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B cell responses to HIV infection

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Summary

The induction of neutralizing antibodies directed against the human immunodeficiency virus (HIV) has received considerable attention in recent years, in part driven by renewed interest and opportunities for antibody-based strategies for prevention such as passive transfer of antibodies and the development of preventive vaccines, as well as immune-based therapeutic interventions. Advances in the ability to screen, isolate and characterize HIV-specific antibodies have led to the identification of a new generation of potent broadly neutralizing antibodies (bNAbs). The majority of these antibodies have been isolated from B cells of chronically HIV-infected individuals with detectable viremia. In this review, we provide insight into the phenotypic and functional attributes of human B cells, with a focus on HIV-specific memory B cells and plasmablasts/cells that are responsible for sustaining humoral immune responses against HIV. We discuss the abnormalities in B cells that occur in HIV infection both in the peripheral blood and lymphoid tissues, especially in the setting of persisting viremia. Finally, we consider the opportunities and drawbacks of intensively interrogating antibodies isolated from HIV-infected individuals to guide strategies aimed at developing effective antibody-based vaccine and therapeutic interventions for HIV.

Keywords

HIV; B cells; HIV-specific humoral immunity; persisting viremia; pathogenesis

Introduction

Over the past several years, a sharp increase in research efforts has been aimed at identifying potent and broad neutralizing antibodies (bNAbs) to the human immunodeficiency virus (HIV) and providing new opportunities for therapeutic and preventative interventions. This renewed focus has led to extraordinary scientific advances that have been highlighted throughout this series of reviews. The vast majority of these advances have derived from studies of the antibody response to HIV in infected individuals, with the hope that similar and hopefully protective responses could be generated in uninfected individuals following vaccination with an antibody-based immunogen(s). However, as discussed in several reviews of this series, the identification of such an immunogen or series of immunogens is a work in progress that would benefit from a more integrated approach, as previously suggested (1). In

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this regard, relatively little attention has been focused on the cells from which antibodies are produced and cloned, namely B cells, and how this population is altered by HIV infection. A better understanding of B cells and their alterations in HIV infection may provide new insights into approaches towards an HIV vaccine. In this review, we focus on B cells, the changes that occur in HIV infection and how such changes affect the functionality of the two main cell populations responsible for sustaining antibody responses, namely long-lived memory B cells and plasma cells.

Populations of human B cells and their relevance to HIV infection

Overview of human B-cell populations as they develop and encounter antigen

In humans, as in other primates and most mammals, the bone marrow is the major site of B-cell development and the repository of returning plasma cells following terminal differentiation of B cells in the periphery (*Fig.* 1). As part of their developmental process, B cells exit the bone marrow at the immature/transitional stage after having successfully completed the rearrangement of both heavy and light chain immunoglobulin (germline) genes to form a fully functional B-cell receptor (BCR). This process involves several checkpoints, designed to evaluate fitness and to eliminate B cells with self-reactive BCRs (reviewed in (2)). In the periphery, immature/transitional B cells develop into naive B cells following further selection, likely in the spleen, a process that is accompanied by a number of distinct phenotypic changes. In humans, a unique set of surface markers has proven useful for tracking maturing B cells in the periphery (*Fig.* 1), in addition to the lineage defining markers of CD19, CD20, as well as IgM and IgD. Bone marrow B-cell emigrants maintain expression of the pre-B cell surface marker CD10, while expressing low levels of the complement receptor CD21 (3, 4). As immature/transitional B cells transition to naive B cells their expression of CD21 increases while levels of CD10 decrease to background and remain undetectable on mature B cells in the circulation, with the exception of a minor population of “germinal center (GC) founder” B cells that can be distinguished by the co-expression of CD10 and the memory B-cell marker CD27 (5).

Human immature/transitional B cells were first described in the peripheral blood of bone marrow transplant patients as the earliest B-cell emigrants involved in immune reconstitution (6). These cells were then further described in the peripheral blood of patients with systemic lupus erythematosus (SLE) (7), and subsequently described in several other lymphopenic or post-lymphopenic settings (*Fig.* 1), including advancing HIV disease (8), idiopathic $CD4^+$ T lymphocytopenia (8), and following B-cell depletion with reagents such as rituximab (9). A detailed discussion of immature/transitional B cells in HIV disease is beyond the scope of this review. However, in the current context, there is the possibility that HIV-specific B cells can develop directly from immature/transitional B cells independently of T-cell help and with a higher than normal level of poly/autoreactivity (10).

As B cells mature and encounter antigen, there are several different pathways they can take, each with different outcomes in terms of functionality and longevity. As discussed below, response to antigen may or may not be accompanied by surface immunoglobulin (Ig) class switching. Furthermore, in disease settings involving a persisting pathogen and/or persistent immune activation, such as in HIV disease, several alterations occur in the B-cell *Table* 1),

many of which can be difficult to identify in terms of the developmental stage being affected.

Despite such challenges, there are helpful molecular tools available for defining the complex developmental stages of B cells, as long as individual populations can be identified by a distinct set of phenotypic markers. One such tool is based on the fact that as B cells mature in the periphery they undergo cell division, which while not a continual or constant process, can nonetheless be used to assess where a phenotypically distinct population lies along the B-cell developmental/differential pathway (*Fig.* 1). The tool for assessing replication histories of B cells that have exited the bone marrow is called the immunoglobulin kappa light chain (Igκ)-deleting recombination excision circles (KREC) assay, which has been shown to accurately determine the number of cell divisions undergone by a wide range of human B-cell populations in the periphery (35, 36).

Once a naive B cell migrates into peripheral lymphoid tissues and encounters a cognate antigen, its response can be divided into two general phases or outcomes: one that occurs in the absence of T-cell help, either because of the nature of the antigen or the phase of the response, and one that occurs with T-cell help, typically within the microenvironment of the GC. Early events of an immune response that occur prior to the establishment of a GC typically involve B cell-T cell interactions in extrafollicular areas and differentiation into short-lived plasmablasts (37, 38). The formation of a GC occurs when antigen-activated B cells migrate into the area of the follicle that is densely packed with follicular dendritic cells (FDC) to undergo cyclic rounds of proliferation and selection of B cells with increased affinity for the antigen (37). Affinity-matured B cells that exit the GC either do so as memory B cells or as long-lived plasma cells. The factors that determine these outcomes remain unclear, although recent modeling suggests a strong temporal component (39). The delineation of pathways and events involved in the induction of antibody responses against pathogens such as HIV is important because outcomes dictate the quality and longevity of such responses, whether they arise following immunization or natural infection. For example, while a successful vaccine or a self-contained infection in healthy individuals will culminate in the generation of long-lived resting memory B cells and plasma cells that home to the bone marrow, both of which are thought to be essential for sustained humoral immunity (37, 38), these processes are altered in the setting of a persisting pathogens (40). The dysregulation of B cells that occurs in the context of chronic immune activation associated with a persistent viral infection such as HIV has been clearly established (reviewed in (41, 42)); however, what remains far less clear is how these alterations affect the antibody response against the persisting pathogen. A better understanding of these processes especially in lymphoid tissues is essential, both for delineating mechanisms of HIV-induced pathogenesis, and perhaps more importantly, for advancing effective antibody-based HIV vaccine strategies based on emulating the rarely occurring potent and broad antibody responses that have been observed in HIV-infected individuals.

Memory B cells

An adequate, comprehensive and useful definition of what constitutes a memory B cell or a population of such cells is challenging and is perhaps best addressed in the context of the

discussion at hand. At its core, a memory B cell is one that has survived and expanded following encounter with antigen; however, to be useful for future effective re-encounters, it should also have undergone somatic mutations in the variable regions of its heavy and light chain Ig genes that increase the affinity of its BCR for antigen. The tracking of memory B cells by this metric of increased affinity has been difficult until recently (*Fig.* 2), when it has been greatly facilitated by the development of protein probes that can directly detect B cells with a given BCR specificity (reviewed in (43)). As discussed more extensively in the following section, probes used to directly identify antigen-specific B cells are now available for several pathogens, including HIV. Despite these advances, immunophenotying with markers that can readily identify memory B cells in the peripheral blood and tissues and that can be adapted to various disease settings remain the most useful approach of identifying antigen-experienced B cells. In humans, CD27 is considered the conventional marker for memory B cells, which may or may not be accompanied by a class-switched BCR (44). Among non-switched CD27-expressing memory B cells, there can also be those that express IgM without IgD, and vice-versa, although both these sub-populations are rare relative to their IgM^+/D^+ CD27-expressing counterparts (45). In addition, several non-conventional memory B cells have been identified in healthy donors, including a minor population of IgG-switched B cells that do not express CD27 (46). In HIV-infected individuals, there are additional receptors that define non-conventional memory B cells and these can represent a plurality of the circulating memory B-cell compartment, especially in HIV-infected individuals with persistent viremia (42). Several years ago, we adopted two markers, namely CD21 and CD27, to identify three memory B-cell populations that circulate in the peripheral blood of HIV-infected individuals and that have since seen been used to identify similar populations in other disease settings (reviewed in (44, 47, 48)). Conventional memory B cells represent the majority of circulating B-cell subsets in healthy donors and express both CD21 and CD27; they are often referred to as resting memory B cells, consistent with their low levels of expression of activation markers (44). Non-conventional memory B cells, which are not seen in substantial numbers in healthy individuals, include tissue-like memory (TLM) B cells that lack expression of CD27 and express low levels of CD21 and activated memory (AM) B cells that are also $CD21^{lo}$, but do express CD27 (*Fig.* 1), in addition to increased expression of markers of activation and markers associated with apoptosis (42). The term “tissue-like” was chosen to describe $CD21^{lo}/CD27^-$ B cells on the basis of similarities with a unique population of memory B cells identified in tonsillar tissues and defined by the expression of the putative inhibitory receptor FCRL4 (49). TLM B cells in HIV-viremic individuals also express FCRL4 and bear other similarities with tonsillar counterparts, including increased expression of CD20 and decreased response to B-cell stimuli (14). In HIV infection, as in other settings of chronic viremia with other pathogens or immune activation related to a variety of conditions (reviewed in (44, 47, 48)), several other inhibitory receptors are over-expressed on TLM or similar B cells, as well as the inflammatory chemokine receptor CXCR3 and adhesion molecule CD11c that have also been associated with LCMV-induced T-cell exhaustion (50). In this regard, HIV-associated B-cell exhaustion among TLM B cells has been described on the basis of cumulative properties and similarities with T-cell exhaustion (14). It is noteworthy that senescent B cells in the absence of any specific disease process, both in mice and humans, also possess several features that are similar to those described for TLM B cells (51).

Ig isotypes are increasingly included in the delineation of various memory B-cell populations associated with HIV, driven in part by recent advances in probe-based flow cytometric techniques to identify HIV-specific B cells (*Fig.* 2), especially those of the IgG class (20, 52). This increasingly popular approach of identifying antigen-specific B cells will be discussed in more detail in the following section. As a consequence of including Ig isotypes, we now refer to $IgG^+/CD21^{hi}/CD27^-$ B cells as intermediate memory (IM) B cells in order to distinguish them from IgG^+ TLM ($CD21^{lo}/CD27^-$) B cells (20). The rational for the IM B-cell terminology is to reflect their intermediate level of somatic hypermutation (SHM) when compared to other IgG-expressing B cells described in healthy donors (36) and more recently, in HIV-infected individuals (53). Of note, CD21 is rarely included in the characterization of different B-cell populations in healthy donors because there are very few $CD21^{lo}$ B cells in the peripheral blood of these individuals (24).

Finally, while the nomenclature described here for memory B cells is not used universally in the description of all diseases, there are many infectious and non-infectious diseases that lead to the over-expression of non-conventional B cells, especially those with features similar to those described for $CD21^{lo}/CD27^-$ TLM B cells (47, 51). The most common denominator of these diseases is chronic immune activation or inflammation.

Plasmablasts and plasma cells

The pathways that culminate in terminal differentiation of B cells are influenced by environment, source of antigen, and somewhat elusive factors that determine outcome and longevity (38). B cells that differentiate into antibody-secreting cells (ASC) while still proliferating are generally referred to as plasmablasts. Whether generated in lymphoid tissues during the early phase of responses to antigen, or released into the peripheral blood following immunization or infection, plasmablasts can be readily identified by their high levels of expression of CD38 and CD27 (*Fig.* 1), as well as by the expression of the cell-cycling marker Ki-67 (54). Within 5–7 days of exposure to a self-resolving pathogen or an immunogen, a transient surge of plasmablasts, most of which are typically antigen-specific, can be detected readily in the peripheral blood (55). This short window of opportunity is often used to isolate and characterize antibodies produced by these fleeting plasmablasts (reviewed in (43)), especially given their ease of identification from other B cells. For example, analyses of antibodies reconstituted from plasmablasts of individuals infected or vaccinated with the 2009 pandemic H1N1 influenza strain revealed that memory B cells established in past exposures were the likely source of rapid response to the newly emergent strain (56). Furthermore, studies in mice have demonstrated that plasmablasts are the precursors of terminally differentiated plasma cells that migrate to the bone marrow for long-term survival (57), although other tissue niches for plasma cells exist (38). A direct link between plasmablasts and long-lived plasma cells has been more difficult to confirm in humans because of the logistic difficulty in obtaining relevant tissue at defined time points.

The dynamics of B cell responses in persisting viral infections such as HIV are very different from those in the self-resolving settings described above. In early HIV infection, the percentage of plasmablasts in the peripheral blood of viremic individuals can be very high, accounting for upwards of 50% of all circulating B cells (58). However, only a fraction

of these plasmablasts are thought to be HIV-specific and it is unclear whether the HIV-specific antibodies secreted by these cells help restrict HIV replication or show evidence of being influenced by ongoing viral replication. In this regard, the reconstitution of HIV-specific monoclonal antibodies (mAbs) from circulating plasmablasts of early HIV-infected individuals revealed that the majority of these mAbs were directed against the gp41 portion of the HIV envelope (Env) and demonstrated limited HIV-neutralizing activity (59). We will have a more detailed discussion on HIV-specific cells in the third section.

During the chronic phase of HIV viremia, frequencies of circulating plasmablasts diminish relative to the early phase; however, they remain elevated compared to healthy donors, and the isotype becomes predominantly IgG. This is in contrast to IgA plasmablasts that predominate in early HIV infection as well as in healthy donors (58). Of note, IgG is the predominant isotype of plasmablasts following parenteral immunization of healthy individuals (60), and likely reflecting the involvement of local draining lymph nodes. A similar tissue source of circulating plasmablasts is suspected in chronic HIV viremia, given the presence of increased IgG^+ plasmablasts/cells in lymph nodes (61). However, mucosal tissues may also be a source of IgG^+ plasmablasts given the increased frequency of this isotype in HIV infection (62, 63).

As has been demonstrated in animal studies (57, 64), and suggested in humans (38), a fraction of plasmablasts induced in lymphoid tissues will survive and migrate to the bone marrow and, during the process, terminally differentiate into long-lived sessile ($Ki\text{-}67^-$) plasma cells that can secrete antibodies over the lifetime of an individual. Other lymphoid tissues such as spleen and gut-associated lymphoid tissues are also repositories for plasma cells, although the bone marrow is thought to be the most prominent and more permanent site of residence (65). A large majority of these tissue ASCs express the transmembrane heparin sulfate proteoglycan syndecan 1 or CD138 (38, 66). Of note, whereas peripheral blood plasmablasts rarely express CD138, plasmablasts in tissues can express both CD138 and Ki-67, especially in inflammatory disease settings (67). Within the human bone marrow there is also recent evidence of plasma cell heterogeneity, with those expressing CD138 in the absence of CD19 being associated with longevity (68). The factors that dictate plasma cell outcomes largely remain unknown in humans. However, despite this paucity of knowledge, such factors should be viewed as potentially highly important to the field of vaccine research as they may be key to the induction of a long-lasting antibody response.

It is also unclear how a persistent infection such as HIV alters the dynamics of plasma-cell development, migration, longevity, and distribution among various lymphoid tissues. Our previous studies have demonstrated that despite the numerous alterations in B-cell dynamics and differentiation that occur in HIV infection, especially in the setting of chronic HIV viremia (*Fig.* 1), there remains a strong correlation between frequencies of HIV-specific plasma cells in the bone marrow and frequencies of HIV-specific antibodies in the peripheral blood (69). These observations suggest that despite HIV-induced aberrant immune activation and dysregulation of B cells, including extensive secondary lymphoid tissue hyperplasia (70, 71), the dynamics of plasma-cell homing to the bone marrow and release of antibody into circulation appear to remain intact. However, the quality of HIV-specific antibodies secreted by plasma cells that reside in the bone marrow of HIV-infected individuals has yet to be

thoroughly assessed and should be the focus of future endeavors. Such knowledge will be important in improving our understanding of and potentially our ability to enhance responses against HIV, as well as against other pathogens, such as influenza, pneumococcus, and hepatitis B virus that are relevant to the HIV community, especially given the increasing age of individuals living with HIV who are facing reduced responses due to immune senescence (72).

Evaluating antigen-specific B-cell responses

Humoral immunity refers to the component of an immune response that is mediated by circulating antibodies. While several other reviews in this series are focusing on the antibody response per se, we consider their cellular source and precursors, namely the plasmablasts/cells that are actively secreting antibodies, and their memory B-cell counterparts that are poised to rapidly differentiate and secrete antibody upon re-encounter with the "memorized" antigen. In this section, we focus on defining, identifying and evaluating HIV-specific responses made by B cells and plasmablasts/cells, and discuss responses against other pathogens in the context of how they compare to those directed against HIV and the extent to which they reflect the general immune competency of HIV-infected individuals.

Approaches for identifying HIV-specific B cells

Both plasma cells and plasmablasts can be directly evaluated in terms of the measurable frequencies of such cells that secrete antibody, hence the terminology of ASC. In contrast to plasmablasts/cells, memory B cells do not actively secrete antibodies and until recently, their specificity could only be assessed following *ex vivo* expansion and differentiation into ASCs. In addition, the tools available for evaluating B-cell responses against a pathogen were limited to assays that measured the quantity, specificity and isotype of secreted antibodies (ELISA) or of ASCs (enzyme-linked immunospot (ELISPOT)). The most common approach taken for measuring antigen-specific memory B-cell responses following *ex vivo* differentiation was to stimulate B cells polyclonally and assess specificity during the ELISA/ELISPOT procedure (73). The reasons why stimulation of B cells with specific antigen is not widely applied vary and depend on the goal of the assay: bulk stimulation is most suitable when evaluating multiple specificities and when viability may be difficult to maintain, for example when culturing B cells or a derived subset alone. In contrast, stimulation with a specific antigen has been reported when PBMCs are used as source of B cells (17). The ELISA/ELISPOT approach also remains the primary assay used to evaluate antigen-specific plasmablasts/cells because the more direct assays now available to evaluate antigen-specific B cells require the BCR to be expressed on the cell surface, a condition that is diminished or lost with terminal differentiation (74).

Since 2009, major advances in protein structure and imaging technologies have provided the B-cell field with tools to visualize BCR specificities similar to those developed over 20 years ago for TCR specificities based on peptide-MHC tetramers (75). It is notable that advances in BCR-directed technologies involving flow cytometry and fluorescent protein-based probes have been instrumental to the recent success of identifying potent and broadly HIV-neutralizing antibodies (*Fig.* 2), especially those directed against the CD4 binding site of the

HIV Env (76–78). A variety of different strategies have been used to design HIV Env protein probes for directly visualizing HIV-specific B cells. The most successful probes in terms of providing high selectivity rely on a multimeric approach, usually with a streptavidin-based tetramer. For the HIV Env, multimerization strategies include tetramer of trimers, for example those based on gp140 (52, 79). While the details regarding the structural elements and immunogenicity of these probes are beyond the scope of this review, what is important here is to discuss the adequacy of probes in terms of the likelihood that the binding of such probes to a given B cell reflects actual binding to the BCR and not to another entity expressed on the surface of the B cell. This consideration is especially important for evaluating HIV-specific B-cell responses per se (left box in *Fig.* 2), as opposed to using probes as tools or "baits" to capture B cells with desired BCRs for the purpose of reconstituting of mAbs bearing its specificity (as described more extensively in review xx of this series). This is because the specificity of the latter but not former approach can be verified with the reconstituted mAb.

Two general approaches illustrated in *Fig.* 2 can help maximize the likelihood that the BCR of a B cell being assessed by flow cytometry with a fluorescently labeled probe is indeed of the intended specificity. One approach we and others have found to be helpful is to include an Ig marker in the staining strategy, typically IgG as it is the isotype most likely to have a high intensity interaction with a probe. The resulting staining pattern will consist of a diagonal, representing the roughly 1:1 ratio between the IgG of the BCR and its cognate antigen (20). A second approach is to use a combination of probes that achieves one of the following outcomes: 1) two similar equimolar probes that bind to the BCR with predictable patterns such as a diagonal in the double-positive quadrant of a flow plot where there is dual binding (20, 80, 81); or, 2) one specific and one irrelevant probe, the latter serving to establish the background binding threshold (82); or 3) two identical probes where only one is conjugated to a fluorochrome and the other is used to compete out the fluorescence from the first probe in a titration curve; or 4) inclusion of B-cell samples from individuals who have never been exposed to the antigen being assessed, for example an HIV Env probe tested against B cells of HIV-negative individuals (20). In addition, we have used an approach of combining probes with different specificities on separate fluorochromes to both verify individual probe specificity and to gain information on the immunologic profiles of our patient population. Given that one BCR cannot be specific for more than one antigen (except for poly/auto-reactive specificities discussed below), a combination of probes, each with a distinct specificity, is expected to yield flow cytometric plots that demonstrate a mutually exclusive binding pattern for each probe, as illustrated in *Fig.* 2. Over the past several years, probes of high specificity and sensitivity have been designed to detect B-cell responses against tetanus (82), influenza (83), malaria (84), dengue (85), rotavirus (86), and the human papilloma virus (87). In addition to providing inference of specificity, the inter-pathogen multi-specificity approach has been informative for immunopathogenesis by allowing us to observe that in our cohort of HIV-infected individuals, memory B-cell responses against HIV are both quantitatively and qualitatively different than those directed against non-HIV pathogens (tetanus and influenza) to which the subjects have been vaccinated (20). Finally, the combination of probes with intra-pathogen specificities can yield useful information regarding immunologic mechanisms associated with response to infection or vaccination

(20, 81). With regard to HIV infection, we interrogated B-cell specificities in a cohort of HIV-infected individuals using the wild-type HIV Env gp140 probe derived from strain YU2 and variants that carried mutations that were critical for binding to CD4 (CD4bs, D368R) or the HIV co-receptor (CoR, I420R) (88). When all three probes were combined in one stain, each with a different fluorochrome, we were able to clearly delineate B cells with specificities for epitopes within the CD4bs or CoR or neither (20). This intra-pathogen multi-specificity approach also allowed us to demonstrate that gp140-specific B cells arise early in HIV infection, first enriched in CoR specificities whereas those against the CD4bs are delayed (20). Our findings of the early antibody response being predominantly directed against epitopes that are not generally neutralizing (CoR) while those more associated with bNAbs (CD4bs) arise later are consistent with previous findings on serum of HIV-infected individuals collected over time (89), and may help explain the late appearance of bNAbs in natural infection.

Evaluating the frequency and quality of HIV-specific B cells

The most appropriate approach to evaluate the frequency and quality of B-cell responses against HIV will depend on a number of considerations (*Fig.* 2), including but not limited to whether the underlying question relates to specific or overall immune competency; whether the focus is on the development of memory or the production of antibody; and, whether the primary interest is to identify B cells of particular specificities that have relevance for therapies or vaccine development (right box of *Fig.* 2). We will address each individually in the following paragraphs but refer the reader to other reviews in this series for a more comprehensive analysis of the last scenario.

As mentioned above, approaches for evaluating HIV-specific plasmablasts/cells are limited to indirect methods that can evaluate Igs being actively secreted by the cells of interest. Given that plasmablasts/cells have reduced levels of or absent Ig on their surface and that intracellular staining with probes, especially the bulky tetramer-based probes, is inefficient, direct probe binding methods are of limited use for these cells unless additional steps are included to restrict and retain the antibody-secretion process. As B cells differentiate into plasmablasts/cells the expression of their surface Ig diminishes as the associated antibody secretion process takes over. For IgG, cell surface expression completely disappears during the evolution to an ASC (74). There are methods of capturing and retaining the IgG being secreted by plasmablasts/cells that can be adapted from approaches used to determine cytokine secretion (90); however, these approaches have yet to be applied to probe-based analyses of antigen-specific plasmablasts/cells.

The approaches available for evaluating HIV-specific memory B cells have dramatically changed over the past six years as the quality of probes for directly visualizing antigen-specific B cells has improved. With a continuously expanding number of markers that can be visualized simultaneously by flow cytometry, probe based analyses now allow for interrogation of multiple B-cell subsets and a variety of different antigen specificities (52, 79, 82–87). Despite the obvious advantages of direct analysis and the increasing number of probes that are available for flow cytometric-based analyses, this approach remains highly susceptible to false positives, especially as a stand-alone method of evaluating the antigen

specificity of a BCR. As such, each probe should be thoroughly evaluated for specificity prior to being used widely for measuring a corresponding antigen-specific B-cell response. This can be accomplished by using a probe to sort single B cells, reconstitute each mAbs with specificity corresponding to the BCR of the sorted cells and determining the frequency of mAbs that bind to the probed used to sort.

In addition to the caveats just mentioned, not all specificities are amenable to direct probe-based analyses, either because probes have yet to be designed for the response of interest or because certain specificities, especially those that recognize quaternary epitopes, have been challenging to design into probes. Until recently, relatively few probes have been designed to cover the different sites of vulnerability (ability to bind bNAbs) that have been identified within the HIV Env (91), with the exception of the CD4bs (*Fig.* 2). The difficulties in expanding coverage within the HIV Env have been attributed to probe designs that do not adequately mimic the native trimers, a challenge that was recently overcome with the design of recombinant Env gp140 trimers such as SOSIP that better retain native conformations (92). With regard to the CD4bs, a variety of different strategies have been used to identify CD4bs-specific B cells, most of which rely on a combination of probes (93, 94). However, while these probes have shown great success in identifying and reconstituting potent bNAbs that recognize unique and highly conserved sites within the CD4bs, relatively few of these probes have been evaluated for selectivity (52, 93, 95). In this regard, the study by Sundling and colleagues gave us the confidence needed to use the same probes in a stand-alone approach to report on frequencies of HIV-specific responses among various memory B-cell subsets in our large cohort of HIV-infected individuals (20, 95). The same strategy led us to more recently report that almost 90% of probe-sorted B cells were specific for the CD4bs, even in the case of TLM B cells that express reduced levels of IgG on their cell surface (53).

As mentioned above, the assessment of HIV-specific memory B cells by flow cyometry has been limited to certain epitopes within the HIV Env. More traditional approaches of *in vitro* expansion and differentiation that were designed to measure frequencies of HIV-specific memory B cells by ELISA or ELISPOT (96–98), have been adapted to identify and isolate the new generation of bNAbs (99–104). The adaptations, illustrated in *Fig.* 2, include a limiting dilution approach that can identify clonal B cells of a given specificity in a high-throughput system (105), and/or EBV transformation (106). However, it should be noted that these approaches, in addition to being labor-intensive and of long duration, also select for those B cells that can best survive and proliferate under the culture conditions used. Such approaches tend to favor the expansion of naive and resting memory B cells, but not more activated or exhausted B-cell subsets that tend to be over-represented in the peripheral blood of HIV-infected viremic individuals (42). An initial selection process for those B cells expressing IgG can help exclude naive B cells; however, it also tends to restrict expansion of other isotypes, including IgM, an under-appreciated source of HIV-responding cells that others have shown to be an important constituent of adaptive immunity (107).

B-cell responses to HIV in infected individuals

Abnormalities in the B lineage of HIV-infected individuals were first described over 30 years ago, almost at the same time as the virus was identified (11). Since then, numerous B-

cell abnormalities have been described at all stages of HIV disease (*Fig.* 2), with two consistently reported observations being hypergammaglobulinemia and defects in memory B cells (*Table* 1). The former defect is most likely a reflection of systemic immune-activating effects of HIV on B-cell differentiation (11, 58), while the latter is likely to also reflect defects in $CD4^+$ T cell help. The scope of memory B-cell defects is quite broad, including changes in the frequencies and functionalities of various subsets of memory B cells that circulate in the blood and populate lymphoid tissues. However, while the impact of changes in the B lineage of HIV-infected individuals on their capacity to respond to various non-HIV pathogens has been widely reported (reviewed in (108)), relatively few studies have addressed the interface between systemic B-cell alterations and their ability to respond to HIV per se. We will consider these issues in the last two sections, including whether there is evidence for antibody-mediated control of HIV disease progression.

Development and regulation of the HIV-specific B-cell response in infected individuals

In individuals diagnosed during primary HIV infection, tracking seroconversion remains an important process of the clinical evaluation (109). The cellular source of these early antibodies has not received much attention, perhaps because these antibodies are known to be non-neutralizing (110), as well as the fact that patients with acute HIV infection are difficult to access for study since most do not come to medical attention until the acute phase has passed. However, given knowledge of other infectious diseases, short-lived plasmablasts elicited prior to GC formation and T-cell help are the most likely source of this initial antibody response (55). This would be consistent with detection of HIV-specific IgM within the first few weeks of infection (110). In addition, early responses to HIV include enrichment of IgG3 antibodies (111), which are also thought to derive from GC-independent class switching of B cells that have not undergone affinity maturation (46, 112, 113). These early responses may also reflect inadequate T-cell help due to rapid destruction of the lymphoid tissue environment where these cognate B-T-cell interactions occur (15). Of note, these early HIV-specific IgG3 antibodies also decline rapidly in early HIV-infected individuals (111), and following vaccination in the RV144 trial (discussed in detail in review xx of this series and (91)), a feature that may more likely reflect a short half-life *in vivo* than factors attributable to HIV itself (114). Nonetheless IgG3 remains an interesting IgG isotype that has relevance to HIV, in part because of its strong association with protection in the RV144 trial and its Fc-mediated effector function (115, 116).

One of the earliest targets of the antibody response following HIV infection is gp41 (110), and again, while the cellular origin of gp41-speicific antibodies that are detected in the serum has not been formally determined, circulating plasmablasts have been identified as a source (59). Frequencies of plasmablasts in the peripheral blood are highest in early HIV infection, although a large fraction of these are not specific for HIV (62). Nonetheless, HIV-specific antibodies cloned from these cells were shown to recognize gp41 and be highly mutated (59). Given that these antibodies were also non-neutralizing, it was speculated that their precursors could be memory B cells that pre-dated HIV infection and may be directed against cross-reactive antigens of bacterial origin, likely from the microbiome of the gut (59). This scenario was recently confirmed in that mutated mAbs derived from B cells in the terminal ileum of early HIV-uninfected individuals were found to cross-react with both HIV

gp41 and antigens of the microbiota (117). Most recently, antigens of the intestinal microbiota were found to cross-react with gp41-specific non-neutralizing antibodies that were elicited as the predominant response in recipients of a failed HIV vaccine trial, suggesting that such a pre-existing pool of cross-reactive B cells may divert the immune response away from one that could be protective (118). However, while the HIV-neutralizing capacity of early infection or vaccine induced gp41-specific antibodies with cross-reactivity to the intestinal microbiota may not be useful for protection, epitopes within the membrane proximal region (MPER) of gp41 have been shown to elicit bNAbs (103, 119, 120). Despite the protective potential of antibodies directed against the MPER, this domain is highly prone to the inactivating effects of immune tolerance designed to prevent autoreactivity that can arise due to mimicry with host proteins (29). There is concern that while self-reactive antibodies can be elicited in the setting of HIV disease where mechanisms of immune tolerance may be compromised, such antibodies may be very difficult to induce following vaccination in normal, non-immunocompromised individuals. Nonetheless, at least one study has shown that potent gp41 MPER-specific neutralizing antibodies can arise in HIV-infected individuals in the absence of autoreactivity (103), thus sustaining the interest in the MPER as a conserved site for bNAb targeting.

Autoreactivity, which is tightly regulated during normal B-cell development (121), is somewhat similar and often confounded with polyreactivity, which relates to promiscuous binding properties of an antibody that typically involve both self and non-self antigens. While the factors that regulate the polyreactivity of B cells in responses to pathogens are poorly understood, there are indications that such features may be beneficial to the host (21, 29, 122). One beneficial mechanism associated with polyreactivity involves enhanced affinity resulting from antibody-mediated heteroligation, as has been shown to occur with certain HIV-specific antibodies that cross-react with host proteins incorporated in lipid membranes of virions (123). Given that HIV Env spikes are sparsely distributed over the surface of virions, the presence of such host proteins in proximity provides bivalent antibodies (shown for IgG) with increased targets to anchor both arms and as such, increase their apparent affinity. A recent survey of over 9000 human proteins found that HIV bNAbs were significantly more poly- and autoreactive than non-neutralizing HIV-specific antibodies leading to the conclusion that such properties represent a selection bias for neutralization and not a general property associated with HIV infection (124). In this regard and discussed in greater detail below, we recently demonstrated evidence of bias in the setting of chronic HIV viremia that involved polyreactivity, VH usage, and memory B-cell subset of origin, and suggested that while certain properties may be beneficial to the host, others may be associated with HIV-induced dysregulation of the immune system (53).

In the majority of HIV-infected individuals who do not initiate antiretroviral therapy (ART) during the early phase of infection, chronic viremia ensues with variable outcomes on time to disease progression and effects on HIV-specific B cell responses. Whereas early infection is dominated by non-neutralizing responses, followed by responses restricted to the autologous virus, the chronic phase has been associated with a broadening of the B-cell response, with a minor percentage of individuals developing a response that is both broad and potent (125). The forces that drive B cells of these infected individuals, often referred to as elite neutralizers, to develop such responses against HIV remain ill-defined, yet may

represent a pathway to the holy grail of immunogen design for an effective vaccine. The screening of sera or plasma from hundreds of HIV-infected individuals has demonstrated that elite neutralizers are not particularly elite in their HIV disease status (126), and few virologic and clinical factors have been conclusively associated with bNAb development. A few studies have associated increased neutralization breadth with high viral load in early infection (127, 128), or later in infection (126), whereas declining or low $CD4^+$ T cells have not, except in one of the studies (128). More recently, longitudinal analyses found associations between neutralization breadth and high viral load, low $CD4^+$ T-cell counts, as well as HIV subtype and host genotype (129). In a somewhat different approach, HIV antibody breadth analyses performed on a cohort of untreated HIV-infected individuals defined as controllers (viral load from undetectable to 2,000 HIV RNA copies/ml for over one year) who were divided into neutralizers and non-neutralizers, the quality of $CD4^+$ T cell responses against HIV was superior in the neutralizer group (130). As will be discussed more extensively in the last section, these different studies may be rather difficult to compare; however, the latter suggests that a strong cellular response may be beneficial for humoral immunity in HIV-infected individuals.

Properties of B cells producing HIV bNAbs

There are a few caveats to the studies that have analyzed serologic activity from large cohorts of infected individuals to evaluate bNAbs potential: 1) the definition of neutralizing breadth and potency vary as do the tools used to evaluate these activities; and 2) relatively few studies have incorporated an assessment of the virologic/immunologic status with both serologic screening and the extent to which the identified bNAbs reconstituted the serologic activity. As such, the neutralization being measured may represent a combination of numerous specificities, as has been demonstrated from analysis of plasma (129, 131), antibodies reconstituted from memory B cells of HIV controllers and slow progressors (132), as well as other HIV-infected individuals (133). In reference to the two latter studies (conducted by the same group), both of these studies performed serologic analyses and mAbs reconstitution from Env probe-sorted memory B cells, with the later study indicating that findings were similar for both controllers and noncontrollers. However, it should be noted that potent HIV bNAbs that were later isolated all belonged to the group of controllers and slow progressors (93). For the majority of potent HIV bNAbs that have been isolated from memory B cells of infected individuals, the most common clinical qualifier is that these were slow progressors (94, 103, 104, 132). As an example, donor 45, from whom VRC01 and several similar antibodies have been isolated, was a slow progressor who maintained a viral load in the range 10,000 HIV RNA copies/ml and a $CD4^+$ T-cell count above 500 cells/μl for over 15 years (134). Careful delineation of the development and evolution of potent HIV bNAbs in donor 45 and others with similar long evolutionary pathways (135, 136), clearly indicate that these specificities took years to develop in individuals whose HIV disease status was stable. However, recent findings from the CAPRISA early infection cohort suggest that rare potent HIV bNAbs can mature in individuals rapidly while disease progression is occurring (102, 128, 137). In the CAPRISA studies, most of the bNAbs that were identified targeted the V1/V2 loops of HIV Env (example CAP256 in *Fig.* 2). Thus, the nature of the target on the viral envelope is likely to be one of many factors that influence the antibody maturation process in HIV-infected

individuals. Another is viral diversification (135). It is striking that in at least two individuals in whom bNAb development has been extensively investigated, diversification by superinfection was likely a major driving force in the induction of the bNAbs (102, 136).

Collectively, the studies on bNAb development have been striking and informative, yet they have been performed on a limited number of individuals and with limited clinical analyses. The field would greatly benefit from more longitudinal analyses of both virus and host from whom bNAbs are isolated, with more emphasis on clinical and immunologic parameters. Several questions come to mind. First, is the development of potent HIV bNAb in the setting of HIV infection more likely to occur in the setting of a stable and competent immune system, as suggested from donor 45? Second, does understanding the components required for a strong antibody response against HIV in infected individuals help inform antibody-based therapeutic interventions and HIV vaccine development? Recent studies would suggest yes on both counts: 1) infusion of antibodies against the CD4bs has been shown to accelerate the broadening of the HIV-neutralizing antibody response in viremic infected individuals by a mechanism that remains unknown (138); and 2), a new systems serology approach for delineating correlates of humoral immunity suggests that the proposed analytical tools used to interrogate responses in natural infection be applied to guide vaccine design (116).

One immunologic consideration regarding the identification and isolation of potent HIV bNAbs from elite neutralizers that has received little attention is the nature of the memory B cells from which the antibodies were cloned. As explained in the section “Evaluating antigen-specific B-cell responses”, when bNAbs are cloned by limiting dilution from B cells that are cultured for several days to weeks, the source population is difficult to identify without compromising yield or survival. In these approaches of *in vitro* expansion with clonal dilution, IgG is typically the only marker used to enrich B cells going into culture (105, 106). However, when bNAbs are generated by means of multi-parameter flow cytometry and single-cell cloning, there is opportunity to perform extensive B-cell profiling without compromising the outcome. As described in the first section, Ig isotype is just one of several markers that can be used to identify memory B cells that typically circulate in the peripheral blood of HIV-infected individuals. In chronically HIV-infected viremic individuals, AM and TLM B cells comprise the majority of circulating B cells, in contrast with the more stable and long-lasting RM B cells that predominate in healthy HIV-uninfected individuals and HIV-infected individuals whose viremia is controlled naturally or by ART (24). Over the past several years, we have used the methods described in the section “Evaluating antigen-specific B-cell responses” to demonstrate that the HIV-specific response is enriched within the AM and TLM B-cell populations of HIV-viremic individuals (14, 20). The latter population predominates as the source of HIV-specific responses when cells are cultured whereas the former predominates when specificity is identified by flow cytometry. These apparent differences in predominance likely reflect the high level of apoptosis associated with AM B cells that reduce their survival and capacity to differentiate into antibody-secreting cells *in vitro* (14, 26), and the paucity of IgG and low intensity of the BCR on TLM B cells that has an impact on probe-based detection by flow cytometry (20, 53). It should be noted that our studies have not focused on individuals with broad and potent antibody responses against HIV and as mentioned above, there are few studies that

have combined screening for potent HIV bNAb with comprehensive immunologic and clinical profiling, including the characterization of B cells from which bNAbs were isolated.

Also as mentioned above, donor 45 was the source of several potent CD4bs-specific bNAbs (88, 93, 139). Several of these and other CD4 supersite bNAbs (VRC13, VRC15, VRC26, and CH103) were isolated from memory B cells that expressed CD27 (53, 139, 140). In these studies, either CD27 was included in the gating strategy or source B cells were found to express CD27 in post-sorting analyses. The observation that CD27 was expressed on the B cells from which these potent bNAbs were derived indicate that TLM B cells were not the source of the bNAbs as these B cells do not express CD27 (14). A paucity of bNAbs originating from TLM B cells would be consistent with our recent findings of low IgG SHM in TLM as opposed to RM B cells, both at the population level and for CD4bs-specific mAbs derived from corresponding B cells (53). Again, the caveat here is that we did not focus on individuals with potent bNAb profiles and few of the CD4bs-specific mAbs that we isolated carried features of bNAbs that target the CD4 supersite (140). Nonetheless, our findings provide insights that may be of interest beyond our primary focus of HIV immunopathogenesis. We found plasticity between memory B cell populations, as evidenced by the presence of shared clonal families between RM and TLM B cells, although this cross-talk was mainly restricted to non VH4 families (53). Furthermore, VH4 usage was enriched among mAbs with high polyreactivity that were derived from TLM B cells and the TLM-derived mAbs with high polyreactivity possessed the weakest maturational profiles and HIV neutralization capacities of all CD4bs-specific mAbs analyzed (53). While these observations are limited in scope and need to be extended to more individuals as well as to more targets on the virus, they nonetheless suggest that, as has been shown for CD4 supersite bNAbs (140), B-cell responses are not simply stochastic. The forces that influence B cells along a restricted or constrained development path, whether driven by the pathogen, host, or disease status, remain largely unknown, yet are likely to heavily impact outcomes in the setting of natural infection and following vaccination and therapeutic intervention.

Correlates of a strong B-cell response against HIV

Currently, there is relatively little evidence that HIV-specific B-cell and antibody responses restrict viral replication and/or prevent disease progression in infected individuals, especially given numerous reports that development of bNAbs is associated with high HIV viremia and low or decreasing $CD4^+$ T cell counts (141). However, there are reports that either dispute these observations or suggest a more complex picture. For example even in the studies reporting that bNAbs are associated with high HIV viremia, there are suggestions that reduced immunologic function may explain why a majority of individuals do not develop bNAbs (129, 142). We explore these ideas in this last section in hope of providing a view of potential correlates of a strong B-cell and antibody response, as reported in certain studies investigating HIV-infected individuals and as should be sought in an effective antibody-based vaccine. We focus on B-cell and T-cell responses in the peripheral blood and lymphoid tissues, but refer to reviews xx in this series for insight into the role of Fc-mediated effector functions of antibodies in restricting or protecting against HIV infection.

Evidence of superior antibody and B-cell responses associated with healthy B-cell profiles in HIV-infected individuals

Over the past decade, we and others have described numerous B-cell abnormalities associated with HIV infection, especially in the setting of ongoing viral replication (reviewed in (41, 42, 143)). Normalization of B-cell populations and functionalities has been shown to occur when HIV viremia is suppressed by therapy (63, 98, 144–151), especially when treatment is initiated early during the course of infection (24, 152). Resting memory B cells, the cells responsible for long-lasting memory in healthy individuals (44), are particularly vulnerable to the deleterious effects of persistent HIV replication and immune activation, with many reports demonstrating only partial restoration following reduction of viremia by ART (24, 144, 149–151). A few studies have reported that a “normal” distribution of the circulating B-cell populations in certain HIV-infected individuals, similar to that observed in healthy uninfected individuals, is associated with broad HIV-specific memory B-cell responses or bNAbs (153, 154). Similar findings of lack of B-cell dysfunction have been reported in the non-pathogenic infection of natural hosts of the simian immunodeficiency virus (SIV), where anti-SIV antibodies in the breast milk of these animals was suggested to contribute to low postnatal transmission of SIV in this setting (155). Furthermore, sustained or evolving HIV bNAbs have also been reported in individuals receiving effective ART (153, 156), with indications or suggestions that low levels of viremia and partial normalization of the B-cell compartment may be necessary for the development of bNAbs. Somewhat related to these findings, we have shown that HIV-specific B-cell responses are enriched among resting memory B cells in individuals whose viremia is suppressed by ART, although the overall frequencies of HIV-specific B cells does diminish with ART (20). We have also demonstrated that in individuals not receiving ART, enrichment of HIV-specific responses among RM B cells is correlated with lower levels of both viremia and immune activation, with the opposite being observed in individuals whose HIV-specific responses were enriched in AM and TLM B cells (20). In more recent findings, we have also demonstrated that HIV-specific mAbs derived from resting memory B cells are superior in maturational and functional properties when compared to those derived from TLM B cells (53). Collectively, these studies suggest that the maintenance of an intact resting memory B-cell compartment may be associated with a superior humoral response to HIV.

Lessons from elite controllers and long-term nonprogressors

The rare HIV-infected individuals who control their viremia for prolonged periods at either undetectable (elite controllers) or low (long-term nonprogressors) levels in the absence of ART have been intensely studied for clues that may lead to immune-based therapies and vaccines. Most of the collective evidence suggests that natural control of HIV infection and disease progression is associated with cellular immunity and that antibodies are likely to play a limited role (reviewed in (157, 158)). While studies that have screened large and diverse cohorts of HIV-infected individuals for HIV-neutralizing activities in sera have shown that few elite controllers are elite neutralizers, it should be noted that one of the most potent CD4bs bNAbs, 3BNC117, was isolated from B cells of an elite controller (93). This study also remains one of very few that investigated and demonstrated related clones of 3BNC117 among circulating memory B cells, plasma cells in the bone marrow and serum-

derived antibodies. The bone marrow is the most likely source of serum antibodies and we have demonstrated that even in the setting of immune activating effects of chronic HIV viremia that lead to increased plasmacytosis in tissues and blood (11, 15, 58, 61, 63, 96, 147, 150, 159), there remains a strong correlation between frequencies of HIV-specific antibodies in the serum and HIV-specific plasma cells in the bone marrow (69). It is noteworthy that we did not find a correlation between serum levels of HIV-specific antibodies and HIV-specific memory B cells in the peripheral blood. While these analyses were not performed on elite controllers, a study that investigated such a cohort also found a discordance between CD4bs-specific memory B cells in the peripheral blood and antibodies in the serum of the same specificity, with the latter being much lower than the former (160). These observations highlight the need to focus on serologic and cellular sources of HIV-specific B-cell and antibody responses. It is also important to note that the bone marrow should be more thoroughly investigated as a source of long-lived plasma cells involved in the sustained antibody response to HIV, especially given recent indications that there are diverse populations of plasma cells in the bone marrow, among which those that express low levels of CD19 have been associated with cellular longevity (67).

With regard to the populations of memory B cells that circulate in the peripheral blood of elite controllers, we recently demonstrated that they more closely resemble those of healthy donors than those of HIV-infected individuals whose viremia is suppressed by ART (161). Furthermore, we demonstrated that while both cohorts of HIV-infected individuals had an HIV-specific response that was enriched within their resting memory B cells, frequencies were significantly higher in the elite controllers, despite a lower cellular HIV burden than in the ART group (161). These findings confirm those of a previous study (162), and extend observations that an intact humoral immune capacity may be responsible for a superior HIV-specific B-cell response.

Role of $CD4^+$ T cells in B-cell responses to HIV

In the context of responses to vaccination or a self-contained infection, affinity maturation of T-cell-dependent B cells is critically dependent on interactions between antigen-specific B cells and T follicular helper (Tfh) cells that take place in GC reactions of secondary lymphoid tissues (163). In this setting, B cells with the highest affinity for the antigen are selected through processes that have not been completely elucidated, but that involve B cells cycling between the GC light zone where they receive positive signals from Tfh cells based on BCR affinity for antigen and proliferation and hypermutation in the dark zone, possibly directed by the strength of the signal received in the light zone (37). How this complex process of selecting for B cells with the highest affinity for cognate antigen evolves in the context of a persistent pathogen such as HIV is far from clear. On the one hand, hyperplasia of lymph nodes typically observed in chronic HIV and SIV viremia is associated with elevated numbers of both Tfh and GC B cells (*Fig.* 1) (61, 164–166), two elements necessary for affinity maturation of an antibody response (37, 163). On the other hand, there are numerous indications that quantity does not equate with quality of response (*Table* 1). These include defects in Tfh cells that are associated with direct and indirect effects of HIV replication (167). Among the indirect effects is increased expression of the ligand PD-L1 on GC B cells that can bind PD-1, an inhibitory receptor expressed at inherently high levels on

Tfh cells, and as such, these interactions can potentially restrict the function of Tfh cells (18). Of note, the increased expression of inhibitory receptors such as PD-1 and many others on both B cells and T cells has been associated with HIV-associated exhaustion of both cellular lineages (reviewed in (168, 169)). However, it remains unclear how alterations in secondary lymphoid tissues associated with chronic HIV viremia affect B-cell responses against the virus given that few studies have addressed this issue directly. While one study has shown that increased frequencies of Tfh and GC B cells in chronic SIV infection were associated with increased serum titers and avidities of SIV-specific antibodies (164), the functionality of these antibodies was not addressed and may simply reflect hypergammaglobulinemia, which was also widely reported in these studies (61, 164, 165).

In recent years, cellular populations akin to tissue Tfh cells have been described within the peripheral $CD4^+$ T-cell compartment, although this population appears to be rather heterogeneous, thus complicating the consensus on how to define and study them (170). Nonetheless, efforts to characterize blood-derived Tfh cells based on phenotypic resemblance to GC-derived counterparts and capacity to provide help to B cells has led to the observation that the development of bNAbs in a large cohort of HIV-infected individuals is associated with increased frequencies of circulating Tfh cells (16). There are also indications that early preservation of circulating Tfh cells is associated with the capacity to develop bNAbs during the chronic phase of infection (171). Loss of Tfh cells and B-cell helper function in chronic HIV viremia has also been described, although in this study there was no correlation between frequencies of Tfh cells and HIV-neutralizing activity (172). These somewhat contradictory findings suggest that more studies are needed to better define and characterize circulating Tfh cells. Finally, the specificity of peripheral Tfh cells has been lacking in these studies. A recent study has addressed this issue in a cohort of untreated HIV-infected controllers (detectable viral load below 2000 RNA copies/ml for over one year), divided into two groups based on HIV neutralization profiles (130). The findings revealed that the group with Env-specific bNAbs also had enhanced HIV Gag- and gp41- but not gp120-specific $CD4^+$ T-cell responses and suggested that intermolecular help between Gag-specific T cells and Env-specific B cells may be occurring (130). While these observations must be tested in larger and more diverse cohorts, they raise the tantalizing notion, with support from the literature (173), that Gag-based immunogens may enhance Env-specific antibody responses. Of note, previous studies on HIV-specific Tfh cells in lymph nodes of chronically HIV-viremic individuals demonstrated a stronger Tfh response to Gag than to Env, although correlation with the antibody response was not addressed (61). Collectively, these findings suggest a need to address HIV-specific responses in both lymphoid tissues and the peripheral blood as they relate to the development of antibody response.

Concluding remarks or Perspectives

Extraordinary progress in HIV antibody research has been made within a relatively short period of time, leading to increased hope and dedicated efforts towards developing an effective antibody-based vaccine. The vast majority of these advances have derived from the investigation of antibody responses in HIV-infected individuals using tools that have been designed to screen large numbers of individuals and to identify rare antigen-specific B cells from which bNAbs can be generated. While the data are striking and informative, leading to

new strategies for both vaccine development and therapeutic interventions in HIV-infected individuals, much remains to be learned regarding the complex interactions between host and virus and how the disease status of the host affects such responses. Immunologically, these issues need to be addressed by investigating all facets of adaptive immunity, as well as innate immunity. It is and will remain challenging to investigate such responses in lymphoid tissues, including evaluating the longevity and specificity of plasma cells in the bone marrow, as wells as the process of antibody affinity maturation in GCs of lymph nodes. Both these processes are poorly understood in the context of persistent viral infections such as HIV. However, such information is likely to be critical to the understanding of how an HIV-specific immune response is generated, matured and sustained.

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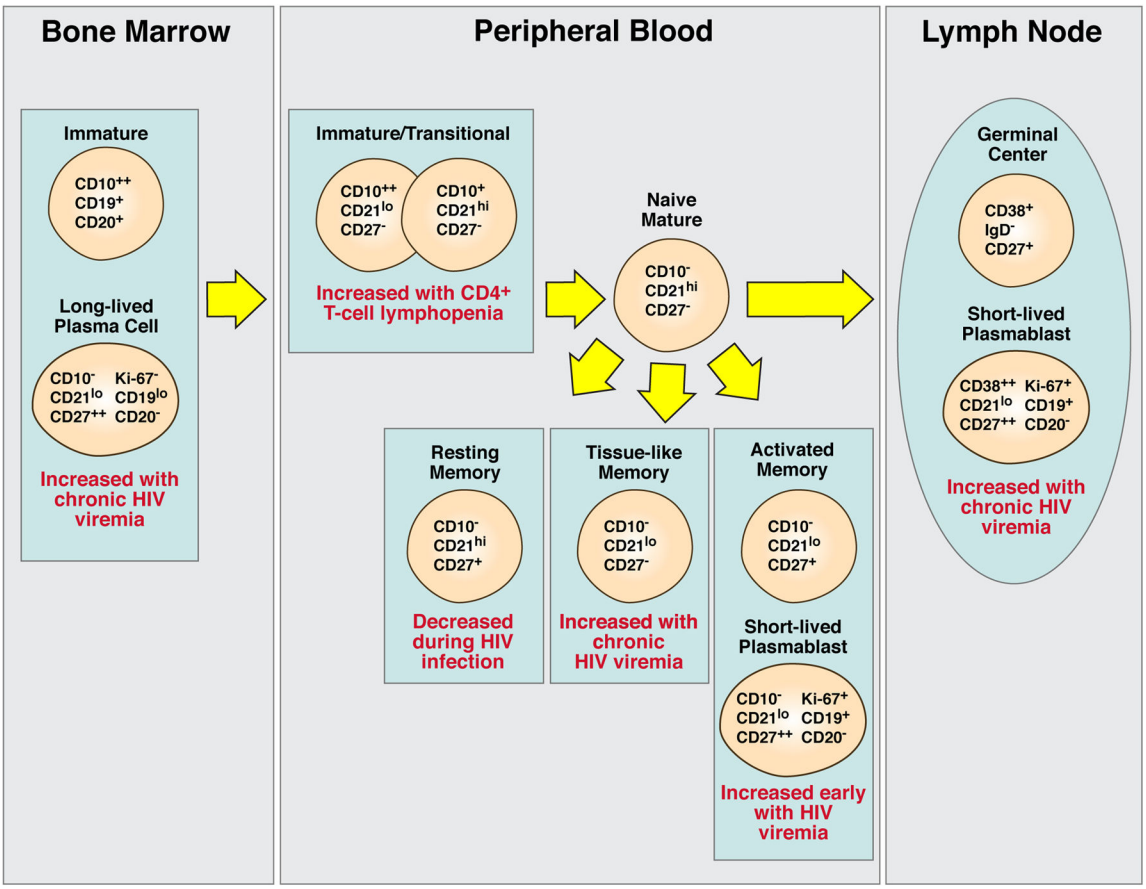

Fig. 1. Changes in B-Cell development and differentiation associated with HIV infection

Different B-cell populations are shown with their defining and/or useful immunophenotypic markers as they begin development in the bone marrow, continue to develop and differentiate in the periphery (peripheral blood and lymph node illustrated), and return to the bone marrow as terminally differentiated plasma cells. Alterations that occur in the various B-cell compartments of HIV-infected individuals are indicated in red text.

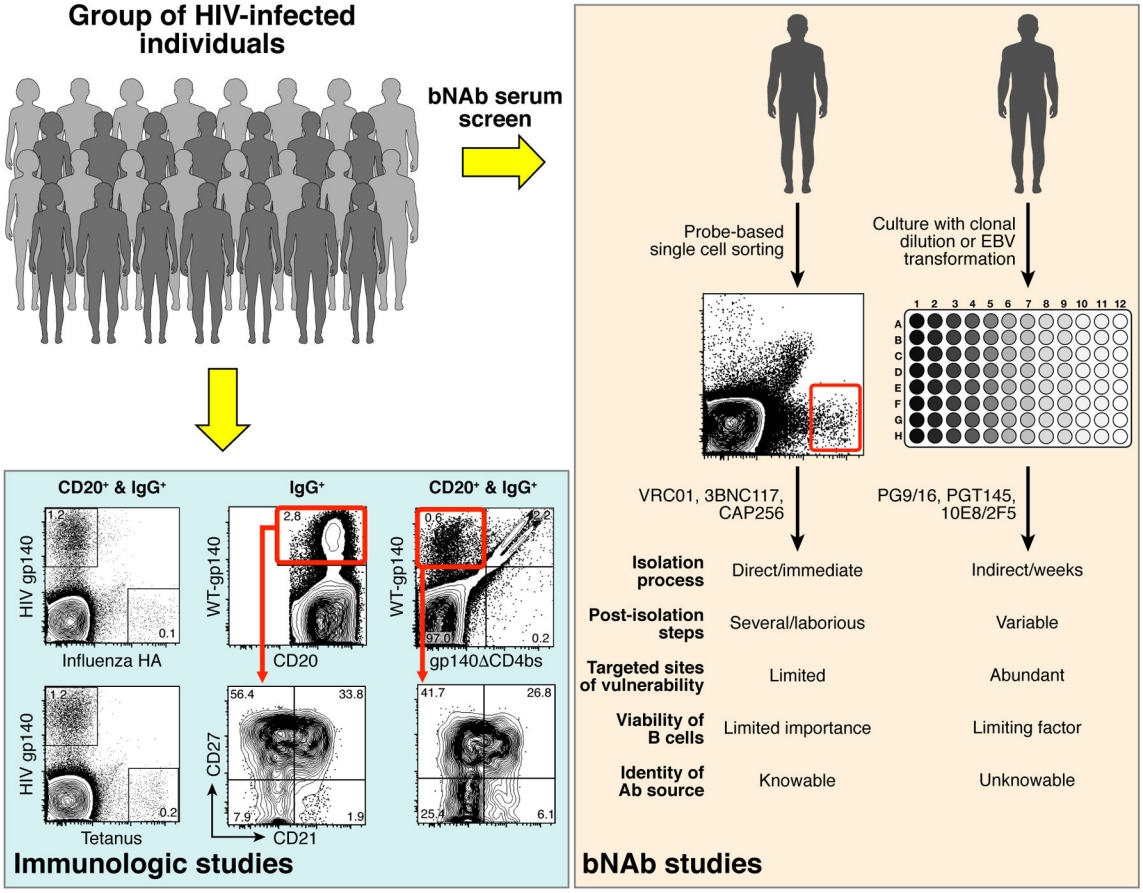

Fig. 2. New strategies for investigating HIV-specific B cells and harnessing their therapeutic potential

Analyses of B cells and sera from large groups of HIV-infected individuals are performed to address immunopathogenic (left box) or therapeutic/vaccine (right box) questions. Left box on immunologic studies: use of probe-based flow cytometry can be used to investigate memory B-cell responses to HIV antigens (WT-gp140), sub-specificities within HIV antigens (upper left quadrant of WT-gp140 by gp140ΔCD4bs gives frequency of B cells specific for gp140-CD4bs), as well as non HIV antigens (influenza and tetanus). Responses are typically reported by gating on cells that express the pan B-cell marker CD20 and the Ig isotype IgG and can be delineated by subset based on various B-cell markers such as CD21 and CD27. Right box on HIV bNAb studies: typically, a few individuals are identified as good candidates by screening serum from a large group of individuals for bNAb activity. Cloning of corresponding bNAbs from candidate IgG^+ B cells proceeds with either probes-base single-cell sorting or a culture method. Examples of bNAbs isolated by each method are given and referenced in the main text, and advantages and disadvantages of each method are listed.

Table 1

Abnormalities in HIV infection impacting B-cell function

Observation	B cells involved	Other factors	Expected/observed outcome	Selected readings
Systemic/indirect effects				
•Increased immune activation	Plasmablast/cell (⬆) Activated memory (⬆) Tissue-like memory (⬆)	Inflammatory cytokines Type I interferons, Foxo3a Persistent HIV viremia	Hyper-Ig - polyclonal > HIV-specific Increased apoptosis Increased exhaustion	(11) (12, 13) (14)
•Lymphoid tissue hyperplasia	GC B cells (⬆) PD-L1$^+$ B cells (⬆)	Inflammatory cytokines	Early loss of morphologic integrity & delayed Ab response Impaired T follicular helper function Increased B-cell neoplasms	(15, 16) (17, 18) (19)
•Altered memory B-cell compartment	T-dependent B cells (⬇) IgM$^+$ memory B cells (⬇)	Loss of T-cell help	Decreased quality of response to HIV Decreased response to immunization	(20, 21) (22–25)
•Disrupted homeostasis/regulation	Immature/transitional B cells (⬆) All B cells	CD4$^+$ T-cell lymphopenia Increased IL-7	Decreased pool of naïve B cells Increased intrinsic apoptosis Autoreactivity	(26, 27) (28, 29)
Direct effects				
•Bound HIV virions	FCR$^+$ & CD21$^+$ B Cells α4β7$^+$ B cells	Follicular dendritic cells TGF-β	Increased extracellular viral reservoir Interfere with B-cell responses	(30–32)
•Nef	Naïve and other B cells	Macrophages, soluble factors	Increased polyclonal activation Decreased virus-specific responses	(33, 34)

Arrows indicate increased or decreased levels of the B cells involved.