Chapter 3
Regulation of B-Cell Self-Tolerance By BAFF and the Molecular Basis of Its Action

Sandra Gardam and Robert Brink

Abstract Signals delivered following the binding of BAFF to its receptor BAFF-R are essential for the survival of mature conventional B cells. In order to maintain self-tolerance, B cells that express antigen receptors with significant reactivity against self-antigens are prevented from receiving or responding to these survival signals. The great majority of B cells produced from bone marrow precursors fail to join the mature peripheral B-cell pool either due to their self-reactivity or due to a stochastic failure to receive adequate survival signals from the limiting levels of BAFF available in vivo. The tight control over BAFF expression plays an important role in enforcing self-tolerance, as is illustrated by the escape of some self-reactive B-cell clones and the production of autoantibodies that accompanies the elevation of BAFF levels in vivo. Recent experiments have identified the molecular basis for the unique dependence of B cells on survival signals delivered by BAFF. In the absence of BAFF, B-cell survival is constitutively suppressed through the cooperative actions of the TRAF2 and TRAF3 signal adapters. BAFF circumvents this activity by triggering the recruitment of TRAF3 to BAFF-R and causing the depletion of TRAF3 from the cell via a TRAF2-dependent mechanism. In this way, critical B-cell survival signals such as the alternative NF-κB pathway are activated. Sustained exposure to BAFF is normally required to maintain the activity of these pathways and B-cell survival. However, this requirement is completely removed in B cells that lack expression of TRAF2 or TRAF3.

Keywords BAFF · B cells · Self-tolerance · Signalling · NF-κB · NIK · TRAF2 · TRAF3

R. Brink (✉)
Garvan Institute of Medical Research, Darlinghurst NSW 2010, Australia

M.P. Cancro (ed.), *BLyS Ligands and Receptors,* Contemporary Immunology,
DOI 10.1007/978-1-60327-013-7_3,

3.1 Regulation of Self-Reactive B Cells by BAFF

3.1.1 The Requirement for B-Cell Self-Tolerance

A fundamental characteristic of B and T lymphocytes is the rearrangement during early lymphopoiesis of the genetic elements that ultimately encode the variable regions of their cell surface antigen receptors. The essentially random nature of this process allows for the development of lymphocyte "repertoires" with the collective ability to recognise and respond to epitopes on virtually any foreign organism or antigen. The price of this diversity is the inevitable production of lymphocyte clones with the ability to recognise components of the host itself. Activation of these cells has the potential to lead to autoimmune destruction of host tissues and must be avoided if the immune system is to protect but not harm the host. In the case of B cells, the imperative is to prevent the differentiation of self-reactive B cells into plasma cells, as these cells secrete soluble copies of the antigen receptor (antibodies). Thus it is the differentiation of self-reactive B cells into plasma cells that leads to the production of potentially pathogenic autoantibodies.

Self-tolerance is ultimately a very efficient process since the production of pathogenic autoantibodies is a relatively rare phenomenon despite the fact that up to 75% of the B cells produced in humans have significant self-reactivity [1]. Because T-cell help is usually required to drive the differentiation of B cells into plasma cells, the production of autoantibodies is avoided to a large extent by maintenance of self-tolerance within the T-cell compartment. However, cross-reactive foreign antigens and T-independent stimuli both have the potential to trigger autoantibody production independently of autoreactive T helper cells [2]. An important component of self-tolerance, therefore, is the purging from the B-cell repertoire of clones that possess significant self-reactivity. This can occur at several different stages during B-cell development depending on the expression of the self-antigen recognised by the self-reactive B cell and the nature of the interaction of self-antigen with the antigen receptor (BCR).

3.1.2 Self-Tolerance Checkpoints During B-Cell Development

3.1.2.1 Immature Bone Marrow B Cells

Both the BCR expressed on the surface of B cells and the antibodies secreted upon their differentiation into plasma cells are encoded by the immunoglobulin (Ig) heavy and light chain genes. Rearrangement of Ig variable region genes occurs in the pro- and pre-B cells located within adult bone marrow. Clones that successfully rearrange their Ig genes and commence BCR expression in the bone marrow are referred to as immature B cells (Fig. 3.1). Immature bone marrow B cells represent the first stage of B-cell development where cellular fate is shaped by the interaction of the BCR with the external antigenic environment. Foreign antigens are not usually encountered within bone marrow. However, B cells that bind strongly to widely expressed

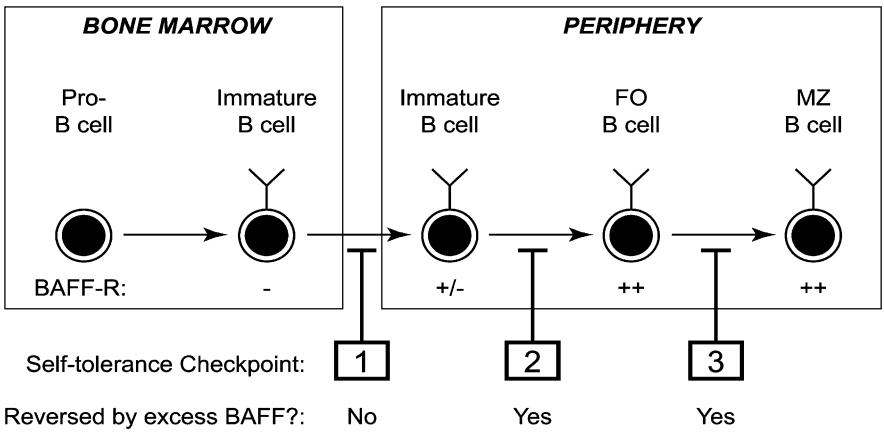

Fig. 3.1 Three major self-tolerance checkpoints during B-cell development and their potential for reversal by BAFF overexpression. A simplified model of B-cell development is shown that also indicates the three major Self-tolerance checkpoints (i.e. points at which self-reactive B cells are eliminated). Antigen receptor (BCR) expression is shown for all cells after the pro-B-cell stage and the relative levels of BAFF-R expression on these B cells is also indicated. The existence of experimental evidence for reversal of self-tolerance at each of these checkpoints is shown ("Yes" in this case indicates that some rather than all self-reactive B cells can be rescued from in vivo deletion by expression of excess BAFF – see Section 3.1.4). Immature B cells in the periphery are also referred to as "transitional" B cells and can be further subdivided into T1, T2, and T3 subsets (see Section 3.1.2.2). To avoid complexity, these subsets have not been included in this Figure. Mature B-cell subsets indicated are the follicular (FO) and marginal zone (MZ) populations

or bone marrow-specific self-antigens typically enter a process that either eliminates their self-reactivity or ultimately results in cell death (Checkpoint 1, Fig. 3.1).

Recognition of self-antigen by immature bone marrow B cells often triggers receptor editing. In this process, Ig gene rearrangements recommence in the immature B cell in an attempt to change the specificity of the self-reactive BCR [3, 4]. If successful, the newly non-self-reactive B cell is able to continue its development and join the peripheral B-cell pool. However, strongly self-reactive B cells that do not manage to change their specificity are blocked from developing further and subsequently die [5, 6] (Fig. 3.1). The elimination of self-reactive clones at this point in B-cell development appears to be the major mechanism by which B-cell self-tolerance is enforced. Thus, whilst 75% of newly generated immature bone marrow B cells are self-reactive, only 35% of the B cells that subsequently emerge from the bone marrow remain so [1].

3.1.2.2 Immature to Mature B-Cell Transition in the Periphery

B cells that survive receptor editing and deletion within the bone marrow eventually emerge from this tissue and migrate into the periphery, predominantly to the spleen (Fig. 3.1). These B cells are still not fully mature but pass through a number of immature "transitional" stages (T1, T2, T3) for 1–2 days before either entering

the mature peripheral B-cell pool or dying [7–9]. The great majority of immature peripheral B cells do in fact die before they fully mature, due at least in part to the further elimination of self-reactive clones at this juncture. Cells that enter the mature B-cell pool are able to survive for weeks to months within the periphery and occupy physiological niches within the peripheral lymphoid tissues such as the primary B-cell follicles of the spleen and lymph nodes as well as the splenic marginal zone (Fig. 3.1).

As was discussed in Section 3.1.2.1, B cells that strongly interact with self-antigen in the bone marrow are eliminated either by receptor editing or by clonal deletion. However, B cells that interact more weakly with self-antigen can evade both of these fates and migrate into the periphery. Nevertheless, weaker interactions with self-antigen during early B-cell development can still have significant consequences, such as rendering the B cell unresponsive (or "anergic") to stimuli that would normally result in cellular activation [10, 11]. The induction of anergy on its own serves as an effective mechanism of B-cell self-tolerance as it greatly reduces the chances of such cells undergoing plasma cell differentiation. In practice, however, anergic self-reactive B cells also fail to mature, survive for only a few days in vivo, and are excluded from the follicular regions of peripheral lymphoid tissues in which mature B cells normally reside [11–13]. Thus, although anergic self-reactive B cells can emerge from the bone marrow, they are unable to join the long-lived mature B-cell pool. Indeed, immature T3 B cells appear to represent the final stage of differentiation for anergic self-reactive B cells [14]. Although it has not been modelled experimentally, it is likely that self-reactive B cells that are "ignorant" of their self-reactivity in the bone marrow but that bind strongly to self-antigen upon their migration into the periphery also fail to make the transition from immature to mature peripheral B cell (Checkpoint 2, Fig. 3.1). The importance of this self-tolerance checkpoint is emphasised by the observation that frequency of self-reactive B cells drops from ∼35% in immature peripheral B cells to ∼15% in the mature peripheral B-cell pool [1].

3.1.2.3 Marginal Zone B-Cell Development

The majority of mature B cells are said to have a follicular phenotype, characterised by high levels of surface CD23 and IgD. These cells circulate around the body through blood and lymph and, together with follicular dendritic cells, occupy the primary follicles within secondary lymphoid tissues. Within the spleen, a separate subset of mature B cells called marginal zone (MZ) B cells is also present. As their name suggests, they occupy a separate MZ niche within the spleen that lies distal to the follicle [15]. MZ B cells do not recirculate, are characterised by low expression of CD23 and IgD and high expression of CD21 and CD1d, and show a greater propensity for activation and rapid antibody production compared to follicular B cells. Although the precise lineage relationship between MZ and follicular B cells remains unclear, it appears that follicular B cells can act as precursors of MZ B cells at least under some circumstances [16, 17] (Fig. 3.1).

A number of reports have suggested that the MZ B-cell compartment is enriched for self-reactive B cells [18]. However, there are at least two instances where a particular self-reactive BCR specificity is selectively eliminated from the MZ B-cell compartment but not the mature follicular B-cell population [19, 20] (Checkpoint 3, Fig. 3.1). Evidently, the development of MZ B cells is also a B-cell self-tolerance checkpoint and possibly the one which operates with the highest stringency of the three checkpoints considered here. The ease with which MZ B cells can be activated and undergo plasma cell differentiation provides a clear rationale for this to be the case.

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 (Fig. 3.1). 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 [15].

3.1.3 Points of Action of BAFF and BAFF-R During B-Cell Development

The constitutive expression of BAFF in secondary lymphoid tissues is essential for sustaining the long-term survival of mature B cells in vivo. Thus mature B cells are rapidly deleted when their access to BAFF is blocked in adult mice [21, 22] and mature B cells are virtually absent in mice that do not express BAFF [23]. On the other hand, both the survival and numbers of mature B cells are greatly increased in transgenic mice that overexpress soluble BAFF [24, 25]. Thus, although BAFF is a potent B-cell survival factor, its normal expression in vivo is limiting and not designed to result in maximal B-cell survival.

BAFF is capable of binding to three receptors: BCMA (B-cell maturation antigen), TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor), and BAFF-R (BAFF receptor, also known as BR3), all of which are expressed on B-lineage cells at various points during development [26]. 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 [27–29], whereas mature B cells numbers are either unaffected or increased in mice lacking BCMA or TACI, respectively [23, 30–32].

In contrast to the situation for mature B cells, immature bone marrow B cells and their immediate peripheral descendents remain unaffected by both the absence of BAFF and its overexpression in vivo [23–25]. 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 [33] (Fig. 3.1). 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 [28, 29].

As discussed above, 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, 34]. The fact that BAFF is both required for the transition of immature to mature B cells and is present in limiting amounts in vivo provides an 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 [33].

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 [22] and the preferential expansion of this mature B-cell subset in mice that overexpress BAFF [24]. 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 [35].

3.1.4 BAFF and the Regulation of B-Cell Self-Tolerance Checkpoints

As well causing B-cell hyperplasia, transgenic overexpression of BAFF in mice is associated with a number of autoimmune phenomena including autoantibody production [24, 25, 35]. In addition, high levels of BAFF have been associated with a number of human autoimmune diseases [26, 35, 36]. The possibility that the overexpression of BAFF may precipitate autoimmunity by circumventing the normal processes of B-cell self-tolerance has therefore received significant attention and is summarised below in relation to the three key B-cell self-tolerance checkpoints described in Section 3.1.2.

3.1.4.1 Immature Bone Marrow B Cells

As described in Section 3.1.3, the immature bone marrow B-cell compartment develops independently of BAFF or BAFF-R and is unaffected by the overexpression of BAFF in vivo. It seems unlikely therefore, that elevation of BAFF levels would interfere with the normal elimination of strongly self-reactive B cells that occurs during this early stage of development. This has been confirmed experimentally by the demonstration that the deletion of B cells recognising membrane-bound

self-antigen in the bone marrow (Checkpoint 1, Fig. 3.1) proceeds normally in transgenic mice overexpressing BAFF [20].

3.1.4.2 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 immature to mature B-cell transition in the periphery can mature and survive if they do not have to "compete" with non-self-reactive B cells [11–13]. Since limiting BAFF expression normally regulates the transition of B cells through this developmental bottleneck, this observation suggests that self-reactive B cells may normally be deleted around the T2 stage due to reduced responsiveness to, and thus inability to compete for, the limiting BAFF-survival signals available in vivo.

This possibility has been examined by reducing the availability of BAFF in vivo via the administration to mice of a soluble version of the BCMA extracellular domain [37]. This treatment reduced the survival of all B cells but particularly affected the survival of self-reactive B cells that recognised soluble 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. This study also showed that these self-reactive cells bound less BAFF per cell when competing non-self-reactive B cells were present [37]. This data therefore supported the idea that contact with self-antigen can reduce B-cell responsiveness to BAFF, and thus make 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 (Checkpoint 2, Fig. 3.1).

This prediction has been directly tested using BAFF transgenic mice. In this case, self-reactive B cells recognising soluble self-antigen in the presence of competing non-self-reactive B cells were rescued from deletion at the T2 transitional B-cell stage and matured into follicular B cells in the presence of excess BAFF [20]. 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 [20]. 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. 3.1), giving the cells that reach this point the best chance of responding to BAFF [20].

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 T-cell area of the spleen [11–13]. Because FDCs are located within the heart of B-cell follicles and are known to express BAFF [38], it was thought 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 has been investigated [39]. It was 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.

3.1.5 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 B cells from entering the MZ compartment (Checkpoint 3, Fig. 3.1) does not require competition from a non-self-reactive B-cell population [19]. 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 self-antigen required for this form of deletion [20]. 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 has been investigated by observing the effects of transgenic overexpression of BAFF on the fate of self-reactive B cells that recognise soluble 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 when their self-antigen was absent [20]. As well as being more easily activated by antigen, the physiological positioning of MZ B cells next to the marginal sinus means that they are more readily exposed than follicular B cells to polyclonal stimuli such as LPS and CpG that are typically associated with blood-borne pathogens [40]. 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.

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 [15, 20] (Fig. 3.1). Equally important, however, is the strength of the interaction between the BCR and the self-antigen. Thus, as is exemplified by the ability of BAFF overexpression to prevent the deletion of intermediate but not high-affinity self-reactive B cells from

the MZ compartment [20], excess BAFF is likely to rescue only peripheral self-reactive B cells that sit relatively close to the normal thresholds (e.g. antigen avidity) that determine whether or not a self-reactive B cell will be deleted.

3.2 Signalling BAFF-Dependent B-Cell Survival

The critical role of BAFF in regulating B-cell homeostasis and self-tolerance has resulted in an extensive investigation of the signalling events triggered by BAFF in B cells. Because primary B cells require BAFF for their survival, analysis of their responses to BAFF has proved difficult. Some of the major insights have come from in vivo mouse models in which B cells either lack expression of or express constitutively active versions of key signalling molecules. These types of analyses have indicated that activation of members of the NF-κB family of transcription factors is critical. Of particular importance appears to be the activation of the alternative (NF-κB2/p52) NF-κB pathway, which is mediated entirely by BAFF signalling through BAFF-R in resting primary B cells [41, 42] (Fig. 3.2). This pathway is essential for normal B-cell survival and recent experiments have revealed that the proximal regulators of the alternative NF-κB pathway in fact determine the BAFF-dependent nature of primary B-cell survival [43]. There is some evidence that BAFF-R can activate the canonical (NF-κB1/p50) NF-κB pathway and that this pathway may also play a role in BAFF-mediated B-cell survival (Fig. 3.2). In fact, like BAFF or BAFF-R-deficient mice, mice doubly deficient for both NF-κB1 and NF-κB2 lack mature B cells [44], whereas the single knockout mice have less severe phenotypes [44–47]. The exact nature of the events downstream of the NF-κB pathways and the relative importance of the BCR and BAFF-R in activating them are yet to be fully resolved.

3.2.1 The NF-κB Signalling Pathways

The operation of the NF-κB transcription pathways in immune cells has been recently reviewed elsewhere [48]. Briefly, the NF-κB transcription factor family consists of five proteins. NF-κB1 and NF-κB2 are synthesised in long precursor forms, p105 and p100, respectively, and are subsequently processed via partial degradation by the proteasome to form the active subunits, p50 and p52, respectively. This occurs constitutively for p105, whilst p100 processing requires the delivery of specific activation signals. The remaining members of the family are the Rel proteins: RelA (also known as p65), RelB, and c-Rel. The Rel proteins contain *trans*-activation domains capable of initiating transcription when they form dimers with p50 or p52 and bind to DNA in the nucleus. In the absence of signalling, pre-formed NF-κB dimers are held inactive in the cytoplasm. Upon signal initiation, nuclear localisation signals in the dimers are revealed, facilitating their migration to the nucleus to activate gene transcription (Fig. 3.2).

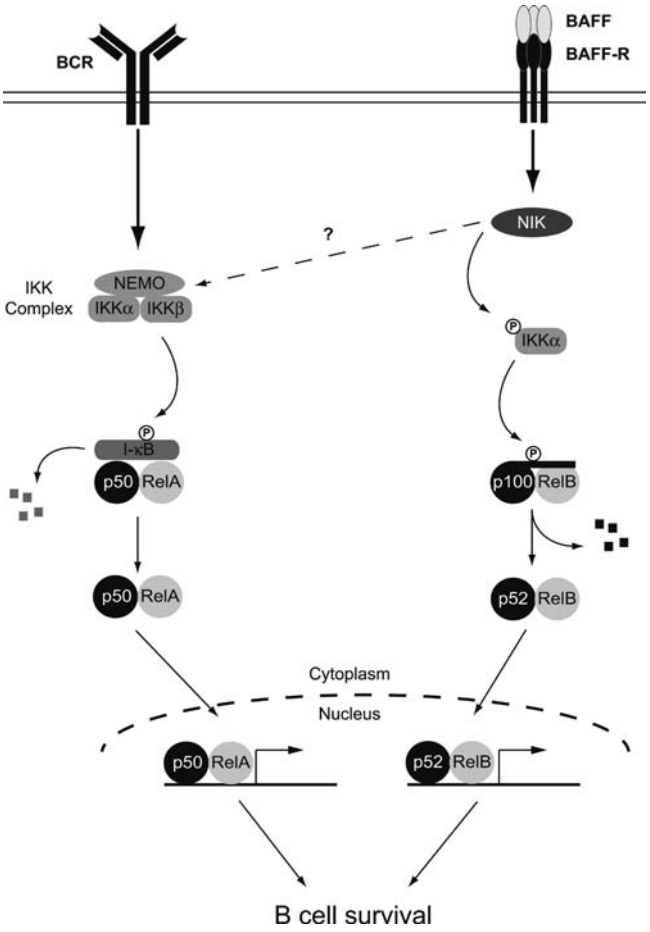

Fig. 3.2 The NF-κB signalling pathways. Canonical (*left*) and alternative (*right*) NF-κB activation in B cells contributes to B-cell survival (see Section 3.2.1 for details). Whilst BAFF-R is capable of initiating both pathways, it is probable in a physiological setting that the BCR is responsible for the majority of activation of the canonical pathway in naive primary B cells

3.2.1.1 The Canonical NF-κB Pathway

Following the constitutive processing of p105, the active subunit p50 forms dimers predominantly with RelA or c-Rel. These dimers are held inactive in the cytoplasm by inhibitors of NF-κB (Iκ-B) proteins, which mask their nuclear localisation signals. Receptors that strongly initiate canonical NF-κB signalling, such as the BCR and CD40, utilise a variety of signal adapters to cause the activation of the Iκ-B kinase (IKK) complex. This complex consists of a regulatory unit (IKKγ, also know as NEMO) and two kinases (IKKα and IKKβ) that are capable of phosphorylating Iκ-B proteins. Once phosphorylated, Iκ-B proteins are degraded by the proteasome,

releasing p50/RelA or p50/c-Rel dimers that can migrate to the nucleus and initiate gene transcription (Fig. 3.2).

3.2.1.2 The Alternative NF-κB Pathway

The precursor form of NF-κB2, p100, contains a carboxy-terminal region that is rich in ankyrin repeats and bestows it with inhibitory properties similar to those of the Iκ-B proteins. Thus p100 forms dimers in the cytoplasm, usually with RelB, and prevents its own nuclear localisation prior to processing. Activation of the alternative pathway requires the serine-threonine kinase NF-κB-inducing kinase (NIK). Accumulation of NIK protein appears to be the critical event in this pathway, with increased levels of NIK subsequently resulting in phosphorylation and activation of IKKα. Neither IKKβ nor NEMO are required for activation of the alternative NF-κB pathway. Rather IKKα alone is thought to be responsible for the phosphorylation of p100 at serines 866 and 870, which then initiates its proteasomal processing to p52 [49]. Liberated p52 remains in a heterodimer with RelB and together they can translocate to the nucleus (Fig. 3.2). The alternative NF-κB pathway is activated with slower kinetics compared to the canonical pathway and is primarily triggered through members of the TNF receptor (TNFR) superfamily such as LT-βR, CD40, and BAFF-R.

3.2.2 The Contribution of the Canonical NF-κB Pathway to B-Cell Survival

BAFF is able to activate canonical NF-κB signalling, but does so weakly and primarily via TACI [50]. However, evidence does exists for a low level of activation via BAFF-R [50, 51] raising the possibility that this pathway may contribute to the ability of BAFF to promote B-cell survival. The kinetics of the NF-κB1 pathway initiated by BAFF-R are considerably slower than is observed for strong activators of this pathway such as TNF and CD40L, and it has been suggested that BAFF-R may utilise NIK to activate the canonical as well as the alternative NF-κB pathway [52] (Fig. 3.2).

The importance of canonical NF-κB pathway in B-cell survival has been demonstrated by the fact that B-cell-specific ablation of NEMO results in a paucity of mature B cells [51]. However, this phenotype is not as severe as BAFF-R deficiency, confirming that the canonical NF-κB pathway is not the only pathway downstream of BAFF-R contributing to survival. Interestingly, NEMO-deficient B cells contained decreased amounts of alternative NF-κB pathway components, p100 and RelB. Processing of this reduced amount of p100 to p52 did occur in these cells, suggesting the alternative pathway was active [51]. There is evidence that the expression of both p100 and RelB is under the influence of canonical NF-κB dimers [53–55]. As such the impaired survival of mature B cells in the absence of NF-κB1 activity could be ascribed at least in part to a role for this pathway in controlling the transcription of alternative pathway components.

To determine if canonical NF-κB signalling alone is sufficient to allow B cells to survive past the immature to mature checkpoint in the periphery, a mouse line was produced with constitutive activation of the canonical pathway in B cells via a constitutively active IKKβ protein [51]. When crossed on to the BAFF-R-deficient background, this facilitated rescue of the mature B-cell compartment in the absence of alternative NF-κB pathway activation.

Whilst activation of the canonical NF-κB pathway appears to be an important factor in the survival of primary B cells, it is difficult to determine the extent to which this is due to the actions of BAFF. B-cell survival also depends on signals that constitutively emanate from the BCR, a known activator of the canonical NF-κB pathway [56]. Given the relatively poor activation of this pathway by BAFF-R, it is probable that it is the BCR that is primarily responsible for the canonical NF-κB activity observed in resting B cells [57]. On the other hand, the primary function of BAFF/BAFF-R signalling appears to be activation of the alternative NF-κB pathway (Fig. 3.2).

3.2.3 The Alternative NF-κB Pathway Is the Major Contributor to B-Cell Survival Downstream of BAFF-R

BAFF-R is responsible for virtually all of the alternative NF-κB pathway activation in mature B cells [41, 42] and it is this pathway which has been most strongly been associated with B-cell survival. Thus *Nfκb2*$^{-/-}$ mice are viable but display a deficiency of peripheral B cells [47, 58] and B cells lacking NF-κB2 fail to survive when provided with BAFF *ex vivo* [42]. In addition, mature NF-κB2-deficient B cells fail to survive in mixed bone marrow chimeras when they are forced to "compete" with wild-type B cells [59]. Taken together, these results confirm that, while the canonical pathway may contribute to survival, the alternative pathway is of primary importance in promotion of B-cell survival by BAFF. At this point, however, it remains unknown what gene targets are activated by the alternative NF-κB pathway in order to promote B-cell survival.

3.2.3.1 TRAF Proteins Are Fundamental Regulators of NF-κB2 Signalling

A phenotype similar to mice, which transgenically overexpress BAFF has recently been described in mice that lack B-cell expression of the signal adapters TNFR-associated factors 2 or 3 (TRAF2 or TRAF3) [43, 60, 61]. These mice display an expanded B-cell compartment, a surfeit of marginal zone B cells and hyperactivity of NF-κB2 in B cells, leading to the proposition that TRAF2 and TRAF3 negatively regulate BAFF signalling and NF-κB2 activation. In the case of TRAF3, this conclusion is consistent with previous in vitro observations. First, TRAF3 is the only TRAF to be recruited to BAFF-R [62]. Second, overexpression studies have shown that TRAF3 inhibits NF-κB2 activation via a number of TNFR members [63]. Third, studies in transformed B-cell lines have indicated that TRAF3

constitutively interacts with NIK and catalyses its proteasomal degradation, thereby inhibiting processing of p100 [64]. Finally, TRAF3-deficient mice display early post-natal lethality [65], a phenotype that is counteracted by crossing to the *Nfκb2*$^{-/-}$ background [66].

More unexpected was the phenotype of the mice containing TRAF2-deficient B cells. Overexpression of TRAF2 or its recruitment to members of the TNFR superfamily has been shown to activate the canonical NF-κB and JNK pathways [67–69] and a negative regulatory role for TRAF2 had not previously been described. Additionally, TRAF2 is not recruited to BAFF-R, so its involvement in signalling by this receptor has not been recognised before. However, it is clear from the conditional deletion approach [43, 61] that TRAF2 negatively regulates NF-κB2 signalling initiated by BAFF-R in primary B cells. In fact, removal of TRAF2 from B cells completely rescued mature B-cell development in BAFF-deficient mice [43]. Thus, the removal of this potent suppressor of B-cell survival pathways on its own was sufficient to activate these pathways and allow peripheral B cells to pass the immature to mature checkpoint. These B cells contained hyperactive NF-κB2 activation, but no significant changes in NF-κB1 activity [61] consistent with the proposition that the primary role for BAFF in B-cell survival is to activate the NF-κB2 pathway.

3.2.3.2 Unravelling the Proximal Signalling Events That Allow TRAFs to Suppress NF-κB2 Signalling

Production of mice with B cells doubly deficient in TRAF2 and TRAF3 revealed a phenotype not more severe than single deletion of either of these genes [43]. This indicated that TRAF2 and TRAF3 play cooperative and non-redundant roles in suppressing B-cell survival pathways. However, there is no precedent for these molecules acting in such a manner, raising the question of what molecular mechanisms underpin this activity.

TRAF2 and TRAF3 are both capable of interacting with NIK but do so at separate sites on NIK [64, 69, 70] and only TRAF3 can bind to BAFF-R. In vitro studies using transformed B-cell lines have indicated that TRAF3 binds NIK and facilitates its degradation in proteasomes [64]. How this occurs and what the role of TRAF2 remains open to question. TRAF proteins contain RING finger domains, zinc-chelated structures that can mark proteins for proteasomal degradation by catalysing the attachment of ubiquitin moieties via their lysine-48 side chains [71]. However, ubiquitin ligase activity associated with the TRAF3 RING finger domain has not been reported. The TRAF2 RING finger does have ubiquitin ligase activity but only appears to catalyse attachment of ubiquitin molecules via lysine-63, a process associated with the building of signalling scaffolds rather than proteasomal degradation [72–74]. However, TRAF2 can interact with cellular inhibitor of apoptosis protein 1 (c-IAP1), a ubiquitin ligase that is capable of catalysing lysine-48 ubiquitination [75]. Recent data showed that both c-IAP1-deficient and TRAF2-deficient MEFs contained higher amounts of NIK and increased levels of p100

processing [76]. Furthermore, c-IAP1 was shown to be capable of mediating degradation of NIK and the TRAF2-binding site present in c-IAP1 was essential for this function [77]. It is likely, therefore, that TRAF2 constitutively suppresses B-cell survival by recruiting c-IAP1 to NIK to facilitate its degradation. TRAF3 binding must also be required for this process although its precise role remains to be elucidated (Fig. 3.3a).

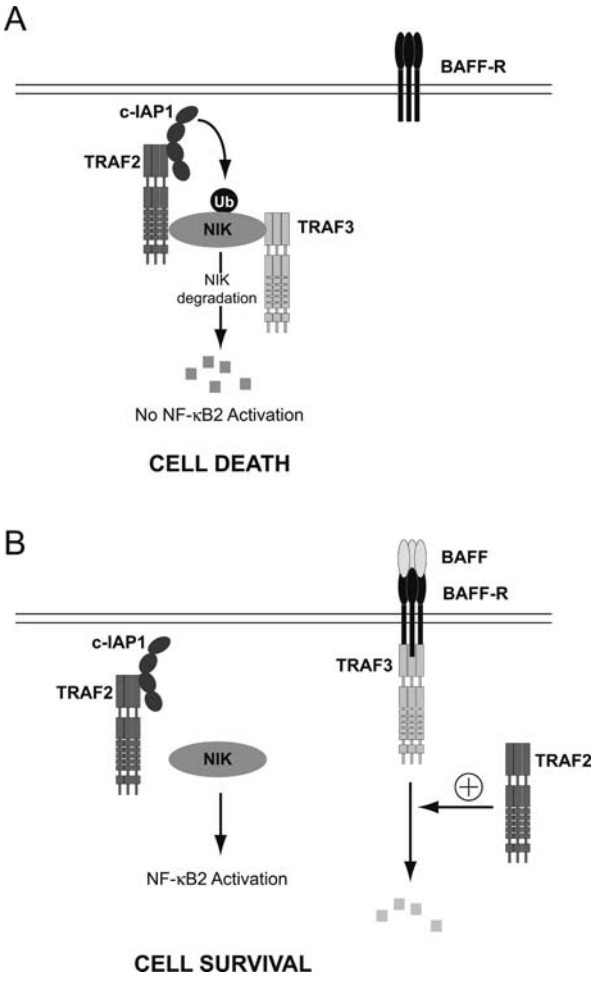

Fig. 3.3 A model of the proximal signalling events that regulate NF-κB2 activation downstream of BAFF-R. (**a**) When BAFF is absent, TRAF2, TRAF3, and c-IAP1 cooperate to ubiquitinate NIK leading to its proteasomal degradation. Under these circumstances NIK levels are too low to initiate NF-κB2 signalling and the B cell dies. (**b**) BAFF binding to BAFF-R recruits TRAF3 to the receptor and it is subsequently depleted from the cell in a TRAF2-dependent manner. Without the contribution of TRAF3, constitutive NIK degradation is reversed. NIK accumulation ultimately initiates NF-κB2 signalling and facilitates B-cell survival

3.2.3.3 BAFF Is an Obligate Survival Factor for B Cells Because It Reverses the Suppression of NF-κB2

Despite the constitutive suppression of NF-κB2 signalling by the actions of TRAF2, TRAF3, and c-IAP1, B cells can survive due to the ability of BAFF to reverse this suppression via the depletion of cellular TRAF3 (Fig. 3.3b). This was first demonstrated in transformed B-cell lines, which showed extensive proteolysis of TRAF3 upon BAFF, consequent accumulation of NIK, and thus promotion of NF-κB2 processing [64]. It was subsequently demonstrated that TRAF3 is depleted during the development of normal primary B cells concurrent with the acquisition of BAFF-R expression and dependence on BAFF for survival. Furthermore, this depletion of TRAF3 failed to occur in BAFF or BAFF-R-deficient mice [43]. An additional role for TRAF2 in this process was indicated by the fact that depletion of TRAF3 was inactivated during the development of TRAF2-deficient B cells [43].

These data therefore reveal dual roles for TRAF2 in regulating BAFF signalling in B cells. TRAF2 on the one hand appears to constitutively recruit c-IAP1 to NIK to facilitate NIK degradation and thus suppress NF-κB2 activation, a role it cooperates with TRAF3 to perform (Fig. 3.3a). BAFF-R signalling lifts this suppression by recruiting TRAF3 to its cytoplasmic domain and initiating its depletion from the cell, a process that also requires TRAF2 (Fig. 3.3b). It is possible that TRAF2 recruits c-IAP1 or another ubiquitin ligase to TRAF3 to facilitate its degradation. However, the exact mechanism by which TRAF3 is depleted from the cell and TRAF2's role in this process remain unclear.

In line with the above description of the proximal signalling events linking BAFF-R with NF-κB2 activation and thus survival in B cells, studies in human patients suffering multiple myeloma have identified mutations in a number of the key regulatory molecules discussed here [78, 79]. Thus, mutations were found which inactivated or deleted TRAF2, TRAF3, or c-IAP1, and also mutations that elevated the expression of or caused increased activation of NIK or NF-κB2. These findings lend support to the theory that these signalling events occur similarly in human and mouse B cells and do indeed impact on B-cell survival to the point of potentially contributing to B-lineage tumours when they are mutated.

3.2.4 Other Intracellular Mediators of B-Cell Survival Initiated by BAFF

Whilst NF-κB2 signalling is the primary survival pathway activated by BAFF and BAFF-R signalling, exactly how it facilitates B-cell survival is not clear. Studies have aimed to identify genes that are up-regulated by BAFF or proteins that are modified and may promote survival. Apart from TRAF3, the only signalling molecule known to be recruited to BAFF-R is the adapter protein Act1 [80]. This protein is thought to act as a negative regulator of BAFF-mediated B-cell survival but its mechanism of action also remains unclear. Either the recruitment of Act1 or the

activation of NF-κB2 signalling or both may modulate the following downstream events that promote B-cell survival.

3.2.4.1 Increasing Glycolysis

BAFF signalling can initiate the phosphorylation of Akt [81, 82], which promotes cell survival by increasing glucose uptake and glycolysis [83]. Microarray analysis has also revealed that BAFF stimulation of mature B cells causes the upregulation of a panel of glycolytic enzymes [81], thus promoting metabolism of glucose and other nutrients [82]. This may represent one direct method by which BAFF signalling can facilitate the survival of B cells. Both phosphoinositide 3-kinase (PI3K) and protein kinase C β (PKCβ) were shown to be important in activating Akt following BAFF stimulation [81].

3.2.4.2 Modulation of Pro- and Anti-apoptotic Proteins

A number of studies have indicated that BAFF upregulates the expression of anti-apoptotic members of the Bcl-2 family of proteins in B cells, including Bcl-2, Bcl-xL, and A1/Bfl-1 [33, 84, 85]. 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 [86]. 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 [24, 25, 86] and that *Bim*$^{-/-}$ B cells are relatively resistant to antigen-induced cell death [87]. Many of the anti-apoptotic Bcl-2 family proteins are regulated by NF-κB transcription factors [33, 85, 88, 89] suggesting that these proteins may represent important targets of the NF-κB pathways stimulated by BAFF.

PKCδ is a pro-apoptotic enzyme that exerts its effects in the nucleus. Mice lacking PKCδ expression exhibit dramatic B-cell hyperplasia and systemic autoimmunity [90], again similar to BAFF-transgenic mice. Further analysis showed that B cells that did not express PKCδ failed to undergo peripheral deletion in response to soluble self-antigen [91]. 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 [92]. Thus, BAFF signalling cannot only regulate the levels of pro- and anti-apoptotic proteins but can potentially modulate their functions within B cells.

3.3 Conclusions

It is clear that the intracellular signalling pathways triggered by BAFF play a critical role in regulating B-cell homeostasis and can have significant effects on the enforcement of B-cell self-tolerance. Whilst not discussed in detail here, it is likely that improper activation of these signalling pathways also makes a significant

contribution to cancer in the B-cell lineage [26]. There still remains much to learn about the critical events involved in the regulation of BAFF-dependent survival signals. A challenge for the future will be to determine whether our increasing knowledge of these pathways can be used to develop more effective treatments for autoimmune and neoplastic diseases involving B cells.

References

1. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science 2003;301:1374–7.
2. Goodnow CC, Adelstein S, Basten A. The need for central and peripheral tolerance in the B cell repertoire. Science 1990;248:1373–9.
3. Radic MZ, Erikson J, Litwin S, Weigert M. B lymphocytes may escape tolerance by revising their antigen receptors. J Exp Med 1993;177:1165–73.
4. Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone marrow B cells. J Exp Med 1993;177:1009–20.
5. Hartley SB, Crosbie J, Brink R, Kantor AB, Basten A, Goodnow CC. Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. Nature 1991;353:765–9.
6. Hartley SB, Cooke MP, Fulcher DA, et al. Elimination of self-reactive B lymphocytes proceeds in two stages: arrested development and cell death. Cell 1993;72:325–35.
7. Allman D, Lindsley RC, DeMuth W, Rudd K, Shinton SA, Hardy RR. Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation. J Immunol 2001;167:6834–40.
8. Forster I, Rajewsky K. The bulk of the peripheral B-cell pool in mice is stable and not rapidly renewed from the bone marrow. Proc Natl Acad Sci USA 1990;87:4781–4.
9. Fulcher DA, Basten A. Influences on the lifespan of B cell subpopulations defined by different phenotypes. Eur J Immunol 1997;27:1188–99.
10. Goodnow CC, Crosbie J, Adelstein S, et al. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature 1988;334:676–82.
11. Phan TG, Amesbury M, Gardam S, et al. B cell receptor-independent stimuli trigger immunoglobulin (Ig) class switch recombination and production of IgG autoantibodies by anergic self-reactive B cells. J Exp Med 2003;197:845–60.
12. Cyster JG, Hartley SB, Goodnow CC. Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire. Nature 1994;371:389–95.
13. Cyster JG, Goodnow CC. Antigen-induced exclusion from follicles and anergy are separate and complementary processes that influence peripheral B cell fate. Immunity 1995;3:691–701.
14. Merrell KT, Benschop RJ, Gauld SB, et al. Identification of anergic B cells within a wild-type repertoire. Immunity 2006;25:953–62.
15. Brink R. Regulation of B cell self-tolerance by BAFF. Semin Immunol 2006;18:276–83.
16. Vinuesa CG, Sze DM, Cook MC, et al. Recirculating and germinal center B cells differentiate into cells responsive to polysaccharide antigens. Eur J Immunol 2003;33:297–305.
17. Srivastava B, Quinn WJ, III, Hazard K, Erikson J, Allman D. Characterization of marginal zone B cell precursors. J Exp Med 2005;202:1225–34.
18. Lopes-Carvalho T, Kearney JF. Development and selection of marginal zone B cells. Immunol Rev 2004;197:192–205.
19. Mason DY, Jones M, Goodnow CC. Development and follicular localization of tolerant B lymphocytes in lysozyme/anti-lysozyme IgM/IgD transgenic mice. Int Immunol 1992;4:163–75.
20. Thien M, Phan TG, Gardam S, et al. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. Immunity 2004;20:785–98.

21. Schneider P, Takatsuka H, Wilson A, et al. Maturation of marginal zone and follicular B cells requires B cell activating factor of the tumor necrosis factor family and is independent of B cell maturation antigen. J Exp Med 2001;194:1691–7.
22. Gross JA, Dillon SR, Mudri S, et al. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease: impaired B cell maturation in mice lacking BLyS. Immunity 2001;15:289–302.
23. Schiemann B, Gommerman JL, Vora K, et al. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science 2001;293:2111–4.
24. Mackay F, Woodcock SA, Lawton P, et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. J Exp Med 1999;190:1697–710.
25. Khare SD, Sarosi I, Xia XZ, et al. Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. Proc Natl Acad Sci USA 2000;97:3370–5.
26. Mackay F, Silveira PA, Brink R. B cells and the BAFF/APRIL axis: fast-forward on autoimmunity and signaling. Curr Opin Immunol 2007;19:327–36.
27. Thompson JS, Bixler SA, Qian F, et al. BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF. Science 2001;293:2108–11.
28. Shulga-Morskaya S, Dobles M, Walsh ME, et al. B cell-activating factor belonging to the TNF family acts through separate receptors to support B cell survival and T cell-independent antibody formation. J Immunol 2004;173:2331–41.
29. Sasaki Y, Casola S, Kutok JL, Rajewsky K, Schmidt-Supprian M. TNF family member B cell-activating factor (BAFF) receptor-dependent and -independent roles for BAFF in B cell physiology. J Immunol 2004;173:2245–52.
30. Xu S, Lam KP. B-cell maturation protein, which binds the tumor necrosis factor family members BAFF and APRIL, is dispensable for humoral immune responses. Mol Cell Biol 2001;21:4067–74.
31. von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. Immunity 2001;14:573–82.
32. Yan M, Wang H, Chan B, et al. Activation and accumulation of B cells in TACI-deficient mice. Nature Immunol 2001;2:638–43.
33. Hsu BL, Harless SM, Lindsley RC, Hilbert DM, Cancro MP. Cutting edge: BLyS enables survival of transitional and mature B cells through distinct mediators. J Immunol 2002;168:5993–6.
34. Crowley JE, Treml LS, Stadanlick JE, Carpenter E, Cancro MP. Homeostatic niche specification among naive and activated B cells: a growing role for the BLyS family of receptors and ligands. Semin Immunol 2005;17:193–9.
35. Groom J, Kalled SL, Cutler AH, et al. Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjogren"s syndrome. J Clin Invest 2002;109:59–68.
36. Pers JO, Daridon C, Devauchelle V, et al. BAFF overexpression is associated with autoantibody production in autoimmune diseases. Ann NY Acad Sci 2005;1050:34–9.
37. Lesley R, Xu Y, Kalled SL, et al. Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. Immunity 2004;20:441–53.
38. Zhang X, Park CS, Yoon SO, et al. BAFF supports human B cell differentiation in the lymphoid follicles through distinct receptors. Int Immunol 2005;17:779–88.
39. Ekland EH, Forster R, Lipp M, Cyster JG. Requirements for follicular exclusion and competitive elimination of autoantigen-binding B cells. J Immunol 2004;172:4700–8.
40. Cyster JG. B cells on the front line. Nature Immunol 2000;1:9–10.
41. Kayagaki N, Yan M, Seshasayee D, et al. BAFF/BLyS receptor 3 binds the B cell survival factor BAFF ligand through a discrete surface loop and promotes processing of NF-kappaB2. Immunity 2002;17:515–24.
42. Claudio E, Brown K, Park S, Wang H, Siebenlist U. BAFF-induced NEMO-independent processing of NF-kappa B2 in maturing B cells. Nat Immunol 2002;3:958–65.
43. Gardam S, Sierro F, Basten A, Mackay F, Brink R. TRAF2 and TRAF3 signal adapters act cooperatively to control the maturation and survival signals delivered to B cells by the BAFF receptor. Immunity 2008;28:391–401.

44. Franzoso G, Carlson L, Xing L, et al. Requirement for NF-kappaB in osteoclast and B-cell development. Genes Dev 1997;11:3482–96.
45. Sha WC, Liou HC, Tuomanen EI, Baltimore D. Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. Cell 1995;80:321–30.
46. Grumont RJ, Rourke IJ, O"Reilly LA, et al. B lymphocytes differentially use the Rel and nuclear factor kappaB1 (NF-kappaB1) transcription factors to regulate cell cycle progression and apoptosis in quiescent and mitogen-activated cells. J Exp Med 1998;187:663–74.
47. Caamano JH, Rizzo CA, Durham SK, et al. Nuclear factor (NF)-kappa B2 (p100/p52) is required for normal splenic microarchitecture and B cell-mediated immune responses. J Exp Med 1998;187:185–96.
48. Beinke S, Ley SC. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. Biochem J 2004;382:393–409.
49. Liang C, Zhang M, Sun SC. Beta-TrCP binding and processing of NF-kappaB2/p100 involve its phosphorylation at serines 866 and 870. Cell Signal 2006;18:1309–17.
50. Enzler T, Bonizzi G, Silverman GJ, et al. Alternative and classical NF-kappa B signaling retain autoreactive B cells in the splenic marginal zone and result in lupus-like disease. Immunity 2006;25:403–15.
51. Sasaki Y, Derudder E, Hobeika E, et al. Canonical NF-kappaB activity, dispensable for B cell development, replaces BAFF-receptor signals and promotes B cell proliferation upon activation. Immunity 2006;24:729–39.
52. Ramakrishnan P, Wang W, Wallach D. Receptor-specific signaling for both the alternative and the canonical NF-kappaB activation pathways by NF-kappaB-inducing kinase. Immunity 2004;21:477–89.
53. Bren GD, Solan NJ, Miyoshi H, Pennington KN, Pobst LJ, Paya CV. Transcription of the RelB gene is regulated by NF-kappaB. Oncogene 2001;20:7722–33.
54. Yilmaz ZB, Weih DS, Sivakumar V, Weih F. RelB is required for Peyer's patch development: differential regulation of p52-RelB by lymphotoxin and TNF. EMBO J 2003; 22:121–30.
55. Derudder E, Dejardin E, Pritchard LL, Green DR, Korner M, Baud V. RelB/p50 dimers are differentially regulated by tumor necrosis factor-alpha and lymphotoxin-beta receptor activation: critical roles for p100. J Biol Chem 2003;278:23278–84.
56. Lam KP, Kuhn R, Rajewsky K. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell 1997;90:1073–83.
57. Vigorito E, Gambardella L, Colucci F, McAdam S, Turner M. Vav proteins regulate peripheral B-cell survival. Blood 2005;106:2391–8.
58. Franzoso G, Carlson L, Poljak L, et al. Mice deficient in nuclear factor (NF)-kappa B/p52 present with defects in humoral responses, germinal center reactions, and splenic microarchitecture. J Exp Med 1998;187:147–59.
59. Miosge LA, Blasioli J, Blery M, Goodnow CC. Analysis of an ethylnitrosourea-generated mouse mutation defines a cell intrinsic role of nuclear factor kappaB2 in regulating circulating B cell numbers. J Exp Med 2002;196:1113–9.
60. Xie P, Stunz LL, Larison KD, Yang B, Bishop GA. Tumor necrosis factor receptor-associated factor 3 is a critical regulator of B cell homeostasis in secondary lymphoid organs. Immunity 2007;27:253–67.
61. Grech AP, Amesbury M, Chan T, Gardam S, Basten A, Brink R. TRAF2 differentially regulates the canonical and noncanonical pathways of NF-kappaB activation in mature B cells. Immunity 2004;21:629–42.
62. Xu LG, Shu HB. TNFR-associated factor-3 is associated with BAFF-R and negatively regulates BAFF-R-mediated NF-kappa B activation and IL-10 production. J Immunol 2002;169:6883–9.
63. Hauer J, Puschner S, Ramakrishnan P, et al. TNF receptor (TNFR)-associated factor (TRAF) 3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF-kappaB pathway by TRAF-binding TNFRs. Proc Natl Acad Sci USA 2005;102:2874–9.

64. Liao G, Zhang M, Harhaj EW, Sun SC. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. J Biol Chem 2004;279:26243–50.
65. Xu Y, Cheng G, Baltimore D. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. Immunity 1996;5:407–15.
66. He JQ, Zarnegar B, Oganesyan G, et al. Rescue of TRAF3-null mice by p100 NF-kappa B deficiency. J Exp Med 2006;203:2413–8.
67. Morrison MD, Reiley W, Zhang M, Sun SC. An atypical tumor necrosis factor (TNF) receptor-associated factor-binding motif of B cell-activating factor belonging to the TNF family (BAFF) receptor mediates induction of the noncanonical NF-kappaB signaling pathway. J Biol Chem 2005;280:10018–24.
68. Rothe M, Sarma V, Dixit VM, Goeddel DV. TRAF2-mediated activation of NF-kappa B by TNF receptor 2 and CD40. Science 1995;269:1424–7.
69. Song HY, Regnier CH, Kirschning CJ, Goeddel DV, Rothe M. Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor-kappaB and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. Proc Natl Acad Sci USA 1997;94:9792–6.
70. Malinin NL, Boldin MP, Kovalenko AV, Wallach D. MAP3K-related kinase involved in NF-kappaB induction by TNF, CD95 and IL-1. Nature 1997;385:540–4.
71. Liu YC. Ubiquitin ligases and the immune response. Annu Rev Immunol 2004;22:81–127.
72. Xia ZP, Chen ZJ. TRAF2: a double-edged sword? Sci STKE 2005;pe7 (http://stke.sciencemag.org/cgi/content/full/OC_sigtrans;stke.2722005pe7)
73. Shi CS, Kehrl JH. Tumor necrosis factor (TNF)-induced germinal center kinase-related (GCKR) and stress-activated protein kinase (SAPK) activation depends upon the E2/E3 complex Ubc13-Uev1A/TNF receptor-associated factor 2 (TRAF2). J Biol Chem 2003;278:15429–34.
74. Habelhah H, Takahashi S, Cho SG, Kadoya T, Watanabe T, Ronai Z. Ubiquitination and translocation of TRAF2 is required for activation of JNK but not of p38 or NF-kappaB. Embo J 2004;23:322–32.
75. Rothe M, Pan MG, Henzel WJ, Ayres TM, Goeddel DV. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell 1995;83:1243–52.
76. Vince JE, Wong WW, Khan N, et al. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell 2007;131:682–93.
77. Varfolomeev E, Blankenship JW, Wayson SM, et al. IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. Cell 2007;131(4):669–81.
78. Annunziata CM, Davis RE, Demchenko Y, et al. Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. Cancer Cell 2007;12:115–30.
79. Keats JJ, Fonseca R, Chesi M, et al. Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. Cancer Cell 2007;12:131–44.
80. Qian Y, Qin J, Cui G, et al. Act1, a negative regulator in CD40- and BAFF-mediated B cell survival. Immunity 2004;21:575–87.
81. Patke A, Mecklenbrauker I, Erdjument-Bromage H, Tempst P, Tarakhovsky A. BAFF controls B cell metabolic fitness through a PKC beta- and Akt-dependent mechanism. J Exp Med 2006;203:2551–62.
82. Woodland RT, Schmidt MR, Thompson CB. BLyS and B cell homeostasis. Semin Immunol 2006;18(5):318–26.
83. Plas DR, Rathmell JC, Thompson CB. Homeostatic control of lymphocyte survival: potential origins and implications. Nat Immunol 2002;3:515–21.
84. Batten M, Groom J, Cachero TG, et al. BAFF mediates survival of peripheral immature B lymphocytes. J Exp Med 2000;192:1453–66.

85. Do RK, Hatada E, Lee H, Tourigny MR, Hilbert D, Chen-Kiang S. Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response. J Exp Med 2000;192:953–64.
86. Bouillet P, Metcalf D, Huang DC, et al. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. Science 1999;286:1735–8.
87. Enders A, Bouillet P, Puthalakath H, Xu Y, Tarlinton DM, Strasser A. Loss of the pro-apoptotic BH3-only Bcl-2 family member Bim inhibits BCR stimulation-induced apoptosis and deletion of autoreactive B cells. J Exp Med 2003;198:1119–26.
88. Zong WX, Edelstein LC, Chen C, Bash J, Gelinas C. The prosurvival Bcl-2 homolog Bfl-1/A1 is a direct transcriptional target of NF-kappaB that blocks TNFalpha-induced apoptosis. Genes Dev 1999;13:382–7.
89. Lee HH, Dadgostar H, Cheng Q, Shu J, Cheng G. NF-kappaB-mediated up-regulation of Bcl-x and Bfl-1/A1 is required for CD40 survival signaling in B lymphocytes. Proc Natl Acad Sci USA 1999;96:9136–41.
90. Miyamoto A, Nakayama K, Imaki H, et al. Increased proliferation of B cells and autoimmunity in mice lacking protein kinase Cdelta. Nature 2002;416:865–9.
91. Mecklenbrauker I, Saijo K, Zheng NY, Leitges M, Tarakhovsky A. Protein kinase Cdelta controls self-antigen-induced B-cell tolerance. Nature 2002;416:860–5.
92. Mecklenbrauker I, Kalled SL, Leitges M, Mackay F, Tarakhovsky A. Regulation of B-cell survival by BAFF-dependent PKCdelta-mediated nuclear signalling. Nature 2004;431:456–61.