B-cell exhaustion in HIV infection: the role of immune activation

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Purpose of review
To discuss a component of the pathogenic mechanisms of HIV infection in the context of phenotypic and functional alterations in B cells that are due to persistent viral replication leading to aberrant immune activation and cellular exhaustion. We explore how B-cell exhaustion arises during persistent viremia and how it compares with T-cell exhaustion and similar B-cell alterations in other diseases.

Recent findings
HIV-associated B-cell exhaustion was first described in 2008, soon after the demonstration of persistent virus-induced T-cell exhaustion, as well as the identification of a subset B cells in tonsil tissues with immunoregulatory features similar to those observed in T-cell exhaustion. Our understanding of B-cell exhaustion has since expanded in two important areas: the role of inhibitory receptors in the unresponsiveness of exhausted B cells and the increasing evidence that similar B cells are found in other diseases that are associated with aberrant immune activation and inflammation.

Summary
The phenomenon of B-cell exhaustion is now well established in HIV infection and other diseases characterized by immune activation. Over the coming years, it will be important to understand how cellular exhaustion affects the capacity of the immune system to respond to persisting primary pathogens, as well as to other microbial antigens, whether encountered as secondary infections or following immunization.

Keywords
exhaustion, HIV, immunopathogenesis, memory B cells, persistent viremia

INTRODUCTION
The immunopathogenesis of HIV infection involves perturbations in both innate and adaptive immunity, including B cells and the antibodies they produce. Hypergammaglobulinemia, first recognized in individuals with AIDS more than three decades ago [1], is a manifestation of the polyclonal activating effects of HIV replication on B cells leading to their terminal differentiation into plasmablasts or plasma cells. Persistent HIV replication is also associated with other B-cell perturbations, including over-representation of memory B-cell subsets that are activated and exhausted, with the latter feature being most prominent during the chronic phase of infection. B-cell exhaustion in HIV infection is very similar to that described for CD4+ and CD8+ T cells in HIV and other persistent viral infections in that they are characterized functionally by a decreased capacity to proliferate in response to de novo stimuli and phenotypically by an increased expression of multiple inhibitory receptors [2,3]. In addition, B-cell exhaustion in HIV infection is manifested by a specific subset of B cells that are present in the peripheral blood, namely tissue-like memory B cells. This name was chosen on the basis of phenotypic and functional similarities between this subset and those described for a unique subset of memory B cells identified in tonsils of healthy individuals [4]. Furthermore, similar subsets of B cells have been described in other infectious and non-infectious diseases in which there is persistent activation and/or inflammation, although not all such cells have been designated as exhausted. This review will focus on recent advances in our understanding of B-cell exhaustion in the setting of HIV infection and other diseases that cause similar perturbations and how these changes in the B-cell compartment affect humoral immunity.
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KEY POINTS

- Persistent HIV replication leads to aberrant immune activation and B-cell exhaustion, mainly among tissue-like memory B cells.
- Increased expression of multiple inhibitory receptors is a prominent feature of B-cell exhaustion; decrease in expression of inhibitory receptors expressed on tissue-like memory B cells by siRNA reverses the unresponsiveness of these cells to stimulation.
- Evidence of phenotypic and functional exhaustion of B cells is observed in many diseases characterized by chronic immune activation.

MEMORY B-CELL ALTERATIONS IN HIV INFECTION

The textbook version of how human naïve B cells respond to T-cell-dependent nonpersisting antigens is through the induction of a germinal center reaction that leads to immunoglobulin class-switching and increased affinity for the antigen by accumulation of somatic mutations in the variable regions of immunoglobulin heavy and light chains. This process culminates in the generation of long-lived resting memory B cells and plasma cells. Circulating resting memory B cells are characterized by the expression of the surface markers CD27 and CD21, as well as reduced expression of markers associated with inductive or effector stages, including HLA-DR, CD38, CD95, and CD80/86. CD27-expressing memory B cells that have not undergone immunoglobulin-class switching are also present in the peripheral blood of healthy individuals at a frequency of ~20% of total B cells [5]; conversely, IgG-expressing circulating memory B cells that do not express CD27 have also been identified, although frequencies are generally below 5% of total B cells [6]. HIV infection is associated with alterations in the ‘healthy’ memory B-cell compartment, in addition to the appearance of additional memory B-cell subsets that are either completely absent or minimally present in the blood of healthy individuals [7]. Resting memory B cells, and in particular those that express IgM [8], are depleted in HIV infection [3,9]. This compartment is especially depleted in individuals who do not initiate antiretroviral therapy in the early phase of infection [7]. Conversely, there is an over-representation of memory B cells that express low levels of the complement receptor CD21, a minor portion of which express CD27 [7]. These CD21hi B cells express high levels of CD20, which is in contrast to CD21hi B cells that express intermediate levels of CD20, without or with CD10 (immature/transitional B cells), or have lost expression of CD20 (plasma cells and plasmablasts). Memory B cells expressing a combination of CD20lo/CD21lo and reduced levels of CD27 were first identified in human tonsils with the added phenotypic property of expressing the immunoregulatory molecule FCRL4 [4]. A few years later, we described a similar population of B cells in the peripheral blood of HIV-viremic individuals [10]. Whereas the tonsil-derived subset of distinct memory B cells was defined by the expression of FCRL4, we chose to define their related counterparts that circulate on the basis of B-cell markers: CD20hi/CD21lo/CD27- and called them tissue-like memory B cells [10]. In contrast to tissue-like memory cells, CD20hi/CD21lo B cells that express CD27 are referred to as activated memory B cells for their increased expression of CD95 and CD86 [10]. Tissue-like memory B cells not only express the highest levels of FCRL4, but also several other inhibitory receptors (discussed below), hence the main reason for not defining them solely by the expression of FCRL4. The expression of multiple inhibitory receptors is a signature feature of exhausted CD8+ T cells in the lymphocytic choriomeningitis virus (LCMV) model [11]. Of note, these T cells also express increased levels of the adhesion marker CD11c and the inflammatory chemokine receptor CXCR3, both also uniquely expressed on tissue-like memory B cells [10].

THE ROLE OF INHIBITORY RECEPTORS IN HIV-ASSOCIATED B-CELL EXHAUSTION

The main phenotypic similarities between exhausted B and T cells include the increased expression of multiple inhibitory receptors, as well as receptors associated with homing to sites of inflammation (CXCR3 and CD11c). Among the inhibitory receptors, there is limited overlap between those associated with B-cell versus T-cell exhaustion [12–15]. This distinction is not unexpected given that even among T cells, the inhibitory receptors expressed on exhausted CD4+ T cells tend to be different from those upregulated on exhausted CD8+ T cells [2]. In addition, the inhibitory receptors expressed on exhausted B cells are not exclusive to the subset associated with exhaustion, namely the tissue-like memory B cells (Table 1). It is the cumulative expression of several inhibitory receptors that distinguishes tissue-like memory B cells from other B-cell subsets. For example, the inhibitory receptors CD22, CD72, and LAIR-1 are expressed at higher levels on naive B cells when compared with ‘normal subsets’ (resting memory or terminally differentiated B cells), yet their expression on tissue-like memory B cells is either
maintained, albeit at lower levels compared with naïve B cells (CD72 and LAIR1) or increased compared with all other subsets (CD22). In contrast, there are certain inhibitory receptors that are distinctly expressed on tissue-like memory B cells, although expression on CD21 \( ^{10} \)/CD27 \(^{11} \) B cells, which we have somewhat arbitrarily defined as activated memory B cells, is also elevated compared with ‘normal subsets’. Inhibitory receptors in this category include CD32b, FCRL4, and Siglec-6, as well as inhibitory receptors normally found on natural killer (NK) cells, namely CD85d, CD85j, and CD85k \[^{10},^{21} \]. Finally, the inhibitory receptor PD-1, which has been associated with T-cell exhaustion in humans and both T-cell and B-cell exhaustion in nonhuman primates \[^{22} \], is equally expressed on all three memory B-cell subsets in humans (Table 1). Others have reported increased PD-1 expression associated with HIV viremia \[^{23} \]; however, whether that reflects merely an increased activation or actual exhaustion remains unknown.

The role of increased expression of inhibitory receptors in HIV-associated B-cell exhaustion was explored \textit{ex vivo} using an siRNA-based approach for reducing the expression of targeted inhibitory receptors \[^{21} \]. Reversal of B-cell exhaustion was assessed following siRNA-mediated reduction of inhibitory receptor expression and included evaluating proliferation in response to various B-cell stimuli, production of various cytokines, and HIV-specific responses, as measured by frequencies of HIV-specific antibody-secreting cells following in-vitro terminal differentiation. Among the inhibitory receptors investigated (listed in Table 1), the downregulation of two, namely FCRL4 and Siglec-6, by siRNA was found to have a particularly strong, although not exclusive, effect on reversing B-cell exhaustion \[^{21} \]. Although it is possible that the effects were strongest because of subtle differences in siRNA-mediated downregulation of expression that were not revealed by transcriptional analysis, it is noteworthy that these two receptors are almost exclusively expressed on tissue-like memory B cells. It should be pointed out that when treatment with siRNA was used to downregulate a variety of inhibitory receptors on a range of B-cell subsets, only the tissue-like memory B cells manifested increased function despite the fact that inhibitory receptors on other B-cell subsets had been downregulated by the siRNA \[^{21} \]. Furthermore, the enhancing effects on B-cell function resulting from siRNA-mediated reduction of expression of inhibitory receptors on tissue-like memory B cells occurred without the addition or presence of ligands for these receptors. Of note, no ligand has been identified with negative signaling effects for either FCRL4 or Siglec-6, yet they remain putative inhibitory receptors. In this regard, it is possible that the inhibitory effects of these receptors do not require triggering by a ligand, and that instead the inhibitory receptors maintain B cells in a state of unresponsiveness through tonic signaling in a ligand-independent process \[^{24} \].

**Table 1.** Inhibitory receptors associated with HIV-associated B-cell exhaustion

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Function</th>
<th>Expression on normal cells</th>
<th>Expression on B-cell subsets in HIV infection</th>
<th>Expression on B cells in other diseases</th>
<th>References (other diseases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAIR-1</td>
<td>Collagen</td>
<td>Negative</td>
<td>Naive B &amp; other immune cells</td>
<td>N &gt; TLM &gt; RM &gt; AM &gt; PB</td>
<td>HCV PBL</td>
<td>[16]</td>
</tr>
<tr>
<td>CD72</td>
<td>CD5</td>
<td>Negative and positive</td>
<td>Naive B cells</td>
<td>N &gt; TLM &gt; AM &gt; RM &gt; PB</td>
<td>HCV PBL; RA/CVID PBL</td>
<td>[16, 17]</td>
</tr>
<tr>
<td>CD22</td>
<td>Sialic acid</td>
<td>Negative</td>
<td>B cells</td>
<td>TLM &gt; N &gt; RM &gt; AM &gt; PB</td>
<td>Malaria &amp; HCV PBL</td>
<td>[16, 18]</td>
</tr>
<tr>
<td>CD32b</td>
<td>IgG</td>
<td>Negative</td>
<td>B cells</td>
<td>TLM &gt; AM &gt; RM &gt; N &gt; PB</td>
<td>RA/CVID PBL</td>
<td>[17]</td>
</tr>
<tr>
<td>FCRL4</td>
<td>IgA</td>
<td>Putative negative</td>
<td>Tonsil B-cell subset</td>
<td>TLM &gt; AM &gt; RM &gt; N = AM</td>
<td>Malaria &amp; HCV PBL; RA SF</td>
<td>[16, 18, 19*, 20*]</td>
</tr>
<tr>
<td>SIGLEC-6</td>
<td>Leptin and</td>
<td>Putative negative</td>
<td>B cells; placental trophoblast</td>
<td>TLM &gt; AM &gt; RM &gt; N &gt; PB</td>
<td>RA/CVID PBL</td>
<td>[17]</td>
</tr>
<tr>
<td>CD85d</td>
<td>HLA-G, other HLA I</td>
<td>Negative</td>
<td>Neutrophils, eosinophils</td>
<td>TLM &gt; AM &gt; RM &gt; N = PB</td>
<td>RA/CVID PBL</td>
<td>[17]</td>
</tr>
<tr>
<td>CD85j</td>
<td>HLA-G, other HLA I</td>
<td>Negative</td>
<td>NK, dendritic and T cells</td>
<td>TLM &gt; AM &gt; RM &gt; N = PB</td>
<td>RA/CVID &amp; Malaria PBL</td>
<td>[17, 18]</td>
</tr>
<tr>
<td>PD-1</td>
<td>PD-L1, PD-L2</td>
<td>Negative</td>
<td>T, B, and NKT cells, monocytes</td>
<td>TLM = RM = AM &gt; N = PB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Review references for normal cellular expression: 12–15. AM, activated memory; CVID, common variable immunodeficiency; N, naïve; PB, plasmablasts; PBL, peripheral blood lymphocytes; RA, rheumatoid arthritis; RM, resting memory; SF, synovial fluid; TLM, tissue-like memory.
Tissue-like memory

CXCR3

Ig

BCR

www.co-hivandaids.com

Y

/C223

1746-630X

475

Differentiate into antibody-secreting cells. Given cells to stimulation and their capacity to terminally affected by factors such as the responsiveness of B orory B cells. In both cases, the analysis is indirect and lation aimed at inducing differentiation of all mem-

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lent. Antigen-specific memory B cells is to detect pathogen. The most common approach for evalu-

receptor (BCR) on a B cell that is specific for a given antigen. In contrast, analysis of B-cell effector function in response to stimulation with the antigen. In contrast, analysis of B-cell exhaustion has been hampered until recently by the inability to directly detect or measure a B-cell receptor (BCR) on a B cell that is specific for a given pathogen. The most common approach for evaluating antigen-specific memory B cells is to detect specific antibodies in the culture supernatant by ELISA or quantify their secreting cells by enzyme-linked immunosorbent spot (ELISPOT) assay following several days of polyclonal stimulation aimed at inducing differentiation of all mem-

ory B cells. In both cases, the analysis is indirect and affected by factors such as the responsiveness of B cells to stimulation and their capacity to terminally differentiate into antibody-secreting cells. Given that exhausted B cells are, by definition, less responsive to stimulation, demonstrating that their antigen specificity has been challenging. Nonetheless, enrichment of HIV-specificity among tissue-like memory B cells of infected individuals was observed by ELISPOT following induction of terminal differentiation with a combination of strong polyclonal B-cell stimuli [10]. Furthermore, as mentioned in the previous section, the HIV-specific response among tissue-like memory B cells was enhanced following siRNA-mediated downregulation of the putative inhibitory receptor FCRL4 [21*]. Collectively, these observations suggest that HIV-specific B-cell exhaustion, manifested primarily by tissue-like memory B cells, occurs in infected individuals, especially during chronic viremia [7].

Several properties of the BCR, including the affinity of its variable regions and the isotype of its heavy chain, determine whether and how B cells respond to stimulation. In a study of HIV-associated B-cell exhaustion, all three immunoglobulin isotypes (IgM, IgG, and IgA) were involved in the HIV-specific responses of tissue-like memory B cells [10], although IgA was a minor constituent of the response. In a followup study, IgA was confirmed as a minor isotype among tissue-like memory B cells and IgG was determined to be more predominant among classical CD27-expressing memory B cells than among tissue-like memory B cells [21*]. Of note, IgM remains the predominant isotype among all memory B-cell subsets, although its proportion is highest among tissue-like memory B cells and lowest among activated memory B cells ([21*]...
and unpublished observations). Given that IgM-expressing memory B-cell subsets of healthy donors have undergone fewer cell divisions in vivo, as measured by kappa-deletion recombination excision circle (KREC) assay, than have their Ig-switched counterparts [25], the differences in immunoglobulin isotype distribution among memory B-cell subsets of HIV-viremic individuals may also explain the lower number of cell divisions reported for this subset [10]. The lower representation of IgG and IgA among tissue-like memory B cells also begs the question of whether the lymphoid tissues, in which these B cells are likely selected, lack the appropriate follicular environment for class-switching. Despite these distinctions, both IgG and IgM-expressing tissue-like memory B cells responded with similar enhancement of proliferation following the down-regulation of inhibitory receptors by siRNA treatment [21*]. In this regard, it was the costimulatory pathways that were differentially modulated following siRNA-mediated FCRL4 downregulation in that enhancement of proliferation was stronger in response to TLR9 than CD40-triggering [21*]. These and other observations are consistent with the possibility that FCRL4 and BCR pathways intersect during tonic signaling, with negative signals involving tyrosine phosphatases SHP-1 and SHP-2 [4,26*], and positive signals involving PI3K [27]. Of note, PI3K is also involved in TLR9-mediated signaling [28]. Interestingly, TLR7-dependent autoimmunity in aged mice is associated with a subset of B cells manifesting many of the same features that have been described for HIV-associated B-cell exhaustion [29*]. The overall effect on B-cell responsiveness is likely dictated at least in part by the balance between the levels of expression of receptors that transduce positive versus negative signals (Fig. 1).

**EXHAUSTED-LIKE B CELLS IN OTHER DISEASE SETTINGS AND TISSUES**

The concept of HIV-associated B-cell exhaustion originated from the convergence of two different observations. The first came from the identification of FCRL4-expressing memory B cells in human tonsil by Cooper and colleagues [4]. The most striking features of FCRL4+ tonsil memory B cells were increased expression of one pan B-cell marker, CD20, contrasted by the reduced expression of another, CD21, as well as variable expression of the classic marker of memory, CD27. This unique combination was something that we had observed among circulating CD21lo B cells of HIV-viremic individuals. We soon realized that the CD27-negative fraction of CD20hi/CD21lo B cells also expressed multiple inhibitory receptors, in addition to CD11c and CXCR3. These three features had also been described for CD8+ T-cell exhaustion in the LCMV model [11], hence the convergence of two observations. Furthermore, both storylines described similar in-vitro outcomes, namely a reduced capacity to proliferate in response to various B-cell stimuli or T-cell stimuli [4,11]. Circulating CD20hi/CD21lo/CD27− B cells of HIV-viremic individuals displayed similar functional impairments [10]. We thus named this subset tissue-like memory B cells, in recognition of similarities with FCRL4+ tonsil memory B cells described by Ehrhardt et al. [4], that have properties of exhaustion, and in recognition of similarities with the LCMV model (and HIV and other infectious diseases, as well as neoplasms) [2].

There are several infectious and noninfectious disease settings that induce chronic or periodic activation of the immune system and lead to the over-representation of B cells with features similar to those described for tissue-like memory B cells in chronic HIV infection. Examples of diseases in which common core features of CD20hi/CD21lo/FCRL4+/CD11c− on circulating B cells have been described include HCV-associated B-cell lymphoproliferative disorder, mixed cryoglobulinemia, and cirrhosis and repeated *Plasmodium falciparum* malaria infection [16,18,19*,30*]. Most of these B cells are also distinguished by the absence of CD27 and presence of several inhibitory receptors listed in Table 1. These core features have also been described for proinflammatory B cells that are present in the synovial fluid of patients with rheumatoid arthritis (RA), although FCRL4 is not expressed on corresponding B cells in the circulation [20*]. Nonetheless, the peripheral blood of RA patients, as well as patients with systemic lupus erythematosus and common variable immunodeficiency, contains an expanded CD20hi/CD21lo B-cell subset that has several other features that are similar to those of tissue-like memory B cells in HIV infection [17,31–33]. Collectively, these observations indicate that B cells with features similar to those of exhausted B cells described in HIV infection have been described in several disease settings that are characterized by chronic immune activation and/or inflammation.

**CONCLUSION**

Chronic immune activation, whether induced by a persisting pathogenic or a noninfectious disease, appears to be a critical component leading to phenotypic and functional exhaustion of B cells.

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Conflicts of interest
The authors declare no conflict of interest.

REFERENCES AND RECOMMENDED READING
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

This study reports HCV-associated B-cell energy, with phenotypic and functional characteristics similar to HIV-associated B-cell exhaustion.
This study reports dichotomous expression of FCRRL4 between tissue and peripheral blood B cells in RA, with similarities to HIV-associated exhausted B cells in both compartments.
This study demonstrates that the function of exhausted B cells of HIV-viremic individuals can be enhanced by siRNA-mediated downregulation of several inhibitory receptors, with strongest effects observed by targeting Siglec-6 and FCRRL4.
This study shows that the expression of FCRRL4 leads to reduced BCR signaling and proposes that the expression of FCRRL4 dampens responses during chronic antigenic stimulation.
This mouse model study reports aged B cells, with phenotypic and functional characteristics similar to HIV-associated B-cell exhaustion.
This study reports malaria-induced B-cell and T-cell exhaustion.