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Antibody Secreting B-cells in HIV Infection

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Abstract

Purpose of review—Several recent advances are permitting a detailed examination of the HIV-specific B cell response. In this review we summarize these advances and their implications for understanding the response to HIV in chronic infection or vaccinees.

Recent findings—In HIV-infected patients, aberrant B cell phenotypes have been associated with diminished humoral responses to other pathogens. HIV-specific B cells are overrepresented in some of these abnormal subsets. Over the past 2 years flow cytometry-based techniques have been developed to stain HIV-specific B cells. These techniques are permitting a re-examination of frequency, phenotype, and function of HIV-specific B cells. They are also permitting the isolation of HIV-specific B cells in high purity. IgG recovered from sorted HIV-specific B cells are oligoclonal, use a limited repertoire of Ig genes, and target multiple epitopes on Env.

Summary—It is likely that the defects found in total B cells in HIV-infected patients also play a role in the poorly effective HIV-specific antibody response. A subset of HIV-infected patients produce broadly neutralizing antibodies. Understanding this antibody response, and the B cells that underlie it, may be critical in efforts to elicit neutralizing antibodies against HIV.

Keywords

B cell; antibodies; HIV; Envelope; ELISPOT

Introduction

Although much work has been devoted to the study of naturally occurring and vaccine-elicited HIV-specific antibodies, until very recently relatively little was known about the B cells that produce them. Defects in global B cell function have been described in HIV infection but the consequences of these defects for HIV-specific B cells have only begun to be explored. Although nearly all HIV-infected individuals make antibodies to HIV, only a fraction of these have documented anti-viral activity such as neutralization, and the neutralizing response only appears months after infection [1,2]. However, up to 20% of B-clade HIV-infected patients with CD4⁺ T cell counts >200 cells/μl mount antibody responses with considerable breadth extending into clades A and C [3]. A detailed understanding of the specificities of the antibodies mediating broad neutralization and the HIV-specific B cells that underlie such a response is likely to provide important information for the design and testing of prophylactic vaccines for HIV.

For many years, only the ELISPOT assay was able to measure HIV-specific antibody secreting cells. Very recently, flow cytometric techniques have been developed to enumerate and

phenotype these cells, permitting an examination of frequency, phenotype, and function of HIV-specific B cells. Because there are several excellent recent reviews on total B cell function in HIV infection, we will only briefly summarize the most recent work in this area. We provide a more detailed summary of studies of HIV-specific B cells permitted by these recent technical advances.

Dysregulation of B cells in HIV infection

Spontaneous activation of IgG secreting B cells and hypergammaglobulinemia were among the first immune dysfunctions reported in AIDS patients [4]. A wide variety of B cell pathologies have been noted since then, recently reviewed in [5]. In the past year, B cells of HIV-infected patients were shown to have an abnormal distribution of various subsets: a decreased frequency of a CD27⁺B220⁻ memory B subset [6] and an increased frequency of CD20^{lo/-}CD27^{hi}CD38^{hi} plasmablasts [3] compared to uninfected controls. Tissue-like memory B cells, which have properties of exhausted cells (e.g. reduced proliferation and a truncated replication history) and the unusual phenotype CD27⁻CD20^{hi}CD21^{lo}, were shown to be increased in HIV-infected patients [7] compared to uninfected controls. In addition, B cells of HIV-infected patients have an altered chemotactic capacity [8]. Some changes in the B cell compartment, such as the proportions of immature/transitional cells, normalize to some extent during antiretroviral therapy although decreased frequencies of IgM memory B cells may persist [9–11].

B cell function in HIV infection has been assessed in studies of vaccination against other pathogens. Several studies, including recent trials of yellow fever vaccine in adults [12] and pneumococcal vaccine in children [13], have observed reduced efficacy of vaccines and lower titers of vaccine-elicited antibodies in those with HIV, as reviewed in [14,15]. One abnormality that was linked to poor vaccine response is a defect in class switching. In response to a vaccination with the neoantigen bacteriophage phiX174, HIV-infected patients had reduced class switching of phage-specific antibodies from IgM to IgG. This effect was most pronounced in those with CD4⁺ T cell counts <200 cells/μl [16].

Vaccine-induced antibody-secreting cells (ASC) also show reduced frequencies in HIV+ patients. Compared to controls, HIV-infected patients had lower frequencies of influenza-specific ASC at peak and fewer ASC in stimulated memory cells two months after vaccination for influenza, with the reduction more pronounced in patients with CD4<350 [17]. T cell-independent antigen-specific responses are also abnormal in HIV+ patients, as noted in a study of pneumococcus vaccination: HIV-infected patients developed lower IgM and IgG titers against pneumococcus after vaccination [10]. Taken together, there is considerable evidence that HIV infection can affect T cell dependent and T cell independent B cell responses. However, it should be noted that some of the effects on B cell subsets or B cell responses observed in the studies described above are likely a consequence of CD4⁺ T cell depletion rather than a direct effect of HIV upon the B cells. In addition, there is considerable overlap between patient groups with some HIV-infected patients developing responses to viral, bacterial, or neo-antigens that are indistinguishable from healthy controls.

HIV-Specific B cells: Frequency

The frequency of HIV-specific B cells and of cells secreting anti-HIV antibodies is quite low. Several approaches to determining these frequencies have been published. Most commonly, B cell ELISPOT has been used to enumerate total or antibody secreting (ASC) antigen-specific cells in PBMC, B cells, or as a fraction of IgG ASC. Two older studies found that the frequency of Env-specific ASC was 0.01% – 0.07% of fresh PBMC [18,19]. Recently, we noted a lower frequency (less than 0.001%) in unstimulated PBMC [3]. We found that gp120-specific ASC

are 0.003% of unstimulated B cells [3] while B cells that were stimulated with CD40L resulted in 0.01% HIV-specific ASC [20]. As a fraction of total IgG secreting ASC, quite different numbers of HIV-specific cells have been reported, ranging from 0.1–7% using assays with varied conditions [3,18–21]. All studies found high patient-to-patient variability, with some patients having HIV-specific ASC in the periphery below the level of detection. Upon HAART treatment, HIV-specific ASC dramatically decrease [18,20] despite continued high titers of circulating anti-HIV antibodies. As ELISPOT can only identify cells that actively secrete antibody, other techniques are needed to account for all circulating HIV-specific B cells.

Flow cytometric techniques have recently been developed to assess the frequency of HIV-specific B cells. Two reagents have been described that permit labeling of HIV-specific B cells with high specificity. Both are based on Env protein and bind to Env-specific antibodies that are displayed on the B cell surface as components of the B cell receptor. A variety of HIV Env epitopes have been incorporated into “B cell tetramers” (A. Moody, reviewed in this issue). A second approach employs a gp140 trimer that can be fluorescently labeled and used in flow cytometric analysis and sorting. The trimer consists of HIV-1 YU2 Env amino acids 1 to 683 fused to the trimerization domain of T4 phage fibrin [22,23]. It is biotinylated at an engineered Avitag sequence in the fibrin domain, which is distal to epitopes that would be recognized by Env-specific B cells. Therefore, the antigenic structure is not affected by biotinylation, as demonstrated by binding of monoclonal antibodies b12, F105, and 17b with or without soluble CD4 (R. Wyatt, unpublished data). After binding of gp140-F trimer to B cells, fluorescent conjugates of streptavidin are used to label the gp140-bound cells. The result is a signal of high strength and low background. Gp140, although an imperfect mimic of gp160 spike, is more similar to the spike than gp120 and also allows labeling of gp41-specific cells. Recently, this approach has been used to study and isolate HIV-specific B cells in the peripheral blood [3, 24,25]. Use of these labeled Env proteins allows enumeration of total Env-specific B cells that complements prior measurements based upon ELISPOT. Using biotinylated gp140-F trimer, we found that a median of 0.1% of total B cells [3] and 0.5% of IgG+ B cells (M. Connors, unpublished data) are HIV-specific. Scheid *et al* found a similar frequency (1%) of Env-specific IgG+ B cells [24,25]. These numbers are much higher than the results from ELISPOT described above. Since ELISPOT only captures the actively antibody-secreting cells, resting memory and other B cells are not counted; in contrast, staining with gp140 can identify all Env-specific B cells regardless of their function at the time of assay.

HIV-Specific B cell Phenotype

Although ELISPOT assays do not allow recovery of HIV-specific B cells, and therefore cannot be used directly to discover phenotypes of the ASC, they can be used to find the frequency of HIV-specific ASC in pre-defined subsets. Using this strategy we examined plasmablasts, which during the acute response to a recall antigen such as influenza vaccine, contain the majority of the antigen-specific ASC, and are 75% antigen-specific [26,27]. We found that 0.05% of plasmablasts secreted gp120-specific IgG. We found that plasmablasts accounted for 58% of all antibody secreting B cells. Within both PBMC and plasmablasts, gp120-specific IgG secreting cells were 0.5% of all IgG secreting cells. Thus, although the ELISPOT assay underestimates the total frequency of Env-specific cells, the numbers of Env-specific cells within the plasmablasts (which are most likely to be ASC) were concordant when measured by gp140 staining or by ELISPOT [3]. Moir and colleagues also measured ASC in several B cell subsets using a restimulated B cell ELISPOT, in which fractionated B cells are cultured with CpG oligonucleotides and *S. aureus* cowan for four days. While only 0.004% of CD27⁺ memory B cells secreted anti-gp120 antibody in this assay, 0.01% of the tissue-like memory cells were gp120-specific ASC, showing an enrichment of HIV-specific cells in these exhausted cells compared to the more functional CD27⁺ memory B cells [7].

Flow cytometric techniques allow direct analysis of the phenotype of HIV-specific cells and B cell subsets. Using the biotinylated gp140-F trimer [3], we found a median of 64% of gp140-labelled B cells expressed the memory marker CD27, compared with 25% of all CD19⁺ B cells. Conversely, the median frequency of gp140-labelled cells was 0.17% in the CD19⁺CD27⁺ cells, higher than in total B cells. We also analyzed the antibody isotypes of the Env-specific cells. Gp140-labelled B cells were highly enriched for surface IgG compared to total B cells (48% vs 9.7%). There was a concomitant reduction of surface IgM⁺ cells (medians 81% and 25% respectively). Surface IgA frequency was 6.0% in the gp140-labelled cells, similar to total B cells. Together these data indicate that the majority of gp140-labelled B cells were class-switched memory cells. In addition, although plasmablasts are increased in HIV-infected patients, only a small fraction were observed to stain with gp140[3]. Thus, it is possible that a large fraction of the plasmablasts that are increased in chronic HIV is not HIV-specific unlike the situation in acute responses to influenza vaccination. Alternatively, the gp140 protein used in these assays is capturing only a subset of HIV antigen-specific cells.

Although it remains incompletely understood, it is possible that some alterations in the total B cell pool in HIV infection are associated with diminished function of HIV-specific B cells. The observation that one third of HIV-specific B cells are CD27[−] by flow cytometry [3] agrees with the finding that HIV-specific cells are overrepresented in the aberrant, CD27[−] tissue-like memory population [7]. The tissue-like memory cells show evidence of reduced rounds of replication and selection. In theory, this could render the antibodies secreted by such cells less effective against HIV. Perhaps more importantly, the diminished functional capacity of B cells in HIV infection demonstrated in vaccination studies outlined above likely reduces the magnitude and breadth of the antibody response to HIV.

HIV-Specific B cells: IgG Genes

An indirect approach to looking at HIV-specific B cells is to examine the individual antibodies that they produce. Although methods have recently been developed to more efficiently clone MAb from human memory B cells [28,29], these methods have been found to be less effective in HIV-infected patients [30] (and M. Connors, unpublished data), possibly due to less efficient transformation by Epstein-Barr virus. An additional hurdle is the low frequency of Env-specific B cells, which requires the screening of thousands of clones to find a small number of Env reactive antibodies. It is therefore useful to enrich for Env-specific cells before cloning. Sorting by FACS for gp140-labelled B cells was used to great advantage by Scheid et al [25]. In this study, single gp140-labelled, IgG⁺ B cells were sorted into individual wells. RT-PCR was used to recover paired heavy and light chain genes, which were subcloned into expression vectors and produced in mammalian cells [31]. 86% of them bound gp140 *in vitro*. An average of 100 antibodies from each of four patients with broadly cross-reactive neutralizing plasma was cloned, expressed, and characterized. There were several interesting findings, which reflect on the B cells from which the monoclonals were derived. The Env-specific response was oligoclonal, consisting of expanded families derived from single B cell clones, as determined by V-D-J and V-J gene usage and junction sequences. Within the 100 antibodies for each patient, there were 22–50 clones with up to 39 related sequences per clone. While IgG from total memory B cells averaged 18 mutations in VH, the Env-specific genes averaged 27, with several exceeding 50 mutations. This suggests that the Env-specific B cells have undergone multiple rounds of somatic hypermutation and selection, consistent with chronic exposure to viral antigen. Diverse epitopes on gp140 were targeted (e.g. gp41EC, CD4bs, CD4i, and variable loops) and the proportion of antibodies specific for each epitope varied by patient. Many of the monoclonals that targeted gp120 epitopes could neutralize sensitive, Tier 1 strains [32] and each patient yielded different proportions of neutralizing monoclonals against the various epitopes. This is consistent with recent serum mapping studies, which indicate that multiple specificities underlie the breadth of neutralization in many sera [33–36]. However,

the individual monoclonals had very limited activity against more resistant Tier 2 [32] primary isolates. When pools of monoclonals were compared to total IgG from the same patient's plasma, only two of the four monoclonal pools could neutralize the Tier 2 isolates, and the pools were less potent than total IgG. Thus, it remains unclear whether all specificities responsible for broad or potent neutralization were recovered from the patients.

A related finding in Scheid *et al* was that gene usage was biased: compared to antibodies derived from gp140-unlabelled B cells, or IgG⁺ B cells from uninfected donors, the Env-specific antibodies were highly enriched for Jk2 and Jk5 and VH1 genes. Light chains were 75–90% kappa, higher than normal for human B cells (average 60%) [31,37] using the same PCR conditions. A similar bias has been noted by other groups for HIV (M. Connors, J. Mascola, unpublished data) and in FACS-sorted ASC obtained seven days after vaccination for influenza or pneumococcus [27]. Several studies have also found preferential use of certain VH genes in antibodies against specific epitopes. Gorny *et al* [38] surveyed published and newly cloned anti-Env monoclonals from multiple patients infected with various clades, and found a limited number of VH genes associated with anti-V3 antibodies, with a particular overrepresentation of VH5-51. Thus, there are some data that suggest that B cells expressing certain Ig genes are preferentially expanded in response to these immunogenic epitopes.

B cell subsets and anti-HIV IgG

In studies of circulating B cells, it has been suggested that plasmablasts [3] or tissue-like memory cells [7] are major sources of circulating anti-Env antibodies based on ELISPOT. The study of Scheid *et al* [25] did not look at fractionated B cells, however their finding of high somatic hypermutation in the cloned IgG genes suggest that tissue-like memory cells may not be a major component of the anti-HIV response, as their genes are less mutated than those of classical memory cells [7]. In contrast, plasmablasts have the highest Ig mutations of any B cell subset [26]. It is likely that circulating plasmablasts along with bone marrow-resident plasma cells are the major sources of anti-HIV IgG production.

Conclusions

Several very recent advances have permitted the detailed study of the HIV-specific B cell response. The ability to stain and isolate HIV-specific B cells and recover HIV-specific antibodies is opening the door to understanding the B cell antigen receptor repertoire and specificities that underlie broad neutralization of HIV. Insights gained from this work will likely provide critical guidance for the goals and design of B cell based HIV vaccines.

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