In the absence of vaccine-elicited NABs in clinical trials, the potential importance of NABs in protection from incident HIV infection remains speculative. Euler et al have added to the growing number of reports showing that broadly cross-reactive NABs provide no benefit to chronically infected patients. Further dissection of the mechanisms by which such antibodies are generated, however, may yield valuable clues for designing vaccines that can elicit antibodies that are protective when present before virus exposure. As shown in Euler et al and similar studies, broad NABs are made by a substantial proportion of HIV-infected patients, at titers in the range shown to be protective in some passive-transfer SHIV experiments [36]. Thus, the human immune system can achieve NAB responses at levels that could be protective. Now, the challenge to the field is to achieve a prophylactic vaccine that elicits them.

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