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Review

Regulation of B cell self-tolerance by BAFF

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Abstract

To avoid the generation of pathogenic autoantibodies, self-reactive lymphocytes are deleted at several distinct checkpoints during B cell maturation. BAFF is required for mature B cell development and survival but causes B cell hyperplasia and autoimmunity when it is overexpressed. Self-reactive B cells have reduced responsiveness to BAFF and therefore die due to the limiting levels of BAFF available in vivo. Elevated BAFF expression subverts B cell self-tolerance by rescuing self-reactive B cells normally deleted relatively late during maturation. Strongly self-reactive B cells are deleted prior to expression of BAFF-R and are therefore resistant to rescue by BAFF.

Keywords: Homeostasis; Marginal zone; Follicle; Hen egg-lysozyme; Transgenic

1. The requirement for immunological self-tolerance mechanisms

Adaptive immunity is characterised by the selective activation of lymphocytes expressing clonal antigen receptors that bind to epitopes associated with invading or foreign antigens. In the case of B cells, activation by foreign antigen results ultimately in the secretion of antibodies, soluble copies of the antigen receptor that can bind to and ultimately eliminate the invading antigen. The incredible diversity of antigen receptor binding specificities within both the B and T cell repertoires is critical for the immune system's ability to mount protective responses against the vast array of pathogenic microorganisms that may infect the host. However, antigen receptor diversity is generated by largely random processes such as V(D)J recombination, meaning that the production of lymphocytes that recognise components of the host organism (self-reactive lymphocytes) is inevitable. Recent data have shown that the generation of self-reactive lymphocytes is by no means a rare event, with up to 75% of the B cells produced in humans every day having significant self-reactivity [1]. The fact that diseases arising from destructive autoimmune responses are relatively rare demonstrates that effective mechanisms exist for preventing immune activation of self-reactive lymphocytes. This phenomenon of self-tolerance is fundamental to the normal functioning of the immune system and is enforced at multiple points during the development of both B and T lymphocytes [2].

2. BAFF and its receptors

In this review we will consider the role of the TNF superfamily molecule BAFF in the mechanisms that control the survival of self-reactive B lymphocytes. BAFF has become the most widely used name for TNF ligand superfamily member TNFSF13B, although it also known by a variety of other names including TALL-1, THANK, BLyS, and zTNF4. BAFF was initially identified through sequence homology searches for unknown TNF superfamily proteins and was subsequently found to bind to and promote the survival of both human and mouse B cells [3–5]. BAFF can be expressed as either a cell surface type II transmembrane protein or in soluble form following proteolytic release by furin-type proteases [3]. BAFF and the related TNF superfamily APRIL (TNFSF13A) both bind to two members of the TNF receptor superfamily, TACI (TNFRSF13B = CD267) and BCMA (TNFRSF17 = CD269). In addition, a third receptor exists, BAFF-R (TNFRSF13C = CD268) that binds BAFF but does not bind APRIL. More detailed discussions of BAFF and its receptors can be found elsewhere [6]. Before considering the specific roles of BAFF, it is pertinent at this point to examine B lymphocyte maturation and migration and how these processes relate to the various mechanisms by which B cell self-tolerance is enforced.

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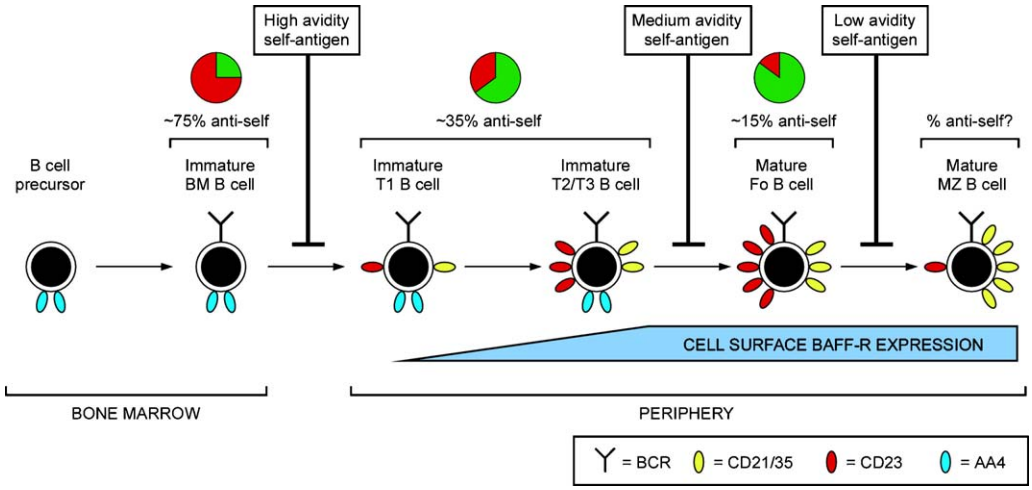

Fig. 1. Cell surface phenotype and self-tolerance checkpoints during B cell maturation. The various stages of B cell maturation are indicated together with the various points at which deletional self-tolerance is enforced as determined in the anti-HEL/HEL transgenic models (see Section 5). Data indicating the proportion of self-reactive B cells at specific points during maturation are from [1]. BCR = B cell antigen receptor.

3. B cell maturation and anatomical localisation

Committed B lymphocyte precursors proliferate and undergo Ig variable region gene rearrangements within adult bone marrow. Following successful rearrangement of both Ig heavy and light chain genes, immature B cells expressing B cell antigen receptor (BCR) of the IgM isotype are generated. From this point on, the fate of maturing B cells is susceptible to the influence of its antigenic environment. As B cells mature they leave the bone marrow and migrate predominantly to spleen. Maturation is associated with changes in a number of cell surface proteins, including the loss of the AA4 antigen, acquisition of CD21/CD35 and CD23 expression (Fig. 1) and coexpression of an additional BCR isotype in the form of IgD. Immature splenic B cells can be divided into the T1, T2 and T3 subpopulations based on differential expression of a number of cell surface markers [7]. Within 3–4 days of their initial production in the bone marrow, transitional cells either die or develop into the long-lived mature B cells that predominate in the periphery [8,9].

Mature splenic B cells can be divided into two distinct subsets. The first of these are the follicular (Fo) B cells which congregate around clusters of follicular dendritic cells (FDCs) within the white pulp (Fig. 2). B cell follicles are arranged around a central T cell rich area known as the periarteriolar lymphatic sheath (PALS). B cell follicles themselves are encapsulated by the marginal sinus, which is in turn surrounded by the splenic marginal zone (MZ) and the red pulp (Fig. 2). The B cells within the MZ represent the second major group of mature B cells in spleen. In addition to being anatomically distinct, Fo (CD21/CD35^{int}, CD23^{hi}, CD1d^{lo}, IgD^{hi}) and MZ (CD21/CD35^{hi}, CD23^{lo}, CD1d^{hi}, IgD^{lo}) B cells also differ phenotypically (Fig. 1). Moreover, MZ B cells show a number of functional differences including a greater propensity for activation and rapid antibody production. Although the precise lineage relationships between Fo and MZ B cells are not clear, they both belong to the B2 lineage and as such are distinct from the B1 B cells that predominate in the peritoneal and pleural cavities [10,11]. Since the survival of B1 B cells is not affected by either the absence or overexpression of BAFF [12,13] the regulation of self-tolerance in this lineage is not considered further here.

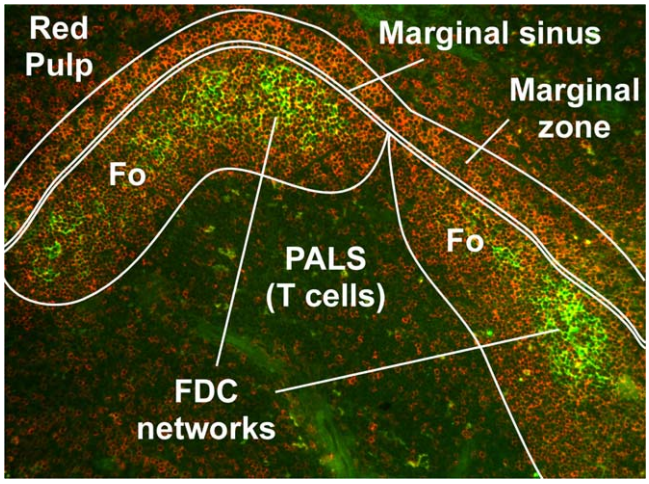

Fig. 2. Organization of B cells within mouse spleen. A section of spleen from a normal C57BL/6 mouse was stained with anti-B220-Texas Red to show B cells (red) and anti-CD21/35-FITC to identify follicular dendritic cells (FDC, green). Lines indicating the borders between the various subregions of the splenic white pulp are shown. PALS: periarteriolar lymphatic sheath, Fo: follicle. See Section 3 for details. Staining was performed by Michelle Amesbury.

4. The BCR and B cell tolerance versus activation

In order to maintain self-tolerance it is necessary that lymphocytes with significant self-reactivity be prevented from initiating immune responses. The mechanism(s) responsible for this must be selective insofar as lymphocytes that do not pose a danger to the host need to maintain their responsiveness in order to provide protective immunity. In the case of B cells, antigen specificity is provided by the cell surface immunoglobulin in the form of the BCR. Unlike T cell receptors (TCRs), BCRs can

bind soluble or cell-associated antigens in their native form without the requirement for presentation by MHC proteins. Binding of antigen to the BCR is the initial step in both immune activation leading to antibody production and the inactivation of self-reactive clones. That BCR ligation can lead to these two opposite outcomes in response to self and foreign antigens is due largely to the different contexts in which these antigens are typically encountered [14]. First of all, B cells that recognise self-antigens usually encounter these host molecules during their relatively short immature phase when their response to BCR ligation either renders them inactive (anergic) or leads to the death of the cell. Also, foreign- but not self-antigens typically deliver costimuli to the antigen-binding B cell (eg cognate T cell help, Toll-like receptor stimuli) required to drive proliferation and antibody secretion by B cells that have received a primary BCR stimulus [14]. Depending on the type of foreign antigen encountered and the costimulus that is delivered, the responses of B cells to foreign antigen can vary in terms of the antibody isotype produced, the site of antibody production and the generation of germinal center and memory cells. What has become apparent is that inactivation self-reactive B cells can also proceed in a number of different ways depending on the nature of the interaction with the self-antigen.

5. Self-tolerance check-points during B cell maturation

Because of the low frequency of cells that recognise a specific antigen within a normal B cell repertoire, it is difficult to track and determine the fate of "natural" self-reactive B cells as they mature. The advent of transgenic mouse technology combined with the allelic exclusion of endogenous Ig genes by rearranged Ig transgenes has facilitated the production of mice in which a large fraction of the B cells express the transgene-encoded BCR specificity [15]. By making the BCR reactive against either a natural self-antigen or transgenic "neo-self-antigen" it is then possible to follow the fate of B cells that recognise different types of self-antigen. One of the most informative systems has been the combination of anti-hen egg lysozyme (anti-HEL) BCR transgenic mice with separate transgenic mice expressing HEL in a number of different forms [15–17]. Double-transgenic mice expressing both HEL and anti-HEL BCR have revealed that B cell self-tolerance can be enforced at different stages during B cell maturation. Whilst these checkpoints are described below in relation to this model system, similar findings have been obtained in several other transgenic systems [2,15].

(i) *Immature bone marrow B cells*. The most stringent mechanism by which self-reactive B cells are removed from the repertoire is when they recognise high-avidity self-antigen immediately upon initial BCR expression (Fig. 2). This is exemplified by the developmental block and subsequent cell death that results when anti-HEL B cells are generated in mice that express HEL ubiquitously as a cell surface molecule [17,18]. Contact with high avidity self-antigen at this point can also trigger the process of receptor editing in which the self-reactive B cell can undergo additional Ig gene rearrangements, thus altering its specificity and potentially losing autoreactivity [19,20]. Strongly self-reactive B cells are therefore eliminated in the bone marrow either by undergoing cell death or receptor editing. Evidence from the analysis of maturing B cells in humans indicates that this type of self-reactive B cell makes up around half of all self-reactive B cells generated in bone marrow and around a third of total B cell production [1]. Thus whilst self-reactive B cells make up approximately 75% of newly generated immature B cells in bone marrow, this fraction drops to around 35% in immature peripheral B cells (Fig. 2) presumably due to the removal of strongly self-reactive clones.

(ii) *Immature to mature B cell transition in the periphery*. B cell tolerance to self-antigens of medium avidity can be observed in the anti-HEL system when HEL is expressed as a soluble protein. In this case the B cells bind the self-antigen in the bone marrow immediately upon BCR expression but neither die nor undergo receptor editing. Instead they survive and migrate into the periphery. Here their fate differs somewhat depending on the extent to which competing non-self-reactive B cells are also present. In the absence of competition, self-reactive anti-HEL B cells enter the long-lived mature Fo compartment, although they persist in a functionally inactive or anergic state [16,21]. When competing non-self-reactive B cells are present at a significant frequency, self-reactive B cells that have bound the medium avidity soluble HEL self-antigen do not mature but instead die around the immature T2 transitional stage (Fig. 2) and are excluded from the B cell follicle [21–23]. This latter case presumably reflects more accurately the normal fate of B cells recognising a self-antigen of medium avidity, since significant numbers of non-self-reactive B cells will inevitably be present within a normal B cell repertoire. Evidence that self-reactive B cells are indeed eliminated near the transition from immature to mature peripheral B cells in the normal repertoire once again comes from analysis of human B cells showing that the frequency of self-reactive B cells drops from ∼35% to ∼15% across this transition [1] (Fig. 2). As we shall in Section 8, the finding that the fate of self-reactive B cells can vary considerably depending on the presence of competition provides an important clue as to how self-reactive B cells are eliminated at this transition point.

(iii) *Marginal zone B cell development.* Although the precise lineage relationship between MZ and Fo B cells remains unclear, it appears that Fo B cells can act as precursors of MZ B cells at least under some circumstances [24,25] (Fig. 2). Consistent with such a relationship, there have been two instances observed using the anti-HEL/HEL transgenic system where self-reactive MZ B cells are eliminated but Fo B cells expressing the same self-reactive BCR are preserved. The first of these is seen when self-reactive anti-HEL B cells comprise >90% of all B cells in the mouse and HEL is expressed as a soluble self-antigen. Under these conditions, self-reactive B cells develop into mature Fo B cells but do not enter the MZ [16,26]. Whilst the lack of competition from non-self-reactive B cells in this case raises the question of whether the selective purging of MZ

B cells is physiologically relevant, the same phenomenon is observed when the anti-HEL B cells comprise <1% of all B cells but they recognise soluble HEL self-antigen with 10–100-fold lower affinity [27] (Fig. 1).

The overall picture to emerge, therefore, is that self-reactive B cells can be removed at three separate checkpoints points during their development depending on the strength of the interaction of their BCR with self-antigen. In this way the self-reactive B cells that pose the greatest threat to the host are eliminated at the earliest checkpoint in the bone marrow, whereas those that are potentially less dangerous are deleted in the periphery at the immature to mature transition or specifically removed from the MZ compartment only (Fig. 1).

6. Points of action of BAFF and BAFF-R during B cell maturation

The constitutive expression of BAFF in secondary lymphoid tissues is essential for sustaining the long-term survival of mature B cells in vivo. This is demonstrated both by the rapid depletion of mature B cells when their access to BAFF is blocked in adult mice [28,29] and the virtual absence of mature B cells in mice that do not express BAFF [12]. Conversely, transgenic overexpression of BAFF in vivo greatly increases the numbers of mature B cells [13,30]. This result not only demonstrates the activity of BAFF as a potent B cell survival factor [5,31] but also indicates that the levels of BAFF expressed under normal conditions is limiting insofar as they do not result in maximal B cell survival.

Analysis of mice specifically deficient in the expression or function of each of the three receptors for BAFF demonstrate that BAFF-R is completely responsible for delivering pro-survival signals to mature B cells by BAFF. Thus mature B cells are largely absent in mice that do not express BAFF-R or express it in a functionally inactive form [32–34] whereas mature B cells numbers are either unaffected or increased in mice lacking BCMA and TACI, respectively [12,35–37].

In contrast to the situation for mature B cells, immature bone marrow B cells and their immediate peripheral descendents (T1 transitional B cells) remain unaffected by both the absence of BAFF and its overexpression in vivo [12,13,30]. Thus B cell development up until the T2 transitional stage does not require BAFF-dependent survival signals. Consistent with this is that fact that BAFF-R, the pro-survival BAFF receptor for mature B cells, is virtually absent from newly generated and T1 B cells and is only expressed at high levels on B cells as they near the mature B cell transition [38] (Fig. 1). Not surprisingly therefore, the absence of BAFF-R expression does not effect the development of immature B cell populations in bone marrow or the periphery [33,34].

It has been long recognised that over half of the immature B cells that enter the periphery do not make the transition into mature long-lived B cell pool but instead die around the transitional T2 stage [8,9,39]. The fact that BAFF is both required for the transition of immature B to mature B cells and is present in limiting amounts in vivo provides a possible explanation for this phenomenon. Thus if the levels of BAFF available in vivo cannot sustain the survival and maturation of all the B cells that enter the periphery, then competition for BAFF should indeed result in the attrition of B cells at the T2 transitional stage. Consistent with this proposition is the observation that raising the availability of BAFF in vivo results in a substantial increase in the proportion of T2 cells that enter the mature B cell pool [38].

In addition to its role in regulating the immature to mature B cell transition, BAFF also appears to be particularly important for the generation and maintenance of the MZ B cell compartment. This is evident both from the particular susceptibility of MZ B cells to depletion of BAFF in adult mice [29] as well as the preferential expansion of this mature B cell subset in mice that overexpress BAFF [13]. Indeed overexpression of BAFF is not only associated with the accumulation of MZ B cells in their natural location in the spleen, but also with the appearance of MZ phenotype cells in other tissues including lymph nodes, blood and salivary glands [40].

7. BAFF overexpression and autoimmunity

As discussed in Section 6, the transgenic overexpression of BAFF results in B cell hyperplasia as a result of the potent pro-survival activity of BAFF and the limiting nature of BAFF expression under normal physiological conditions. Animals that express high levels of BAFF also suffer from a number of autoimmune manifestations including high levels of circulating autoantibodies, immune complexes in serum and kidneys, and proteinuria due to immune complex-mediated glomerulonephritis [13,30]. Older BAFF transgenic mice also show hallmarks of the autoimmune disorder Sjogren's syndrome, including sialadenitis and decreased saliva production [40]. Interestingly, a causal relationship between BAFF overexpression and human autoimmune disease is also suggested by the high levels of serum BAFF found in patents with Sjogren's syndrome [40] and the association between high serum BAFF and autoantibody production in several other autoimmune diseases [41].

Although BAFF may potentially act on multiple immune cell types, its prominent role as a B cell survival factor has led to the widespread theory that the presence of high levels of BAFF in vivo potentiates autoimmunity at least in part by preventing the normal deletion of self-reactive B cells. This theory is given more credence when it is considered that the two principal points of action of BAFF during B cell maturation, the immature to mature B cell transition and MZ B cell development (see Section 6) coincide with two out of the three major self-tolerance checkpoints (Fig. 1, see Section 5). Whilst a key role for BAFF in the enforcement and/or breakdown of B cell self-tolerance is an attractive theory, it was necessary to return to the transgenic models of B cell self-tolerance in order to confirm whether such an association does in fact exist.

8. BAFF and the regulation of B cell self-tolerance checkpoints

The influence of BAFF in the various B cell self-tolerance checkpoints has been primarily studied using the anti-HEL/HEL

transgenic systems described in Section 5. To mirror the discussion from that section, the three B cell self-tolerance checkpoints (Fig. 1) are again considered separately here, this time in terms of the potential roles played by BAFF.

(i) *Immature bone marrow B cells.* The evidence documented in Section 6 would indicate that these earliest of B cells are unlikely to be influenced by BAFF due to their lack of BAFF-R expression. This prediction was borne out by experiments performed by Thien et al. [27] in which they demonstrated that there was no effect on the deletion of anti-HEL B cells recognising membrane-bound self-antigen in the bone marrow in transgenic mice that expressed excess BAFF.

(ii) *Immature to mature B cell transition in the periphery.* Several earlier studies have shown that self-reactive B cells that would normally be deleted at the T2 transitional stage in the periphery following recognition of soluble HEL self-antigen can survive in the absence of competing non-self-reactive B cells [21–23]. As discussed in Section 6, the limiting amounts of BAFF normally present in vivo acts to regulate the transition of B cells through this developmental bottleneck. Together these observations suggested that self-reactive B cells may be deleted around the T2 stage due to reduced responsiveness to, and thus inability to compete for, limiting BAFF survival signals.

Lesley et al. [42] examined this possibility firstly by reducing the availability of BAFF in vivo by administering mice with a soluble version of the BCMA extracellular domain. This treatment reduced the survival of all B cells but particularly affected the survival of self-reactive B cells recognising soluble HEL self antigen with high affinity. In other words these cells were indeed more dependent on BAFF for their survival than the majority of B cells. The authors were also able to show that these self-reactive cells bound less BAFF per cell when competing non-self-reactive B cells were present [42]. Their data therefore support the idea that contact with medium avidity self-antigen reduces B cell responsiveness to BAFF, and thus makes the self-reactive B cells incapable of obtaining sufficient survival signals within a normal repertoire due to the limiting levels of BAFF present in vivo. Because these self-reactive B cells do survive and mature in the absence of competition, their responsiveness to BAFF is reduced rather than eliminated. This model predicts, therefore, that elevation of BAFF levels in vivo may indeed rescue self-reactive B cells that are normally deleted at the immature to mature B cell transition.

This prediction was directly tested by Thien et al. using BAFF transgenic mice. They showed that self-reactive B cells recognising soluble HEL self-antigen in the presence of competing non-self-reactive B cells were indeed rescued from deletion at the T2 transitional B cell stage, matured into Fo phenotype cells and efficiently colonized the splenic follicle in the presence of excess BAFF [27]. Interestingly however, if the self-reactive B cells were deleted slightly earlier during their maturation, they were resistant to rescue by the increased levels of BAFF expressed in these mice [27]. It appears, therefore, that self-reactive B cells that are normally deleted prior to entering the mature compartment can be rescued by increased expression of BAFF, but only if their normal point of deletion is close to this transition. This is likely to be due to the fact that the expression of the pro-survival BAFF-R increases during early maturation and peaks just prior to the mature transition (Fig. 1), giving the cells that reach this point the best chance of responding to BAFF.

An interesting aspect of the deletion of self-reactive B cells at the immature to mature transition is that these cells are prevented from entering the follicle and are primarily found in the PALS [21–23]. Because FDCs are located within the heart of B cell follicles (Fig. 2) and are known to express BAFF [43], it was possible that these or some other cells localised within the follicle may provide a critical source of B cell survival signals that cannot be accessed efficiently by self-reactive B cells excluded from the follicle. The possibility that such a mechanism may underlie the reduced ability of self-reactive B cells to compete for survival signals was investigated by Ekland et al. [44]. These authors found that self-reactive B cells that lacked expression of the chemokine receptor CCR7 were not excluded from the follicle but were still deleted prior to entering the long-lived mature B cell pool. Thus the inability of such self-reactive B cells to compete for BAFF-mediated survival signals does not result from reduced access to BAFF brought about by follicular exclusion. It is more likely that contact with self-antigen renders these B cells intrinsically hyporesponsive to BAFF survival signals, possibly via upregulation of the expression of pro-apoptotic protein Bim (see Section 9).

(iii) *Marginal zone B cell development.* Unlike the deletion of self-reactive B cells at the immature to mature B cell transition, the prevention of self-reactive anti-HEL B cells from entering the MZ compartment does not require competition from a non-self-reactive B cell population [26]. This on the one hand indicates that deletion of self-reactive B cells prior to MZ differentiation is relatively stringent, an assertion supported by the relatively low avidity of the HEL self-antigen required for this form of deletion [27] (Fig. 1). What this also means, however, is that competition for limiting BAFF is unlikely to be the mechanism for deletion of self-reactive B cells prior to their entry into the MZ compartment. Nevertheless, the potent activity of BAFF in expanding the MZ B cell compartment when it is overexpressed in vivo suggests that deletion of self-reactive B cells at this point may indeed be compromised by the presence of excess BAFF.

This question was investigated by Thien et al by observing the effects of transgenic overexpression of BAFF on the fate of self-reactive B cells that recognised soluble HEL self-antigen with relatively low affinity. Whilst these cells are normally excluded from the MZ B cell compartment, overexpression of BAFF restored them to this compartment in similar numbers to those present in the absence of their

self-antigen [27]. As well as being more easily activated by antigen (see Section 3), the physiological positioning of MZ B cells next to the marginal sinus (Fig. 2) means that they are more readily exposed than Fo B cells to polyclonal stimuli such as LPS and CpG that are typically associated with blood-borne pathogens [45]. Thus the promotion into the MZ compartment by excess BAFF of self-reactive B cell specificities that are normally restricted to the follicle may well contribute to the autoimmunity associated with BAFF overexpression.

Whilst self-reactive B cells are normally prevented from becoming MZ B cells in the anti-HEL/HEL transgenic model, a substantial amount of evidence indicates that some self-reactive specificities are in fact enriched in this B cell compartment [46]. It may be that such specificities do not undergo sufficient interaction with autologous structures to be eliminated from the MZ. Alternatively, the nature of their interaction with self-antigen may differ in some way to that between HEL and its transgenic BCR such that these specificities are positively selected into the MZ rather than being deleted. In either case, the expansion of these "natural" self-reactive MZ B cells by excess BAFF may contribute to the autoimmunity associated with BAFF overexpression over and above the rescue of normally deleted self-reactive clones.

In summary, the ability of BAFF overexpression to rescue self-reactive B cells from deletion is limited to those cells normally deleted relatively late in their maturation. The ability of self-reactive B cells to be rescued by BAFF is most likely determined by their expression of BAFF-R, which peaks around the point during B cell maturation where BAFF-mediated rescue begins to operate [27] (Fig. 1). It is probable that the expression of BAFF-R is delayed during B cell maturation to ensure that B cells with strong self-reactivity will not reach the point where they express this pro-survival receptor. If this were not the case then the autoimmunity associated with increased BAFF expression could well be significantly more catastrophic than it is.

9. Intracellular mediators of BAFF signalling and B cell self-tolerance

It is clear that the pro-survival signals delivered by BAFF through BAFF-R play an important role not only in the breakdown on B cell self-tolerance in the presence of excess BAFF, but also in the normal deletion of B cells at the immature to mature B cell transition (Section 8). It stands to reason, therefore, that the intracellular molecules that deliver survival signals from BAFF-R will also play an important role in B cell self-tolerance. Some of the molecules involved will be touched briefly upon here, particularly with regard to what is known about their roles in the regulation B cell self tolerance.

A number of studies have indicated that BAFF upregulates the expression of several of the anti-apoptotic members of the bcl-2 family of proteins in B cells, including Bcl-2, Bcl-xL, A1/Bfl-1 [5,31,38]. At the same time, BAFF signalling also downregulates the expression of pro-apoptotic family member Bim and so counteracts the upregulation of this molecule induced by BCR signalling [47]. These combined actions of BAFF almost certainly play an important role in sustaining B cell survival. The importance of Bim downregulation in particular is suggested by the fact that BAFF-transgenic and $bim^{-/-}$ mice both exhibit B cell hyperplasia and autoimmunity [13,30,48] and that $bim^{-/-}$ B cells are relatively resistant to antigen-induced cell death [49]. Direct evidence of a connection between BAFF and Bim in the regulation of B cell self-tolerance comes from the observation that peripheral self-reactive B cells express increased levels of Bim and that these levels increase further when competing non-self-reactive B cells are present [42]. Thus the reduced ability of these B cells to access BAFF under these circumstances and therefore counteract self-antigen-mediated upregulation of Bim may explain their elimination by competing non-self-reactive B cells in vivo [42].

Another intracellular signalling event triggered by BAFF is the processing of the p100 form of NF-κB2 to its active p52 form [50,51]. This non-canonical pathway of NF-κB activation, as opposed to the canonical (I-κB degradation-mediated) NF-κB activation pathway, is the predominant method by which BAFF activates this family of transcription factors [52,53]. It is likely that NF-κB2 processing contributes to the pro-survival signals delivered by BAFF since the survival of B cells lacking functional NF-κB2 is compromised [54] and many of the anti-apoptotic Bcl-2 family proteins are regulated by NF-κB transcription factors [31,38,55,56]. The serine/threonine kinases NIK and IKK-α are each required for NF-κB2 processing, whilst recent evidence indicates that the TNF receptor superfamily signalling proteins TRAF2 and TRAF3 both act as negative regulators of this process [53,57]. It is likely that all of these molecules are involved in the regulation of B cell self-tolerance although direct evidence of this is yet to be shown.

The final signalling molecule that will be mentioned here is protein kinase Cδ (PKCδ). The potential role of this enzyme in the regulation of B cell self-tolerance is evident from mice lacking PKCδ expression since these animals exhibit dramatic B cell hyperplasia and systemic autoimmunity [58]. Further analysis using the anti-HEL/HEL transgenic system showed that B cells that did not express PKCδ failed to undergo peripheral deletion in response to soluble HEL self-antigen [59]. A connection with BAFF-mediated survival signals was subsequently established when it was found that the pro-apoptotic translocation of PKCδ to the B cell nucleus could be inhibited by BAFF signalling [60]. It will be of great interest to determine whether natural mutations in any of the genes encoding molecules involved in BAFF signalling might be associated with human autoimmune diseases.

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