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B-Cell Development and Differentiation During HIV Infection: Tolerance *Versus* Immunity

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Abstract: Early after HIV infection, B-cell homeostasis is disrupted which results in autoantibody production and autoimmunity. The systemic inflammation induced during chronic HIV infection relaxes B-cell tolerance mechanisms that normally suppress this autoimmune pathology. Tolerance to self antigens is established during the development of B cells; however, rare, broadly neutralizing antibodies to HIV cross-react with host cellular antigens, suggesting that mechanisms of B-cell tolerance limit protective humoral responses to HIV. The association between HIV infection, induction of autoantibody and the emergence of neutralizing antibodies directed at HIV suggests that protective humoral immune responses may only occur after B-cell tolerance control mechanisms are relaxed.

Keywords: B-cell development, tolerance, autoantibody, HIV.

INTRODUCTION

Subsequent to establishing cellular reservoirs of virus and chronic infection, HIV elicits generalized immune dysregulation, including B-cell activation regardless of antigen specificity. This polyclonal B-cell activation does not result from direct viral infection as HIV-infected B cells are rarely observed in patients [1]. Instead, chronic HIV infection drives B-cell activation *via* systemic inflammatory signals [1], including but not limited to interferons, TNF-family members and interleukins (reviewed in [2]). This sustained B-cell dysregulation during HIV infection elicits serum autoantibody that results in autoimmune disease [3]. Thus, the milieu of inflammatory cytokines during chronic infection relaxes B-cell tolerance mechanisms that normally suppress autoimmune pathology [4].

Interestingly, recent evidence demonstrates that a subset of broadly neutralizing antibodies to HIV cross-react with host cellular antigens, raising the possibility that immune tolerance mechanism limit the development of some HIV-reactive B cells [5]. HIV infection commonly induces neutralizing antibodies, many of which are directed against strain-specific and highly variable regions of the viral envelope [6]. Therefore, these antibodies exhibit a restricted capacity to control the rapidly mutating HIV virus [7]. In addition, vaccines designed to induce protective B-cell responses have failed to elicit broadly neutralizing antibodies, suggesting that the B-cell repertoire is limited in inducing HIV-neutralizing specificities. The hypothesis is that mechanisms of B-cell tolerance that block autoimmune pathology can limit humoral immune responses to pathogens; however, reduced immune recognition of these pathogens can also result in chronic inflammation and the relaxation of immune tolerance (Fig. **1**).

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In this review, we focus on mechanisms that promote a diverse repertoire of antibody specificities during mouse and human B-cell development. Furthermore, we discuss mechanisms of tolerance that purge the B-cell repertoire of self-reactive clones and, as a consequence, limit the recognition of pathogens which express "host-like" antigens. In addition, we explore mechanisms that can be used by pathogens to subvert immune recognition, including the elicitation of autoantibody. We discuss the "tolerance hypothesis" of Haynes *et al.* [5] as a potential mode of immune evasion utilized by HIV to subvert neutralizing B-cell responses.

B-CELL DEVELOPMENT

B-lymphocytes arise from committed lymphoid progenitors present in the bone marrow (BM) [8]. Commitment to the B lineage requires expression of specific transcription factors, such as E2a, EBF and Pax5 [9]. These transcription factors drive the expression of B cell-specific genes and maintain B-cell identity [10]. E2a is required for initiating EBF and Pax5 expression [11] and E2a$^{-/-}$ mice exhibit developmental defects in the BM resulting in a lack of mature B cells, while ectopic expression of EBF in E2a$^{-/-}$ cells restores B-cell development potential [12].The loss of Pax5 expression in mature B cells, either due to plasma cell differentiation or experimental manipulation, results in the decreased expression of definitive B-cell surface markers [10].

In mice, B-cell progenitors occupy discrete BM niches depending upon their developmental stage and maturity [13, 14]; however, the investigation of B-cell development is confounded by heterogeneous BM stromal cell populations [15]. In part, the expression of cytokines that are essential for B-cell development and survival define these distinct BM niches [16]. IL-7 is a key cytokine required during the stromal-independent phase of mouse B-cell development in the BM [17]. IL-7$^{-/-}$, IL-7R$^{-/-}$ mice or mice deficient in IL-7 signal transduction components (i.e. common γ-chain) are unable to efficiently produce lymphocytes, including B cells [18-20].

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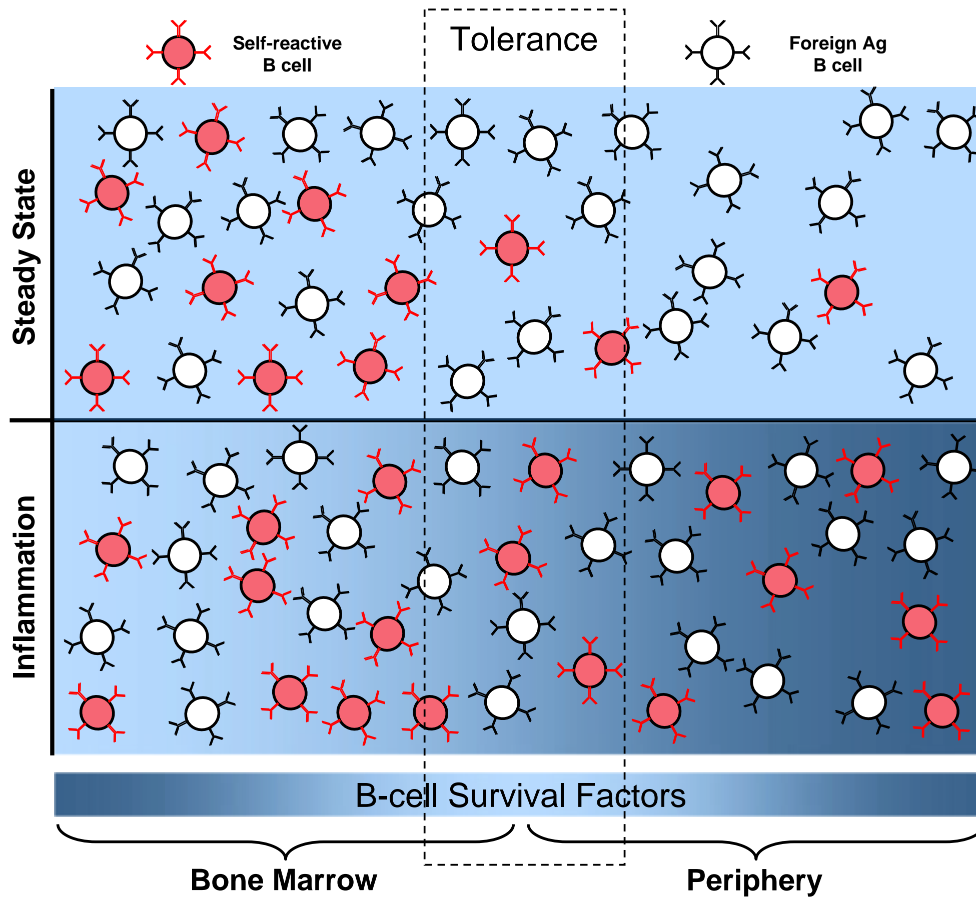

Fig. (1). Inflammation can reduce the effectiveness of tolerance by increasing the production of B-cell survival factors. During their development in the bone marrow, newly formed B cells may express membrane receptor antibodies that are specific for self- (*red circle*) or foreign (*white circle*) antigens. The several mechanisms of central tolerance (*boxed area*) efficiently remove self-reactive B cells, decreasing their frequency in the mature, peripheral B-cell compartments. In part, B-lymphopoiesis and the maintenance of peripheral B-cell compartments are controlled by the availability of B-cell survival factors (*light blue*). During inflammation, increased production of these survival factors (*darker blue*) may promote the survival and/or retention of self-reactive B cells and thereby increase their frequency in peripheral B-cell compartments.

While it is assumed that development of human B cells mirrors that of murine B cells, fundamental differences do exist in B-cell development between each species. For example, human B-cell development is observed in patients with primary immunodeficiency resulting from the loss of IL-7 or signal transduction components within this pathway (IL-7R and common γ-chain) [21].

While many cues, internal and external, drive the production of B cells, we will focus on the assembly of antibody molecules to describe early stages of B-cell development. It is important to note that antibody production is controlled, in part, by *i*) the expression of specific transcription factors and *ii*) signals derived from the cytokine mileu [22, 23]. Furthermore, the expression of transcription factors control molecules that promote diversity within the antibody repertoire during the assembly of antibody molecules [24].

Immunoglobulin and B-Cell Diversity

The immunoglobulin (Ig) heavy chain (HC) and light chain (LC) gene loci (*Igh* and *Ig*κ/λ respectively) encode the B-cell receptor (BCR), which is necessary for B-lymphocyte survival and specific recognition of antigens during humoral immune responses [25, 26].During Ig HC rearrangement, gene segments of variable- (V), diversity- (D) and joining- (J) regions of *Igh* are assembled in an ordered fashion (D-J, then V-DJ) [27]. After the completion of HC rearrangement, V- and J-gene segments from the *Ig*κ or *Ig*λ loci are combined to form the LC. Together, the HC and LC form complementarity determining regions (CDRs) that bind antigenic structures in their native conformation [25]. Of note, the BCR is created in the absence of its cognate antigen, producing an un-selected pool of B cells capable of recognizing both foreign and self antigens (reviewed in [28]).

The combinatorial association of V(D)J gene segments and pairing of unique Ig HC + LC increase the diversity of antibodies available for antigen recognition [27]. This diversity within the HC antibody repertoire is enhanced beyond the DNA sequence encoded within *Igh*. Terminal deoxynucleotidyl transferase (TdT) is an enzyme responsible for the template-independent addition of N-nucleotides at *Igh* gene

segment junctions increasing the diversity of CDR3 regions [24].

V(D)J recombination utilizes many enzymes and cellular processes to promote DNA rearrangement while ensuring genomic stability. RAG1/-2 and DNA-protein kinases (PKcs) are enzymes required for rearrangement process as they permit double-stranded (ds) DNA cleavage and resolution of dsDNA breaks, respectively (reviewed in [24, 29]). Other proteins, such as Artemis and DNA ligase IV, in the nonhomologous end-joining DNA repair pathway are important for the completion of V(D)J recombination [30]. During resolution of these dsDNA breaks, imprecise joining of V(D)J gene segments permit unique patterns of template-encoded P-nucleotide additions, yet another mechanism that contributes to BCR diversity [31].

Combined, the molecular mechanisms of Ig recombination can generate frame-shift mutations, resulting in truncated or mis-folded non-functional Ig proteins [32]. In-frame rearrangement of the HC and LC loci occur when V(D)J rearrangement maintains the proper reading frame through the constant (C-) regions of each polypeptide [33]. The usage of these different C-regions (Cμ, Cδ, Cα, Cγ, and Cε) of *Igh* determines the isotype of antibody, each with unique biological properties [34]. Differential mRNA splicing of the HC VDJ-Cμ-Cδ transcript creates antibody of IgM or IgD isotype; whereas, the induction of IgA, -G and -E isotype antibody is regulated by genomic recombination events termed class-switch recombination (CSR) [34].

Pro- and Pre-B-Cell Development

Early stages of B-cell development are classified based on rearrangement status of *Igh* and *Igκ/λ* [35]. In mice, prepro-B cells initiate the rearrangement of D-gene segments to J-gene segments of the *Igh* loci (reviewed in [36]). Pro-B cells continue the rearrangement process by adding an *Igh* V-gene segment to the DJ rearrangement previously created (reviewed in [36]). Successful, in-frame rearrangement of the *Igh* locus is required for pairing of HC with surrogate LC to form the pre-BCR [37]. The pre-BCR contains Igα/Igβ signal transduction components and is necessary for B-cell survival beyond the pro-B cell stage [38] by delivering tonic signals that increase expression of bcl-2 family proteins, which inhibit the intrinsic mitochondrial death pathway [39].

The earliest stages of human B-cell development are defined by expression of RAG1/-2 and D- to J- rearrangements at the *Igh* loci (reviewed in [40]). As in mice, functional VDJ rearrangements in human pro-B cells result in the generation of Ig HC polypeptides that associate with the surrogate LC to generate the pre-BCR. The human pre-BCR provides survival and proliferation signals necessary for continued B-cell development through the large and small pre-B stages (reviewed in [41]). Developmental arrest at the pre-B cell stage has been observed in humans with mutations of pre-BCR components, including HC, surrogate LC and Igα [42].

Pre-B cells are divided into cycling large pre-B and non-cycling small pre-B compartments [43]. Proliferation at the large pre-B stage expands the frequency of cells with in-frame HC rearrangements which *i*) mitigates the apoptotic loss of pro-B cells with non-functional HC rearrangement and *ii*) gives rise to many small pre-B cells that independently rearrange their Igκ or -λ LC promoting diversification of the BCR repertoire.

In mice and humans, rearrangement of the Igκ LC loci tends to occur prior to the Igλ LC loci [44-46] but the in-frame rearrangement of V- and J- gene segments of either loci leads to expression of "mature" antibody (HC+LC) [47]. IgM antibody is first observed in the cytoplasm of small pre-B cells [47] and in the cytoplasm and surface of immature B cells [48] while the IgD isotype is expressed later during development.

Alternatively, early B-cell subsets are identified by flow cytometry using classification systems based on differential expression of surface antigens [49, 50]. In mice, Hardy *et al.* compare the VDJ rearrangement of Ig HC loci with the surface phenotype of prepro-B (fraction A-$CD43^{hi}B220^{lo}HSA^{lo}$ $BP\text{-}1^{neg}IgM^{neg}$), early pro-B (fraction B-$CD43^{hi}B220^{lo}HSA^{int}$ $BP\text{-}1^{neg}IgM^{neg}$), late pro-B and large pre-B (fraction C-C`-$CD43^{hi}B220^{lo}HSA^{int}BP\text{-}1^{hi}IgM^{neg}$) and small pre-B (fraction D-$CD43^{lo/neg}B220^{lo}HSA^{hi}BP\text{-}1^{lo}IgM^{neg}$) cells. The identification and recovery of mouse B cells at distinct stages of development, independent of Ig rearrangement, is a valuable tool to investigate the changing BCR repertoire during maturation.

The earliest stages of human B-cell development are defined by surface expression of CD34, CD38, and CD10 (reviewed in [51]). Subsequently, pro-B cells may be identified by their expression of CD19 (reviewed in [51]) and pre-B cells by the acquisition of CD20 [reviewed in [52]]. In addition to characterization by surface molecules and Ig gene rearrangements, human B-cell progenitors, pro-B and pre-B cells can be defined by distinctive patterns of gene expression that offer an independent gauge of developmental maturity [53].

Immature and Transitional B-Cell Development

Immature and transitional B-cell compartments are the targets of central tolerance mechanisms to purge self-reactive B cells upon antigen stimulation (reviewed in [54]). Similar to the characterization of progenitor B-cells, Ig^{+} B-cell compartments are defined by the expression of surface antigens, antibody isotype and distinct patterns of localization within secondary lymphoid tissues. In part, their unique pattern of surface antigen expression permits the removal of antigen activated B cells by tolerance mechanisms. In mice, immature and transitional B-cell populations are distinguished based on differential expression of fetal stem-cell antigen (CD93), IgM, IgD, complement- (CD21) and Fc-receptors (CD23) [55, 56].

Immature B cells are present in both the BM and spleen and express a surface antigen profile ($B220^{int}CD93^{hi}IgM^{lo}$ $IgD^{neg}CD21^{neg}CD23^{neg}$) that is low for BCR and other receptors (CD21 & CD23) utilized by mature B cells to promote activation [57]. Low expression of BCR and these co-activating receptors limits B-cell activation upon antigen encounter; in fact, immature B cells are induced to undergo apoptosis upon cognate antigen exposure [57].

The phenotype of human immature ($CD19^{+}CD10^{+}IgM^{+}$) B cells [48] has not been extensively characterized compared to the mouse immature B-cell compartment. However, *in vitro* culture systems routinely support $CD19^{+}IgM^{lo}$ B-cell

development from hematopoietic stem cells [58, 59], suggesting that a detailed phenotypic analysis of human immature B cells can be performed.

Immature B cells become mature B lymphocytes after passing through transitional (T) stages of development [60, 61]. Mouse transitional B cells are present in BM and spleen, increase expression of BCR and initiate expression of surface antigens (CD21 & CD23) that define mature B-cells [62]. T1 B cells ($B220^{int}CD93^{hi}IgM^{hi}IgD^{neg}CD21^{neg}$ $CD23^{lo/neg}$) do not express IgD BCR, CD21 or CD23 antigens that are present on T2 B cells ($B220^{int}CD93^{int}IgM^{hi}$ $IgD^{lo/hi}CD21^{int}CD23^{hi}$). In part, B-cell activating factor belonging to the TNF family (BAFF) has a role in regulating B-cell maturation by increasing the expression of CD23 and CD21 on mouse transitional B lymphocytes [63]. T3 B cells ($B220^{int}CD93^{int}IgM^{lo}IgD^{lo}CD21^{int}CD23^{hi}$) are anergic, "post-selection" cells that do not substantially contribute to the mature B-cell repertoire [64, 65].

Human transitional B cells ($CD19^{+}CD10^{+}CD38^{hi}CD24^{hi}$) express antigens similar to mouse transitional B cells ($IgM^{hi}IgD^{lo}CD21^{lo/neg}CD23^{lo}$) and have been isolated from blood and BM; however, expression of human CD93 is not used as a B-cell developmental marker as in mice [66].Similar to their murine counterparts, Wardemann *et al.* demonstrate that human transitional B-cell compartments contain self-reactive cells that must be purged by tolerance mechanisms [48].

The induction of B-cell tolerance is confounded by some observations that transitional B cells are capable of survival and proliferation upon BCR ligation [67]. Other studies demonstrate that mouse T2 B cells respond to BCR ligation by increasing expression of survival signals, proliferating and differentiating; whereas T1 B cells do not [61]. Similar to mature B-cell compartments, transitional B cells of mice can participate in immune responses if their BCR has affinity for the pathogen/antigen and they receive additional stimulating (CD40L) and survival (BAFF) factors [67].

Human $CD10^{+}$ transitional B cells isolated from blood do not proliferate in response to BCR ligation alone [68]. However, human transitional B cells will proliferate in response to BCR activation coupled with IL-4 or CD40L stimulation [68]. Unlike their murine homologues, BAFF stimulation cannot rescue human transitional B cells after BCR ligation [68].

The induction of B-cell tolerance is influenced by external cues that promote B-cell survival. BAFF is a cytokine that promotes transitional B-cell survival [69] *via* up-regulation of Bcl-2 family members (Bcl-xL or Bcl-2) as well as down-regulation of the pro-apoptotic molecule Bim [70, 71]. Moreover, studies of mice with impaired BAFF signaling but ectopic expression of pro-survival molecules (Bcl-2 and Bcl-xL) have shown that peripheral B-cell development can be restored [72, 73]. These observations suggest that environmental cues, such as elevated BAFF, promote B-cell survival and contribute to autoimmunity by mitigating the induction of B-cell tolerance.

Mature B-Cell Compartments

In mice and humans, mature splenic B cells are located in follicles with closely associated T-cell zones that surround a central arteriole (reviewed in [74]). The marginal zone (MZ) areas of the spleen are located at the border of the white pulp (B- and T-cell zones) and the red pulp [75, 76]. The MZ of mice is comprised of cells, including metallophilic macrophage ($MOMA\text{-}1^{+}$), dendritic cells (DC) and B cells, that surround mature follicular (MF) B cells (reviewed in [74]); whereas in humans, MF B cells are encompassed by two areas of MZ cells (inner and outer) that are themselves surrounded by a perifollicular zone containing blood vessels wrapped with macrophages (reviewed in [74]).

In mice, the expression of IgM, IgD, CD23 and CD21 are used to identify splenic MF and MZ B cells [67]. MF B cells express high levels of IgD and CD23, but low levels of IgM and CD21 ($B220^{+}IgM^{lo}IgD^{hi}CD23^{hi}CD21^{lo}$). Cells with this phenotype are also found in the BM, blood, lymph nodes (LN) and mucosal areas (reviewed in [77]). Alternatively, MZ B cells express high levels of IgM and CD21, but low levels of IgD and CD23 ($B220^{+}IgM^{hi}IgD^{lo}CD23^{lo}CD21^{hi}$) and are only found in the spleen [75, 78]. The responses to BCR cross-linking, Toll-like receptor (TLR) stimulation [79, 80] and regulation of molecules that promote T-cell activation (CD80 and CD86) are also used to distinguish MF and MZ B-cell subsets [81].

In humans, naïve mature B cells ($CD19^{+}IgM^{lo}IgD^{hi}CD27^{-}$ $CD38^{-}CD10^{-}$) are present in the BM, blood, spleen, LN and mucosal areas (reviewed in [82]). As with mice, the initial identification of human MZ B cells was defined by anatomical location and morphology in the spleen [83, 84]. Currently, there is no clear consensus on a unique phenotype for human MZ B cells due to high inter-individual variability (reviewed in [85]). Histological analysis has been used to demonstrate that most human MZ B cells are $IgM^{hi}IgD^{lo}$ $CD27^{+}$ and can be further characterized by differential expression of CD38, CD10, CD1c, CD21 and CD23 (reviewed in [85]). However, it is clear that the MZ can contain many B-cell populations beyond the MZ phenotype described above, including IgA^{+} B cells [86]. In addition, Weller *et al.* suggests that $IgM^{+}IgD^{+}CD27^{+}$ cells in human blood correspond to circulating "splenic" MZ B cells [87]; further illustrating that mouse and human MZ B-cell biology differs.

Lastly, B1 B cells are a third type of mature B cell that are identified by a unique pattern of surface antigen expression [88]. Mouse B1 B cells ($B220^{lo}IgM^{hi}IgD^{lo}CD23^{lo}$ $CD21^{lo}CD11b^{lo}$) are further subdivided into B1a ($CD5^{+}$) and B1b ($CD5^{-}$) subsets [89] while human B1 B cells are defined by their expression of CD5 [90]. In mice, these B cells utilize a restricted set of V gene-segments to form their antibody [91]. Mouse B1 B cells rarely contain N-nucleotide additions, presumably due to the lack of TdT expression [92].

Both mouse and human B1 B cells express low-affinity antibody against conserved bacterial components [93, 94] and can express "polyreactive" receptors that have low affinities for many different antigens including other immunoglobulins and self antigens [93, 95]. Mouse and human B1 B-cell populations contribute to circulating natural antibody present in the serum [93, 96, 97] and require complement fixation for effective clearance of many pathogens (reviewed in [98]). Mouse B1 B cells promote host survival, *via* natural antibody, by limiting the initial dissemina-

tion of pathogens and enhancing antigen delivery to secondary lymphoid tissue [99].

In mice and humans, B-1 cells are produced primarily during fetal and peri-natal development [100, 101]. The frequency of B1 B cells is low in the spleen and secondary lymphoid tissues; however, the pleural and peritoneal cavities are enriched for B1 B cells [102, 103].

Currently, there is debate as to whether B1 B cells arise from a precursor that is independent from progenitors that give rise to other mature (B2) B-cell compartments (reviewed in [104]). This contention arose from transplantation experiments demonstrating that hematopoietic tissues of fetal and adult mice were distinct in their ability to reconstitute the B1 B-cell compartment of recipient mice [100, 105]. Donor cells from fetal liver were able to repopulate both B1 and B2 B-cell compartments. In contrast, adult BM cells efficiently reconstituted B2 B cells but were poor at B1 B-cell reconstitution. Recently, a progenitor cell ($CD19^{+}CD45R^{-/lo}$) that does not express lineage-specific surface antigens (Lin^{-}) has been identified in the fetal liver and adult BM that specifically reconstitutes B1 B-cell development in mice [106]. However, other research suggests that commitment to either the B1 or B2 lineage is determined by BCR specificity and density [107] and that distinct niche(s) are present during fetal B-cell ontogeny [108] that promote B1 B-cell development.

BAFF and Mature B-Cell Numbers

The number of mature peripheral B-cells is regulated, in part, by the systemic availability of BAFF (reviewed in [109]. Studies in BAFF transgenic ($BAFF^{tg}$) animals have demonstrated an increase in numbers of MF and MZ B cells [110]; while $BAFF^{-/-}$ and $BAFF\text{-}R^{-/-}$ mice exhibit an almost complete loss of T2, MF and MZ B cell compartments [111, 112].

A proliferation-inducing ligand (APRIL) is another TNF-family member with partially overlapping functions as BAFF; however, much less is known about its unique role as a B-cell cytokine in either mice or humans [109]. APRIL is unable to restore wildtype levels of mature B cells in $BAFF^{-/-}$ mice.

Both BAFF and APRIL can be membrane-bound or cleaved and released as soluble ligands [113]. While many cell types express BAFF, including neutrophils, monocytes, macrophages and dendritic cells [109], less is known about the regulation and expression of APRIL. The differential ability of BAFF and APRIL to bind receptors may account for their divergent biological functions. BAFF binds to three TNF receptor family members: BAFF-receptor (BAFF-R), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA). APRIL, however, binds to only two of these receptors - TACI and BCMA [114, 115]. Further investigation is necessary to elucidate the distinct roles of BAFF and APRIL during mouse and human B-cell development.

However, humans B cells are less dependent on BAFF for their development and survival despite expressing BAFF-R. One common variable immunodeficiency (CVID) patient has been identified with mutations in BAFF-R that results in hypogammaglobulinemia; however, B-cell development appears to occur but at reduced numbers than healthy individuals [116].

Clearly, factors that promote B-cell survival alter B-cell selection and result in autoimmunity [117, 118]. As such, $BAFF^{tg}$ mice contain high levels of rheumatoid factors, circulating immune complexes, anti-DNA autoantibodies and immunoglobulin deposition in the kidneys [110]. These observations predict that B-cell autoimmunity could be modulated by controlling BAFF availability in the system (organism) and, in fact, BAFF is a current target for therapeutic intervention in some human autoimmune disorders [119, 120].

B-CELL ACTIVATION

Normally, the B-cell repertoire comprises a diverse array of BCR molecules, perhaps $>10^7$ distinct specificities, that allow antigen recognition and promote host survival (reviewed in [30]). The initial B-cell response to microbes is initiated by genetically diverse populations of B cells that recognize their cognate antigen, even with very low affinities [121, 122]. These antigen-activated B cells migrate to nearby T-cell areas to interact with antigen-activated helper T cells and receive survival, proliferation, and differentiation signals in a process termed “linked-recognition” (reviewed in [123-125]). Generally, this “extra-follicular” interaction between antigen-specific T and B lymphocytes initiates two distinct pathways of B-cell differentiation: *i)* production of short-lived plasmacytes that provide early, low affinity antibody responses and; *ii)* the germinal center (GC) reaction [123, 124]. Therefore, humoral immune responses are constrained by the qualities, frequencies, and locations of antigen-specific, naive B cells.

The Germinal Center Reaction

The GC reaction is the foundation of T_d humoral responses by promoting the development of higher affinity antibody and memory B-cell compartments (reviewed in [126, 127]). Antigen-activated B cells enter GCs and initiate a complex cellular program that promotes proliferation, somatic hypermutation (SHM), isotype switching and differentiation into antibody forming cells (AFC) and memory cells [128]. The interruption of T-B collaboration, by disruption of CD40-CD40L interaction, blocks the formation of GCs [129]. Genetic mutation in the mouse and human *CD154* gene (CD40L) results in type-1 hyper-IgM syndrome characterized by elevated serum IgM, the absence of class-switched antibodies and reductions in affinity maturation and B-cell memory [130].

Early during the initiation of GCs, a network of follicular dendritic cells (FDC) is filled with proliferating B cells. FDCs capture antibody/antigen complexes on their surface, *via* CD21, and secrete cytokines that support B-cell activation and proliferation [131] . GCs contain two distinct zones of B-cell morphology, the dark and the light zone. The dark zone is comprised of rapidly dividing B cells called centroblasts; while the light zone consists of B cells, or centroctyes, that express surface antibody and compete for antigens present on FDCs [132, 133]. Differential expression of chemokine receptors, CXCR4 and CXCR5, controls migration of B cells between these distinct GC areas [134].

Importantly, the GC alters the repertoire of antigen-specific B cells so that high affinity clones are preferentially expanded, a process termed affinity maturation (AM) [135, 136]; while low affinity clones are eliminated by apoptosis [137]. AM is driven by activation-induced cytidine deaminase (AID), a B cell-specific enzyme that is produced upon B-cell activation [138]. Mutation in the variable, antigen-binding regions of Ig HC and Ig LC is much greater (~10^6 fold) than mutation in cells outside of the B-lineage, a process termed SHM (reviewed in [139]). The accumulation of mutations in the Ig loci alters the binding specificity and affinities of the resultant antibodies [140]. B cells that have undergone SHM must compete for antigen and limited growth resources for survival [136]. Clonal expansion and SHM occur in a cyclical process, each round increasing the affinity of antibody for the cognate antigen [135]. From the GC process, high-affinity memory and AFC are formed that provide long-lived protection against secondary pathogen challenge [141, 142].

Class Switch Recombination

CSR is an AID-mediated DNA recombination event which diversifies antibody effector functions by replacing the Ig HC Cμ/Cδ gene segments (IgM/IgD antibody) with the Cα, Cγ, or Cε genes segments (IgA, IgG or IgE antibodies) [143, 144]. CSR was initially thought to occur only in GCs and to be highly dependent on the interaction of CD40 on B cells with CD40L on T cells. This stems from observations that IgA, -G, and -E antibody production is severely reduced in patients with a mutation in the CD40L gene [145, 146].

This dogma has recently been revised as CSR is shown to occur *in vivo* in $CD40^{-/-}$ mice, in T-independent splenic MZ and in intestinal lamina propria immune responses [147, 148]. These findings suggest that molecules other than CD40 are involved in initiating CSR. In addition to B-cell maturation and survival, BAFF has recently been implicated in Ig HC CSR in the absence of CD40 co-stimulation [111, 149, 150]. *In vitro*, BAFF stimulation increases AID expression and is involved in promoting α-germline transcript (αGT) and Iα-Cμ transcript (αCT) production, all of which are hallmarks of CSR [151-153].

Some antigens can activate B cells in the absence of helper T cells (T_H); a process termed T-independent (T_i) B-cell responses [147]. T_i antigens are divided into Type-1 and Type-2 antigens as their mechanism for B-cell activation differs. Most T_i Type-1 antigens drive polyclonal B cell activation *via* TLR ligation regardless of BCR specificity, while T_i Type-2 antigens activate B cells by extensive cross-linking of the BCR which alleviates the requirement of T_H cytokines necessary to induce B-cell proliferation [154].

Regardless of species, MZ B cells are constantly exposed to blood-borne pathogens/antigens and contribute to T_i B cell responses that result in *i*) rapid production of low-affinity antibody or *ii*) migration to T-cell zones as antigen-presenting cells (APC) (reviewed in [78, 83]). It is widely agreed that B-cell memory is the result of T_d B-cell responses [155]; however, recent work suggests that some T_i type-2 antigen immunizations can form antigen-specific memory B cells [156].

B-CELL SELECTION

As discussed earlier, rearrangement of Ig gene segments that form the BCR are combined in the absence of their cognate antigen [36]. This process generates a population of immature B lymphocytes that express receptors reactive to self antigens. Early in the 1950s, Drs. Billingham, Brent and Medawar demonstrated that immunological tolerance was an "actively acquired" process while investigating the mechanism of allogeneic skin transplant rejection [157, 158]. Later, it was determined that the mature B-cells repertoire is purged of this pool of self-reactive clones through at least three mechanisms: deletion, anergy and receptor editing [159, 160].

These processes were further characterized using transgenic mouse lines that express B cell receptors (BCR) for authentic [161, 162] or neo-self-antigens [163]. These experimental models demonstrated that immature and T1 B-cells receive "tolerizing" apoptotic signals [164] and identified anergy [65, 165] and receptor editing [166, 167] by characterization of B-cell populations that escape apoptotic deletion.

The escape of self-reactive B cells from central tolerance does not guarantee survival since autoreactive B cells can be eliminated from the mature repertoire by peripheral tolerance mechanisms [163, 168]. Indeed, there is an inherent danger of acquiring self-reactivity as a result of DNA mutation within the BCR loci during the GC reaction and AM. However, mechanisms are present within the GC to limit the emergence of "neo-self-reactivity" [169, 170]. Furthermore, the regulation of BCR co-receptors, such as CD19, CD22 and CD21 [171, 172], that modulate the intensity of signal transduction is essential to block the activation of anergized autoreactive B cells (reviewed in [173]).

Recently, Wardemann and colleagues demonstrated the mitigating effects of tolerizing processes in humans by expressing Ig HC and LC rearrangements from single immature, transitional, and mature B cells and determining the frequencies of antibodies that reacted with self-antigens [48, 174]. Wardemann *et al.* determined that 55-75% of antibody expressed by early immature B cells is autoreactive [48], a pattern that holds not only in the BM but for immature cells in peripheral sites as well [175, 176].

However, the processes of tolerance are incomplete as some (~20%) naïve MF B cells express autoreactive receptors [48] and the MZ and B1 B-cell pools are further enriched for self-reactivity. Thus, immunological tolerance purges 30-50% of the potential antibody repertoire of humans. Undoubtedly, the spectrum of antigenic determinants produced by a host organism must overlap, to some degree, with the spectrum of antigenic determinants produced by pathogenic organisms. Therefore, a portion of exogenous antigen recognition must be lost while establishing self-tolerance; whether this loss significantly impacts the host's immunity depends on the frequency and extent to which microbial and host antigens cross react.

AUTOIMMUNITY

Failure of tolerance mechanisms result in organ-specific and/or systemic autoimmunity depending on the nature of the defect [177]. In many cases, B-cell mediated autoim-

mune diseases are characterized by long-lived plasma cells that secrete autoantibody [178]. Furthermore, many autoantibodies are IgGs containing V-gene mutations consistent with SHM and, consequently, have very high affinity for their cognate autoantigen [179, 180]. Autoantibody is a key component in the pathogenesis of several autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) [181] and multiple sclerosis (MS) [182].

RA is a chronic inflammatory disease affecting many tissues, but is primarily targeted to the synovial spaces of joints resulting in the destruction of the cartilage and surrounding soft tissue. Rheumatoid factor (RF), a common autoantibody directed against the Fc portion of IgG, contributes to the pathogenesis of RA. During the initiation of RA, immune complexes of RF and IgG are deposited in the synovial joints of the synovial membrane, eventually resulting in inflammation and tissue destruction (reviewed in [183]). In humans, $CD5^+$ B cells produce most RF present in serum [90], demonstrating that human B1 B cells express autoantibodies similar to their murine counterparts.

SLE is a chronic inflammatory disease that targets connective tissue(s) present in multiple organs, such as skin, lungs, heart and kidneys. There are many factors that contribute to SLE susceptibility including gender, major histocompatibility complex (MHC) haplotype and mutations in complement components (reviewed in [184]). The classification of SLE severity is described using the American College of Rheumatology criteria [185] which measures many parameters including joint swelling, neurologic and hematologic disorders rash and autoantibody tiers. As one of many disorders resulting from SLE, immune complexes are deposited in the kidney resulting in glomerulonephritis and kidney failure [186].

As evidenced by the severity of autoimmune diseases, the immune system must be tightly regulated throughout the progression of an immune response [187]. Homeostatic conditions are compromised when innate cell activation and antigen-specific lymphocyte proliferation occur after pathogen challenge (reviewed in [4]). Additionally, inflammation disrupts BM B-cell development to accommodate for expanded granulocyte production, termed "emergency granulopoiesis" [188, 189]. This process releases developing B cells to peripheral lymphoid tissue where their fate is undetermined [67]; however, these B cells may provide a source of self-reactive clones that eventually arise during chronic inflammation. Clearly, factors that promote B-cell survival, such as enhanced expression of pro-survival molecules, alter B-cell selection and result in autoimmunity [117, 118].

In addition to autoantibody production, B cells serve as efficient antigen presenting cells (APC) to autoreactive T cells in RA [190], MS and type-1 diabetes (T1D) [191]. The expression of surface autoantibody allows B cells to internalize and present autoantigen far more efficiently than other APC.

For some autoimmune patients, the health benefit imparted by humoral immunity is mitigated by the damage done by aberrant autoimmune B-cell responses. To that end, Rituximab is a successful B-cell depleting antibody therapy used to treat autoimmune diseases (SLE, RA and MS) by reducing clinical symptoms and minimizing tissue damage [192]. In addition, other therapeutic interventions that alleviate clinical symptoms of autoimmune diseases target pro-inflammatory cytokines [193, 194], but their mechanism(s) of action is less well understood.

IMMUNE EVASION

Many pathogenic organisms including protozoan parasites, bacteria, and viruses actively avoid immune surveillance to increase their proliferation and dissemination. The evolutionary strategies for this subversion are remarkably diverse; for example, many viruses have evolved mechanisms to suppress MHC expression, antigen processing, endocytosis, expression of immune co-stimulator molecules, apoptosis, cytokine production and signaling, and/or complement dependent cytotoxicity [reviewed in [195]]. Often the mechanism responsible for these suppressive pathways is a viral protein(s) that mimics and subverts the function of a host analogue [195].

Antigenic mimicry has also been suggested as a component – or even the cause – of certain autoimmune diseases by the (inflammatory) rescue of self-reactive B cells normally appropriated for apoptosis or anergy [196]. The rescue of self-reactive B-cell development by virus decorated with host antigen has been demonstrated in transgenic mice [197] and suggested by the strong linkage of autoimmune disease to certain infections. For example, *Trypanosoma cruzi*, the protozoan responsible for Chagas' disease and its characteristic cardiomyopathy, expresses at least three antigens that are cross-reactive with cardiac muscle [198]. Infection with *Campylobacter jejuni* elicits IgG antibody that strongly reacts with both *C. jejuni* LPS and the GM-1 gangliosides abundant at the neuromuscular junctions and nodes of Ranvier. These IgG antibodies induce a neuropathy with conduction block in rabbits and are strongly associated with Guillain-Barré syndrome (GBS) in humans [199]. Additional examples of autoimmune diseases that appear to be induced by microbial antigens include streptococcus-induced carditis, herpes stromal keratitis [200], and lung damage mediated by antibody to corona virus [201].

In contrast to immune cell activation, antigenic mimicry often acts to suppress immune responses to microbial pathogens. Some microbial pathogens subvert protective immunity by mimicking antigens of their hosts, thereby suppressing immune control. For example, in most mice immunized with *C. jejuni* LPS, GM-1 serum antibody titers are low and restricted to the non-pathogenic IgM isotype. However, in mice deficient for GalNAc transferase activity (and, consequently, lack complex ganglioside self-antigens) LPS immunization leads uniformly to high titers of pathogenic αGM-1 IgG and robust memory responses [202].

Immune responses to dengue virus (DENV) are especially complex, as humoral immunity is not only protective but also plays a major role in the pathogenesis of the most severe forms of the disease. DENV, a flavivirus that infects 50- to 100 million annually [203], contains a single-stranded RNA genome that encodes a single polyprotein precursor. This precursor is cleaved into three structural proteins, C (capsid), precursor membrane (prM), and E (envelope), and seven non-structural (NS) proteins, NS1 NS2A and -2B, NS3, NS4A and –B, and NS5 [204]. Primary infections by

one of the four DenV serotypes elicit long lasting, strain-specific humoral immunity (homotypic immunity) and a more transient protection against other dengue serotypes (heterotypic immunity). Humoral protection is largely conferred by neutralizing antibody (NAb) specific for E glycoprotein epitopes although antibodies to prM and NS proteins are also elicited during infection. The most severe forms of DENV infection are linked to the waning of heterotypic immunity when secondary infections may result in dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) characterized by high fever, thrombocytopenia, bleeding disorders, increased vascular permeability, and leakage of intravascular fluid [204]. DHF and DSS are associated with extraordinary viremias resulting from antibody-dependent enhancement (ADE) of heterotypic infections, a process whereby complexes of DENV and non-neutralizing antibody bind to $Fc\gamma R^+$ cells, increasing viral up-take and replication [205]. ADE can be mediated *in vitro* and *in vivo* by low doses of monoclonal NAb; ADE *in vitro* is FcγR-dependent and *in vivo* ADE is abolished by a $C_H2\gamma1$ mutation that disables FcγR binding [205].

This model for ADE viremias as the causative agent of DHF and DSS has been disputed by a recent prospective study showing that enhancing antibody activity (EAA) present in DenV immune plasma does not correlate with the viremia/severity of subsequent, heterotypic infections, indicating the presence of additional factors important in DenV immunopathogenesis [203]. At least one of these factors may be the induction of autoimmune antibody. Several mouse mAb generated to purified NS1 protein cross react with fibrinogen and platelets, bind to human endothelial cells, and induce moderate to severe intraperitoneal hemorrhage [206]. Serum antibody from dengue patients also exhibits rNS1 inhibitable binding to human platelets and endothelial cells and induces endothelial cell death by a zVAD-fmk sensitive mechanism [207-211]. The effects of these crossreactive murine and human NS1 antibodies suggests a potentially significant role in DHF and DSS [212].

HIV-Mediated Disruption of B-Cell Homeostasis

Overwhelming viral replication can result in systemic immune dysregulation and pathogen dissemination. During primary HIV infection, viral load can reach as high as 10^5 to 10^7 copies/ml in peripheral blood [213]. The primary immune response to HIV usually reduces serum viral load within 3 months; however, viral titers can rebound (by 6-12 months) to levels greater than that observed during initial infection in untreated individuals [214].

Early in the HIV epidemic and prior to the advent of effective anti-retroviral therapy (ART), disruption of B-cell homeostasis was commonly observed and initially characterized by hyperactivation and faulty immunoregulation [215-217]. B-cell dysregulation in the presence of HIV infection include: hypergammaglobulinaemia, polyclonal B-cell activation, aberrant maturation, elevated plasmacytosis and enhanced B-cell malignancy formation (reviewed in [2]). Due to chronic antigen stimulation *in vivo* and the lack of additional AFC formation after treatment with B-cell mitogens [217, 218], it is suggested that B cells in HIV-infected patients are "hyper-activated" and spontaneously differentiate to plasma cells without cognate T-cell help. In fact, polyclonal activation of human memory B cells by mitogens and "bystander" T cells can drive short-lived plasma cell differentiation [219], indicating that inflammation circumvents mechanisms that limit cellular activation.

Several reports indicate that HIV infection results in the rapid loss of memory B cells [220, 221], including cells specific for non-HIV-1 antigens [222]. This loss of memory B cells impacts serologic memory to some HIV antigens (*e.g.*, p24) [223] and to pre-existing memory induced by routine childhood vaccination (*i.e.*, measles virus and tetnus toxoid) [222]. This phenomenon has important implications for vertically-infected children, as control of HIV by ART must occur within the first year of life to ensure the establishment of humoral memory to childhood vaccination [224]. These data suggest that HIV affects humoral immune responses by suppressing the induction of *novel* memory cells and reducing *existing* memory B-cell compartments.

While direct interaction of HIV with B cells has been observed [225], sometimes *via* complement-coated viral particles and CD21 [226], HIV-infected B cells are rarely observed in the peripheral blood of patients. These and other observations suggest that persistent HIV infection drives B-cell activation *via* systemic inflammatory signals [1], not through direct infection of B cells by virus. These inflammatory mediators include but are not limited to interferons, TNF-family members and interleukins (reviewed in [2]). Analogous to the mobilization of developing B cells in mice by pro-inflammatory cytokines [188], transitional B cells are expanded in the periphery of HIV-infected individuals [68] and may contribute to another prevalent disorder, the presence of serum autoantibodies [3].

The frequency and distribution of lymphocytes (T- and B-cells) within gut-associated lymphoid tissue (GALT) is severely impacted early after HIV infection [227, 228], further contributing to systemic inflammation by disrupting homeostatic control of commensal flora. By contrast, vaccination of healthy individuals with influenza vaccine only activates influenza-specific memory B cells [229], demonstrating that fundamental aspects of B-cell biology differ between immunization and infection.

AUTOANTIBODY AND HIV INFECTION

Pathogens that are successful in establishing long-term infections promote chronic inflammation, sometimes resulting in granuloma formation and the induction of tertiary lymphoid tissues (reviewed in [230]). The milieu of cytokines present during inflammation can enhance cell survival and contribute to the breaking of B-cell tolerance [4]. Low levels of non-pathogenic serum autoantibody are routinely observed during HIV infection and reports of HIV infection with coincident rheumatological disorders began to appear in the mid-1980s [231, 232].

Depending upon the cohort examined, RF has been observed in 5-60% of HIV^+ patients [233-236]. Reactive arthritis, previously named *Reiter's syndrome*, is initiated by infection of a distal anatomical location (intestines, genitals or urinary tract) that results in arthritic inflammation of large joints, eyes and skin (references within [237]). Not surprisingly, HIV infection can induce reactive arthritis [238] similar to many other pathogens (reviewed in [239]). Whether direct recognition of HIV or the destruction of GALT and

microbial dissemination drives the induction of reactive arthritis remains unclear. Other rheumatic disorders have been described in HIV$^+$ patients including: septic arthritis, myalgias, Sjogren's syndrome, avascular bone necrosis and dermatomyositis.

Anti-nuclear antibodies (ANA) are autoantibodies reactive with various nuclear antigens including DNA, RNA, nuclear pore complex, histones and combinations of these molecular components. ANA are readily detected in systemic autoimmune diseases including SLE, Sjogren's syndrome, autoimmune hepatitis, scleroderma, T1D, polymyositis, and mixed connective tissue disease [240]. In some HIV$^+$ patients (>25%), ANA are present at low, but detectable concentrations (>1:40 titer) in serum [234, 241, 242]. The frequency of HIV$^+$ patients with ANA is much lower than individuals with active SLE disease (~75-85%) but very similar to patients with inactive SLE disease (~10-25%) [243]. Furthermore, the serum titer of ANA in patients with active SLE is far greater (1:100 – 1:10,000) than observed during HIV infection [244]. At present, it is unknown whether B1 B cells contribute to ANA production; however, it is reasonable to speculate that HIV-mediated dysregulation of B1 B cells could contribute to the presence of ANA.

Elicitation of autoantibody against hematopoietic cells and their cellular components has been previously characterized as a part of HIV pathogenesis. Neutropenia, or a lack of neutrophils, has been associated with HIV infection and is present in ~40% of patients with AIDS [245, 246]. Neutrophils are a target of autoantibody which, in association with production defects, contributes to neutropenia [247, 248]. Furthermore, autoantibody has been observed against granule- (elastase, lactoferrin and myeloperoxidase), cytoplasmic- and membrane-associated components of neutrophils [241, 249].

Red blood cell (RBC) and platelet autoantibodies are readily detected during HIV infection [250-253]. The frequency of platelets tends to inversely correlate with the amount of platelet autoantibody in HIV$^+$ patients, suggesting a pathologic effect. Compared to HIV$^-$ controls however, platelet numbers are already significantly reduced in HIV$^+$ subjects, indicating that the presence of anti-platelet antibody is only one of several factors contributing to platelet deficiency.

Anemia, or insufficiency of RBC, is a common hematologic abnormality after HIV exposure and becomes progressively worse in late-stage disease, affecting most patients (60 to 80%) [245, 252]. Currently, it is unclear whether autoantibodies contribute to anemia *via* hemolysis, enhanced phagocytosis and/or antibody-dependant cell-mediated cytotoxicity (ADCC) of RBCs. Other cellular targets of autoantibody during HIV$^+$ infection include: natural killer (NK) cells [254], CD4$^+$ and CD8$^+$ T cells [255-257].

Cellular infection by HIV can alter the lipid content of cell membranes [258, 259]. Anti-lipid autoantibodies arise in many forms and may, in part, represent binding to "neo-self" antigens or newly exposed autoantigens [260]. Anti-cardiolipin antibodies are *i*) present in diseases such as SLE and primary anti-phospholipid syndrome (APS) (autoimmune-derived) [261] and *ii*) result from polyclonal B-cell activation induced by Epstein-Barr virus, leishmaniasis and HIV (infection-derived) [262]. HIV-infected patients (~40%) make anti-cardiolipin antibodies likely as a result of polyclonal B-cell activation. In this regard, Moody *et al.* [291] recently demonstrated that select anti-lipid antibodies from autoimmune patients inhibit binding of CCR5-utilizing HIV-1 to peripheral blood CD4 T cells. This inhibition is mediated by the antibody binding to blood monocytes and inducing their production of CCR5-blocking chemokines. In this example, a neutralizing antibody acts without binding the virion [291]. Whether autoimmune disease patients with defective tolerance make broadly neutralizing, envelope antibodies after infection with HIV-1 remains unknown.

Research suggests that protective, HIV-neutralizing antibodies (NAb) inhibit viral entry into target cells through interaction of both the lipid membrane and proteins of virus [263, 264]. Furthermore, there is evidence that the normal formation of membrane microdomains, such as lipid rafts, is required for efficient HIV infection [265, 266]. As such, novel anti-cholesterol antibodies have been used to limit viral replication by preventing "the proper spatio-temporal juxtaposition of HIV-1 glycoproteins with CD4 and chemokine receptors, thus negatively interfering with virus attachment/entry" [267]. Therefore, it is reasonable to study these lipid-reactive antibodies identified in HIV-1 infected patients as precursors to efficacious anti-HIV envelope antibody responses. It is important to recognize that not all anti-lipid antibodies are pathogenic as anti-lipid antibodies are thought to serve as critical mediators of cholesterol homeostasis [268].

THE TOLERANCE HYPOTHESIS

Exploitation of immunological tolerance by viruses and other human pathogens in order to mitigate humoral immunity can be detected by comparing the antibody repertoires of B-cell populations before and after their tolerization. If the antibody repertoires for self- and microbial antigens do not substantially overlap, then the extensive losses of autoreactive cells typical of B-cell maturation [48, 176, 269] will not diminish the numbers of B cells specific for viral, bacterial, etc. antigens (in fact, it will increase their relative frequencies). On the other hand, if pathogens commonly express antigens that cross-react with their host, self-tolerance will also reduce the size and diversity of the antibody repertoire to these foreign determinants (Fig. **2**). Indeed, the frequency of neutralizing antibodies reactive to broadly conserved portions of Influenza hemagglutinin (HA) is extraordinarily low (~10^{-9}) [270]. Future experiments should test if this rarity results from the induction of B-cell tolerance in the progenitor pool that is reactive to these conserved regions of HA.

HIV-1 may offer another example of antigenic mimicry as a protective adaptation [5], indicating an immunological advantage for retaining a limited pool of mildly self-reactive lymphocytes. Protective antibodies that neutralize multiple HIV-1 clades have been identified but are rare [271]. HIV-1 infection commonly induces primarily antibodies that are non-neutralizing or are against highly variable envelope regions that result in restricted type- or isolate-specific neutralization [6]. Many theories have been offered for the scarcity of HIV-1 NAb, including the complex nature and genetic plasticity of HIV epitopes, the shielding of crucial antigen sites by glycosylation, competitive suppression by

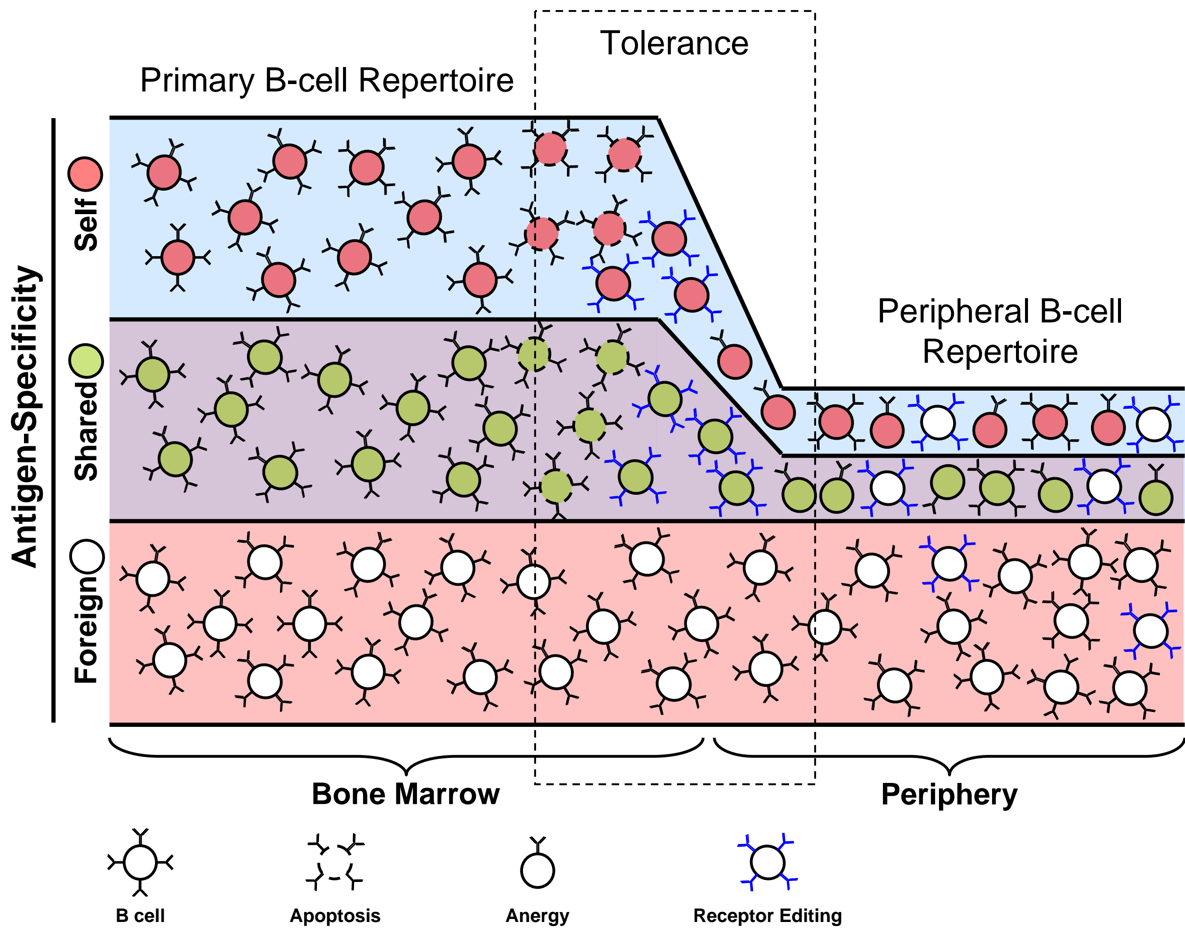

Fig. (2). Mechanisms of tolerance block the development of B cells that bear receptors specific for cross-reactive antigens present on microbes and host tissues. The primary B-cell repertoire is composed of cellular clones specific for self-antigens (*red circles*), foreign or non-self antigens (*white*), and antigen subsets that are present on both foreign-, *e.g.* microbial, and self structures (*green*). During development, tolerance mechanisms (*boxed area*) limit the maturation of B-cell clones that react with "self-" and "shared" antigens, whereas B cells specific for foreign determinants are not affected. The fates of these two classes of self-reactive B cells include *i*) apoptotic deletion (*broken lines*), *ii*) induction of anergy (*single receptor*) or *iii*) receptor editing (*blue receptors*). Therefore, the frequencies of B cells specific for "self"- and "shared-antigens" are minimal in the mature, peripheral B-cell compartments compared to those B lymphocytes specific for foreign antigens.

highly immunogenic but non-neutralizing surface antigens, and insufficient diversity in the primary repertoire of Ab specificities [reviewed in [271]].

Although each of these arguments is plausible, the recent finding that most HIV-1 NAb react with host antigens offers an alternative explanation for their rarity [5, 272]. These recent reports indicate that the broadly neutralizing anti-HIV antibody repertoire may be lost due to cross-reactivity to self-antigens [5, 272], *via* the induction of B-cell tolerance. Two antibodies, 2F5 and 4E10, neutralize HIV's ability to infect cells and show considerable binding to HEp-2 cells, a measurement commonly used to show ANA reactivity. These antibodies also bind other self-antigens such as cardiolipin and phosphotydlserine (PS).

Recent demonstrations that most HIV-1 NAb exhibit weak but crucial binding to host membrane antigens [273-275] add additional support to the hypothesis that the HIV Nab repertoire might be lost as a consequence of B-cell tolerance mechanisms. Indeed, a 2F5 Ig heavy chain "knock-in" (2F5 HC-KI) mouse line was recently generated to determine whether 2F5 HC encoded receptors are sufficiently cross-reactive to activate B-cell tolerance mechanisms [276]. Verkoczy *et al.* [276] clearly demonstrate that expression the 2F5 HC elicits a developmental blockade that prevents the maturation of small pre-B cells to immature B cells, a defect first observed for B cells expressing receptors reactive with DNA- or MHC I [166, 277]. This demonstration is unequivocal proof that at least one broadly neutralizing, human antibody for HIV-1 is sufficiently cross-reactive to self-antigen(s) to elicit immunological tolerance.

Therefore, the prediction is that most B cells specific for HIV-1 epitopes that also react to self-antigens will be subject to tolerization during development. Given that many HIV-1 infected patients and vaccinees generate non-neutralizing antibodies [6], the hypothesis is that these antibodies - unlike the rare, broadly neutralizing antibodies - may not exhibit substantial autoreactivity and do not invoke B-cell tolerance.

Recent longitudinal studies have monitored the emergence of neutralizing autologous and heterologous antibody responses in recently HIV-infected individuals [278-280].

Early after infection (~3-6 mo), strain-specific neutralizing antibodies can be formed to reduce viral burden; however, these antibodies rarely contain heterologous neutralizing capacity. After brief interruption of ART, viremia rapidly ensues with a concomitant autologous antibody response that eventually limits viral replication [281]. Unfortunately, HIV usually evolves rapidly to circumvent neutralization, indicating that NAb exerts selective pressure(s) that the virus is well equipped to avoid. This is precisely why novel immunogens must elicit NAb responses to conserved regions of HIV that cannot easily be modified without severe repercussions to replication "fitness".

In the infrequent instance that broadly NAb are formed, it is only after months (10-20) of chronic HIV-1 infection, antigen exposure and induction of autoantibody [278]. Unfortunately but expectedly, patients that make Nab after viral reservoirs have been established do not control viral load [278], since humoral immunity is not effective for clearing intracellular pathogens.

The MZ and B1 B cell compartments are candidates to target for efficacious HIV vaccine design as both types of B cells are enriched for self-reactive clones compared to MF B cells [282], the normal targets of B-cell vaccines. The retention of some autoreactive B cells and the conservation of the MZ and B1 B-cell compartments may represent an "evolutionary compromise" for the capacity to respond to microbial antigens that are structurally similar to those of the host. Expansion of these B-cell pools or the utilization of autoreactive strains of mice may provide insight into HIV humoral immunity upon immunization. Alternatively, whether the inflammatory release of developing, immature B cells to the periphery increases the "precursor pool" for rare broadly neutralizing B cells should be explored.

SLE and HIV Infection

As noted, a number of rheumatological disorders can arise after the establishment of a chronic HIV infection. Presumably, these autoimmune syndromes are the result of T- and B-cell dysregulation and it may be that these late events are important in understanding the complex biology of HIV-1 infection. We note that the occasional production of broadly reactive NAb comes only after months (10-20) of chronic HIV-1 infection, antigen exposure and induction of autoantibody [278]. Could it be that the immune dysregulation that follows HIV-1 infection creates conditions that are permissive for both autoreactivity and NAb production?

The tolerance hypothesis predicts that individuals with active humoral autoimmunity might have a lower incidence of HIV infection. SLE is understood to be a disease of impaired early B-cell tolerance [283] and if developing NAb B cells are lost to immunological tolerance [276], then NAb B cells will be more frequent in SLE patients. It follows that patients with active SLE may contain antibodies that provide a degree of protection from HIV.

Several investigators have described a lower-than-expected frequency of coincident SLE and HIV-1 infection and suggest this is related to the spectrum of antibodies that SLE patients can make [284]. As of 2004, only 32 cases of combined SLE and HIV-1 disease have been reported [285, 286]. Of the 32 SLE/HIV-1^+ individuals, only 21 cases met full diagnostic criteria for SLE, suggesting that coincidence of SLE and HIV infection is even more rare. Based on the prevalence of each disease, this observed frequency of SLE/HIV-1^+ patients is much lower (<10%) than the expected frequency of coincidence that was predicted (~400 cases) in the early 1990's.

Production of broadly Nab by HIV-1^+ autoimmune disease patients would provide strong evidence for the suppression of NAb by B-cell tolerance mechanisms. Prospective studies in autoimmune patients (SLE and APS) have been initiated to *i*) determine the incidence of HIV-1 infection and *ii*) characterize anti-HIV-1 NAb that are elicited after HIV-1 infection (Moody, MA, Haynes, BF, unpublished). Furthermore, the presence or absence of 2F5 and 4E10-like B cells are being determined using gp41 antigen-loaded liposomes and B-cell tetramers to identify and determine the frequency of HIV-reactive B-cell populations. If present, it will be informative to determine their B-cell subset of origin (MF, MZ and/or B1) as potential targets of future B-cell vaccine design. However, if either HIV-1 infected autoimmune patients or autoimmune animal models do not make broadly NAb, then other mechanisms that control these antibody species will have to be explored.

It should be noted that the elevated level of IL-16 in SLE patients has been suggested as the mechanism behind reduced HIV infection [287], since IL-16 has previously been reported to limit viral infection [288] and viral promoter activity [289]. Our future studies will examine the incidence of HIV-1 within multiple cohorts of patients with SLE and other autoimmune diseases (RA, MS and/or Sjogren's) to determine whether autoimmunity limits infection with HIV.

In addition to the 2F5 HC-KI mice, the tolerance hypothesis can be studied in mouse models since many autoantigens are conserved throughout phylogeny. We have determined that HIV-1 MPER-specific B cells appear to be differentially regulated in both normal (BL/6 and BALB/c) and autoimmune (MRL/$lpr^{-/-}$) mice ([290] and unpublished results Verkoczy and Haynes). For example, antibodies that target the 2F5 gp41 epitope, but not the 4E10 peptide epitope, are constitutively produced in MRL/$lpr^{-/-}$ mice. After cloning, sequencing and re-expression of 2F5-epitope reactive antibodies, many questions can be asked about the capacity for viral neutralization, bias in V(D)J usage in IgH and Igκ/λ and whether these Abs are cross-reactive with host cellular antigens.

SUMMARY

In conclusion, B-cell development in mice and humans share many fundamental aspects that promote the generation of a diverse repertoire of antigen-specific lymphocytes. Mechanisms of B-cell tolerance that limit autoimmunity can purge up to half of the initial BCR repertoire and, as a consequence, remove some portion of foreign antigen-reactive lymphocytes. Obviously, many microbial antigens are unique and, consequently, highly immunogenic. On the other hand, antigenic similarities do exist as evidenced by the capacity of some infections to elicit autoimmunity. Many mechanisms are used by pathogens to subvert immune rec-

ognition, but complete understanding of how HIV-mediated B-cell dysregulation occurs *in vivo* requires further investigation. The "tolerance hypothesis" as a mechanism to subvert neutralizing B-cell responses has been supported by *i*) a lower frequency of HIV infection in SLE patients and *ii*) the loss of 2F5 HC-KI B-cell development in the BM. Regardless of whether the immune system has exerted selective pressure on HIV-1 or this antigen mimicry is a random phenomenon, future vaccine strategies may need to account for these observations to promote robust B-cell responses against HIV.

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