Mechanisms of allergic diseases

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Pathogenic mechanisms of B-lymphocyte dysfunction in HIV disease

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Activity Objectives

1. To understand the effect of HIV infection on B lymphocytes in the setting of $CD4^+$ T-cell lymphopenia.
2. To understand the effect of antiretroviral therapy (ART) on B-cell dysfunction in HIV disease.
3. To understand the effect of early versus late ART in HIV infection.
4. To understand the role and mechanisms of B-lymphocyte apoptosis in HIV disease.

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HIV disease is associated with abnormalities in all major lymphocyte populations, including B cells. Aberrancies in the B-cell compartment can be divided into 3 broad categories: changes that arise as a result of HIV-induced immune activation, changes that arise as a result of HIV-induced lymphopenia, and changes that arise independently of these 2 parameters. We review recent developments in all 3 categories of abnormalities and highlight how observations made in the early years of the HIV epidemic are better understood today in large part because of the advent of effective antiretroviral therapy. Insight into the mechanisms of B-cell dysfunction in HIV disease has also been achieved as a result of increased knowledge of the B-cell subpopulations as they exist in healthy individuals, compared with their abnormalities in HIV-infected individuals. A better understanding of the pathogenic mechanisms of B-cell abnormalities in HIV disease can potentially lead to new strategies for improving antibody responses against opportunistic pathogens that afflict HIV-infected individuals and against HIV itself, in the context of both HIV infection and an antibody-based HIV vaccine. (J Allergy Clin Immunol 2008;122:12-9.)

Key words: *HIV, B cells, immunopathogenesis, immune activation, lymphopenia, apoptosis*

HIV infection leads to persistent viral replication, immune activation, loss of $CD4^+$ T cells, and disease progression in a majority of infected individuals who do not receive antiretroviral therapy (ART). Although $CD4^+$ T cells represent the primary target for HIV in terms of both direct and indirect effects of viral replication, varying degrees of perturbations related to HIV infection are observed in virtually all lymphocyte populations.[1-4] Apart from the obvious $CD4^+$ T lymphocytopenia, B cells were among the first dysfunctional lymphocyte populations to be described in patients with AIDS.[5] These initial observations revealed that patients with AIDS exhibited hypergammaglobulinemia, and their B cells showed evidence of polyclonal B-cell hyperactivity *in vivo*, yet poor responsiveness to neoantigens *in vivo* and B-cell stimuli *in vitro*.[5] These observations were seminal in establishing the concept that HIV disease leads to defects in B-cell function despite intense polyclonal B-cell activation.

Before the era of effective ART, insight into mechanisms of B-cell pathogenesis in HIV-infected individuals proved to be difficult because of the paucity of adequate controls of aviremic

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Terms in boldface and italics are defined in the glossary on page 13.

Abbreviations used
ART: Antiretroviral therapy
V_H3: Variable heavy-chain family member 3

HIV-infected individuals; in addition, the process could not easily be modeled *in vitro*. The advent of effective ART in the mid 1990s not only provided a means of slowing and reversing disease progression but also provided new opportunities for investigating mechanisms of HIV pathogenesis longitudinally in the same patient during viremia and after therapy-induced suppression of virus replication. In this regard, the majority of advances regarding mechanisms of B-cell pathogenesis in HIV disease have been based on longitudinal as well as cross-sectional studies that compare the effects of ART on B cells. This review focuses on these studies by addressing the direct and indirect effects of HIV on B cells, the phenotypic and functional alterations of B cells associated with ongoing viral replication and $CD4^+$ T-cell lymphopenia, and changes in B cells that appear to be independent of ongoing viral replication. The delineation of mechanisms of B-cell pathogenesis associated with various stages of HIV disease may help address some of the clinical consequences of B-cell dysfunction, including the increased incidence of B-cell neoplasms, increased autoimmune manifestations, and decreased humoral responses to specific antigens.

B-CELL HYPERACTIVATION IN HIV DISEASE

Many features of B-cell dysregulation in HIV disease suggest a prominent role for aberrant immune activation. These features include elevated serum levels of immunoglobulins and autoantibodies,[6] extensive expansion in B-cell areas of lymphoid tissue,[7,8] and an increased expression of activation, proliferation, and terminal differentiation markers on circulating B cells.[5,9-11] As illustrated in Fig 1 and in data not shown, terminal differentiation of B cells is associated with a loss in the expression of ***CD20*** and ***CD21***, an increase in the size of B cells with prominent plasmacytoid features, and an increase in the expression of CD38 and ***CD27***. In addition, HIV-induced immune activation of B cells is thought to be a contributing factor to the increased frequency of B-cell malignancies observed in HIV-infected individuals, which were especially observed before the widespread use of effective ART.[12] Evidence for B-cell hyperactivity as a direct consequence of ongoing HIV replication came from studies demonstrating that

GLOSSARY

B-CELL TERMINAL DIFFERENTIATION: B-cell development can be simplified as (1) *proB*, H-chain rearrangement begins; (2) *preB*, H-chain rearrangement complete, pre–B-cell receptor expressed; (3) *immature B*, H and L–chain rearrangement complete, surface IgM expressed; (4) *mature B*, surface IgM and IgD expressed; (5) *activated B cells*, antigen responsive; (6) *plasma cells*, no longer express surface immunoglobulin but secrete large quantities of antibody. Memory B cells are generated in germinal centers, are $CD20^+CD27^+$, and can rapidly differentiate into plasma cells.

CD10: CD10 is also known as the common acute lymphoblastic leukemia antigen (CALLA) and is expressed on early lymphoid precursors, as well as some epithelial cells, and neutrophils. CD10 can be a marker associated with cancers of the skin, pancreas, and kidney.

CD19: An immunoglobulin superfamily member, CD19 is present on B-cell precursors and mature B cells but decreased on plasma cells. CD9 can be considered a marker of B-cell maturation.

CD20: CD20 is a calcium channel protein present on B cells but not plasma cells that is involved in B-cell activation and proliferation. Rituximab targets CD20 and is used to treat non-Hodgkin lymphoma, rheumatoid arthritis, and other autoimmune disorders such as autoimmune cytopenias and SLE.

CD21: Also known as complement receptor 2, CD21 is present on mature B cells and binds complement fragment C3d as well as EBV. CD21 is involved in B-cell proliferation and activation and IgE production on interaction with CD23 (the low-affinity IgE receptor).

CD27: CD27 is a TNF-α receptor family member present on activated and memory B cells and plasma cells that mediates costimulatory signals. HIV-infected individuals can have specific deficiency of $CD27^+$ memory B cells, especially IgM memory B cells. This finding could correlate with poor polysaccharide response seen in HIV-positive patients.

CD80/86:CD28, CD40:CD40 ligand: Interactions between T cells and B cells or antigen-presenting cells that are required for T-cell activation can occur through CD80/86:CD28 or CD40:CD40L. T cells that do not get a second (or costimulatory) signal become anergic. CD40 is expressed on mature B cells, and CD40 signaling results in increased CD80/86 expression with subsequent interaction with CD28 and signaling through the phosphatidylinositol (PI) 3 kinase–Ras–mitogen-activated protein kinase pathway. CD40 triggering of B cells is essential for their ability to class-switch and make high-affinity antibodies. Blockade of the CD80/86 with abatacept or belatacept is used in treating autoimmune diseases such as rheumatoid arthritis and transplant rejection.

CD95, CD95 ligand: CD95 and CD95L are also known as Fas and Fas ligand, respectively. CD95 is expressed mainly on activated T cells, B cells, and eosinophils and is a member of the TNF receptor superfamily. The ability to induce apoptosis lies in the death domain. Binding of Fas-associated death domain (FADD) protein recruits caspase 8 and caspase 10, which are proapoptotic signaling molecules to the death-induced signaling complex (DISC). Mutations in Fas, Fas ligand, caspase 10, and caspase 8 are associated with immune deficiency associated with autoimmunity syndromes, autoimmune lymphoproliferative syndrome 1a, 1b, 2a, and 2b.

DNA MICROARRAY: Also known as *gene chips*, DNA microarrays look simultaneously at changes in expression in a large number of genes. DNA oligonucleotides are fixed on a solid matrix. mRNA is reverse-transcribed to cDNA and incubated with the array.

gp120: Initial binding of HIV to target cells occurs via gp120 and CD4. Binding results in a conformational change in the viral envelope, exposing binding sites for other coreceptors involved in viral entry. Essential chemokine coreceptor CCR5 or CXCR4 interacts with HIV, which allows the envelope protein gp41 to complete viral-host cell membrane fusion.

IL-7: A proliferation signal for T and B cells, IL-7 signals through the IL-7 receptor, which is composed of the IL-7 receptor α and the common gamma chain (γc). The γc is also used by IL-2, IL-4, IL-9, IL-15, IL-21, and IL-46, and mutations in γc as well as in the IL-7 receptor lead to severe combined immunodeficiency. IL-7 promotes the survival of lymphocytes by regulating the Bcl-2 family of proapoptotic and antiapoptotic factors and may be therapeutically useful in conditions associated with lymphopenia.

SUPERANTIGEN: B-cell superantigens interact with immunoglobulin outside of the antigen binding site and are capable of activating large numbers of B cells, resulting in a polyclonal response. Superantigens for B cells include *Staphylococcus aureus* protein A and HIV gp120. Superantigens for T cells bind to the Vβ region of the T-cell receptor and cross-link it to MHC independent of specific antigen recognition.

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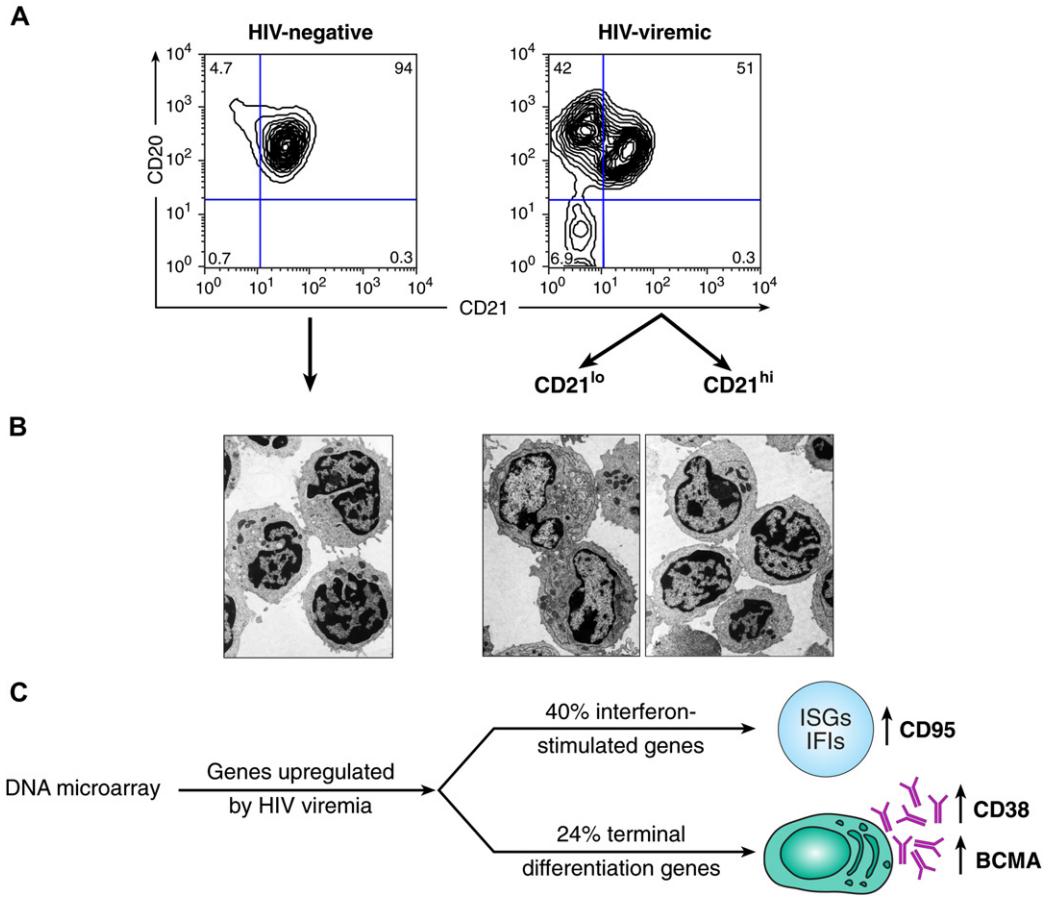

FIG 1. Phenotypic and genotypic aberrancies associated with HIV viremia. **A,** Phenotypic profile of peripheral blood–derived B cells isolated from representative HIV-negative and HIV-viremic individuals illustrating decreased CD21 expression on B cells of the HIV-viremic individual. **B,** Electron micrograph illustrating presence of cells with plasmacytoid features in the $CD21^{lo}$ B-cell fraction of a representative HIV-viremic individual. Micrograph originally appeared in Moir S, Malaspina A, Ogwaro KM, Donoghue ET, Hallahan CW, Ehler LA, et al. HIV-1 induces phenotypic and functional perturbations of B cells in chronically infected individuals. Proc Natl Acad Sci U S A 2001;98:10362-7.[9] **C,** Main findings from DNA microarray analyses performed on blood-derived B cells isolated from HIV-viremic individuals and compared with B cells isolated from HIV-aviremic and HIV-negative individuals.[18] *BCMA,* B-cell maturation protein; *IFI,* IFN-induced family of genes; *ISG,* IFN-stimulated gene.

many of the features associated with B-cell hyperactivity become attenuated after the reduction of HIV plasma viremia by ART.[10,13-16] One of the first studies to illustrate this point clearly used both longitudinal and cross-sectional approaches to demonstrate that ART decreased both hypergammaglobulinemia and the frequency of B cells in the blood that spontaneously secreted immunoglobulins.[17] An increased frequency of B cells undergoing terminal differentiation, as evidenced by phenotypic, functional, and morphologic changes consistent with the differentiation of B cells into plasma cells (Fig 1), has also been linked to HIV viremia.[9] In addition, ***DNA microarray*** analyses performed on B cells isolated from HIV-viremic, HIV-aviremic, and HIV-negative individuals revealed that 24% of the genes found to be upregulated in HIV-viremic individuals but not the other 2 groups were associated with ***B-cell terminal differentiation*** (Fig 1).[18] In this study, the potentially confounding effects of $CD4^+$ lymphopenia in HIV-viremic individuals were controlled for by recruiting HIV-viremic and HIV-aviremic individuals with similar $CD4^+$ T-cell counts. These findings thus underscore the direct role of HIV viremia in B-cell terminal differentiation. Of note, and as discussed in more detail below (see "Changes in B-cell subpopulations in HIV disease"), advanced HIV disease and profound $CD4^+$ T-cell lymphopenia is associated with a waning of HIV-induced immune activation[19] and a shift toward overexpression of immature/transitional B cells.[20]

DIRECT INTERACTIONS BETWEEN HIV AND B CELLS

Although there is little evidence that HIV productively infects B cells *in vivo*, we have shown that B cells isolated from the blood and lymph nodes of HIV-infected individuals carry replication-competent virus on their surface.[21] The interaction is mediated primarily through the binding of complement-opsonized HIV virions to CD21 expressed on the surface of B cells. These findings are consistent with other *in vivo* and *in vitro* studies demonstrating a prominent role for CD21 in the trapping of HIV virions coated with antibody and complement,[22] the form of virus that is likely to predominate *in vivo*. The potential consequences of the direct binding of HIV to B cells include enhanced infectivity

TABLE I. Alterations in B-cell subpopulations associated with HIV infection

Subpopulation	Phenotype	Properties	In HIV disease	Reversed by ART	References
Immature/ transitional	$CD10^{+}/27^{-}$	• High susceptibility to intrinsic apoptosis • Low proliferative response	Expansion associated with lymphopenia and increased IL-7	Yes	20, 43, 48
Activated/mature	$CD21^{lo}/10^{-}$	• High susceptibility to extrinsic apoptosis • Plasmacytoid features • Spontaneous secretion of immunoglobulins • Increased expression of Ki-67	Expansion associated with immune activation	Yes	9, 15-18, 48
Resting/memory	$CD21^{hi}/27^{+}$	• Long-lived • Induced response to antigen	Contraction	No	30, 31, 35-37, 63-65

through virologic cross-talk between virion-bound B cells and target $CD4^{+}$ T cells,[23] and potential effects on B-cell responses stemming from the triggering of CD21 by bound virions.[24,25] However, given the relatively low frequency of B cells carrying HIV in infected individuals contrasted with the high frequency of B-cell dysfunction, it is likely that B-cell dysfunction is predominantly driven by indirect effects of HIV on B cells. It is noteworthy that similar arguments have been made with regard to direct and indirect effects of HIV on $CD4^{+}$ T cells.[3]

HIV has also been shown to bind to B cells through ***superantigen*** interactions between the viral envelope ***gp120*** and the immunoglobulin variable heavy-chain family member 3 (V_H3).[26] Some investigators have shown depletion of V_H3-expressing B cells in HIV-infected individuals,[27] whereas others have either not confirmed these findings or found defects in the V_H3 repertoire that appear unrelated to interactions with gp120.[28,29] Furthermore, few studies were performed in the era of effective ART, and thus, evidence of changes in V_H3-expressing B cells relative to ongoing viral replication and disease progression is lacking.

CHANGES IN B-CELL SUBPOPULATIONS IN HIV DISEASE

Many of the B-cell aberrations that have been reported in HIV disease are likely to reflect alterations in the frequencies of the various subpopulations of B cells that are present in the human body, or at least that are detectable in the peripheral blood (Table I). Given that the vast majority of these studies have been performed on B cells isolated from the peripheral blood, we restrict our comments to alterations in this compartment. Naive B cells constitute the largest B-cell subpopulation in the blood, followed by memory B cells, the frequency of which varies considerably among healthy individuals, yet appears to be surprisingly constant over time for a given healthy individual. Several studies have shown that the frequency of memory B cells is decreased in HIV-infected individuals.[30,31] However, several confounding factors should be considered, in addition to the high variability among healthy donors that inherently makes it more difficult to compare groups of HIV-infected and HIV-negative individuals. Human memory B cells are most commonly defined by the expression of the CD27 cell-surface marker. However, CD27 is also a marker of B-cell activation and terminal differentiation,[32] 2 features that are overrepresented in HIV disease and not generally considered to represent true memory,[33] especially given the short lifespan of most activated and differentiated lymphocyte populations circulating in the blood. Accordingly, additional markers should be included in studies on HIV-infected individuals to distinguish between resting memory B cells and other activated/differentiated subpopulations of B cells that also express CD27. One such marker is CD21, which can be used to distinguish between activated/differentiated ($CD21^{lo}$) and resting ($CD21^{hi}$) B cells (Fig 1). B cells undergoing terminal differentiation also lose expression of CD20 and express reduced levels of ***CD19***, 2 additional features that need to be considered when assessing the totality of B cells expressing CD27, especially in HIV-viremic individuals. In these individuals, it is common to find similar levels of CD27 expression on both $CD21^{lo}$ and $CD21^{hi}$ B cells,[18] and a total CD27 percentage that is not significantly different than the average 35% to 40% of $CD27^{+}$ B cells in HIV-negative individuals.[34] The significant differences arise once HIV-viremic individuals initiate ART. The CD27 component on activated B cells decreases substantially as these cells disappear because aberrant immune activation diminishes with therapy, whereas the CD27 component on resting B cells increases slightly.[35] However, the percentage and number of CD27-expressing resting B cells (classic resting memory B cells) remain low in ART-treated individuals.[30,36,37] Of note, there are also indications that early treatment of ART may prevent loss of memory B cells.[38]

Several B-cell subpopulations that are normally present at low frequencies in the peripheral blood of healthy individuals are expanded in HIV-infected individuals (Table I). Whereas the percentage of plasma cells ($CD20^{-}/CD21^{lo}/CD27^{++}/CD38^{+++}$) circulating in the blood of healthy individuals is typically below 1%, this percentage is increased several-fold in HIV-viremic individuals.[18] In addition to plasma cells, mature/activated B cells, which have a similar phenotype to the recently described tonsil-derived memory B cells ($CD20^{++}/CD21^{lo}/CD27^{-/+}/CD38^{-/+}$),[39] are overrepresented in the peripheral blood of HIV-viremic individuals (Fig 1).[40] The percentage of these B cells is typically below 5% in healthy individuals and HIV-infected aviremic individuals, compared with more than 25% in HIV-viremic individuals. In a recent longitudinal study on chronically HIV-infected individuals,[35] we found that before ART, the mean percentage of activated and terminally differentiated B cells in the peripheral blood was 29%. After 1 year of effective ART, that percentage dropped to 12%.

Finally, we and others have recently characterized immature/transitional B cells in the peripheral blood of healthy individuals and found their frequency to be significantly increased in various disease settings of immune deficiencies, including HIV infection (Table I).[20,41,42] This subpopulation of B cells can be defined by the expression of ***CD10*** in the absence of CD27. B cells

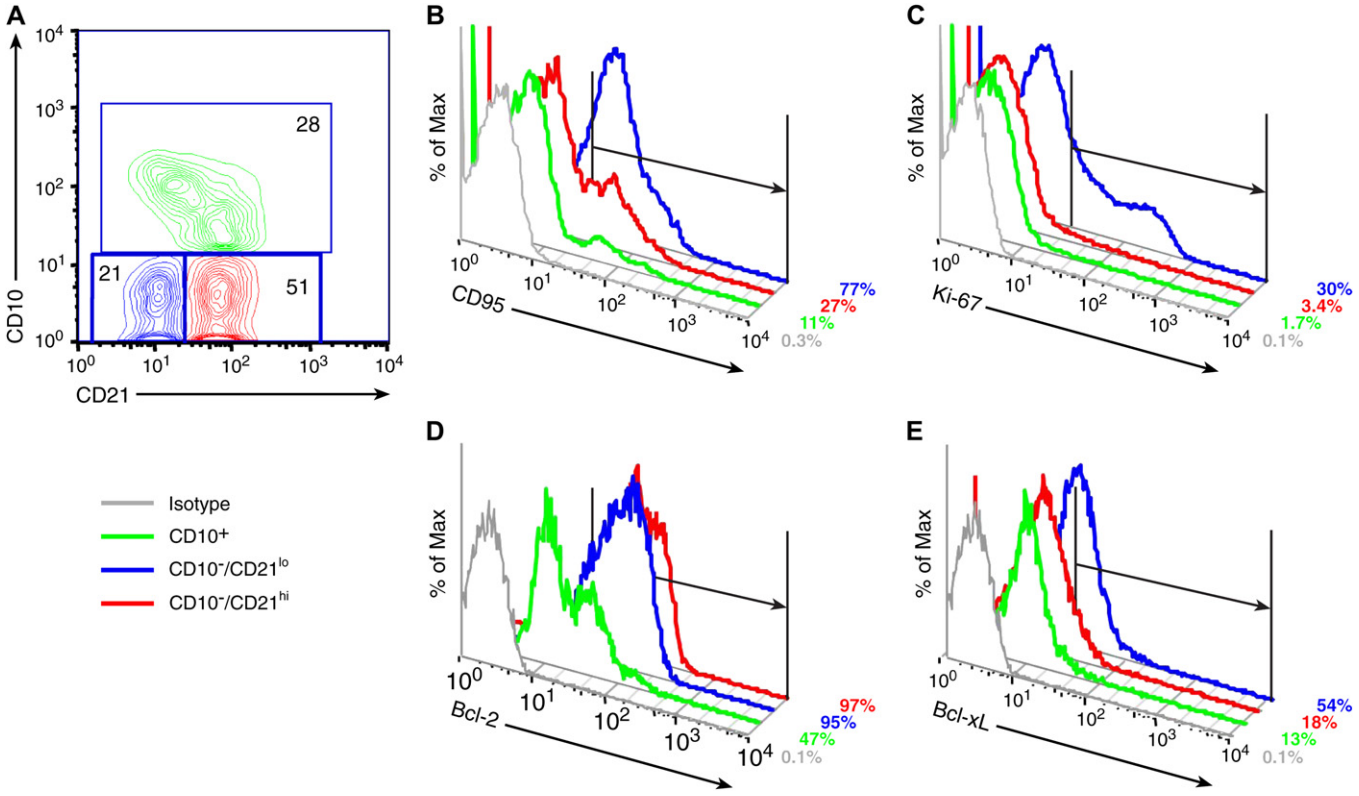

FIG 2. HIV disease is associated with increased B-cell turnover and increased B-cell death by intrinsic and extrinsic apoptosis. **A,** Identification of B-cell subpopulations in the peripheral blood of a representative HIV-infected individual with active disease. **B,** Increased expression of CD95 on $CD10^-/CD21^{lo}$ mature/activated B cells correlates with increased susceptibility to CD95-mediated extrinsic apoptosis. **C,** $CD10^-/CD21^{lo}$ mature/activated B cells also express increased levels of the cell-cycling marker Ki-67. Decreased expression of **(D)** Bcl-2 and **(E)** Bcl-xL in $CD10^+$ immature/transitional B cells is associated with increased susceptibility to intrinsic apoptosis. These profiles originally appeared in Ho J, Moir S, Malaspina A, Howell ML, Wang W, DiPoto AC, et al. Two overrepresented B cell populations in HIV-infected individuals undergo apoptosis by different mechanisms. Proc Natl Acad Sci U S A 2006;103:19436-41.[48] *Max,* Maximum.

coexpressing CD10 and CD27 represent mature germinal center B cells that normally circulate in the blood at a frequency of approximately 2%, a percentage that is not affected by disease status.[20] In contrast, immature/transitional B cells account for more than 30% of peripheral blood B cells in active HIV disease, compared with approximately 10% in healthy individuals.[20] In addition, this immature/transitional B-cell subpopulation can be further divided into less immature ($CD21^{hi}/CD10^+$) and a more immature ($CD21^{lo}/CD10^{++}$) B cells, the latter of which are rarely observed in the blood of healthy individuals, yet are very common in HIV-infected individuals with advanced disease. These individuals typically have very low $CD4^+$ T-cell counts. A similar expansion of immature/transitional B cells in individuals with idiopathic $CD4^+$ T lymphocytopenia indicates that $CD4^+$ T-cell lymphopenia in HIV disease drives the overrepresentation of immature/transitional B cells and not HIV viremia *per se*.[43] The association between immature/transitional B cells and $CD4^+$ T-cell lymphopenia also correlates with increased serum levels of ***IL-7***, a cytokine involved in homeostatic compensation of lymphocyte subsets in HIV disease.[44,45]

INCREASED B-CELL DEATH IN HIV DISEASE

Cell death by apoptosis is an important component of immune activation and lymphocyte depletion in HIV disease.[46] Two major pathways of apoptosis exist: the intrinsic pathway arises from an insufficiency in survival factors that result in mitochondrial-driven apoptosis, whereas the extrinsic pathway arises from the triggering of a death receptor.[47] In HIV disease, both these pathways likely contribute to increased B-cell death,[18,48] and B-cell depletion that we and others have documented.[35,49,50] On the one hand, immature/transitional B cells are highly susceptible to intrinsic apoptosis as a result of low expression of members of the Bcl-2 family associated with survival, including Bcl-2 and Bcl-xL (Fig 2).[48] On the other hand, mature/activated B cells are highly susceptible to extrinsic apoptosis as a result of increased expression of ***CD95*** and increased apoptosis in the presence of ***CD95 ligand*** (Fig 2).[18,48] Considering that both immature/transitional and mature/activated B cells are overrepresented in HIV-infected individuals with ongoing viral replication and that effective ART results in a decrease in these overrepresented apoptosis-prone B-cell subpopulations concomitant with an increase in B-cell numbers,[35] it is fair to suggest that the B-cell lymphopenia observed in HIV disease is in part a result of increased B-cell death by apoptosis.

The high levels of immune activation and cell turnover induced by ongoing HIV replication contribute to increased cell death by extrinsic apoptosis. In HIV and SIV disease, increased cell turnover is well documented for $CD4^+$ and $CD8^+$ T cells and to a lesser extent for B cells.[51-56] Within the B-cell compartment, mature/activated B cells express increased levels of the cell cycle

marker Ki-67 (Fig 2),[48] suggesting that this subpopulation of B cells arises as a result of HIV-induced B-cell turnover. In addition, mature/activated B cells express increased levels of activation markers, including CD80, CD86, and CD38, collectively suggesting that these would be the cells most prone to proliferation-induced and activation-induced extrinsic apoptosis. Of the numerous death receptors that can mediate extrinsic apoptosis, CD95 was the most overexpressed death receptor on B cells of HIV-viremic individuals, as determined by DNA microarray analysis.[18] CD95 is also one of many genes shown to be induced after type I IFN treatment.[57] Given that IFN-induced genes are prominently featured in the list of genes upregulated in HIV infection, which includes the genes in B cells of HIV-viremic individuals,[18] it is not surprising that CD95 is among these upregulated genes. Phenotypic analyses have also revealed that the increase in CD95 expression is concentrated on mature/activated B cells, the same B-cell subpopulation expressing increased levels of Ki-67 and activation markers. Furthermore, functional analyses have demonstrated that levels of CD95 expression on B cells of HIV-infected individuals correlate with susceptibility to CD95 ligand–mediated apoptosis and that this extrinsic form of apoptosis is directly correlated to HIV viremia.[18] Collectively, the data from our studies and other reports strongly suggest that ongoing HIV replication is associated with the appearance of apoptosis-prone subpopulations of B cells that arise from increased cell turnover and activation.[18,38,48,58,59] On balance, these events are likely to contribute to B-cell lymphopenia in the peripheral blood of HIV-viremic individuals that we and others have reported.[35,49,50]

FUNCTIONAL ALTERATIONS OF B CELLS IN HIV DISEASE

The effects of HIV infection on B-cell function can be divided into 2 broad categories. The first category relates to changes that directly reflect *in vivo* phenomena, such as hypergammaglobulinemia, increased autoantibody levels, and poor antibody responses to specific antigens. The second category relates to changes that are inferred from *ex vivo* analysis of B cells isolated from HIV-infected individuals. There have been substantial advances in the latter category over the period of the past 25 years as new techniques and a better understanding of B-cell development have helped to dissect the various elements of HIV-induced B-cell dysfunction.

Several studies have confirmed the early observation that although B cells of HIV-infected individuals with active disease exhibit numerous signs of increased activation *in vivo*, they respond poorly *ex vivo* to B-cell stimuli.[5] All the early *ex vivo* observations were based on analyses performed on unfractionated B-cells, and the precise effects of HIV viremia were difficult to assess. With more recent data based on B-cell fractionation and control of HIV viremia by ART, the early observations can now best be explained by HIV viremia inducing the expansion of terminally differentiated B cells with secretion of high levels of immunoglobulins, loss of responsiveness to stimuli, and a high propensity to cell death.[9,18] In addition, the overrepresentation of immature/transitional B cells, especially in patients with advanced $CD4^+$ T-cell lymphopenia, can also help explain the unresponsiveness of B cells *ex vivo* to B-cell stimuli because immature/transitional B cells have been shown to respond poorly to stimulation and to die rapidly by intrinsic apoptosis.[20,48] Given that more than 50% of peripheral blood B cells isolated from chronically HIV-viremic individuals are composed of immature/transitional and mature/activated B-cell subpopulations,[35] such a skewing of the B-cell compartment provides a compelling explanation for the poor overall B-cell responses that have been reported *in vivo* and *ex vivo*.

Loss of B-cell function can also be investigated by reconstituting the events of cognate interactions that occur between B cells and $CD4^+$ T cells after antigenic stimulation. Once stimulated, B cells acquire antigen-presenting cell capacities, which then enable them to provide help to $CD4^+$ T cells. This occurs in part through stimulatory interactions between ***CD80/CD86*** receptors, which are upregulated after B-cell activation, and ***CD28*** on responder $CD4^+$ T cells.[60] B-cell antigen-presenting cell function is inefficient in HIV-viremic individuals, as evidenced by the inability of activated B cells to provide CD80/CD86-mediated stimulatory signals to autologous $CD4^+$ T cells.[61] Furthermore, $CD4^+$ T-cell help given to B cells has also been shown to be defective in HIV-viremic individuals as a result of an impaired interaction between ***CD40 ligand*** on the T cells and ***CD40*** on the B cells.[62] In both of these studies, the reduction of HIV plasma viremia by ART was associated with a normalization of responses resulting from bidirectional interactions between B cells and $CD4^+$ T cells. Given that normalization of the representation of B-cell subpopulations also occurs during ART, it is reasonable to suggest that impaired bidirectional interactions between B cells and $CD4^+$ T cells in HIV-viremic individuals are at least in part a result of skewing toward unresponsive B-cell subpopulations.

One aspect of B-cell dysfunction in HIV disease that does not appear to be normalized by ART is the loss of memory B cells. This loss correlates with a reduced frequency of immunogen-specific memory B cells after immunization in HIV-infected individuals that does not normalize with ART.[63,64] Many of these reported defects in antigen-specific memory B-cell responses, particularly those that are T-cell–dependent, might result from defects in the $CD4^+$ T-cell compartment of HIV-infected individuals. However, there is also evidence for memory B-cell deficiencies in HIV-infected individuals against $CD4^+$ T-cell–independent immunogens such as pneumococcal polysaccharides.[65] These defects have been associated with a reduced frequency of IgM^+ memory B cells, the subpopulation of B cells thought to be important for memory B-cell responses against pneumococcal infection.[66] In pediatric HIV infection, deficiencies in B cells and humoral responses to various childhood vaccines have also been described. Decline in $CD19^+$ B-cell counts has been reported in pediatric HIV disease, and there are some indications that an irreversible loss of $CD27^+$ memory B cells also occurs in pediatric HIV infection.[49,67,68] These observations are consistent with poor antibody and B-cell memory responses in pediatric HIV infection to both T-cell–dependent and T-cell–independent antigens that are not fully reversed by ART.[69,70] These observations may also help explain the high risk of bacterial infections that is seen in pediatric HIV disease.[69,71,72] Thus, several defects in the memory B-cell compartment of HIV-infected individuals appear to occur independently of ART. However, one open question is whether initiation of ART during acute HIV infection rather than after prolonged periods of viremia can prevent the lost of memory B-cell frequency and function.

Finally, one very important aspect of B-cell function in HIV disease that has received relatively little attention is the induction of HIV-specific B cells in infected individuals. High frequencies of B cells actively secreting antibodies against HIV are observed

in the peripheral blood of HIV-viremic individuals, along with high levels anti-HIV antibodies in the serum.[11,17] However, as polyclonal B-cell activation and hypergammaglobulinemia decrease with the reduction of HIV viremia by ART, so do frequencies of HIV-specific B cells and anti-HIV antibodies. Given the strong indications from SIV models that antibodies can contribute to the control of viral replication,[73-75] it is important to understand the mechanisms involved in the rise and fall of HIV-specific B-cell responses in infected individuals and whether early intervention can lead to an effective antibody response. Ideally, insights from these observations can help in the design of an effective antibody-based HIV vaccine. Vaccine strategies that elicit B cells to produce broadly neutralizing antibodies against HIV are being investigated, along with a variety of approaches that fall beyond the scope of this review (see reviews[76,77]). The characterization of broadly neutralizing antibodies isolated from HIV-infected individuals and related advances in immunogen design represent some of the more recent approaches being considered for an antibody-based HIV vaccine.[78-80] However, whether the elusive process by which neutralizing anti-HIV B-cell responses arise in infected individuals can be reproduced with a vaccine strategy remains to be determined.

CONCLUSION

In summary, HIV infection in a majority of untreated individuals leads to persistent viral replication and progressive $CD4^+$ T-cell lymphopenia. Persistent HIV replication is associated with increased immune activation that manifests itself in the B-cell compartment as hypergammaglobulinemia, polyclonal B-cell activation, induction of terminal differentiation of B cells, increased levels of autoantibodies, and increased frequency of B-cell malignancies. $CD4^+$ T cell lymphopenia and increased serum levels of IL-7 in HIV-infected individuals with advancing disease are associated with an expansion of immature/transitional B cells. In addition, HIV infection is associated with a decrease in $CD27^+$ resting memory B cells. The overall effect of these changes in the B-cell compartment is a decreased capacity to proliferate in response to B-cell stimuli, a decreased capacity to respond to neoantigens and recall antigens, and a decreased capacity to generate antigen-specific memory B cells.

Future efforts should include the following: 1) evaluating whether early initiation of ART leads to a normalization of memory B-cell function in HIV-infected individuals, 2) increasing our understanding of the anti-HIV antibody response relative to B-cell function, and 3) evaluating new vaccine strategies aimed at enhancing antibody responses to a variety of immunogens in immunocompromised, HIV-infected individuals. These strategies include the addition of adjuvants such as Toll-like receptor agonists, which may both lower the response threshold to immunogens and modulate the quality of the response. As individuals living with HIV grow older, it will become important to consider these strategies in light of an immune system that is affected by both HIV and age. Finally, there are also concerns that rates of cancer, including those of B-cell origin, remain high in the HIV-infected population, indicating the need for a better understanding of pathways of immune surveillance and regulation in HIV disease.

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