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B cells and Autoimmunity

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Abstract

There is a growing appreciation for the role for B cells in autoimmune disorders in which inflammation is driven by T cells, in addition to the well-established role for B cells in autoimmune disorders characterized by pathogenic auto-antibodies. Current information on tolerance checkpoints in B cells, B cell depletion, BAFF blockade, regulatory B cells and clonal ignorance mediated by the SIAE/Siglec pathway will be reviewed.

The mechanisms of immune mediated injury have long provided a basis for categorizing most autoimmune disorders into two broad sets of diseases – one set of disorders in which inflammation is driven by T cells and another group of conditions in which auto-antibodies play a key role, either by binding to tissue antigens or by forming immune complexes. This approach to segregating autoimmune disorders into T cell mediated and so-called B cell mediated diseases has eroded quite dramatically in recent years.

It is now recognized that T cell help for B cells during adaptive immune responses is reciprocated by a critical role for B cell help during CD4+T cell activation, as discussed below. While auto-antibody linked diseases such as systemic lupus erythematosus, myasthenia gravis, and Goodpasture's syndrome, among many others, likely involve a break in tolerance in antigen-specific B cells, an important role for T cells in this category of diseases has also long been recognized. Most disease related auto-antibodies are IgGs that are somatically mutated suggesting that helper T cells drive the autoimmune B cell response [1]. More recently it has also been recognized that B cells play important roles in inflammatory conditions such as rheumatoid arthritis, multiple sclerosis, and type I diabetes, disorders that have long been considered to be mediated primarily by T cells. It is clear that in most autoimmune disorders cells of both lymphocyte lineages cooperate closely in disease pathogenesis.

Although studies on the role of B cells in autoimmunity have focused primarily on the mechanisms of B cell tolerance and how tolerance may be abrogated in auto-antibody mediated diseases, it is possible that a loss of B cell tolerance might occur in almost all autoimmune disorders. In diseases in which specific autoimmune T cell clones drive the process of inflammation, auto-antibody production may represent a marker for the expansion of auto-antigen specific B cells that capture and present self-antigen peptides to inflammatory T cells. In other disorders, T cell help appears to be a crucial component in driving self-reactive B cells to make pathogenic auto-antibodies.

In this review we will first attempt to provide an overview of what is known regarding B cell tolerance checkpoints during development and in germinal centers, both in rodents and humans, and discuss the postulated role of B regulatory cells in tolerance and autoimmunity. We will then consider why B cell depletion facilitates the remission of some autoimmune disorders; in this context we will examine the role of B cells in antigen presentation and the induction of CD4+ T cell memory and/or effectors in the context of autoimmunity. We will finally consider the phenomenon of clonal ignorance in B cells mediated by the Siglec/SIAE inhibitory pathway.

Tolerance checkpoints during B cell development – lessons from rodents

B cell tolerance has been studied extensively using BCR transgenic and knockin mice as well as mice in which critical recognition or signaling molecules of developmental relevance have been mutated.

A major tolerance checkpoint in the bone marrow occurs just after small pre-B cells (surface IgM negative cells that are actively rearranging at the κ light chain locus) transition into the immature B cell stage. Immature B cells are the first cells to express the BCR which at this stage is made up the of μ heavy chain (and not the δ chain), a newly rearranged and synthesized κ light chain (not λ, which is rearranged later) and an Igα/Igβ heterodimer. For multivalent antigens such as membrane proteins, membrane glycolipids and nucleic acids, this checkpoint is mediated primarily by the process of receptor editing wherein immature B cells that recognize self-antigens with high avidity are apparently induced to revert to a pre-B cell like phenotype, express Rag genes and induce additional κ light chain gene rearrangements [2–7]. Novel Vκ-Jκ rearrangements, involving upstream V segments and downstream J segments, delete the original Vκ-Jκ rearrangement that had produced a self-reactive light chain. The new rearrangement may be out-of- frame, or could be in-frame but perhaps still produce a κ light chain that is self-reactive. Continuing BCR signaling because of self-reactivity at this stage presumably results in ongoing receptor editing on both κ light chain chromosomes, followed by rearrangements at the λ light chain gene locus, accompanied by deletion of the κ loci. The presence of a heptamer (CACAGTG) containing RS (recombining sequence) element downstream of Cκ [8] permits the deletion of the κ locus during receptor editing and appears to be crucial for the induction of λ light chain gene rearrangement [9]. RS element knockout mice have both tolerance defects and defective Igλ gene rearrangement [9]. In humans the analogous sequence similar to the RS element is called the κ deleting element or KDE [10]. An assay for receptor editing has been developed that identifies the frequency of RS/KDE recombination events in B cells [11]. This assay has revealed defects in receptor editing in human subjects with lupus and type I diabetes.

While multivalent antigens appear to primarily mediate receptor editing, soluble antigens mainly induce anergy [12,13]. Some deletion of self- reactive B cells also probably occurs in immature and transitional B cells in vivo. Evidence for deletion and anergy was originally obtained from pioneering and elegant studies [12,14–16] employing conventional BCR transgenic models. More “physiological” BCR knockin models however provide strong evidence supporting the view that receptor editing rather than deletion is the dominant mechanism of tolerance induction by multivalent antigens in developing B cells [4–7]. In BCR knockin models rearranged Ig heavy and κ light chain genes are inserted respectively into the endogenous heavy chain locus on mouse chromosome 12 and the κ light chain locus on mouse chromosome 6, and not into random sites on other chromosomes. It is therefore possible for receptor editing at the κ locus to delete the original self-reactive Vκ-Jκ exon that had been inserted in the correct position in the κ locus. In contrast, in conventional BCR transgenic mice, the “offending” self-reactive light chain is expressed by a transgene inserted in some random chromosomal location and this cannot be deleted by a new Vκ-Jκ rearrangement on chromosome 6. In such situations the self-reactive light chain persists

even if receptor editing does occur, and continuing signaling might result in cell death. Indeed while the original conventional BCR transgenic studies using membrane bound hen egg lysozyme and MHC class I as self antigens revealed an important role for deletion, knockin BCR experiments with the same antigens revealed that tolerance was mediated primarily by receptor editing [5,7].

Clonal deletion may occur at some frequency in B cells that recognize multivalent self antigens even though receptor editing may well be the dominant mechanism. Deletion may be crucial when all editing options are exhausted, and may be the major mechanism that drives pre-BCR censoring (see below) when most of the self-recognition is mediated by the heavy chain alone. It could be argued that multivalent antigens that do not have access to the bone marrow but which can encounter transitional B cells in the spleen might mediate deletional tolerance since receptor editing is no longer an option. There is no evidence at present to support this speculation. The relevance of deletion as a central tolerance mechanism receives support from studies in which transgenic expression of Bcl-2 preserves self-reactive B cells beyond those tolerized by receptor editing [9].

The earliest tolerance related checkpoint during B cell development may occur at the pro-B to pre-B cell transition and is a phenomenon known as pre-BCR censoring [17]. Assembly of the pre-BCR results in positive selection of pre-B cells in which heavy chain gene rearrangement has been productive and signals are generated in a ligand independent fashion [18]. In contrast, during pre-BCR censoring, it is possible that specific self-ligands do interact with some pre-BCRs. In surrogate light chain deficient mice, some autoreactive Ig heavy chains are found in increased frequency in the periphery and auto-antibodies are generated [17]. Some pre-B cells that express potentially self-reactive Ig heavy chains are culled by a poorly understood mechanism, possibly involving self-antigen recognition by the pre-BCR coupled to deletion. It is difficult to assess exactly how important pre-BCR censoring is in terms of tolerance. Indeed it has been shown that pre-BCR signaling resembles the analogous process initiated by auto-reactive BCRs: both categories of receptors initiate light chain gene rearrangement, primary light chain gene rearrangement in the case of the pre-BCR and secondary rearrangements, as discussed above, when signals are initiated by autoreactive BCRs [19].

Immature B cells migrate from the bone marrow to the spleen as part of the maturation process although some maturation can continue in the bone marrow as well [20,21]. Splenic cells with a phenotype that resembles that of immature B cells ($IgM^{+}IgD^{-}$ $CD23^{-}$ $CD21^{lo}CD93^{hi}$ $CD24^{hi}$) are recent emigrants from the bone marrow that are assumed to have not yet entered the follicular niche and are called T1 cells. Splenic B cells that retain markers of recent generation ($CD93^{hi}CD24^{hi}$ B cells) and which are CD23+ begin to express IgD (they can be IgD^{-} or IgD^{+}), are called T-2 transitional B cells and represent recent emigrants that have entered the follicular niche and have acquired the ability to recirculate. T-1 and T-2 B cells that encounter self-antigen that is multivalent may be clonally deleted. Immature, T-1, and T-2 B cells that are repeatedly stimulated by high affinity self antigen (likely monovalent protein antigens for the most part) may be anergized and acquire an $IgD^{hi}IgM^{lo}CD93^{+}$ T-3 B cell phenotype. Most transitional cells however are not as strongly self-reactive as cells that will be deleted or anergized and these less obviously self-reactive B cells differentiate either into long-lived follicular B cells or into MZ B cells. Long-lived recirculating follicular B cells can be divided into $IgD^{hi}IgM^{hi}CD93^{-}$ $CD21^{int}$ FO-II cells that do not require self- antigen and Btk during ontogeny and $IgD^{hi}IgM^{lo}CD93^{-}$ FO-I B cells that require Btk for their development [22,23]. The possible role of BAFF in regulating anergy and deletion will be discussed in a subsequent section.

In rodents splenic B cells that are $IgM^{hi}IgD^{hi}CD21^{hi}CD1d^{hi}$ are categorized as marginal zone precursor B cells or MZP cells. Mutations that block MZ B cell development almost always result in the loss of MZP cells [24]. Although MZP B cells have sometimes been called T2-MZP cells (and we are responsible in part for this older terminology, [25]), it is more appropriate to define T2 cells as a distinct population as described above. In in vitro stimulation studies MZP cells are capable of making high levels of IL-10, have been called B regulatory cells, and in certain models these cells can suppress the disease process when introduced into recipient mice. B regulatory cells will also be discussed separately below.

B cell tolerance in the context of the germinal center reaction

Epitopes on the surface of microbial protein antigens are recognized by specific B cells in the follicle and the B cell activation that results typically does not suffice to induce cell cycle progression but can contribute to endocytosis via the BCR and initiate the expression of CCR7 on these B cells that can now begin to migrate out of the follicle towards the T cell zone. The same protein antigen when initially internalized and presented by activated dendritic cells results in specific CD4+ T cell activation and expansion, induction of CXCR5 expression on these T cells and their migration towards the B cell zone. CD4+ T cell mediated activation of specific B cells via CD40L –CD40 interactions induces B cell activation and expansion initially at extra-follicular sites. Activated B cells in turn can trigger activated CD4+ T cells, in part via ICOSL-ICOS interactions to differentiate into T follicular helper (T_{FH}) cells (reviewed in [26,27]). Interactions between activated B cells and activated CD4+ T cells in the inter-follicular zone can be prominently observed using multi-photon microscopy and B-T interactions are important beyond T_{FH} cell differentiation for many CD4+ T cell responses as will be discussed below.

T_{FH} cells express high levels of CXCR5, migrate into the follicle and help set up the environment for germinal center formation [26,28,29]. Activated B cells undergo rapid proliferation and somatic mutation in the dark zone of the germinal center [30,31] and in the light zone antigen bound to FDCs may be captured by high affinity B cells which may then be able to present peptides to T_{FH} cells, receive help and survive [26,27,32].

IL-21 secreted by T_{FH} cells may contribute to the high levels of Fas and the low levels of Bim expressed by GC B cells which might in turn render these cells prone to apoptotic death by both the mitochondrial pathway and death receptor signaling. It is possible that high affinity B cells may be induced to express c-FLIP, an antagonist of caspase-8, either though the BCR or because they more readily capture and present antigen in order to receive T cell help. These cells may thus not be killed by FasL expressed on T_{FH} cells resulting in the predominance of CD40 mediated survival, proliferation and differentiation signals. Some regulation of B cells in the germinal center may be mediated by a subset of regulatory T cells that resemble T_{FH} cells and express CXCR5 [33]. Whether these T follicular regulatory cells contribute to self-tolerance in the germinal center reaction remains to be determined. There are two broad issues to be considered in the context of B cell tolerance in the germinal center reaction.

Firstly, in responses to a microbial/foreign antigen, somatic hypermutation may cause a B cell clone that was specific for a foreign epitope to mutate towards specificity for a self antigen. Such a self antigen, being ubiquitous, could potentially select a self-reactive B cell in the light zone and contribute to its expansion. How are such self-reactive B cells tolerized? It could be argued that if T_{FH} derived signals are essential for B cell selection and survival in the light zone, most often self-antigens will not yield peptides that can be recognized by T_{FH} cells and help will not be provided to autoimmune clones. It is possible that on occasion the very rare self-reactive B cell generated by somatic hypermutation is specific for a self –antigen that can complex with a foreign antigen and thus break tolerance.

How exactly such a scenario may be avoided is unclear. Single cell cloning studies have revealed a tolerance checkpoint between the germinal center B cell and plasma cell stages [34] that is regulated in part by FcγRIIb. In the absence of FcγRIIb an accumulation of self-reactive GC B cells synthesizing somatically mutated IgGs was observed suggesting that this inhibitory receptor contributes to the elimination of strongly self-reactive GC B cells [34]. It is possible that IgG immune complexes containing low affinity self antigens produce relatively weak BCR signals that are overpowered by negative signals from FcγRIIb, leading to deletion of self-reactive B cells. Alternatively perhaps inhibitory signaling through FcγRIIb that is induced by IgG immune complexes must be overcome by positive signals received from a T_{FH} cell in order for a GC B cell to survive in the light zone; self-reactive B cells presumably fail to receive the requisite T cell help to survive.

The second issue relates to weakly self-reactive B cells that are not of sufficient affinity for self to be tolerized by receptor editing, deletion, or anergy. Such clones may sometimes be activated by self-antigen complexed with a microbial protein. Such a scenario could result in T-B collaboration and somatic mutation. How tolerance may be regulated in such a scenario will be discussed in a later section on clonal ignorance in B cells.

B cell tolerance checkpoints in humans

Studies on tolerance checkpoints in humans have typically depended on an approach originally developed in the Nussenzweig laboratory that involves single cell cloning of B cells at different stages of development or activation, expression of matched Ig heavy and light chain genes in non-lymphoid cells and assays for self-reactivity or poly-reactivity of the expressed antibodies [35–37]. Using such an approach it has been shown that “Early Immature B cells” – phenotypically surface IgM negative pre-B cells in the bone marrow that are actively rearranging their κ light chain genes – contain rearranged μ heavy chain and κ light chain genes that are about 75% self-reactive, many encoding anti-nuclear antibodies. These early immature B cells also have an enhanced frequency of polyreactive B cells, containing immunoglobulins that can bind to structurally diverse antigen such as DNA, LPS and some protein antigens including insulin. There is a significant drop in self-reactive and polyreactive B cells at the immature B cell stage. This checkpoint is sometimes referred to as the “central” checkpoint and most likely reflects ongoing receptor editing.

A second “peripheral” checkpoint has been described at which a loss of autoreactive B cells is observed when the frequencies of self-reactive B cells at the transitional B cell stage and in mature B cells are compared. Based on analogies with tolerance mechanisms in mice it would be reasonable to speculate that this second checkpoint reflects B cell deletion at the transitional B cell stage or anergy, or both. Individuals lacking MHC class II (the bare lymphocyte syndrome) or with CD40L defects causing the hyper IgM syndrome have a defect at this checkpoint. It has been suggested that naïve B cells present self-antigen to T cells, likely T regulatory cells, and that these Tregs specifically suppress the antigen presenting self-reactive B cells [38]. Defects in the central checkpoint as well as in the peripheral checkpoint have been observed in subjects with rheumatoid arthritis and systemic lupus erythematosus [39–42].

A third potential checkpoint has been observed in human subjects. A significant decrease in autoreactive B cells is seen between naïve B cells and IgM^+CD27^+ B cells – also known as IgM memory B cells or marginal zone B cells. This population of cells lacks self-reactive or polyreactive B cell receptors [43]. The mechanisms that regulate this third checkpoint are not understood.

In human subjects the IgG^+CD27^+ presumed class switched memory B cell population exhibits both some auto-reactivity as well as some polyreactivity even in healthy subjects,

suggesting that a break in tolerance occurs during the germinal center reaction [44]. Since this polyreactivity is observed in healthy subjects it is likely that these self-reactive IgGs are not pathogenic.

Regulatory B cells

An inflammatory colitis seen in TCRα knockout mice was shown to be exacerbated in the absence of B cells and ameliorated by the introduction of wild type B cells in transfer experiments [45]. IL-10 producing $CD1d^{hi}$ B cells were found to accumulate in the intestine and to suppress inflammation suggesting that a $CD1d^{hi}$ subset of B cells may represent regulatory B cells [46]. A similar regulatory role for IL-10 producing B cells was described in a collagen induced arthritis model [47] and in EAE models [48–50].

The in vivo source of regulatory B cells is unclear and the existence of a single defined population in an in vivo setting remains to be established. Many B cell populations, including B-1 B cells [51] and MZ B cells [52,53] have been suggested to possess regulatory properties, but two independently described but likely overlapping populations have been most thoroughly examined. $IgM^{hi}IgD^{hi}CD21^{hi}CD1d^{hi}$ splenic T2-MZP B cells and $CD5^{hi}CD1d^{hi}$ splenic B cells are the best studied putative regulatory B cell populations [54,55]. Both these $CD1d^{hi}$ populations secrete IL-10 when stimulated. Transfer of putative regulatory B cells (Bregs) with an MZP B cell phenotype (that are capable of secreting IL-10 upon activation in vitro) into mice with collagen arthritis and other inflammatory models have been shown to reduce inflammation [54]. In the arthritis model, transfer of in vitro activated MZP/Breg cells that are capable of secreting IL-10 results in the induction of FoxP3 expressing Tregs and a reduction in Th1 and Th17 $CD4^+$ T cells [56]. Suppression of inflammation by Bregs may however also be observed in mice that lack Tregs, and the exact mechanism of Breg action remains unclear. Studies involving B lineage specific deletion of MyD88 indicate that Bregs may be activated via TLR ligands in order to suppress other immune cells that are activated via TLRs [57].

There is some indirect evidence that Bregs might be relevant in human disease. Exacerbation of ulcerative colitis has been observed in subjects in whom B cells have been depleted with Rituxumab and colitis has been induced in subjects receiving Rituximab for lymphoma and Graves disease [58–61]. These and other case reports suggest that Bregs that dominantly inhibit intestinal inflammation might have been depleted resulting in an ulcerative colitis like presentation. Human $CD19^+CD10^+CD38^+$ B cells, that correspond to a transitional B cell population [62,63] can be induced in vitro to make IL-10 and to suppress T cell responses [64]. Cells with the same phenotype from lupus subjects were less efficient at suppression arguing that a defect in Breg function might be relevant in the pathogenesis of this disease [64]. A $CD24^{hi}CD27^+$ human B cell population that can be induced to secrete IL-10 in vitro has also been identified as a putative Breg population [65]. The relationship between these two putative human Breg populations remains to be determined.

Significant gaps remain in our appreciation of the in vivo role of Bregs. Bregs, as commonly defined, overlap with cells that are precursors of marginal zone B cells. The absence of MZP B cells is always correlated with an absence of MZ B cells. Mice with engineered genetic mutations in which MZP and MZ B cells are absent do not present with florid autoimmunity - certainly any comparison of relevance in a functional sense with Tregs would be premature. Detailed studies of Breg function in genetic mutants that cannot make MZP and MZ B cells need to undertaken.

While mice lacking B cells (μMT mice) have been reconstituted with mixtures of $IL\text{-}10^{-/-}$ and μMT bone marrow to study the role of IL-10 secreting B cells in vivo [56], conditional deletion of IL-10 in B cells, and eventually, when the technology becomes available, in

defined subsets of B cells should be undertaken to further understand the in vivo relevance of IL-10 secretion by B cells. Indeed while evidence for cytokine secretion by B cells activated in vitro is strong [66], in vivo evidence for the relevance of B cell mediated cytokine secretion must be ascertained by B cell specific conditional knockout studies, and this is still lacking.

B cell help for $CD4^+$ effector and memory T cell generation

As mentioned earlier, in the first day or two after immunization with a protein antigen, multiphoton microscopy reveals a large influx of activated helper T cells into the inter-follicular area [67]. A number of studies have established that B cells are required for the generation of $CD4^+$ memory cells in most antigenic contexts with some increasingly rare exceptions [68–70]. It appears therefore that although dendritic cells are the key antigen presenting cells for the initiation of $CD4^+$ T cell activation, B cells are required for subsequent activation of the $CD4^+$ T cells [71], particularly for memory generation. Although evidence for an antigen presentation function for B cells was not observed in earlier studies, it is possible that B cells that have expanded in response to T cell help via CD40 activation, present antigen to T cells during $CD4^+$ memory generation. $CD4^+$ effector and memory cells that drive the pathological changes in specific autoimmune disorders may therefore require B cells for their generation.

Lessons from Rituximab and Bemilumab: revisiting BAFF dependent tolerance checkpoints

CD20 is expressed on mature B cells and memory cells of the B lineage but not on long-lived plasma cells. Clinical trials have established that B cell depletion using Rituximab (anti-CD20) significantly ameliorates the disease process in a large number of autoimmune and chronic inflammatory diseases [72–74]. Its efficacy in diseases in which autoantibodies are not though to play a key role (multiple sclerosis and type I diabetes for instance) may reflect the importance of B cells as APCs and their role in sustaining $CD4^+$ effector and memory responses. It has been shown that remission in certain diseases can be linked to a striking reduction in B cells so it is perhaps less likely, as it has been sometimes argued, that an increase in regulatory B cells seen during the early phases of bone marrow recovery contributes to a suppression of disease symptoms [62]. In subjects with systemic lupus erythematosus Rituximab has been of variable efficacy. One possible explanation may be linked to the role of BCR – TLR collaboration during the process of lupus B cell differentiation [75]. In some autoimmune states autoantibodies arise from short-lived plasma cells in extra-follicular foci perhaps because auto-antigens generally lack the ability to provide conventional costimulatory stimuli [76]. Since lupus pathogenesis is driven by chromatin derived autoantigens that can both engage the BCR and activate endosomal TLRs, auto-antigen specific B cell differentiation into long-lived plasma cells may occur more readily in this disease than in many other auto-immune disorders. Since Rituximab cannot typically deplete these long-lived cells, the secretion of pathogenic auto-antibodies might not be attenuated by this biological therapy.

T cell responses have been monitored in Rituximab-treated patients with pemphigus vulgaris (PV). Although B cell depletion had no effect on the total T cell pool in these subjects, the frequency of desmoglein-specific autoreactive T cells in peripheral blood that produced interferon-γ (IFNγ) or interleukin-4 (IL-4) declined significantly following B cell depletion. In addition a reduction in desmoglein specific autoantibodies also accompanied the decrease in desmoglein-reactive T cells [77]. Similar results were obtained in patients with immune thrombocytopenic purpura (ITP); although autoantibodies contribute largely to the pathogenesis of ITP, disease subjects also present with predominantly monoclonal or pauciclonal T cell repertoires that are skewed towards the production of TH1 type cytokines

like IFNγ [78]. These T cells from ITP patients express higher levels of Bcl-2 and lower levels of BAX, suggesting the development of a memory or memory-like phenotype. These T cell changes were reversed in ITP subjects who responded positively to B cell depletion therapy.

Rituximab treatment has also been reported to promote the expansion of Treg populations [79,80]. These results have been reproduced in autoimmune mouse models. When B cells were depleted before the onset of disease in NOD mice expressing a human CD20-transgene the numbers of $Foxp3^+$ T cells increased, and $CD4^+$ T cells from these animals suppressed diabetes in adoptive transfer experiments [81].

BAFF/BLyS is a TNF family protein that functions as a growth factor for B cells after the T-1 stage of differentiation, essentially in the follicular milieu. Its receptor on developing B cells is BAFF-R [82]. BAFF and a related protein, APRIL, can interact with two other receptors, TACI and BCMA, not considered further in this review. BAFF is also required for the differentiation in the spleen of transitional and FO-II cells into MZP B cells and MZ B cells. High level overexpression of BAFF in transgenic mice results in a lupus like disorder, and it has been shown in transgenic BCR models that engineered high levels of BAFF can prevent the death of cells that would be otherwise anergized or deleted by self-antigen encounter [83,84]. This has led to the view that high levels of BAFF can contribute to autoimmune disease pathogenesis by preventing the tolerization of self-reactive B cells thus allowing them to enter B follicles and survive [85]. Subjects with lupus and Sjogren's syndrome have elevated levels of BAFF but these levels are considerably lower in relative terms than the BAFF levels in BAFF transgenic mice. The elevated levels of BAFF appear to be secondary to inflammation and activation of myeloid cells in autoimmune subjects.

Blockade of BAFF using antibodies or TACI-Ig in mice has ameliorated disease in animal models of autoimmunity [86], but Bemilumab (an anti-BAFF monoclonal antibody) therapy in human subjects with SLE has been disappointing, although it does reduce peripheral B cell numbers without reducing numbers of memory B cells or long-lived plasma cells [87,88]. BAFF levels may not be high enough to break tolerance in lupus subjects. Even if, at best, BAFF plays a secondary role in pathogenesis and contributes to a break in tolerance, Bemilumab would be unlikely to eliminate the cells that are the source of the pathogenic auto-antibodies in lupus subjects. Bemilumab would be expected to perhaps cause an increase in transitional and presumed Breg cells but such increases, if they do occur, do not appear to be effective in controlling disease at least in subjects with SLE.

Given the fact that Bemilumab only targets BAFF, while Atacicept (TACI-Ig) blocks both BAFF and APRIL, it might be assumed that the latter reagent could possibly phenocopy Rituxan when used in subjects with autoimmunity, given the requirement for BAFF and/or APRIL for B cell survival. BAFF and APRIL, however, are required for naïve follicular B cell and marginal zone B cell survival but may not be essential for memory B cells. While Rituxan can lead to the elimination of both naïve and memory B cells, Atacicept does not contribute to a significant reduction in the memory B cell pool. As a result, in disorders such as rheumatoid arthritis and multiple sclerosis, while Rituxan has proven to be therapeutically effective, Atacicept therapy has not proven to be useful (89–91). In subjects with multiple sclerosis Atacicept led to an unexpected increase in inflammation, and trials in subjects with this disease were suspended (91). Although with Rituxan, naïve and memory B cells are depleted, it is possible that with Atacicept the memory B cell pool is preserved while perhaps regulatory B cells may actually be lost (along with naïve B cells), thereby perhaps leading to disease progression. The phenotype of bona fide human regulatory B cells remain to be unequivocally established, their requirement for BAFF and APRIL are unknown, and the relevance of BAFF blockade in autoimmune therapy remains questionable.

B cell immunodeficiencies and autoimmunity

Immunodeficiency states can result in autoimmunity for a number of reasons. Robust BCR signaling is required for receptor editing, clonal deletion and anergy, and defective BCR signaling could therefore contribute to autoimmunity. In milder cases of X-linked agammaglobulinemia, attenuated BCR signaling because of defective Btk molecules results in a breakthrough of self-reactive B cells at the bone marrow and early peripheral B cell tolerance checkpoints [92].

Hypomorphic Rag mutations causing the Omenn syndrome for instance could contribute to defective receptor editing, and an increase in BAFF levels that occurs when B cell numbers are depleted, and these phenomena (as well as T cell defects) may explain the autoimmune phenomena observed in this syndrome [93].

Examination of individuals with the bare lymphocyte syndrome and with the X-linked hyper-IgM syndrome has revealed a requirement for T cells in peripheral B cell tolerance as discussed earlier [38]. In subjects with mutations in activation induced cytidine deaminase (AID), the hyper-IgM syndrome may also be accompanied by autoimmune manifestations. Somatic mutation and isotype switching both depend on AID, and the possibility has been raised that estrogen receptor mediated regulation of AID transcription might help explain the gender bias in autoimmunity [94]. AID has been shown to be expressed not only in activated B cells but also in ES cells and in developing B cells in the bone marrow. In patients with defective AID autoimmune B cells accumulate both at the late pre-B checkpoint bone marrow checkpoint as well as at the peripheral naïve B cell checkpoint [95]. The exact function of AID in terms of tolerance in developing B cells is unclear. Since AID can contribute to DNA demethylation by deaminating methylated cytidines, it might possibly contribute to the regulation of V(D)J recombination and receptor editing at the bone marrow checkpoint. AID could potentially contribute to the induction of DNA lesions and contribute to the process of apoptosis during clonal deletion in the peripheral B cell checkpoint. AID knockout mice exhibit a defect in central tolerance which may be linked to defective apoptosis (96).

Some interesting information on the pathogenic role of TLR signaling in autoimmunity has been obtained from studying human subjects with genetic defects in IRAK-4, MyD88 and UNC93B. Signaling downstream of all TLRs except TLR3 makes use of the MyD88 adaptor and the IRAK-4 kinase. UNC93B is a polytopic membrane protein that is required for localization and signaling mediated by nucleic acid specific endosomal TLRs. Although all subjects with these disorders accumulated auto-reactive B cells at the bone marrow and peripheral B cell tolerance checkpoints, the patients did not exhibit the presence of auto-antibodies or develop any other features of autoimmunity [97]. TLRs are required for tolerance in part perhaps because TLRs on B cells contribute to tolerance induction, but TLRs are also required for auto-immune B cell activation and disease progression.

Clonal Ignorance in B cells – the SIAE/Siglec pathway

Weakly self-reactive B cells that fail to induce receptor editing, deletion, or anergy, mature and differentiate into follicular or marginal zone B cells. While such weakly self-reactive cells were once considered to be innocuous it is clear that these cells have the potential to be dangerous if they are permitted to receive T cell help and undergo somatic mutation.

It appears to be important for weakly self-reactive B cells to be controlled by inhibitory signaling and maintained in a state of clonal ignorance in order to protect against the development of autoimmunity. If a weakly self-reactive B cell were to be engaged by a self-antigen that is physically complexed to a foreign antigen, activation of such B cells could potentially facilitate the endocytic process, induce the expression of CCR7 and drive the

migration of such a B cell towards the T cell zone where it could present foreign peptides to previously activated $CD4^+$T cells. This could result in T-B collaboration between a T cell specific for a foreign peptide and a B cell specific for a self epitope. Somatic mutation at either extra-follicular sites or in the germinal center could result in the generation of strongly self-reactive B cells and auto-antibodies.

Although *defects* in BCR signaling would be expected to lead to a failure of receptor editing, or deletion or anergy, *enhanced* BCR signaling is observed in mutations of certain negative regulators of the BCR which can contribute to the development of autoimmunity. CD22/Siglec-2 is a negative regulator of the BCR which binds to α2, 6 linked sialic acid containing N-glycans and is then phosphorylated on cytoplasmic tail ITIM tyrosines by the Lyn tyrosine kinase, resulting in the recruitment of the SH2 domain containing tyrosine phosphatase SHP-1. Activated SHP-1 contributes to the attenuation of BCR signaling. Mice with a mutation in Lyn develop autoimmunity as do mice in which SHP-1 is conditionally deleted in B cells [98–101]. CD22 knockout mice develop anti-DNA antibodies as they age [102]. Some sialic acid containing glycoconjugates are modified in the Golgi by the addition of an acetyl moiety at the 9-OH position. This acetyl group can be removed by an enzyme known as sialic acid acetyl esterase or SIAE. CD22 cannot bind to 9-O-acetylated sialic and siae knockout mice present with enhanced BCR signaling and an autoimmune phenotype [103]. SIAE may regulate more than one Siglec on B cells and $CD22^{-/-}$/Siglec-$G^{-/-}$ mice present with an autoimmune phenotype although single mutant mice do not develop autoimmunity [104]. Defective rare variants of SIAE are enriched in human subjects with autoimmunity suggesting that the SIAE/Siglec pathway protects against autoimmunity in both mice and men [105].

CD22 and SIAE are expressed primarily in mature B cells and the Lyn knockout autoimmune phenotype is recreated when Lyn in knocked out only in the periphery [106]. The overall evidence suggests that the SIAE/Siglec pathway maintains peripheral tolerance by setting a threshold for B cell activation, and presumably prevents weak self-antigens from drawing cognate B cells into T-B collaboration and the risk of somatic mutation [107]. SIAE, Siglecs, Lyn and SHP-1 may function primarily to maintain B cells in a state of clonal ignorance.

Conclusions

Considerable progress has been made in understanding the role of B cells in autoimmunity from studies in mice as well as from therapeutic interventions, especially antibody mediated B cell depletion studies in human subjects. These analyses have revealed unexpected roles for B cells in large numbers of human inflammatory and autoimmune disorders previously thought to be primarily T cell mediated diseases. Novel tools have been developed in the field to identify tolerance checkpoints in human subjects that probably correspond to more functionally defined checkpoints in mice. Emerging roles for regulatory B cells and inhibitory mechanisms that mediate B cell clonal ignorance will likely contribute to and be integrated with the explosive growth in knowledge regarding human autoimmunity that is certain to result from numerous ongoing studies on rare human genetic alterations that make individuals more susceptible to autoimmune disorders.

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Highlights

- B cells contribute to both auto-antibody linked and helper T cell mediated inflammatory disorders
- Tolerance checkpoints in murine and human B lymphocytes are discussed
- Lessons from Rituximab mediated B cell depletion and Bemilumab mediated BAFF antagonism in human autoimmune subjects are considered
- Current views on Regulatory B cells has been described
- The contribution of the SIAE/Siglec pathway and clonal ignorance in autoimmunity have been discussed

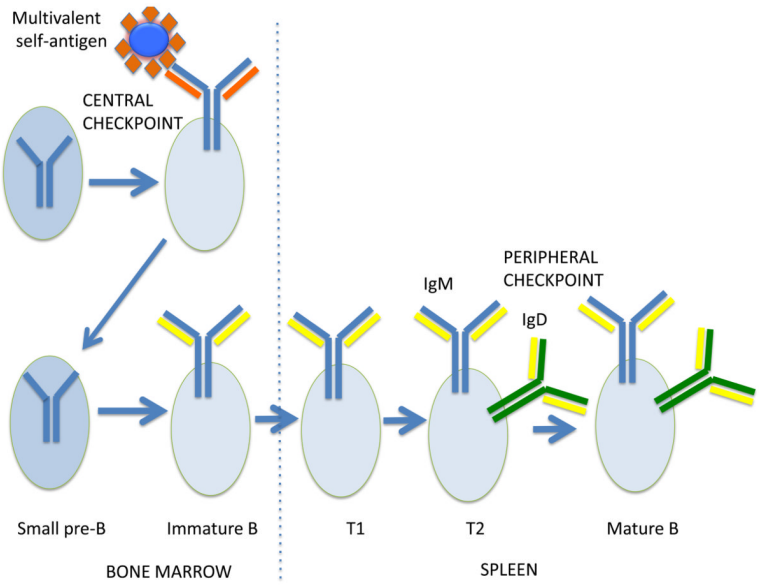

Figure 1. Receptor editing is a major mechanism of central tolerance in B cells

Immature B cells in the bone marrow that encounter multivalent self antigens revert to the small pre-B stage, continue to rearrange κ and if necessary λ light chain genes and generate newly generated B cells that have a novel light chain that is no longer self-reactive. Immature B cells with novel light chains that are no longer part of a self-reactive B cell receptor, then migrate to the periphery as T1 B cells where they mature into newly generated IgM and IgD expressing recirculating T2 B cells and then into mature recirculating B cells.

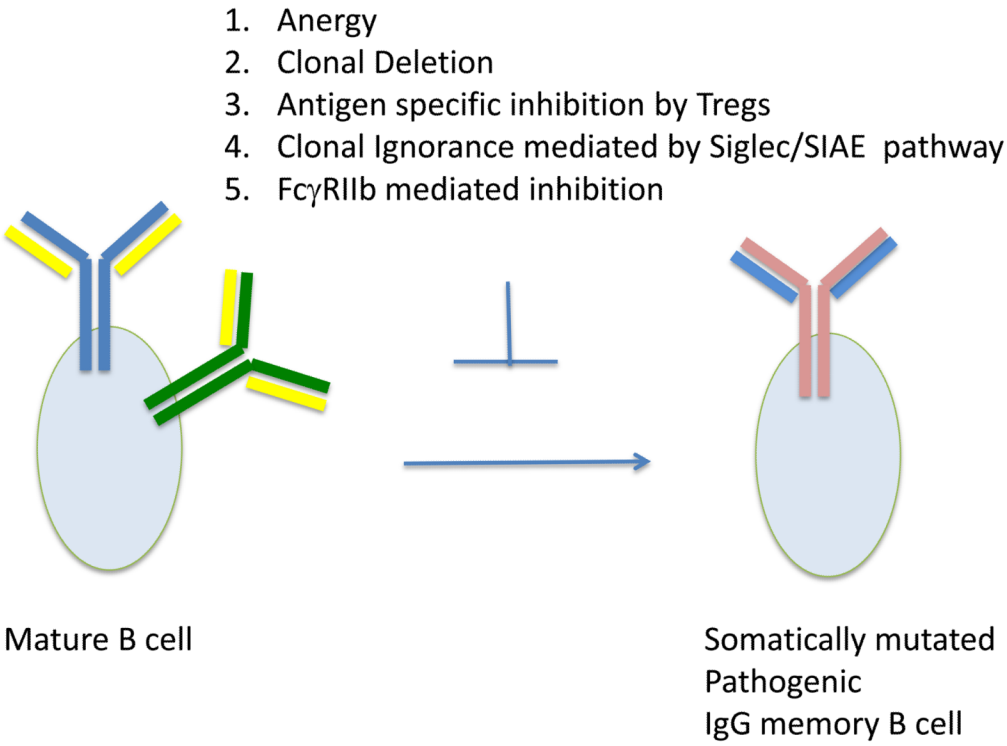

Figure 2. A number of tolerance mechanisms prevent mature peripheral B cells from developing into pathogenic class switched and somatically mutated auto-antibody producing B cells

These mechanisms include anergy, clonal deletion, specific inhibition of naïve B cells by Tregs in an MHC class II and CD40L dependent manner, clonal inhibition by the Siglec/SIAE pathway, and inhibition by FcγRIIb.