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B cells take the front seat: Dysregulated B cell signals orchestrate loss of tolerance and autoantibody production

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Abstract

A significant proportion of autoimmune-associated genetic variants are expressed in B cells, suggesting that B cells may play multiple roles in autoimmune pathogenesis. In this review, we highlight recent studies demonstrating that even modest alterations in B cell signaling are sufficient to promote autoimmunity. First, we describe several examples of genetic variations promoting B cell-intrinsic initiation of autoimmune germinal centers and autoantibody production. We highlight how dual antigen receptor/toll-like receptor signals greatly facilitate this process and how activated, self-reactive B cells may function as antigen presenting cells, leading to loss of T cell tolerance. Further, we propose that B cell-derived cytokines may initiate and/or sustain autoimmune germinal centers, likely also contributing, in parallel, to programming of self-reactive T cells.

Introduction

The importance of B cells in the pathogenesis of human autoimmunity is well established. Specific autoantibody profiles serve as serologic hallmarks of multiple autoimmune diseases, and B cell directed therapies have demonstrated therapeutic benefit in clinical trials for rheumatoid arthritis (RA) [1], type 1 diabetes (T1D) [2], ANCA vasculitis [3], multiple sclerosis (MS) [4], and systemic lupus erythematosus (SLE) [5]. Notably, B cell targeted therapies frequently provide lasting clinical benefit without significantly impacting autoantibody levels, suggesting that other B cell functions, including antigen presentation and cytokine production, play important roles in autoimmune pathogenesis.

While the mechanisms promoting B cell activation during autoimmunity have not been completely defined, multiple genome-wide association studies (GWAS) of human

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autoimmune disease risk have implicated genetic polymorphisms that impact lymphocyte activation responses [6-8]. In this context, it is known that even modest alterations in B lymphocyte signaling thresholds can promote autoimmunity in the appropriate environmental setting [9]. Based on emerging data, we propose a model wherein altered B cell signals are sufficient to promote spontaneous activation of self-reactive B cell clones via self-antigen, allowing B cells to function as antigen presenting cells that trigger a loss in T cell tolerance and facilitate spontaneous germinal center (GC) reactions that promote development of high-affinity, class-switched autoantibodies.

The importance of dysregulated GC responses in autoimmunity is reinforced by the observation that anti-dsDNA (and RNA-associated) autoantibodies cloned from SLE patients are typically class-switched and somatically hypermutated [10]. Similarly, high-affinity anti-insulin and islet-specific antibodies are present in the majority of pre-diabetics, including very young subjects. Although B cells can also undergo somatic hypermutation at extrafollicular sites in murine autoimmune models [11], spontaneous GCs are frequently observed in B cell-driven murine models and in human autoimmune patients, implicating antigen-driven, GC selection in autoantibody production [12]. Tertiary lymphoid follicles and ectopic GCs have also been demonstrated within inflamed RA joints, lupus nephritis kidneys and meninges in MS, further reinforcing the importance of B:T cross-talk in the pathogenesis of systemic autoimmunity [13].

B cells express both clonally-rearranged antigen receptors (BCR) and innate pattern-recognition receptors (including toll-like receptors, TLRs), and have a unique propensity for activation via integrated signaling through these pathways [14]. Robust anti-viral antibody responses are dependent on B cell-intrinsic TLR signals via the adaptor protein MyD88, emphasizing the evolutionary advantage of this arrangement [15]. However, dual BCR/TLR activation also increases the risk of autoimmunity, since B cell TLRs can also respond to endogenous ligands [14,16,17]. Because dual BCR/TLR activation serves protective functions during infection, and also carries the potential to promote autoimmunity, these signaling pathways must be tightly regulated.

In this review, we describe recent animal studies in which genetic manipulation of B cell signaling has been shown to promote T cell activation, spontaneous GC responses and systemic autoimmunity. In particular, we will focus on genetic changes that exert both a B cell-intrinsic impact on autoimmunity, and have direct relevance to our understanding of how human candidate risk variants may promote disease.

Dysregulated B cell signals promote spontaneous autoimmunity

Wiskott-Aldrich syndrome—In addition to recurrent infections, eczema and bleeding diathesis, patients with the primary immunodeficiency disorder, Wiskott-Aldrich syndrome (WAS), experience high rates of humoral autoimmunity [18]. In contrast to marked attenuation of T cell receptor signaling, WAS protein (WASp)-deficient B cells are modestly hyper-responsive to both BCR and TLR ligands [19]. To model the impact of this dysregulated signaling on autoimmunity risk, we generated mixed bone marrow chimeras in which B cells, but not other cellular lineages, lack WASp. Strikingly, hyper-responsive *was*^{-/-} B cells were sufficient to promote wild-type CD4⁺ T cell activation and spontaneous

GCs, resulting in class-switched autoantibody production and immune-complex glomerulonephritis. Further, B cell-intrinsic MyD88 deletion abrogated CD4⁺ T cell activation and spontaneous GC formation [19]. Together with other murine models showing a similar role for B cell MyD88 signals in disease pathogenesis [20,21,22•,23•], this observation emphasized the critical importance of dual BCR/TLR-activation in driving autoimmunity and lends support to our model whereby autoreactive B cells directly promote CD4⁺ T cell responses.

We recently utilized the *was*^{-/-} chimera model to further dissect the B cell-intrinsic signals responsible for spontaneous autoimmunity. The MyD88-dependent receptors TLR7 and TLR9 have long been known to be required for anti-nuclear antibody production. In murine lupus models, global deletion of TLR7 abolishes autoantibodies to RNA-associated proteins and limits systemic autoimmunity, while TLR9 deletion prevents anti-dsDNA and anti-chromatin antibodies but exacerbates disease [24-27]. Whether B cell or myeloid TLR signals explain these opposing phenotypes had not been addressed. For this reason, we directly tested the impact of B cell-intrinsic TLR7 vs TLR9 deletion in the *was*^{-/-} chimera model. B cell-intrinsic TLR7 and TLR9 deletion exert opposing impacts on the generation of RNA- and DNA-associated autoantibodies, respectively. In addition, B cell TLR7 deletion prevented, while TLR9 deletion exacerbated, systemic autoimmunity, recapitulating the phenotype of global TLR7- and TLR9-deficient lupus strains [28•].

This study emphasized the critical importance of B cell TLR7 signaling in the pathogenesis of murine lupus. The importance of TLR7 is additionally shown using models in which TLR7 is overexpressed, including male mice with the spontaneous *Yaa* translocation or TLR7-transgenic (TLR7-Tg) animals [29]. Although enhanced TLR7 signals likely impact several immune lineages, TLR7-Tg B cells are preferentially recruited into spontaneous GCs in competitive chimeras suggesting that B cell-intrinsic TLR7 signals preferentially drive autoimmune GCs [30]. Interestingly, B cell TLR7 signals were also shown to promote, and TLR9 to limit, the development of low-level spontaneous GCs in non-autoimmune C57BL/6 mice housed within specific pathogen free environments. Further, this process relies upon B cell intrinsic TLR signaling and is not impacted by myeloid specific loss of MyD88 [31•]. Finally, over-expression of soluble RNase ameliorated autoimmunity in TLR7 transgenic mice, implicating endogenous RNA in disease pathogenesis and suggesting a possible therapeutic strategy in SLE [32].

In human genetic studies, SNPs within TLR7 have only been associated with SLE in a small subset of patients [33-35]. However, polymorphisms genes encoding proteins and transcription factors downstream of TLR activation, including *TNFAIP3*, *TNIP1* and *IRF5*, correlate with lupus risk [36,37]. Further, variants in *SLC15A4*, a histidine transporter involved in lysosomal TLR signaling, are associated with SLE [36]; and B cell-intrinsic *SLC15A4* deletion limits autoimmunity in murine models [38]. With the exception of *SLC15A4*, whether these GWAS signaling variants impact B cells vs. other immune lineages during disease pathogenesis remains to be determined. However, the murine studies described above support a model whereby dysregulated TLR (in particular TLR7) signaling, together with altered BCR signals, is sufficient to promote B cell activation and autoantibody production in the appropriate environmental context.

LYN deficiency—Deficiency of the Src family tyrosine kinase, *Lyn*, results in spontaneous autoimmunity characterized by splenomegaly, anti-dsDNA autoantibodies and glomerulonephritis [39]. In B cells, *Lyn* promotes proximal BCR signaling but also inhibits B cell activation by phosphorylating immunoreceptor tyrosine-based inhibition motifs on CD22, a sialic acid-binding immunoglobulin-type lectin (Siglec), and the inhibitory FC-receptor FcγRIIB. Consistent with this, BCR-mediated calcium flux is enhanced in *Lyn*^{-/-} B cells [40].

To address whether B cell-intrinsic deletion of *Lyn* is sufficient for development of autoimmunity, Lamagna et al. utilized a Cre-recombinase strategy to delete *Lyn* specifically in B cells [41•]. B cell-intrinsic *Lyn* deficiency promoted development of class-switched autoantibodies to dsDNA and RNA-associated antigens, as well as splenic immune activation and immune-complex glomerulonephritis. Although total numbers of GC B cells were not increased (likely secondary to the B cell lymphopenia in this model), IgD⁻FAS⁺GL7⁺GC B cells as a percentage of CD19⁺ cells appear expanded in *CD79a*^{cre}-*Lyn*^{fl/fl} mice. Consistent with this interpretation, deletion of the SAP adaptor protein required for interaction between T follicular helper cells (T_{FH}) and GC B cells markedly decreased anti-dsDNA Ab in *Lyn*^{-/-} mice, implicating GCs in the genesis of autoantibodies in this model [23•].

Deletion of the signaling adaptor MyD88, either globally [41•] or only in B cells [23•], prevented activation of *Lyn*^{-/-} B cells, class-switched antibody formation and systemic autoimmunity. Similar to the *was*^{-/-} chimera model, this suggests that, by enhancing BCR signaling, *Lyn* deletion promotes B cell activation via dual BCR and TLR signals.

In addition to these murine data, SNPs in *Lyn* and a related Src family kinase *BLK* are associated with human lupus risk [42-44]. Further, rare loss-of-function mutations in sialic acid acetyl esterase (*SIAE*), which is required for function of the inhibitory siglec, CD22, markedly increase the risk of RA, T1D and SLE [45]. These data implicate the *SIAE*, CD22, *Lyn* inhibitory axis in regulating B cell activation and the prevention of humoral autoimmunity.

PTPN22—Protein tyrosine phosphatase nonreceptor 22 (*PTPN22*), encodes the tyrosine phosphatase, LYP, that negatively regulates lymphocyte activation downstream of both the BCR and T cell antigen receptor (TCR). Population-based studies have demonstrated that a 1858C→T single-nucleotide polymorphism in *PTPN22* (resulting in R620W amino acid substitution in LYP) is associated with susceptibility to multiple humoral autoimmune diseases including SLE [6], T1D [7], and RA [46]. Prior studies have reported contradictory findings as to whether the *PTPN22* variant serves as a gain- or loss-of-function allele. Further, whether LYP R620W predisposes to autoimmunity primarily via impacting T vs. B cell function had not been addressed.

To address these questions, two groups, including our own [47••,48••] independently generated murine knock-in models containing the equivalent amino acid change, R619W, in the murine orthologue protein PEST domain phosphatase, PEP. T and B cells from knock-in mice exhibited enhanced antigen receptor signaling, expansion of memory/effector CD4⁺

and CD8⁺ T cells, and spontaneous GCs in aged mice. While significant autoimmunity was not observed in knock-in mice on the C57BL/6 background [48••], aged knock-in mice on a mixed C57BL/6J and 129/Sv genetic background developed autoimmune disease characterized by a broad range of autoantibodies, vasculitis and immune complex renal injury [47••].

Since the *PTPN22* variant impacts both T and B cell activation, we tested whether B cell-intrinsic expression of PEP R619W might be sufficient to induce autoimmunity. We utilized an alternative model wherein the murine variant is expressed via the *Rosa-26* promoter specifically within B cells [47••]. Strikingly, aged mice developed splenomegaly with spontaneous GCs, anti-dsDNA autoantibodies, and glomerulonephritis. Finally, B cell intrinsic MyD88 deficiency abrogates spontaneous GCs and autoantibody production mediated by variant expressing B cells (X Dai, DJ Rawlings; unpublished data). Taken together, these findings indicate that dysregulated PTPN22-dependent signals are sufficient to promote systemic autoimmunity in a B cell-intrinsic manner.

BANK1—The B cell scaffolding protein, *BANK1*, binds Src family protein tyrosine kinases and promotes BCR-mediated calcium flux via *Lyn*-mediated phosphorylation of the inositol trisphosphate receptor (IP₃R) in B cell lines [49]. In contrast, while *Bank*^{-/-} primary B cells display normal BCR triggered calcium flux, *Bank*^{-/-} mice exhibit enhanced T-dependent antibody responses and GC formation. *In vitro*, Bank-deficiency results in enhanced CD40-mediated proliferation, survival and Akt activation, implying a key role in regulating B cell responses to T cell help [50].

In humans, two nonsynonymous variants in *BANK1*, leading to Arg61His and Ala383Thr substitutions, are highly associated with SLE susceptibility [51]. While the relationship between these variants and disease pathogenesis has not been determined, the increase in CD40 mediated Akt signals in *Bank1* deficiency suggests that *BANK1* variants may promote autoimmunity via modulation of B:T interactions within autoimmune GCs.

B cell antigen presentation in autoimmunity

In addition to producing antibodies, B cells process antigens and present them to CD4⁺ T cells as peptide fragments in the context of MHC Class II. As described above, dysregulated B cell signaling may be sufficient to promote CD4⁺ T cell activation and expansion, suggesting a role for B cell antigen presentation in promoting initial breaks in T cell tolerance during humoral autoimmunity. Direct evidence for B cell antigen-presenting cell (APC) function driving autoimmunity is limited to a few murine studies. For example, in non-obese diabetic (NOD) mice, B cells expressing surface, but not secreted, IgM develop penetrant diabetes despite absence of islet autoantibodies [52]. In addition, B cell-intrinsic deletion of MHC Class II I-A^{g7} prevents CD4⁺ T cell activation and T1D in the NOD background [53]. Skewing the BCR repertoire towards insulin reactivity also accelerates diabetes, despite lack of antibody production by BCR heavy chain transgenic strains, further implicating B cell APC function in diabetes pathogenesis [54].

Similar findings were recently documented in the experimental autoimmune encephalitis (EAE) model of multiple sclerosis, when Molnarfi and colleagues elegantly dissected the

impact of B cell APC function vs. antibody production on disease progression. Transgenic mice expressing a myelin oligodendrocyte glycoprotein (MOG)-specific BCR developed acute EAE, even though these B cells were engineered to be unable to secrete antibody. In contrast, mice with MHC Class II-deficient B cells failed to develop pro-inflammatory T_H1 and T_H17 cells and were resistant to EAE induced by immunization with full-length MOG [55•].

Together, these studies highlight an important role for B cell antigen presentation in promoting T cell activation in autoimmunity. Although not yet addressed, we predict that activated effector/memory CD4⁺ T cells in the B cell-driven murine lupus models described above, likely target self-epitopes derived from nuclear autoantigens. Confirming this hypothesis will lend further support to the model whereby activated autoreactive B cells initiate breaks in T cell tolerance.

B cell cytokines in autoimmune GC responses

B cells can also produce cytokines, suggesting another mechanism whereby B cells can impact autoimmune pathogenesis. On the basis of cytokine profiles, B cells have traditionally been divided into effector and regulatory subsets. Effector B cells were originally designated as IFN- γ /IL-12 (“B effector 1”) and IL-2/IL-4/IL-6 (“B effector 2”) subsets. In contrast, B cells can suppress immune responses via the production of IL-10 and TGF β [56]. Recently, we demonstrated that B cells can produce IL-17 during *Trypanosoma cruzi* infection [57•], and Fillatreau’s group showed B cells to be a primary source of immunosuppressive IL-35 [58•,59]. These recent findings expanded the mechanisms whereby B cells regulate both infectious and autoimmune models. An important caveat is that effector and regulatory B cell subsets may exhibit significant plasticity depending on environmental and inflammatory context. In addition, unlike effector and regulatory T cell subsets, cytokine-producing B cells have not been shown to fulfill the requirements of classic immune lineages, such as defining transcription factors.

There is broadening evidence that key cytokines markedly influence GC biology, including dysregulated GCs in the setting of autoimmunity. In this context, cytokines promote the generation and accumulation of T follicular helper cells (T_{FH}), regulate B cell activation, survival and class-switch recombination and similar events presumably coordinate formation and outcome of ectopic GCs in autoimmunity [60].

Whether B cell cytokines directly impact GC responses, however, has been less extensively addressed. As detailed above, TLR signals promote B cell activation and autoantibody production. In addition, TLR ligation synergizes with CD40 signaling to drive B cell cytokine production, including the regulatory cytokines IL-10 and IL-35. It therefore is likely that B cell-derived cytokines produced during antigen and TLR mediated activation, may function to limit spontaneous autoimmune GCs. Conversely, additional B-intrinsic cytokines likely enhance these events. By promoting T_{FH} accumulation, excess IFN- γ drives dysregulated GCs and autoimmunity in the *sanroque* lupus model [61]. Since activated B effector 1 cells produce IFN- γ , it is possible that such cells represent a key IFN- γ source during humoral autoimmunity. Supporting this hypothesis, we have observed IFN- γ -producing *was*^{-/-} B cells in diseased chimeras (SW Jackson, DJ Rawlings; unpublished

data). It remains to be determined whether loss of B cell-intrinsic IFN- γ can restrain autoimmune GCs or whether alternative IFN- γ sources may compensate *in vivo*.

Conclusions

Recent data provide direct evidence that B cells can serve as primary drivers of autoimmunity. Figure 1 summarizes our proposed model in which, after encountering self-antigen, dysregulated B cell signaling is sufficient to promote B cell activation. This leads to recruitment and activation of autoreactive CD4⁺ T cells initiating GC development. Within developing GCs, B cells present antigen to T cells in the context of MHC Class II and likely also modulate the GC program via cytokine production.

We have summarized the limited studies to date where B cell-intrinsic dysregulated signals leading to autoimmunity have been directly tested. Importantly, we predict that altered B cell function is likely to exert a much broader impact on human autoimmune pathogenesis than is currently appreciated. Indeed, examination of published GWAS in SLE, T1D, RA and MS demonstrates that a significant percentage of putative risk genes are expressed in B cells (Figure 2). Although not yet tested, we anticipate that a subset of these variants will exert B cell-intrinsic impacts in autoimmunity.

Several important questions remain regarding how disease-associated signaling variants promote autoimmunity. For example, in addition to driving mature B cell activation and GC events, risk variants might directly impact the naïve B cell repertoire. Self-reactive B cells are removed from the repertoire via deletion, receptor editing and anergy. In addition, peripheral positive selection also modulates the naïve repertoire. Therefore, it will be important to address whether signaling variants that impact BCR, TLR, CD40 and/or BAFF (B cell activating factor of the TNF family) responses skew the repertoire toward greater baseline poly- and self-reactivity. In addition, as autoreactive B cells persist in the naïve repertoire even in healthy individuals, the mechanisms that constrain their initial activation and the impact of genetic risk variants on these events remains to be addressed.

Finally, an improved understanding of B cell mechanisms in autoimmunity carries the potential to develop new targeted therapies. Although B cell depletion has provided clinical benefit, its efficacy has been limited in other trials [62,63]; and long-standing B cell depletion carries a risk of infectious complications. Recent studies testing inhibitors of the BCR signaling effector, Bruton's tyrosine kinase, have shown utility in murine lupus models [64-66]. Thus, targeting dysregulated B cell signals using this or analogous agents directed at effectors downstream of BCR, TLR or CD40 may prove an effective therapeutic strategy.

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Highlights

1. B cells play multiple roles in autoimmune pathogenesis
2. A large proportion of autoimmune disease GWAS risk genes are expressed in B cells
3. Altered B cell signaling may be sufficient to initiate autoimmune germinal centers
4. B cell-intrinsic, dual BCR/TLR (especially TLR7) signals generate autoantibodies
5. B cell-derived cytokines may facilitate or sustain autoimmune GC responses

Figure 1. Model for how altered B cell intrinsic signals may function to orchestrate autoimmune GC responses

Schematic showing how alterations in signaling effectors in BCR and other co-stimulatory signals (including candidate GWAS risk variants or gene disruption) function to increase susceptibility to autoimmune disorders. In response to self-antigens (such as apoptotic cells) dysregulated signals downstream of the BCR, TLR and/or CD40 pathways, including exaggerated dual TLR and self-antigen specific BCR signals, function to facilitate spontaneous GC formation. Activated B cells promote activation of self-reactive T cells via self-antigen presentation - leading to a loss of T cell tolerance. Cytokine mediated feed-forward signaling likely further amplifies this process. The emerging GC reaction facilitates both affinity maturation of autoreactive B cells and generation of plasma cells secreting pathogenic class-switched autoantibodies. Examples of candidate risk alleles are displayed in red and include GWAS variants in *PTPN22* (protein tyrosine phosphatase nonreceptor 22); Src family kinases, *LYN* and *BLK*; *SLC15A4* (solute carrier family 15, member 4); *TNFAIP3* (tumor necrosis factor, alpha-induced protein 3); *TNIP1* (TNFAIP3 interacting protein 1); *BANK1* (B-cell scaffold protein with ankyrin repeats 1); and loss of function in *WAS* (Wiskott Aldrich Syndrome).

Figure 2. Autoimmune GWAS risk alleles include a large array of genes expressed in B cells
Disease-associated risk variants in SLE, T1D, RA and MS were identified using a catalog of published GWAS alleles (www.genome.gov/gwastudies). B cell expression of risk genes was evaluated using the Immunological Genome Project database (<http://www.immgen.org/databrowser/index.html>). The total number of risk genes in each disease is shown, with the percentage of genes expressed in B cells listed and displayed in red.