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Autoreactive B Cells in SLE, Villains or Innocent Bystanders?

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SUMMARY

The current concepts for development of autoreactive B cells in SLE focus on extrinsic stimuli and factors that provoke B cells into tolerance loss. Traditionally, major tolerance loss pathways are thought to be regulated by factors outside the B cell including autoantigen engagement of the B cell receptor (BCR) with simultaneous type I interferon (IFN) produced by dendritic cells, especially plasmacytoid dendritic cells (pDCs). Later, in autoreactive follicles, B cells encounter T follicular helper cells (Tfh) that produce interleukin (IL)-21, IL-4 and pathogenic cytokines, IL-17 and IFN gamma (IFNɣ). This review discusses these mechanisms and also highlights recent advances pointing to the peripheral transitional B cell stage as a major juncture where transient autocrine IFNβ expression by developing B cells imprints a heightened susceptibility to external factors favoring differentiation into autoantibody producing plasmablasts. Recent studies highlight transitional B cell heterogeneity as a determinant of intrinsic resistance or susceptibility to tolerance loss through the shaping of B cell responsiveness to cytokines and other environment factors.

Keywords

SLE; lupus; BXD2; autoantibodies; autoimmunity; IFNβ; type I IFNs; lymphotoxin beta; IL-17; IL-21; regulator of G-protein signaling; transitional stage 1 B cells; marginal zone-precursor B cells; germinal center B cells; plasmacytoid dendritic cells; marginal zone macrophages; follicular T-helper cells

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“Look like the innocent flower, but be the serpent under it.”

William Shakespeare ~

1 INTRODUCTION

Autoreactive B cell development in SLE is often considered in terms of the extrinsic stimuli and factors that drive B cells into autoantibody production. This review focuses on the novel concept that autoreactive B cells are initially programmed at the transitional stage for heightened susceptibility for development into autoantibody secreting plasmablasts (Figure 1). During peripheral B cell development in the spleen, transitional B cells expressing elevated levels of endogenous intracellular interferon-β (IFNβ) exhibit enhanced survival

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CONFLICT OF INTEREST

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and activation through the B cell receptor (BCR), a feature associated with more active SLE, particularly in African American (AA) patients[1]. Following passage through the transitional stage, a second major defect is the failure of B cells to maintain the integrity of tolerogenic splenic marginal zone macrophages (MZM), which normally function to remove apoptotic cell debris in a non-inflammatory fashion through upregulation of indoleamine-pyrrole 2,3-dioxygenase (IDO)[2]. This failure is amplified by the type I IFN-driven migratory properties of marginal zone-precursor (MZ-P) B cells as they lose their tethering to sphingosine-1-phosphate (S1P) and drift across the follicular exclusion barrier[3–8]. Here, they act as potent antigen-carrying, costimulatory cells expressing inflammatory cytokine IL-6 and low levels of the anti-inflammatory cytokine IL-10[9]. In addition, Ag transporting MZ-P B cells express high levels of membrane lymphotoxin β (mLTβ) that can potently stimulate follicular dendritic cells (FDCs) to initiate germinal center (GC) formation[5,6,10]. A third B cell defect occurs during the T-dependent GC or extra GC stage of development in which B cells respond not only to IL-4 and IL-21, but also upregulate IL-17 receptor A (IL-17RA) and type II interferon-$_{\gamma}$ receptor (IFNγR). Simulation by IL-17 on IL-17RA^{+} B cells induces up-regulation of regulator of G-protein signaling (RGS)13 and RGS16, leading to prolonged retention in the GC, upregulation of activation-induced cytidine deaminase (AICDA), and migratory behavior that can prolong the interaction between follicular T-helper cells (Tfh) and GC B cells[11–15]. Alternatively, upregulation of IFNγR in the presence of IFNγ producing Tfh (Tfh-IL-IFNγ) can lead to T-bet^{+}CD11c^{+}CXCR5^{-}IgD^{-}CD27^{-} double negative 2 (DN2) autoreactive B cells[16–20]. This review will highlight the high degree to which autoreactive B cells both directly and indirectly self-regulate their development through autocrine cytokine signaling during development and promoting the disintegration of critical tolerance barriers. This creates an environment ripe for pathogenic T cells to drive differentiation of B cells into pathogenic autoAb production.

2 PRE-GERMINAL CENTER STAGE B-CELL AUTOREGULATION

2.1 Control of the Pre-Immune Repertoire

B cell development in the bone marrow occurs in a progressive series of events leading to the assembly, expression, and signaling of the B cell antigen receptor (BCR). During early B cell genesis, a cascade of microenvironmental and stromal-derived signals including IL-7 induce the rearrangement of the immunoglobulin (Ig) locus and surface expression of a pre-B cell receptor comprised of an IgM heavy-chain with a surrogate light-chain, which does not efficiently bind antigen[21–23]. B cells then activate the light-chain locus and express a bonafide light-chain gene that enables production of a mature BCR capable of binding antigen. Tonic signaling through the BCR at this stage promotes positive selection, while high-affinity immunoglobulin binding to self-antigen triggers apoptotic cell death, contributing to the elimination of highly self-reactive and polyreactive B cells[24,25]. Autoreactive B cells can be rescued for survival through induced alteration of the structure and specificity of their antigen receptors, resulting in a less self-reactive immunoglobulin receptor, a process known as receptor editing[26,27]. B cells that are not clonally deleted leave the bone marrow as transitional B cells and migrate to the peripheral lymphoid organs. Studies by Nussenzweig and co-workers[28] have shown that approximately 75% and 60% of bone marrow emigrant B cells are HEP-2 autoreactive or polyreactive, respectively, but this

is reduced to approximately 40% and 7% as B cells transition from the immature new emigrant stage to the mature naïve stage within lymphoid organs.

In addition to clonal deletion and receptor editing, developing B cells that encounter low avidity self-antigen also may enter into an anergic state. Early studies using the HEL-anti-HEL mouse system by Goodnow and colleagues demonstrated that strong BCR crosslinking by membrane-bound HEL resulted in clonal deletion at the early transitional of peripheral B cell development[29,30]. In contrast, lower affinity and sustained signaling through the BCR by soluble HEL resulted in induction of B cell unresponsiveness or anergy, as defined by unresponsiveness to antigen stimulation[29,30]. Anergic B cells exhibit impaired signaling, proliferation and differentiation to antibody-secreting cells after antigen receptor stimulation either during immunization with exogenous HEL or in response to the innate Toll-like receptor (TLR) agonists CpG-containing DNA and lipopolysaccharide[31,32]. Mechanistically, Cambier and colleagues have shown that anergic B cells exhibit impaired $Ca2^{+}$ mobilization and impaired phosphorylation events downstream of BCR crosslinking in part by recruitment of phosphatases to the BCR apparatus which counteracts strong BCR signaling[33]. Adoptive transfer of anergic B cells with subsequent induction of gene expression further showed that the inhibitory signaling pathways involved a tyrosine phosphatase SHP-1 and inositol phosphatase SHIP-1, which are both required to maintain anergy[34]. Activation events that disrupt anergy leads to rapid cell activation, proliferation and short-lived plasma cells that reside in extra-follicular foci[34]. Previous studies by Goodnow and co-workers showed similar anergy loss in mice lacking Lyn, SHP-1 and CD22. The mechanistic basis was found to be phosphorylation of CD22 by Lyn, which then recruits SHP-1 to the CD22/BCR complex[35]. More recently Dorner and Lipsky have proposed that the anti-B cell therapy Epratuzumab binds CD22, interfering with CD22 function and BCR signaling.

In addition to direct modulation of BCR structure and function, other extrinsic and intrinsic factors can affect activation, survival and development of autoreactive B cells. The transitional T1 stage in the periphery has been identified as a major tolerance checkpoint where autoreactive B cells are highly susceptible to clonal deletion. Following bone marrow selection, the earliest B cell stage to enter the circulation in humans is defined as $CD10^{+}CD24^{Hi}CD38^{Hi}CD27^{Neg}$ [37]. The most immature peripheral T1 stage is distinguished by high levels of surface IgM, but low surface IgD[38–41]. In mice, T1 B cells exhibit a similar phenotype and are commonly classified as $CD93^{+}IgM^{Hi}IgD^{Lo}CD23^{Lo}$. These immature T1 B cells from the BM percolate into the spleen marginal sinuses and red pulp where they must migrate along chemokine and integrin-mediated signals for positive selection into the T-cell-rich periarteriolar lymphoid sheath (PALS) of the white pulp[42,43]. During this process, BCR-mediated negative selection serves to remove self-reactive B cells that encounter strong BCR stimulation[44], and the remaining T1 B cells acquire IgD and CD23 expression and become T2 B cells, which lose sensitivity to negative selection and become responsive T-cell help[43,45]. T1 B cell negative selection is consistent with their inability to induce c-Myc, cyclin D2, BclxL and A1 following BCR stimulation[46,47]. Positive selection also occurs at the T1 stage, as suggested by the phenotypes of mice deficient in various components involved in BCR signal transduction. Syk-deficient mice, for example, exhibit a developmental block at the T1 stage[48]. In general, weak BCR signaling is thought to favor

positive selection, whereas strong BCR signaling encountered by autoreactive clones leads to negative selection.

2.2 T1 B cell regulation by autocrine/paracrine IFNβ

Autocrine/paracrine-acting regulatory cytokine production in B cells is often associated with a transitional B cell phenotype[49–51]. Consistent with this, the transitional B cell stage has been associated with outcomes in various immunological settings, including allograft survival, tumor suppression responses and progression of autoimmune responses[51–54] and thus appears to be a critically important developmental checkpoint that determines the fate and function of peripheral B cells. In both SLE and RA patients, the transitional B cell checkpoint is characterized by a failure to exclude new autoreactive BM emigrant B cells from the naïve pool[41,55,56], underscoring the importance of identifying intrinsic and extrinsic factors that modulate T1 B cell survival in SLE. Among the potential mediators of T1 B cell survival, BAFF signaling has been a major subject of studies and clinical trials due to its critical role in B cell survival. However, the earliest $IgM^{hi}IgD^{lo}$ T1 B cell stage manifests low BAFF responsiveness, and BCR- and BAFF-mediated signals are not well integrated at this stage[57,58]. belimumab treatment in human SLE did not affect T1 B cell frequencies or numbers, but led to dramatic reductions in T2, T3 and mature B cell subtypes[59] consistent with studies in mice in which T1 B cells are largely unaffected by BAFF manipulation[60,61]. It remains unclear whether BAFF promotes T1 progression to the T2 stage in vivo. However, several lines of evidence clearly pin-point the T2 stage as the stage where productive BAFF signaling begins. At the T2 B cell stage, BAFF-R expression is dramatically upregulated and associated with preferential responses to BAFF in vivo[61,62]. Spleen T1 B cells in contrast express low levels of BAFF-R and are not expanded in BAFF transgenic mice nor in mice administered exogenous BAFF[63]. Together, available data suggest BAFF is not the main survival factor for immature T1 B cells arriving the spleen.

We recently showed that transitional T1 B cells possess a propensity for type I IFN production and responses, including a primary role for high-affinity IFNβ. In contrast to BAFF-R, IFNAR1 is highly expressed on both T1 and T2 B cells and is down-regulated on mature B cells[61]. Similarly, the highest levels of type I IFN transcripts, including *Ifna4*, *Ifna7* and *Ifnb*, are observed in T1 B cells with subsequent down-regulation in mature MZ, FO and GC subsets, suggesting that transitional B cells are poised for responses to autocrine and/or paracrine type I IFN signaling. Single cell gene expression analysis of type I IFN and IFN response genes (IRGs) along with developmental markers *Cd93* and *Cd23* revealed distinct gene expression patterns in T1 B cells suggesting a model where autocrine IFNβ signaling leads to an ordered unfolding expression of genes, including *Tlr7*, other type I IFNs and IRGs during the T1 to T2 progression[61]. This is reminiscent of the priming role that IFNβ can elicit in other cell types, whereby low levels of IFNβ leads to the accumulation of antiviral factors enabling rapid amplification of subsequent antiviral responses[64]. Indeed, T1 B cells isolated from $Ifnb^{+/+}$:$Ifnb^{-/-}$ BM chimeras revealed impaired expression of *Tlr7*, *Tlr3*, *Ifna1*, *Ifna7* and *Pkr* in $Ifnb^{-/-}$ T1 B cells, and specific neutralization of IFNβ impaired TLR7 responses in transitional B cells[61]. This action of type I IFNs at the transitional B cell stage is notable, in that it provides a link between the reduced stringency of counter-selection at this early stage[58] with later development of

autoAbs specific for DNA, RNA and associated nuclear autoAg[65,66]. Within the same environment, IFNβ deficient B cells were less able to survive and develop into GC B cells, compared to IFNβ competent B cells (Figure 2).

Examination of type I IFN production in circulating B cell subsets from SLE patients revealed expression of multiple type I IFNs, including IFNβ, beginning with the transitional B cell stage[1]. Interestingly, single cell gene expression studies in circulating SLE transitional B cells revealed distinct subpopulations, including type I IFN producers, IFN responders, and mixed IFN producer/responder clusters[1]. At the protein level, increased B cell IFNβ levels were positively associated with renal disease, anti-DNA and anti-Sm autoantibodies and African American race[1]. The transcription factor ARID3a which regulates accessibility of the IgH enhancer region was recently associated with increased expression of IFNα in transitional and naïve B cells from SLE patients[67]. Other potential candidates include components of the IFNβ enhanceosome, such as IRF7 and other *trans*-acting regulatory factors, which have been previously implicated in SLE[68]. A challenge for future studies will be identification of genetic and non-genetic factors promoting aberrant endogenous type I IFN production by SLE B cells.

2.3 Pathogenic effects of pDCs

pDCs are often considered the principal source of type I IFNs *in vivo*; however it is becoming clear that production of type I IFN, especially the rapidly induced high-affinity IFNβ, is accomplished a variety of cell types and cannot be mainly attributed to pDCs[69]. Our studies and others similarly suggest that pathological type I IFN production in SLE is not limited to pDCs[1,67,70–72]. pDCs have long been prime suspects owing to a large body of literature confirming their high capacity for type I IFN production following viral infection or stimulation with viral nucleic acid mimics[73]. Indeed, injection of Poly(I:C) into BXD2 mice induces pDCs to produce >10 fold more IFN transcripts compared to other cell types. However, BXD2 mice develop type I IFN-dependent autoantibodies targeting DNA and nuclear Ag in the absence of exogenous pDC stimulation, further suggesting additional complexity in type I IFN dysregulation in SLE. Interestingly in humans, pDC *in vitro* depletion studies have shown that while pDCs are responsible for the majority IFNα produced by healthy PBMCs, they account only for 57% of IFNα produced by SLE PBMCs[70], further supporting that pathological IFNs in autoimmune disease derive from additional cell types. Such complexity is also implicated by observations that type I IFN signaling is not concordant across immune cell populations of an individual patient and tends to vary among patients[74].

The cellular sources of type I IFN production depend on many factors, including the nature and multiplicity of the stimulus, temporal factors, as well as genetic factors[69,71]. In human studies, pDCs clearly have a significant presence in cutaneous lupus[75] and are increased in tissues in later stages of SLE where immune complexes are available for deposition and stimulation[76,77]. Autonomous IFNβ production by T1 B cells developing within the MZ microenvironment may prime surviving autoreactive clones for subsequent responses to other sources of IFNα and nucleic acid sensing[64], thus “imprinting” heightened antiviral-like responses onto the B cell compartment. Further interrogation of mechanisms associated

with T1 B cell development and cytokine production, including single-cell analyses of molecular features and BCR usage will be necessary to obtain a better understanding of this critical subset.

2.4 The IFNβ-TLR7-IFNα loop of autoreactive B-cell development

Priming by IFNβ is especially important in the regulation of TLR7 responses[61,78]. Intracellular nucleic acid sensors are important in the pathogenesis of SLE, and TLR7 in particular is associated with the development of antibodies targeting RNA associated antigens in SLE[79,80]. Similarly, in autoimmune BXSB and TLR7 transgenic mice, overexpression of TLR7 causes severe lupus and antibodies to RNA-containing complexes[81,82]. TLR7 stimulation of B cells from wild type C57BL/6J is known to induce upregulation of type I IFNs including IFNβ, which has a pro-inflammatory role to promote TLR7 responses[78]. Our analyses of *Ifnb*$^{-/-}$ B cells extended this initial finding and further showed that B cell endogenous IFNβ and autocrine signaling is required for *Tlr7* and *Ifna* gene induction in T1 B cells[61]. Experiments in chimeric mice revealed that B cell endogenous IFNβ exhibited an intrinsic survival advantage for B cells, where although IFNβ$^{+/+}$ and IFNβ$^{-/-}$ B cells localized to the same regions of the spleen, IFNβ$^{+/+}$ B cells survived to the mature and GC B cell stage in greater numbers (Figure 2)[51]. Functionally, genetic deficiency of IFNβ resulted in impaired transitional B cell upregulation of CD69 and CD86 in response to TLR7 stimulation *in vitro*, suggestive of an autocrine/paracrine feedback loop in transitional B cells[61]. Similar mechanisms of transitional B cell autocrine regulation have been observed in human transitional B cells, where transitional B cell produced IL10 resulted in CD86 down-regulation which functionally reduced T cell responses. Future studies will be necessary to determine if SLE non-responders to IFNAR blockade treatment is due to an intrinsic survival advantage that results from endogenous IFNβ[83].

2.5 IFNα vs. IFNβ

There are 13 distinct type I IFN species in mice and humans, all of which bind the same heterodimeric receptor composed of IFNAR1 and IFNAR2, as distinct from receptors for type II and type III interferons[84]. SLE is highly associated with a type I IFN stimulated gene (ISG) signature[85], but little is understood regarding the specific species of IFN subtypes underlying the signature. Recently gene expression analyses identified the potential roles of multiple interferons in SLE, including prominent expression of IFNβ-specific signatures[86]. The majority of type I IFN studies in SLE have focused predominantly on the effects of IFNα as the main driver of lupus pathogenesis. This focus on IFNα is partially due to differences in the relative abundance of the type I IFN subtypes, where some IFNα subtypes are more readily detectable in circulation vs. IFNβ[87–89]. Consistent with this, the induction of IFN-response genes *PRKR*, *IFIT1*, *IFI44*, *MX1* in WISH cells by SLE plasma was inhibited >90% by anti-IFNα antibody, but not by anti-IFNβ or anti-IFNγ antibodies suggesting that IFNα is the dominant IFN in many SLE plasma samples[90]. In addition, a positive correlation between serum IFNα and various disease parameters has also been established[91]. However, these studies have been limited by an inability to detect IFN species less abundant in circulation including IFNβ. The relative lack of detectable IFNβ in serum is consistent with its much higher affinity for cell-surface bound IFNAR1 and IFNAR2, as well as a lower absolute abundance[92]. Functionally, the higher affinity of IFNβ for IFNAR1 and

IFNAR2 increases the efficiency of ternary complex formation and stability, especially at low receptor surface concentrations[92,93]. Indeed, the regulation of IFNAR1 and IFNAR2 expression levels is a major mechanism controlling the potency of cell responsiveness to IFNβ[94]. Several studies have demonstrated that IFNβ induces endocytosis and degradation of IFNAR1 to regulate the cell signaling[92,95,96]. Down-regulation of IFNAR1 appears to occur rapidly in a tunable fashion, while IFNAR2 expression displays a slower basal turnover[97]. Additionally, both IFNAR2 and IFNAR1 can interact with IFNβ leading to signal transduction and changes in gene expression (IFNβ/IFNAR1 and IFNβ/IFNAR2)[98], a property that may explain differences in the response to IFNβ treatment[94].

2.6 What drives transitional B cell IFNβ production?

The driving mechanisms behind increased type I IFN production in the absence of detectable chronic viral infection is a long-standing and critical question in SLE, as precision targeting of these mechanisms will likely be prerequisite for therapeutic success (Figure 3A). As with most features of SLE, elucidation of these mechanisms is challenging due the complex contributions of genetic and environmental factors as well as inter-patient heterogeneity. Some evidence suggests that the aberrant transitional B cell type I IFN production observed in SLE may have earlier origins incipient during development in an inflammatory BM niche. IFNβ has been shown to promote autoreactive IgM expression by developing B cells in the BM by altering stringency of negative selection[65,66]. More recently, elevated IFNβ expression in bone marrow–derived mesenchymal stem cells from SLE patients was associated with increased production of reactive oxygen species and expression of mitochondrial antiviral signaling protein (MAVS), suggesting cell intrinsic mitochondrial dysfunction and type I IFN signaling in the BM hematopoietic microenvironment[99]. Increased oxidative stress and decreased glutathione (GSH) levels are frequently associated with SLE[100,101] and are implicated as a trigger for spontaneous MAVS oligomerization and type I IFN production by circulating B cells, T cells and monocytes from SLE patients[102]. These mechanisms are associated with increased disease activity, as both oxidative stress and increased plasma levels of IFNβ have been associated with impending disease pre-flare[103,104].

A second and complimentary model holds that selection pressures due to exposure to pathogens endemic to Africa may account for increased type I IFN production and responses in the AA population. Although studies are limited, there are reports that SLE incidence is lower in Africa compared to North America and genetic studies have demonstrated the impact of genetic admixture on SLE risk[105–108]. Other population-level studies have shown that genetic variants across populations play a major role in shaping an individual's immune response[109], including variability in cytokine responses to various stimuli[110–112]. Extensive studies into the genetic associations in SLE identified that aberrant regulation of innate and adaptive immunity including increased type I IFN is a genetically conferred risk factor for development of SLE[113,114]. As B cell IFNβ was significantly increased in AA patients, this further suggests that polymorphisms in the IFNβ enhanceosome genes or other upstream genes may predispose some patients to dysregulated IFNβ production[1]. Future studies will be needed to determine if increased B cell endogenous IFNβ in African American patients can be a useful predictor of predisposition to development of SLE.

The IFNβ promoter contains four positive regulatory domains (PRD I-IV), which are occupied by overlapping transcription factor complexes[115]. IRF3 and IRF7 bind PRDI and III; the AP-1 heterodimer of ATF-2 and c-Jun binds PRDIV; and the NF-κB p50-RelA heterodimer binds PRDII[115]. Both IRF3 and IRF7 are critical for type I IFN induction and amplification following viral infection and activation of cytosolic nucleic acid sensing pathway (Figure 3A)[116]. As such, genetic variation in such loci regulating cytokine release could affect expression of type I IFNs and IFN signature genes as commonly observed in SLE. Indeed, the *IRF7* locus has been identified as a trans-response expression quantitative trait locus (*trans*-reQTL) that regulates expression of multiple type I IFN genes following influenza expression[117], and several studies have indeed identified variations in the IRF7 gene that confer elevated activation of IRF-7 and predisposes to the development of SLE[68,118]. Recent population level studies found marked differences between transcriptional immune responses in individuals of African vs. European ancestry[109,119] and showed that genetic variants including a regulatory hotspot in the *IFNB1* gene itself explain a substantial proportion of these population differences, concluding that adaptation and a genetic admixture shaped the immune responses of human populations[109]. Increased colocalization of NF-κB p65 and IRF7 has been identified in the nucleus of B cells derived from BXD2 mice (Figure 3B). Future studies will be needed to further delineate the multiple independent interacting loci and molecular mechanisms that account for aberrant type I IFN production and tolerance loss in SLE.

3 BREAKDOWN OF THE FOLLICULAR EXCLUSION BARRIER

3.1 Follicular migration of autoreactive B cells

Regulation of B cell migration into follicles, termed “follicular exclusion,” represents a second level of pre-GC B cell tolerance[120], as B cell access to follicles is associated with entry into the long-lived repertoire of re-circulating B cells. Developmentally, B cell potential for follicular migration is concurrent with progression beyond the T1 stage[45]. B cells that survive T1 negative selection progress to the T2 stage characterized by CD93, IgM and CD23 co-expression and regulatory properties. T2 cells expressing a high level of CD21 ($CD21^{hi}$) and can further differentiate into MZ or FO B cells[53]. The MZ-P stage, an intermediate subset between T2 and MZ B cells, is marked by heightened responsiveness to BCR signals, T cell help, cytokines and signals promoting entry into the B cell follicle[5,45]. Phenotypically, MZ-P B cells are distinguished by expression of both the MZ B cell marker, CD21 (complement receptor 2), and the follicular B cell marker, CD23 (high-affinity IgE receptor) and can be further defined as $CD93^{neg}CD1d^{hi}IgM^{hi}$ $CD21^{hi}CD23^{hi}$.

In the spleens of BXD2 mice, MZ-Ps are notable for their presence within the follicle and their extensive migratory properties[5] (Figure 4). Normally, MZ B cell localization is under the influence of local levels of the red blood cell product sphingosine 1-phosphate (S1P) which stabilizes S1P receptor ($S1P_1$)-positive MZ B cells in the spleen MZ region[3]. Cyster and colleagues found that type I IFN can rapidly down regulate the $S1P_1$ leading to follicular inward migration of MZ B cells[121]. In the context of autoimmunity, we have shown that two key cell types in the MZ play counteracting roles to regulate MZ and MZ-P B cell

localization and tolerance to autoantigens[9,122]: These are the plasmacytoid dendritic cell (pDC) and the marginal zone macrophage (MZM), as further discussed below.

3.2 Breakdown of the follicular exclusion barrier by pDCs

Seminal work on follicular exclusion of autoreactive B cells was carried out by Cyster et al. in a paper which showed that HEL autoAg-engaged B cells were excluded from entering B cell follicles and instead remained at the T-cell–B-cell border[120]. The relevance of defective follicular exclusion in SLE patients was shown by Sanz and colleagues in a study showing that $9G4^+$ B cells successfully participated in GC reactions and expanded within the IgG memory and plasma cell compartments[123]. Our studies in lupus-prone BXD2 mice which develop spontaneous autoreactive spleen GCs revealed a breakdown in follicular exclusion of MZ-P B cells in the spleen[9,122]. The formation of aggregations of MZ-P B cells in the follicle is associated with the presence of type I IFN producing pDCs in the MZ, providing a connection between defective follicular exclusion of B cells and pDC-derived type I IFN in the regulation of the localization of B cells at the MZ-P stage.

Plasmacytoid dendritic cells are present in the splenic T cell area and in the red pulp[124] of mice and humans where they are poised for abundant type I IFN production[125]. Apoptotic cell debris can readily interact with and can be taken up by pDCs to stimulate TLR3, 4, 7 and 9, in the endosome resulting in migration toward the MZ and local production of high levels of type I IFN[124]. In autoimmune BXD2 mice, pDCs are clustered in the MZ and outside the FO and peak in frequency at about 3 months of age, coinciding with the induction of anti-Ro and anti-SSA and slightly preceding induction of anti-dsDNA IgG by several weeks[6,126]. CpG-induced type I IFN production by pDCs also peaked in 3-mo-old mice, a trend which correlated with increased frequencies of MZ-P B cells[5,6,14]. Genetic disruption of type I IFN signaling using *Ifnar*$^{-/-}$ mice led to fewer PNA^+ GCs, fewer autoAbs and reduced frequencies of MZ-P B cells. Mechanistically, type I IFN has multiple stimulatory effects on B cells, including rapid upregulation of CD69 which down-regulates $S1P_1$ expression, leading to inward migration of MZ B cells[121]. In an autoimmune setting, freshly isolated MZ-P B cells exhibited the highest surface expression of CD69 and decreased levels of S1P transcripts[5] suggesting MZ-P involvement in the follicular directed flux. Consistent with this, $CD1d^{hi}CD23^{hi}$ B cells from IFNAR intact mice exhibit the highest expression of CD86, the highest capacity for T cell costimulation and are situated adjacent to the GC and opposite from the $CD4^+$ T cell area, suggesting localization near the GC light zone[5,6].

Compounding the strong effect of type I IFN on B cell migration, MZ-Ps in BXD2 mice also serve as potent antigen-presenting cells which can carry apoptotic cell debris into the follicle in a “Trojan horse” fashion[6]. MZ-Ps express high levels of class II MHC which can present autoantigens, as well as high levels of mLTβ which can activate FDCs to promote a strong GC response[6,122]. Consistent with this, histological analyses revealed accumulation of TNP-Ficoll carried primarily by MZ-P B cells in proximity to the GCs in BXD2 mice (Figure 4). Although MZ B cells could trap TNP-Ficoll, the majority of TNP^+ MZ B cells were located in the MZ and not adjacent to the developing GC[6]. This pattern of Ag-loaded MZ-P localization was significantly reduced in BXD2-*Ifnar*$^{-/-}$ mice as evidenced by retention to

the FO–MZ border (Figure 4). Together, these results suggest a mechanistic model in which the production of type I IFNs by pDCs in the marginal periphery of B cell FOs can upregulate CD69 expression on MZ-P B cells to induce intra-follicular aggregations that may facilitate GC induction. As described later in Section 4, inside the GC, other cytokines, including IL-17A promote autoAb formation through upregulating the expression of regulators of G protein signaling (RGS) in B cells, desensitizing responses to CXCL12 and CXCL13. This fosters a prolonged stable interaction of B and T cells in the GC, high levels of activation-induced cytidine deaminase (AICDA) and the development of pathogenic autoantibody-producing B cells[11]. These studies illustrate how multiple, sometimes antagonizing cytokines can co-exist in SLE by being produced and active at different sites during a GC B cell development.

AutoAbs in SLE are known to occur years before disease onset, suggesting a gradual pathogenic process that occurs over a long period[127,128]. The expansion of transitional and MZ-P B cells and their dysregulation by type I IFN appear to be early events that occur prior to disease onset and, when combined with later immune defects, eventually culminates in clinical disease. It remains unclear whether pDC-produced type I IFN is a major SLE initiator and at what stages of disease its pathological effects occur. Stimulation of pDCs with apoptotic cells induces type I IFN production in a FcγRIIa (CD32)-dependent mechanism pDCs[129]. This and the fact that pDCs lack a specific Ag receptor, unlike B cells, make pDCs unlikely primary sensors of nuclear antigens[130]. As there is a constant outpouring of B cells from the BM, type I IFN stimulated MZ-Ps have the potential to provide a continuous supply of autoAg to the GCs, which may ultimately support the generation of autoantibody-producing plasma cells.

3.2 Breakdown of the follicular exclusion barrier to autoantigens by MZM disruption

The MZ barrier receives large amounts of blood from the general circulation and thus constitutes a major site of Ag capture and regulation of immunity to circulating Ag. Although MZMs were first described in the 1980s, their Ag capture function was not fully appreciated until the identification of specific scavenger receptors[131]. Their ability to clear blood-borne antigen and AC-Ag is a complex process requiring recognition by scavenger receptors including macrophage receptor with collagenous structure (MARCO) and specific intracellular adhesion molecule-grabbing non-integrin receptor 1 (SIGN-R1), which send signals to the cytoskeletal apparatus to promote proper engulfment and vesicular trafficking to phagolysosomes as well as induction of tolerogenic signals[132,133]. Defective serum-dependent, Rho-mediated mechanosensing cytoskeletal reorganization has been identified in macrophages obtained from 6 strains of lupus mice prior to disease onset[134].

Multiple cell types are resident in the MZ where they engage in interactive activities critical for immune surveillance and tolerance[135]. In addition to MZ-Bs, MZMs are also prominent in the spleen MZ where they have a specialized role to interact with and regulate responses to circulating apoptotic cell material[4]. MZ-B cells and MZMs form a close network and sustain each other through several mechanisms, including LT-β signaling whereby membrane LTβ (mLTβ) expressing MZBs signal to LTβR+ MZMs, promoting proper localization and function of MZMs[7,122,136,137]. MZMs in turn produce BAFF[138] which is

necessary for maintenance of mature B cells[139]. This reciprocal survival signaling in MZ-B and MZMs is central to self-tolerance, as MZMs are specialized for disposal of apoptotic cells and immune complexes in a non-inflammatory fashion involving the expression of TGF-β1 and IL-10[2,140]. T2/MZ-P B cells can also contribute to local IL-10 production (sometimes referred to as B10 B cells) and can produce other tolerogenic factors including TGF-β1[141,142]. Together, MZ-Bs and MZMs form a “follicular exclusion” barrier in which autoimmune complexes cannot obtain access to the follicle, which may account for the use of extra-follicular pathways for generation of some short-lived autoreactive B cells. These mechanisms are of interest in SLE, as they provide a link between extra-follicular and intra-follicular B cell response to apoptotic cell debris.

3.3 Breakdown of the MZM follicular exclusion barrier in SLE

Systemic lupus erythematosus (SLE) and mouse models of lupus both exhibit central features of increased circulating apoptotic cell autoantigens (AC-Ags) and, in most cases, elevated type I IFN signaling[9,143,144] (Figure 5). The most accepted model for development of SLE is that the presence of uncleared ACs or AC-autoantibody immune complexes with DNA- or RNA-containing proteins signals the production of type I IFNs by pDCs. This in turn drives T cell activation and B cell differentiation into autoantibody-producing B cells[145–149]. Although it is well accepted that increased circulating ACs can be due to reduced clearance of ACs in SLE, the underlying mechanism for the clearance defect is not completely understood[9,150–152]. Marginal zone macrophages (MZMs) surrounding the splenic follicles have been reported to promote efficient clearance of ACs accompanied by the induction of tolerance to AC-Ags[2,140,153,154]. Because of their advantageous anatomic location of MZMs, they act as the final follicular AC-Ag exclusion barrier[9].

Understanding the sequence of events that leads to breakdown of the follicular exclusion barrier provides insights into the chain of events that can lead to follicular translocation of B cells and autoantigens in SLE. One of the most fundamental observations in SLE is the increased circulation of apoptotic cell debris[2,140,143,152]. Related to this is the well-established interaction of such apoptotic cell debris with disposal mechanisms including uptake by DCs including plasmacytoid dendritic cells (pDCs), and with B cells through their BCR antigen receptor[20,61,78,80,81,155–157]. In SLE, such clearance mechanisms can lead to localization of apoptotic cell debris in the endosome and induction of type l interferons via stimulation through the endosomal TLRs including TLR7 and TLR9[156–160]. Secondary events of this process are two-fold. First, there is increased production of type l interferon, which is well established as being associated with SLE[113,145,146,161–163]. Second, there is the opportunity for autoantigens to be presented by class II MHC and up-regulating of CD86[5,6] leading to T cell tolerance loss[164–167]. As described above, type I IFNs can signal through the type I interferon receptor which is expressed on virtually all cells, leading to enhanced pro-inflammatory activity by the cell. Thus, the simplest explanation for breakdown of the follicular exclusion barrier by AC debris is that type I interferon acts directly on B cells to result in follicular translocation, as described above, and on MZMs that take up apoptotic cell debris to further reinforce a proinflammatory activity upon uptake of this debris.

However, adding to the complexity of this process, we also observed that uptake of apoptotic cell debris by MZMs not only changes the phenotype of these macrophages, but also results in dissipation of the MZMs. To investigate if MZM dissipation is a direct effect of type I interferon, *Rag1*$^{-/-}$ BXD2 mice were reconstituted with bone marrow from GFP^{-}BXD2-IFNαR$^{-/-}$ and GFP^{+}BXD2-IFNαR$^{+/+}$ mice[122]. Four months after reconstitution, both MZ B cells and MZMs were repopulated with cells that did and did not express the IFNAR. As previously reported, there was enhanced follicular translocation of IFNAR+ MZ B cells, and only IFNAR- B cells remained in the MZ. However, both IFNAR+ and IFNAR- MZMs populated the MZ at equivalently, indicating that type I interferon does not directly impact MZM numbers or structure. Building upon previous observations that membrane lymphotoxin-β (mLTβ) expression on MZ B cells sustained MZMs[7,122,136,137], we demonstrated that abrogated mLTβ expression on B cells usng LTβ$^{flox/flox}$ CD19-Cre-B6 mice led to loss of MZMs[122].

As one of the major functions of MZMs is to efficiently take up apoptotic cell debris in a non-inflammatory fashion, a potential link between LTβ receptor and molecules associated with this function were investigated. Using high-throughput transcriptomics, we investigated how signaling through LTβ receptor on MZMs might affect their function. Since *in vitro* studies could result in artifacts due to disruption of the MZM from its environment during cell preparation, studies were carried out *in vivo* using BXD2 mice that were treated with either control PBS or LTβR-Fc to block LTβR signaling. The results indicated that in the absence of LTβ receptor, there is a defect in the megakaryoblastic leukemia 1 (MKL1) mechanosensing pathway which resulted in failure of LTβR deficient MZMs to retain their efficient phagocytosis clearance function and anti-inflammatory function. MKL1 functions with serum response factor (SRF) as an important transcription factor regulating cytoskeletal gene expression in macrophages[168].This important role of LTβR signaling to maintain MZMs was observed in BXD2 mice[9,11,14,15,61,126,169–172] and B6.SLE1.SLE2.SLE3 (B6.TC mice)[8]. There was also a decreased expression of MKL1 and loss of MARCO $^{+}$CD11c^{+}MZ-B cells by histological section of spleens of SLE patients.

The LTβR-MKL1 mechanosensing pathway regulates endocytic uptake, actin polymerization and RhoA, which is required for proper cytoskeletal activities that regulate both uptake of apoptotic cells and the organization of intracellular compartments to efficiently process the apoptotic cell debris after it enters the MZM[173,174]. Future investigations into molecular mechanisms of proper uptake of apoptotic cells and their non-inflammatory digestion within a phagocytic cell may shed further insights into this as a common pathogenic mechanism in SLE and may explain such defects in other tissues. For example, defects in apoptotic cell debris uptake and elimination are a major pathogenic mechanism in mesangial proliferative glomerulonephritis which involves mesangial cells[175] and also, membranous glomerulonephritis which involves defects in apoptotic cell disposal by the podocytes lining the outside of basement membranes in the capillary loop of the glomerulus[176,177]. Importantly, such apoptotic cell uptake defects may be related to several under-explored mechanisms as described in this section, and studies should extend beyond effects of proinflammatory cytokines (such as type I interferon, interferon-$_{\gamma}$, IL-6 or IL-17)[178], to include intracellular defects in the organization of membrane contact molecules and within internal organelles associated with proper apoptotic cell debris.

4 TOLERANCE LOSS IN THE GC

4.1 STIMULATION OF RESTING B CELLS WITH AUTOANTIGENS AND CO-STIMULATORY MOLECULES

Despite the existence of BM and transitional B cell stage negative selection and anergy induction mechanisms, mature autoreactive B cells do find their way into GC reactions, as inferred from the high levels of mutations observed in the IgVH genes of circulating plasma cells (PCs) in SLE[179]. For activated of naive autoreactive B cells escaping central tolerance, the nature of secondary signals during B cell antigen presentation to T cells is a major gate-keeper of GC initiation. GC formation begins with antigen recognition and acquisition by resting B cells[180], followed by B cell migration to the T:B border[181]. Here, B cells receive co-stimulatory signals from $CD4^+$ T cells[182,183]. This interaction triggers full activation, proliferation, the initiation of differentiation[182], as well as a coalescence in close association with a network of stromal cells known as follicular dendritic cells (FDCs)[184,185]. B cells compete for limited antigen in an affinity-dependent manner, such that B cells with higher-affinity BCRs can gradually outcompete lower-affinity B cells[184,185]. After autoAg stimulation, co-stimulatory molecules, including B cell expressed inducible T cell co-stimulator (ICOS) ligand, B7 molecules (CD80 and CD86), and CD40 provide a positive co-stimulatory signal to T cells through ICOS, CD28 and CD40L, respectively[182]. An additional crucial level of control is achieved by the expression of inhibitory receptors that can transduce negative signals. B cell PD-L1 provides a negative signal through interaction with T cell PD-L, which is linked to an immuno-tyrosine inhibitory motif (ITIM) and an immuno-tyrosine domain switching motif (ITSM)[186]. This inhibitory motif can dampen signaling through TCR-CD3-ICOS family and dominates during a low-affinity CD86 class II MHC and TCR interaction.

CTLA-4 is another well-studied inhibitory receptor which binds both CD80 and CD86 and effectively opposes B cell interaction CD28[182,187]. An important role of CD86 to promote SLE is suggested by increased expression of CD86 on circulating B cells in SLE subjects[188] and its association with renal disease[51]. Inhibition of the CD28-CD86 interaction in BXD2 mice through administration of an adenovirus expressing CTLA4-Ig resulted in a long-term normalization of AICDA expression in the B cells, suppression of both autoAb producing B cells and IgG autoAbs[189]. Similarly, knockout of CD86 prevented the co-stimulatory activity of MZ-P B cells, even though they retained their ability to translocate into the FO[6]. As CD86-CD28 engagement induces both AICDA and signals for SHM and CSR within autoreactive B cells, high levels of CD86 has the potential to fully potentiate follicular naïve (or transitional/MZ-P B cells) to stimulate TFHs. Moreover, these signals can potentially overcome the inhibitory effects of PD-L1, even in the context of low affinity binding of class II MHC plus antigen to TCR on the T cell[190–192].

4.2 STIMULATION OF AUTOREACTIVE B CELLS BY TFH

GC-dependent B cell responses are initiated similar to GC-independent B cell responses in which there is an initial contact of an antigen activated B cell at the T/B border[193]. The T-follicular helper cell that initiates this response does so using interactions to initially promote survival with an interaction with the GC B cell. Whether this GC reaction is normal

or pathogenic is determined by the cytokines produced by the Tfh and the cytokine receptors expressed by the GC B cell[194–196]. After successful interaction with T cells, the B cell has a potential to encounter a spectrum of T-follicular helper cells each of which possess a different transcription factor that drives a different program and different cytokines that can promote B cell development. The canonical T-follicular helper cell is one in which STAT3 signaling, either through IL-6 or IL-21, can upregulate Bcl6, the canonical transcription factor for Tfhs (Figure 6)[197–202]. We have also identified that IL-21 selectively inhibits $Foxp3^+Bcl6^+CXCR5^+ICOS^+$ follicular regulatory T cell (Tfr) commitment without influencing total Treg cells (Figure 6). Bcl6 turns on a program of Tfh genes that can promote B cell development and at the same time, turns off the Tfh's ability to develop into other Tfhs. However, this suppression has been shown to be incomplete and also, subject to a synchronous development. Weinstein and co-workers have shown that Tfhs exhibit synchronous development from Bcl6-IL21 producing Tfh to a IL-4 producing Tfh[196]. IL-4 producing Tfhs have not been shown to be especially pathogenic in SLE but may play a role in enhancing AICDA in B cells. In contrast to these normal GC encounters, Tfhs that express IL-17 or IFNɣ (Tfh-IL-17, Tfh-IFNɣ) (with the underlying T cell program transcription factors being RORγt and T-bet) then interact with the corresponding receptors, IL-17R or IFNγR on B cells to induce development of autoantibody producing B cells[11,15,171,194,203]. The underlying mechanism for production of such autoantibodies is by definition due to a loss of tolerance at some step before or at this GC stage of development.

4.3 IL-17, RGS13, AND PROLONGED RETENTION IN THE GC

In SLE, pathogenic Tfhs include those that produce interferon-$_\gamma$ (Tfh-IFN_γ) or IL-17 (Tfh-IL-17). In 2008, we first showed in BXD2 mice that GC T cells expressed high levels of IL-17 and that GC B cells expressed high levels of IL-17RA[11]. IL-17 did not have a direct proliferative or survival effect on B cells but could stabilize the T-B interaction by upregulation of RGS13 and RGS16 which desensitized their migratory response to a CXCL-13 chemokine gradient from the follicular dendritic cell (Figure 7)[12,204]. IL-17 effectively induces expression of regulator of G protein signaling 13 (RGS13), a GTPase accelerator (GTPase-activating protein) for Gα subunits that can control the magnitude and duration of chemotactic responses to CXCL12 and CXCL13[13,205]. The resulting stabilization of T-B interaction is similar to stabilizations that have previously been described by CTLA4 which suppresses T cell migratory behavior or PD-L1[186,206]. However, instead of inhibiting migration signaling outside the GC, these interactions now inhibit migration of B and T cells inside the GC[14]. We showed that these interactions lock B and T cells into a state of persistent or prolonged interaction. RGS13 expression in BXD2 mice occurred exclusively in GC B cells, and its expression was stimulated by IL-17, but not IL-21. Importantly, IL-17 induction of RGS13 enabled retention and close contact of GC B cells and CD4 T cells, which was necessary for AICDA up-regulation[11]. Although BXD2-*Rgs13*$^{-/-}$ mice exhibited equal numbers of GC B cells surrounding CD4 T cells, their levels of AICDA expression were significantly attenuated. We subsequently showed that IL-17 can directly activate through the IL-17 on B cells through the ACT1/Traf6 pathway and directly activate the p50/p65 NF-kB pathway[15]. This activation pathway has turned out to be a central mechanism for regulating B cell activation responses through multiple signaling pathways. B cells lacking IL-17RA upregulate the chromatin transcriptional repressor,

p50/p50 homodimers which can block NF-κB signaling through multiple molecules including IRF7, IgG, CD40, and others[207]. These results show the broad-spectrum importance of IL-17RA signaling toward autoreactive B cell development and support that such IL-17 signaling is an important contributor to development of autoantibody producing B cells. In the Tfh cells, we have further identified that IL-17 is an extrinsic stop signal that it acts through the induction of RGS16 on post-differentiated IL-17RA(+) Tfh to enable interaction with responder B cells in the LZ niche. These data suggest a novel concept that Tfh differentiation and its stabilization in the LZ are two separate checkpoints and that IL-21 and IL-17 act at each checkpoint to enable pathogenic GC development (Figure 7).

Two major decision points for proliferating GC B cells is whether and when their progeny undergo (1) Ig class-switching and (2) the switch to Blimp1 and differentiation into plasma cells. The reduced AICDA expression and lower titers of autoantibody production in RSG13-deficient mice suggest a role for RGS13 in this fate decision choice. Indeed, while RGS13 deficiency was associated with a significant reduction in the frequency of GC B cells, there was a significant increase in $CD138^{+}B220^{+}$ plasmablasts[14]. High-power microscopy further confirmed higher numbers of IgM^{bright} plasmablasts located in bridging channels and outside of the marginal zone in the BXD2-*Rgs13*$^{-/-}$ mice compared to the BXD2 mice. This increase in plasmablast numbers was associated with changes in gene expression in sorted GC B cells, including increased PC program genes Blimp1, IRF-4, and XBP-1 and significantly lower expression GC program genes *Bach2*, *Pax5*, and *AICDA*, but not Bcl6[14]. Functionally, immunization of wild-type BXD2 and BXD2-*Rgs13*$^{-/-}$ with NP21-CGG, a T cell–dependent antigen revealed that, from week 2 to week 4, the development of anti-NP7 (high-affinity) and anti-NP33 (all-affinity) IgG1 and IgG2b antibodies were significantly reduced in BXD2-*Rgs13*$^{-/-}$ mice, consistent with a reduction in GC-mediated SHM in the BXD2-*Rgs13*$^{-/-}$ B cells. Taken together, IL-17–induced RGS13 favors the GC program and delays the transition of GC B cells to PC, leading to increased CSR, SHM, and production of pathogenic autoantibodies in a model of “delay-driven diversity” (Figure 7)[208,209]. In this model, antibody affinity maturation processes, including SHM and CSR, can be controlled at the point at which GC B cells initiate the transition to PC differentiation[208,209]. This model provides a framework for the understanding of IL-17–mediated promotion of GC formation and autoantibody production in autoimmune disease. Factors such as IL-17 that can directly regulate the expression of RGS13 have the ability to influence the size of GCs, as well as temporal differences in expression of key GC-PC differentiation genes and the extent of affinity maturation and CSR in the GC which in turn can affect disease severity. Future studies will be needed to determine if regulation of B cell migration can be safely use to prevent autoimmune disease.

4.4 TYPE II IFN AND AUTOREACTIVE B CELLS IN SLE

A second pathogenic cytokine in the GC B cell development is IFNγ produced by Tfh-IFNγ. Such Tfhs express the transcription factor, T-bet and drive the development of $T\text{-}bet^{+}$ B cells[16,18–20,210,211]. This B cell phenotype is similar to that previously found age-associated B cell (ABC) and overlap with memory populations[17,18]. As described above, this can occur outside the GC to produce short-lived responses or inside the GC to produce higher affinity and more pathogenic B cells and lead potentially to a longer-lived memory B

cell. These interactions are best characterized using viral GC responses which promote a strong Tfh-IFNγ response consistent with the Th1 response to a virus. These interactions produce IFNγ and promote development of high-affinity B cells to undergo SHM and class switching to produce neutralizing antibody to the virus. In the context of autoimmunity, B cells require additional helper factors including TLR7, strong IgG immunoglobulin crosslinking, IL-21 and other factors, as described above.

In SLE, it has been long-recognized that there is an increased population of circulating activated B cells that have been characterized by a $CD19^{hi}CD21^{-}CD38^{lo}IgM^{lo}$ $CD23^{-}$ phenotype[212]. There was also a lower expression of CD25 (IL-2Ralpha) in both naïve and memory B cells in SLE patients, suggesting defects in development of immunomodulatory $CD25^{+}$ B cells[213]. One of the fundamental B cell defects in SLE is the inability to maintain naïve B cells in a resting state, either due to an incomplete tolerance or anergy induction during transitional B cell development or exposure to stimulatory T cell cytokines or an inflammatory environment (potentially BAFF from macrophages). Especially important for SLE is the exposure of resting naïve B cells to type II IFNγ[17]. This results in upregulation of the Th1 transcription factor, T-bet, and down regulation of the BACH2 suppressor. BACH2 is one of the key transcription factors leading to a program preventing development of antibody-secreting B cells, whereas reprogramming by IFNγ in the context of TLR7 stimulation can upregulate T-bet and reprogram the B cells to become activated naïve which can then, in the presence of IL-21 and further stimulation, upregulate Blimp1 and lead to antibody-secreting cells[16,214]. This development pathway is not dependent on a GC but is dependent upon T cell cytokines. As both IL-17 and IFNγ can promote development of autoreactive B cells, further trials with an anti-interleukin-12/23 (IL-12/23) monoclonal antibody such as Ustekinumab (Stelara), which is blocks the p40 common chain[215,216] will be important[217,218]. Such therapies might inhibit development of T cells that produce IL-17 or IFNγ, and be effective to prevent the T-dependent phase of pathogenic B cell development in SLE.

5. CONCLUSIONS

A focus of this review has been that pathogenic autoAb development in SLE is not merely an outcome of mis-directed B cell responses in which the driving Ags are self-derived; rather pathogenic autoAbs are the outcome of a gradual dissolution in tolerance checkpoints and barriers realized in large part by the B cells themselves. Thus, B cells are not viewed as bystanders “caught up” or recruited into responses as a consequence of extrinsic factors such as exposure to excess autoantigens, type I IFN production, innate environmental factors such as BAFF, and T cell-derived factors such as IL-21, IL-17 or IFNγ. Moreover, the rapidly advancing frontier of single-cell technologies and our own single-cell studies suggest that B cells at the early transitional stage are endowed with a unique spectrum of gene expression destined to follow certain trajectories[1,61,219]. We propose that these endogenous properties such as IFNβ expression are maintained to guide subsequent B cell responses at the MZ-P stage and beyond in which responses to apoptotic cell debris, type I IFNs, and mLTβ are integrated in a balancing act to either maintain a tolerogenic MZM barrier or initiate to a cascade of follicular translocation, stimulation of FDCs and initiation of an early GC response. The development of autoreactive GCs thus possesses a component of spontaneity,

as their development is in a sense continuous with prior defects, rather than the result of a strong immunization, such as by an infectious agent. Unresolved questions are whether the initial propensity of early T1 stage B cells to produce IFNβ is due to polymorphisms in transcription factor components of the IFNβ enhanceosome or is secondary to stimulation through cytosolic and/or endosomal DNA/RNA sensors. The finding that IFNβ production is enhanced in AAs compared to EAs suggest a genetic contribution, but does not exclude other possibilities.

Another complexity in SLE autoreactive B cell development is the opposing effects that immunologic factors have depending on their localization (e.g. outside or inside the follicle). For example, outside the follicle, B cell expression of mLTβ has a protective role to maintain a tolerogenic MZM barrier. Yet, these same mLTβ^{+} B cells, when translocated into the follicle (through the action of type I IFNs to induce CD69), can stimulate FDCs to initiate an active GC response[10,122]. BAFF is another factor with dual roles. Produced by MZMs, it maintains MZ B cells and is necessary for MZ barrier integrity[122,220]. However, when present and signaling at the T2 or the GC stage of development, BAFF can promote survival and tolerance loss of autoreactive B cells[221–224].

Similarly, regulation of chemotaxis can either enhance or inhibit immune responses. Interruption of a chemokine signaling is not always beneficial. In the case of T and B cells in which contact is established by chemokine gradients, IL-17 can function to induce chemokine unresponsiveness, effectively stabilizing and prolonging T:B interactions. Thus, turning off motion or chemotaxis can accentuate development of an immune response and autoreactive B cells. Fingolimod represents a practical example of this principle; interruption of $S1P_1$ responses to high levels of extra-follicular S1P may prevent egress of immune cells from a follicle, and at the same time, promote compartmentalization and an intense immune response within the follicle. Thus, termination of fingolimod treatment may exacerbate multiple sclerosis in certain patients[225,226], presumably due to the release of follicular inflammatory cells into the circulation or local tissues.

Another unresolved question is whether the transitional B-cell checkpoint can influence B and T-cell interactions at the GC stage. While transitional B cell properties can influence intrinsic responsiveness to TLRs and T cell polarization[51,61], it is less clear whether transitional stage events imprint specific patterns of cytokine receptor expression. The regulation of cytokine receptor expression is important, as the pathogenic cytokines produced by Tfh-IL-21, Tfh-IL-17 or Tfh-IFNγ cells are insufficient for adaptive immunity and require the expression of specific receptors in order to drive GC B cell responses. The molecular basis for when and how specific profiles of cytokine receptor expression are acquired is poorly defined for B cells, but ongoing advances in single-cell technologies are providing the tools and approaches to answer these questions. Such studies will be needed to probe relationships between developmental transitions (i.e. T1-T2-mature B), chromatin states, gene expression, and specific pathogenic phenotypes.

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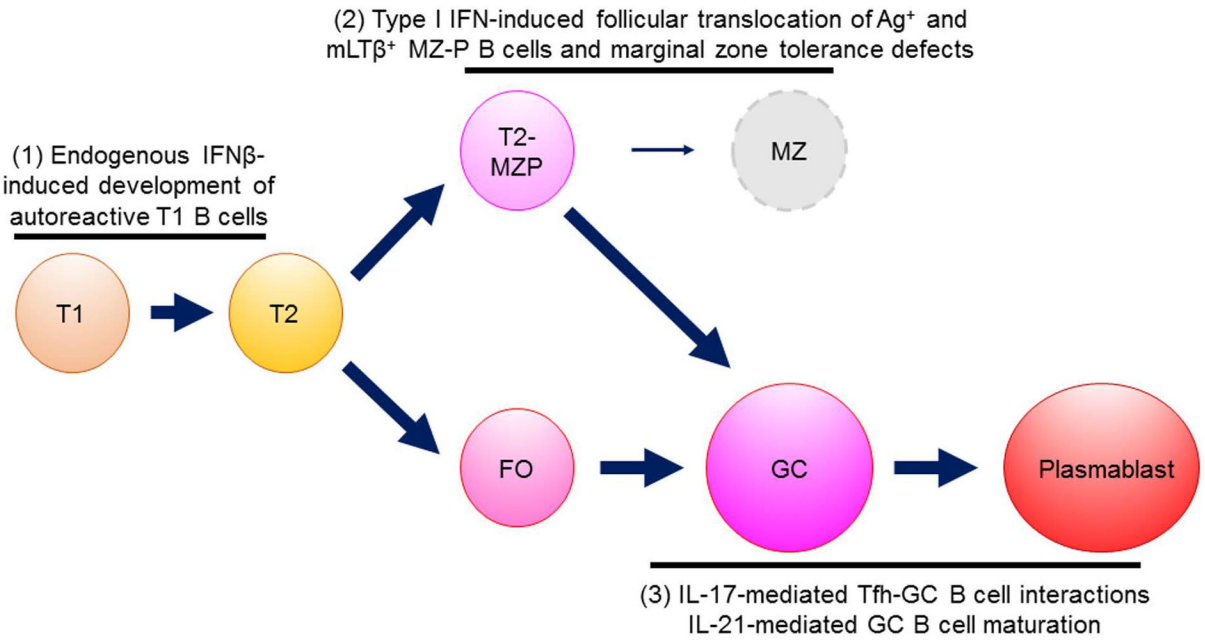

Figure 1.

Overview of three key mechanisms for development of autoreactive B cells. (1) At the early T1 transitional stage, endogenous IFNβ promotes activation and survival of B cells through enhancing responses to TLR signaling, including TLR7. (2) At the T2/MZ-P stage, B cells are susceptible to type I IFN induced follicular translocation. This event disrupts the tolerogenic MZM barrier which depends on mLTβ+ B cells which further enhances defective apoptotic cell clearance and increased type 1 interferon in the MZ. The translocated B cells can serve as excellent antigen-presenting cells with high levels of CD86 and class II MHC, and in the context of mLTβ can strongly stimulate FDCs to initiate a spontaneous autoreactive GC development. (3) During the GC or extra GC T-dependent phase of response, Tfh-IL21 initiates the T-B interaction and survival of GC B cells, but subsequent stimulation of B cells by Tfh-IL17 or Tfh-IFNɣ promotes development of either highly pathogenic and prolonged T-B interactions due to increased RGS13 and RGS16 or IFNɣ-induced upregulation of T-bet leading to development of the autoreactive DN2 B cell ($T\text{-}bet^{+}CD11c^{+}IgD^{lo}CXCR5^{lo}$). We hypothesize that not all B cells are equally as susceptible to each of these pathogenic mechanisms; Rather, early events such as endogenous IFNβ and ensuing changes in chromatin organization influence individualized developmental trajectories.

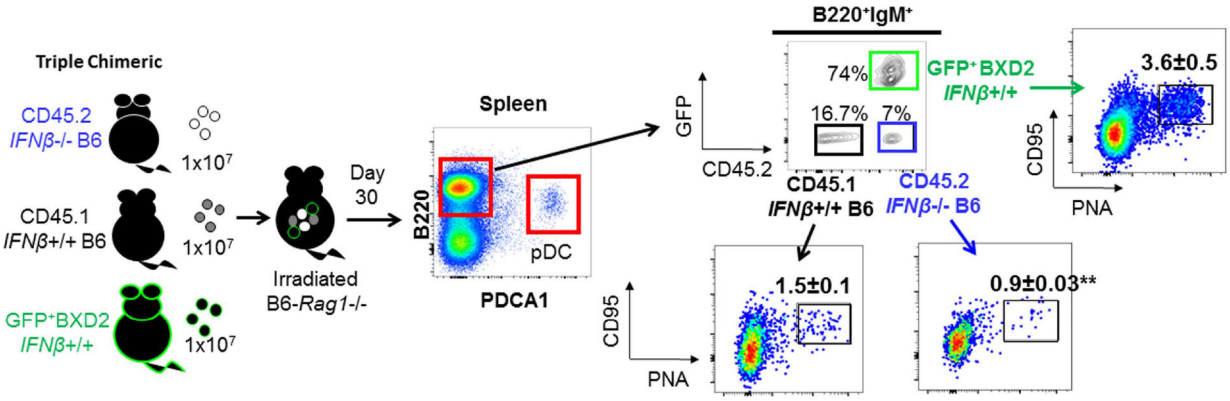

Figure 2.

Lupus susceptible B cells exhibit a higher potency to become GC B cells within the same environment. BM-chimeric mice were generated by reconstitution of CD45.2 *Rag1*$^{-/-}$ mice with equal numbers of BM derived from CD45.2 B6-*Ifnb*$^{-/-}$ mice, CD45.1 *Ifnb*$^{+/+}$ (WT) B6 mice and GFP^{+} BXD2 mice. Recipient mice were sacrificed at day 30 after BM transfer. FACS analysis of pDCs, gated as PDCA1^{+}B220^{lo} cells or PNA^{+}CD95^{+} GC B cells revealed that despite the identical developmental environment, B cells derived from BM of lupus prone BXD2 mice and those derived from *Ifnb*$^{+/+}$ (WT) B6 mice exhibited a higher survival advantage and higher potency to develop into GC B cells vs. CD45.2 B6-*Ifnb*$^{-/-}$ mice.

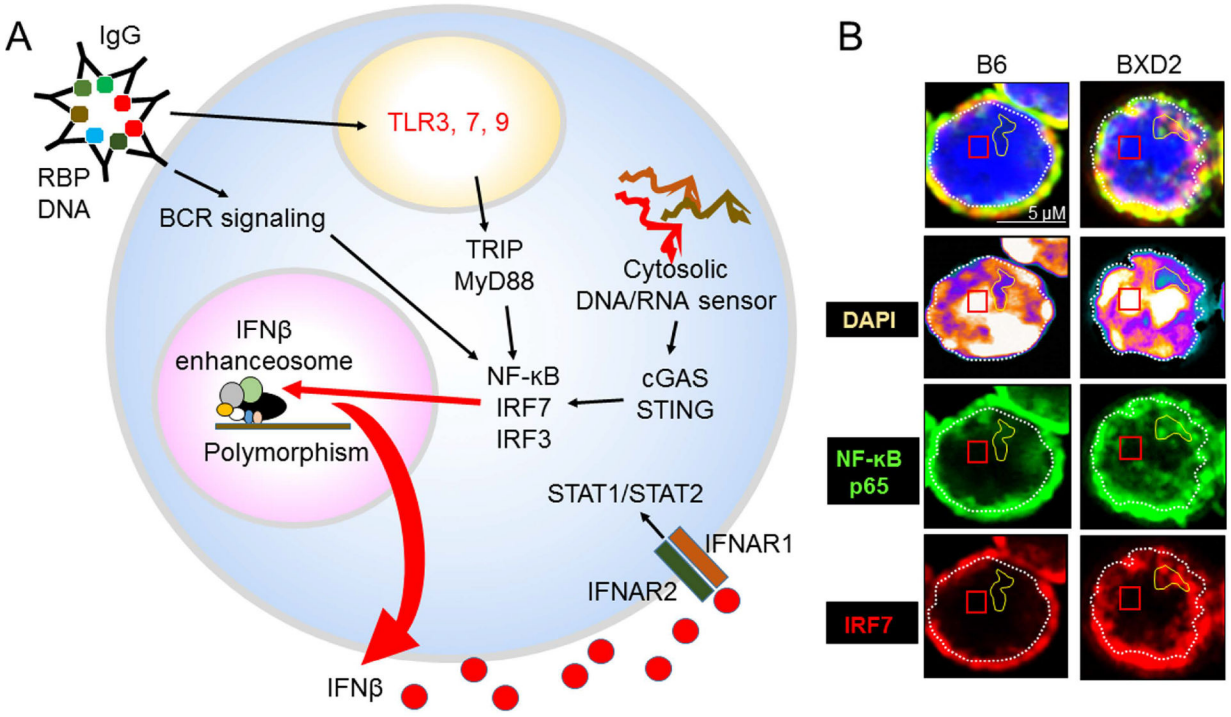

Figure 3.

Increased IFNβ enhanceosome components in lupus B cells. **A**. Uncleared ACs or AC-autoantibody immune complexes with DNA- or RNA-containing proteins signals BCR and the TLR3, 7, 9 endosome in B cells. Both IRF3 and IRF7 are critical for type I IFN induction and amplification following TLR signaling. Alternatively, defects in clearance of endogenous DNA/RNA could activate cytosolic sensors through the cGAS/STING pathway. This all leads to increased NF-kB, IRF7 and IRF3 which are important co-factors for the IFNβ enhanceosome. The IFNβ promoter contains four positive regulatory domains (PRD I-IV), which are occupied by overlapping transcription factor complexes[115]. IRF3 and IRF7 bind PRDI and III; the AP-1 heterodimer of ATF-2 and c-Jun binds PRDIV; and the NF-κB p50-RelA heterodimer binds PRDII[115]. Polymorphisms in IFNβ enhanceosome genes and aberrant induction of nucleic acid sensing pathways may contribute to elevated IFNβ expression, which leads to production of other IFN subtypes and amplification of nucleic acid sensing responses. **B**. Increased expression of IFNβ enhanceosome components in lupus B cells. Super-Resolution Structured Illumination Microscopy (SIM) imaging analysis showing the colocalization of NF-κB p65 and IRF7 in DAPI^{dim} open chromatin region in B cells derived from lupus prone BXD2 mice but not normal B6 mice. As SLE B cells express endogenous IFNβ, these results suggest there is increased stimulation of the IFNβ enhanceosome.

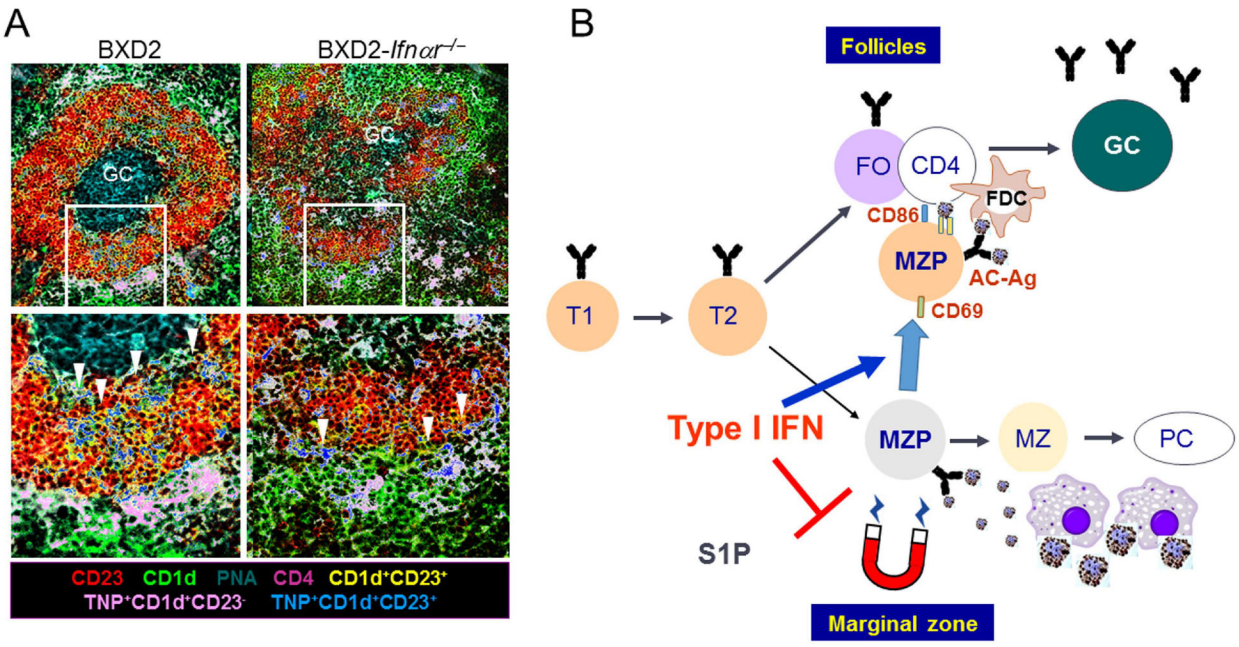

Figure 4.

A. Type I IFN-stimulated follicular translocation of antigen-delivery MZ-P B cells in lupus. In BXD2 mice, there was a significantly larger population of MZ-P B cells in the spleens of autoimmune BXD2 mice compared with B6 mice and IFNAR deficient BXD2 mice. In a TNP-ficoll injection experiment, TNP^+ MZ-P B cells (blue) were aggregated inside the follicle and were in proximity with a GC. In contrast, TNP^+ MZ-P B cells in BXD2-*Ifnar*$^{-/-}$ mice were found to reside predominantly in the periphery and had a lower ability to transport TNP into the GCs (adapted from Wang et al, J Immunol. 2010 Jan 1;184(1):442–51)[5]. **B**. Model where type I IFN driven follicular translocation of MZ-P B cells promotes apoptotic cell autoantigen transport into the GC region and ultimately B-cell tolerance loss. Generation of type I IFNs, generated by pDCs in the MZ, induced the expression of CD69 and suppressed the sphingosine-1-phosphate-induced chemotactic response, thereby promoting FO-oriented Ag transport by MZ-P B cells.

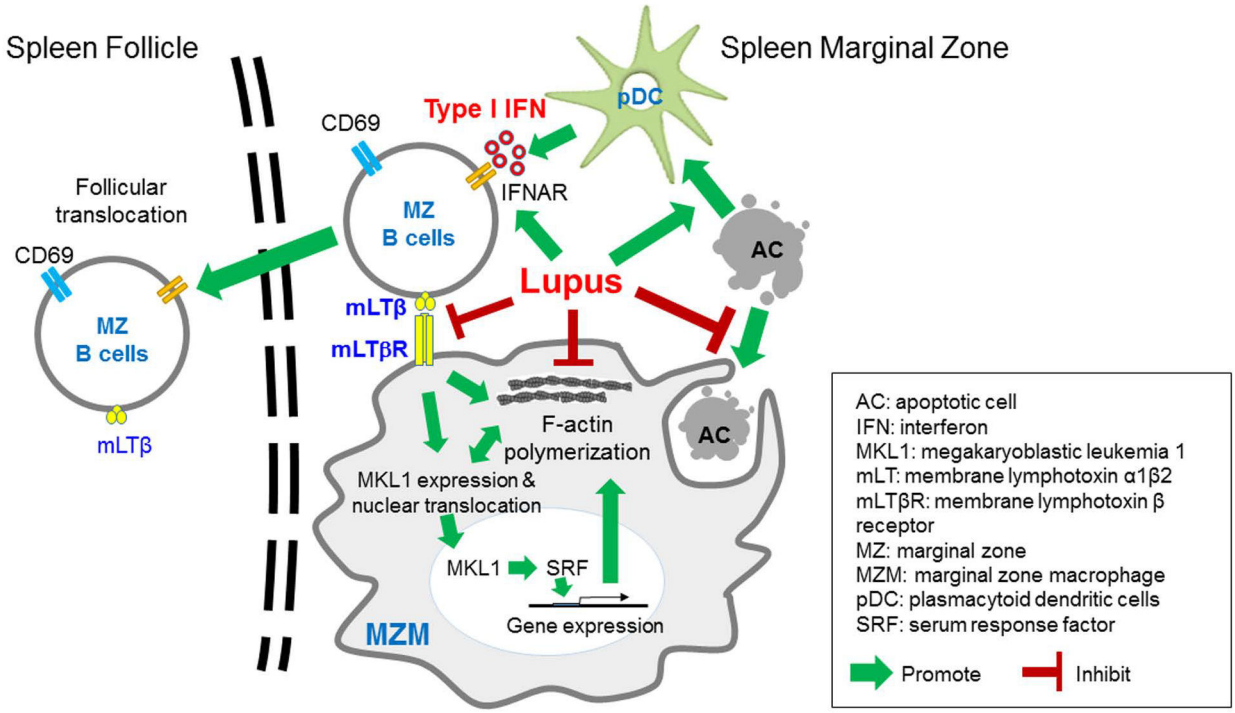

Figure 5.

Type I IFN indirectly induces MZM tolerance loss through follicular translocation of mLTβ expressing MZ B cells. SLE is characterized by increased type I IFN and circulating apoptotic cell-derived autoantigens (AC-Ags), both of which drive autoantibody production by B cells. We have found that in murine SLE models, type I IFN increased follicular translocation of MZ B cells in the spleen, which disrupted the interaction between these B cells and MZMs. The interaction between MZ B cells and MZMs was shown to activate the MKL1–mediated mechanosensing pathway, which was essential for MZMs to phagocytize ACs and thereby prevent follicular entry of AC-Ags. Moreover, these defects were also present in spleens from patients with SLE. The results of this study suggest that strategies to maintain this mechanosensing pathway may block follicular entry of AC-Ags and prevent the development of autoantibodies against these antigens (adapted from Li et al, J Clin Invest. 2015 Jul 1;125(7):2877–90)[122].

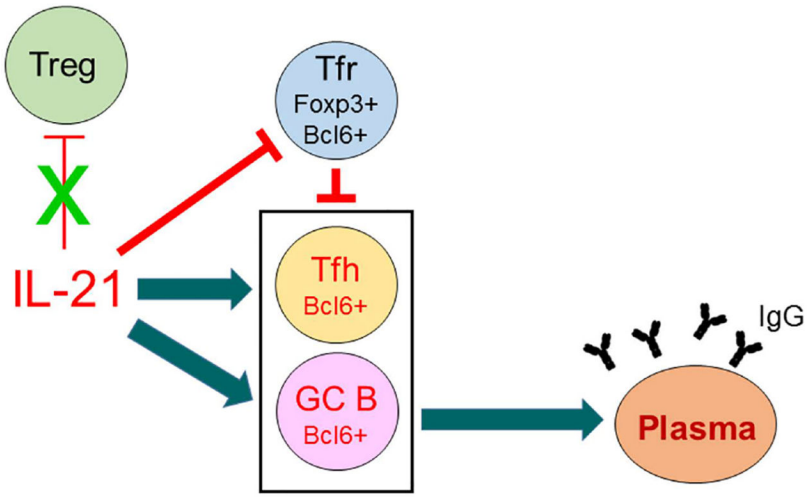

Figure 6.

IL-21 breaks GC B-cell tolerance through the stimulation of Tfh and GC B cells and the inhibition of Tfr CD4 T cells. In BXD2 mice, IL-21 regulates GC formation by preferential expansion of Tfh cells, relative to Tfr cells. These $CXCR5^{+}ICOS^{+}$ subpopulations of CD4 T cells are located in the GC as we previously described[171,172]. In BXD2-*Il21*$^{-/-}$ mice, the frequency of $Foxp3^{+}$ Tfr cells was increased, whereas there was a significant decrease in the frequency and the number of $Foxp3^{-}$ Tfh cells, resulting in an increase in the ratio of $Foxp3^{+}/Foxp3^{-}$ $CXCR5^{+}ICOS^{+}$CD4 T cells. There was no change in the frequency and the ratio of conventional Treg to total CD4 T cells. Together, these results suggest that IL-21 regulates GC development through an effect that is limited to the relatively small subpopulations of Tfh and Tfr cells, which underscores the important role of these two small subpopulations in regulating GCs.

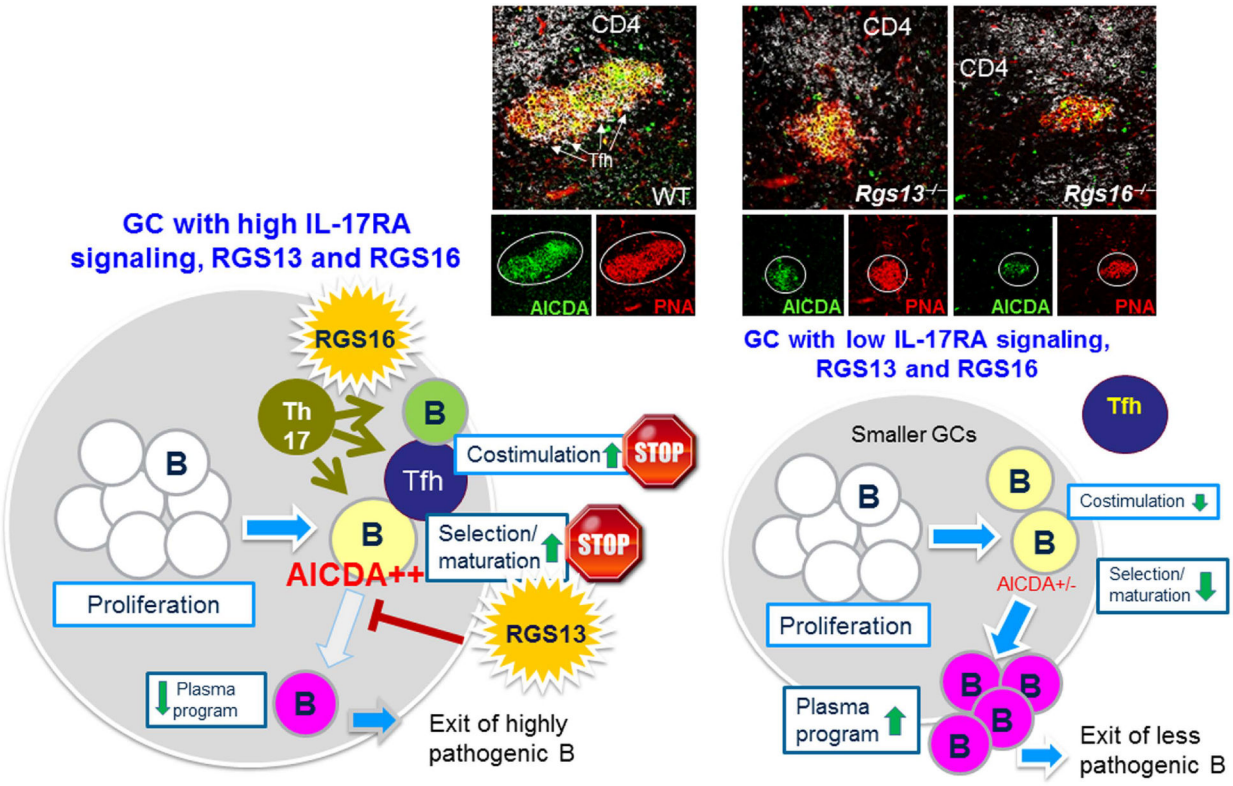

Figure 7.

Prolonged Tfh and GC B cell interactions through the effects of IL-17-mediated upregulation of RGS13 and RGS16. Chemotactic responses play a key role in orchestrating the cell-cell interactions in the GCs. This process involves active shuttling of the antigen-carrying B cells between the marginal zone and the GCs. We have shown that within the GCs of autoimmune BXD2 mice, IL-17A upregulates the expression of regulators of G protein signaling (RGS) in B cells to desensitize the G protein-coupled receptor (GPCR) signaling pathway of CXCL12 and CXCL13 chemokines. Consistent with this, IL-17 also stimulated the expression of RGS16 in Tfh to promote Tfh migration arrest at the GC light zone to facilitate Tfh and GC B cell interactions. This promotes a prolonged stable interaction of B and T cells in the GC that induces high levels of activation-induced cytidine deaminase (AICDA) thereby enabling development of pathogenic autoantibody-producing B cells.