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BAFF signaling in health and disease

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BAFF is a critical cytokine supporting the survival of mature naïve B cells, acting through the BAFFR receptor. Recent studies show that BAFF and BAFFR are also required for the survival of memory B cells, autoimmune B cells as well as malignant chronic lymphocytic leukaemia (CLL) cells. BAFFR cooperates with other receptors, notably the B cell antigen receptor (BCR), a process which is critical for the expansion of autoimmune and CLL cells. This crosstalk may be mediated by TRAF3 which interacts with BAFFR and with CD79A, a signalling subunit of the BCR and the downstream SYK kinase, inhibiting its activity. BAFF binding to BAFFR leads to degradation of TRAF3 which may relieve inhibition of SYK activity transducing signals to pathways required for B cell survival. BAFFR activates both canonical and non-canonical NF-κB signalling and both pathways play important roles in the survival of B cells and CLL cells.

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Introduction

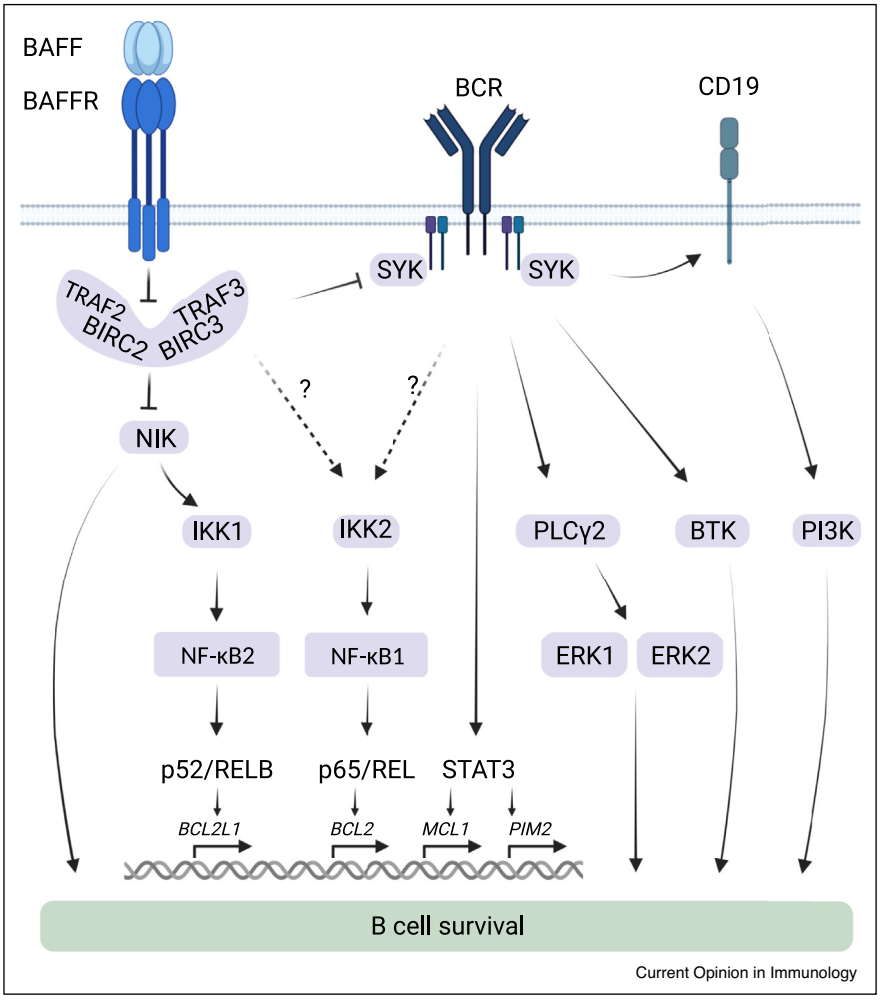
BAFF (TNFSF13B), a TNF-family cytokine essential for the survival of mature B cells, is a ligand for three receptors: BAFFR (TNFRSF13C), TACI (TNFRSF13CB) and BCMA (TNFRSF17) [1]. TACI and BCMA also bind APRIL (TNFSF13), a BAFF-related cytokine. Genetic ablation of BAFF or BAFFR, or blocking of BAFF binding to BAFFR cause death of most mature B cells, demonstrating that BAFF binding to BAFFR induces signalling that is essential for B cell survival [2,3]. As a consequence, BAFF–BAFFR signalling has profound consequences on both immune responses as well as pathological conditions such as autoimmunity and B cell malignancy.

Elevated levels of BAFF have been reported in several autoimmune conditions including systemic lupus erythematosus (SLE), Sjörgen's syndrome (SS) and rheumatoid arthritis [4]. Importantly, Belimumab, an anti-BAFF antibody showed clinical efficacy in SLE and has been approved for use in this condition [5]. However, around 40% of SLE patients do not show clinically meaningful responses to Belimumab treatment, highlighting the need for other therapeutic approaches [6]. Chronic lymphocytic leukaemia (CLL) is a B cell malignancy for which small molecule inhibitors of B cell antigen receptor (BCR) signalling are now a mainstay of treatment. Despite their success, the therapy is not curative and some patients relapse, thus attention has focussed on other therapeutic targets, notably BAFF, BAFFR and signalling pathways from this receptor.

The best described signalling process from BAFFR is the activation of the non-canonical NF-κB pathway. In the absence of BAFF, a complex of the TRAF2, TRAF3, BIRC2 (c-IAP1) and BIRC3 (c-IAP2) E3 ligases ubiquitinates the NIK kinase leading to its degradation. Binding of BAFF to BAFFR results in recruitment of TRAF3 to the receptor, redirecting the TRAF2–TRAF3–BIRC2–BIRC3 complex to ubiquitinate TRAF3 instead of NIK, causing degradation of TRAF3 and stabilisation of NIK (Figure 1). NIK then phosphorylates and activates IKK1 which in turn phosphorylates NF-κB2 (p100) leading to its processing to p52, which binds RELB, translocates to the nucleus and regulates gene transcription [7]. However, the relevance of this pathway to B cell survival was cast into doubt by a study showing that inducible loss of IKK1 in mature B cells did not affect their survival [8].

BAFFR also transduces signals to several other pathways, including phosphoinositide 3-kinase (PI3-kinase) and ERK1 and ERK2 kinases [8,9]. BAFFR signalling to PI3-kinase and ERK1 and ERK2 is transduced via the BCR and the associated SYK kinase, as well as CD19, a co-receptor for the BCR (Figure 1). These pathways are clearly important, since loss of BCR, SYK or CD19 impairs BAFF-induced B cell survival [8,9]. However, it remains unclear how BAFFR signalling is coupled to these pathways, since TRAF3 is the only signal transduction molecule known to bind to BAFFR, and it has not been shown to transduce signals to the BCR or CD19. Furthermore, BAFFR signalling activates the canonical

Figure 1



Signal transduction pathways from BAFFR.
In unstimulated cells, a TRAF2–TRAF3–BIRC2–BIRC3 complex of E3 ubiquitin ligases ubiquitinates the NIK kinase leading to its degradation. The complex may also inhibit the SYK kinase which signals from the BCR. Binding of BAFF to BAFFR results in recruitment of TRAF3 to the cytoplasmic domain of BAFFR, redirecting the TRAF2–TRAF3–BIRC2–BIRC3 complex to ubiquitinate TRAF3 causing its degradation. This stabilises NIK and relieves inhibition of SYK. NIK phosphorylates IKK1 causing its activation, which in turn phosphorylates NF-κB2 (p100) leading to its processing into p52, which binds RELB, translocates to the nucleus and regulates gene transcription (non-canonical NF-κB pathway). NIK also signals independently of IKK1 to support B cell survival. The disinhibited SYK binds to the phospho-tyrosines in the cytoplasmic domains of CD79A and CD79B, causing its phosphorylation, activation and signalling to downstream pathways including BTK, Phospholipase Cγ2 (PLCγ2), ERK1, ERK2, STAT3 and PIM2, and, via CD19, to the activation of PI3-kinase (PI3K). BAFFR signalling also activates the canonical NF-κB pathway via IKK2 and NF-κB1, p65 and REL. However, the biochemical mechanism leading to this is unclear. These multiple pathways support survival by inducing expression of anti-apoptotic proteins such as BCL2, BCL2L1 (BCL-xL) and MCL1, and of the PIM2 kinase. Created with Biorender.com.

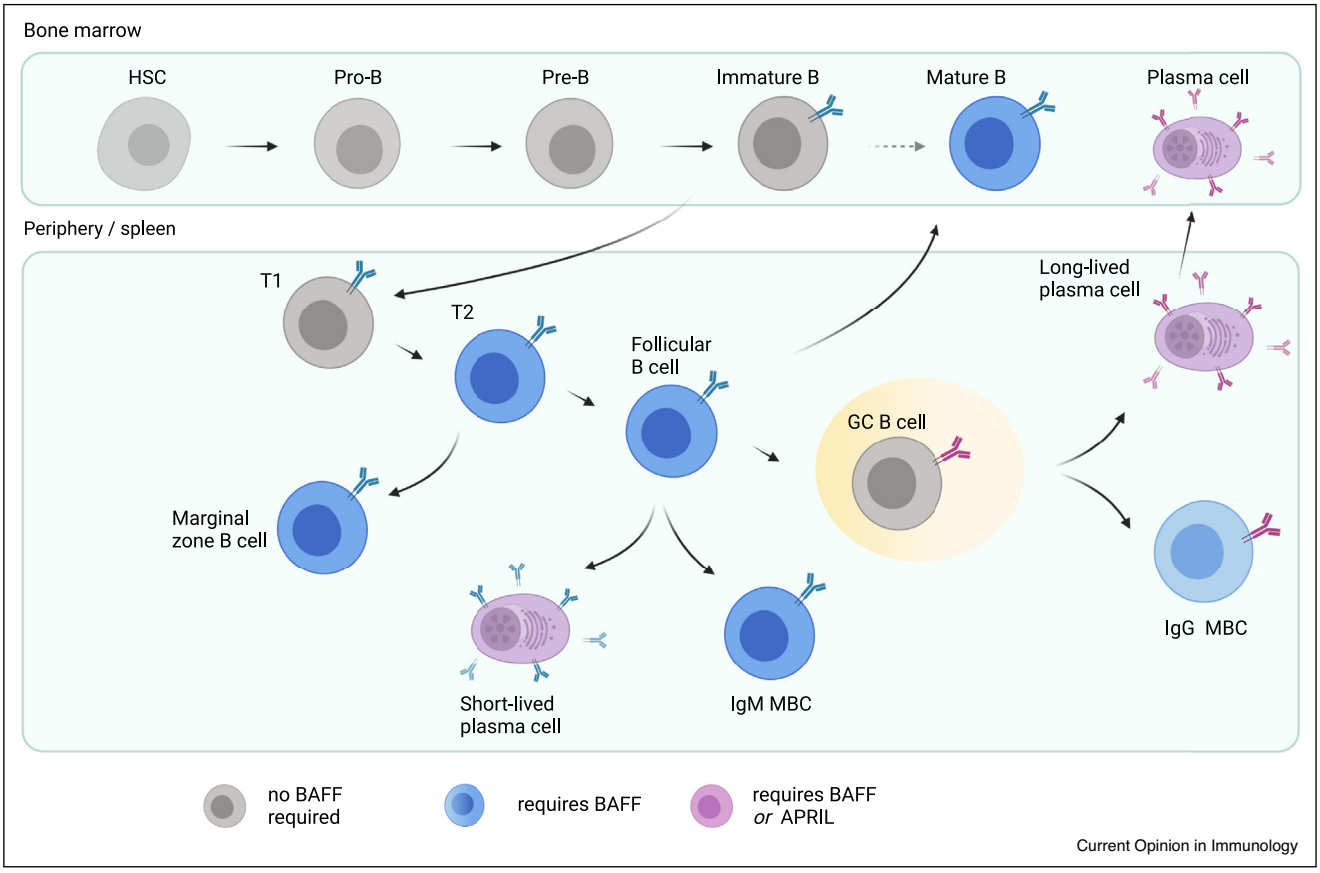
IKK2-dependent NF-κB pathway by an unknown mechanism [10,11]. However, while canonical NF-κB signalling is important in B cell development, its requirement for B cell survival had not been established, for example by inducible ablation of the gene encoding IKK2 in mature B cells.

In this review we focus on recent advances relating to the B cell subsets within which BAFF signalling plays a role, the role of BAFF in survival of CLL cells, crosstalk between BAFFR and BCR signalling, function of TRAF2 and TRAF3, and BAFF-regulated NF-κB signalling.

BAFF-dependent survival of memory B cells

Not all B cell subsets require BAFF or BAFFR for survival. The earliest stages of B cell development, pro-B, pre-B and immature B cells in the bone marrow and transitional type 1 (T1) B cells in the spleen, are

Figure 2



Differential requirement for BAFF or APRIL in survival of B cell subsets.
B lymphocytes develop from haematopoietic stem cells (HSC) in the bone marrow, giving rise to pro-B, pre-B and immature B cells, which emigrate to peripheral lymphoid tissues such as the spleen, where they become transitional type 1 (T1) and T2 B cells, eventually maturing into naïve follicular and marginal zone B cells. The survival of the bone marrow B cell subsets and of T1 B cells in the spleen is not dependent on BAFF or APRIL. However, a requirement for BAFF is seen in T2 B cells and in follicular and marginal zone B cells, but not the B1 cells of the peritoneum. Once B cells are activated and differentiate into germinal centre (GC) B cells, they are no longer dependent on BAFF, but regain this requirement when they differentiate into MBC. Finally, the survival of antibody-secreting plasma cells depends on BAFF or APRIL. Diagram indicates subsets that do not require BAFF for survival (grey), or that require BAFF (blue) or either BAFF or APRIL (purple). Created with Biorender.com.

found in normal numbers in mice deficient in either BAFF or BAFFR [1]. In contrast, T2 B cells in the spleen and two main subtypes of mature naïve B cells, follicular and marginal zone B cells, are critically dependent on BAFF and BAFFR for survival, whereas B1 cells of the peritoneal cavity are largely BAFF-independent (Figure 2). The requirement for BAFF and BAFFR in activated B cells subsets has been less clear. Loss of both BAFF and APRIL but not either alone causes plasma cells to die [12–14]. Similarly, plasma cell numbers are substantially reduced in the absence of BCMA [15], demonstrating that the survival of plasma cells requires either BAFF or APRIL acting through BCMA. Two studies in 2008 reported that memory B cells (MBC) did not express BAFFR and survived in the absence of BAFF, although one study showed a partial dependence of IgM$^+$ MBC on BAFF [12,13]. Finally, it was not known if germinal centre (GC) B cells required BAFF or BAFFR.

Two recent studies have revealed key roles for BAFF and BAFFR in MBC survival. In one study, IgM$^+$ and IgG1$^+$ MBC were found to express levels of BAFFR similar to those on naïve follicular B cells, and inducible genetic ablation of BAFFR or blockade of BAFF caused substantial loss of both IgM$^+$ and IgG1$^+$ MBC, while numbers of plasma cells were unaffected [16••]. Furthermore, analysis of MBC in the lung, generated following influenza infection, showed that survival of lung-resident MBC is also BAFF-dependent. Treatment with anti-BAFF not

only caused a large decrease in MBC numbers, but also substantially impaired a secondary recall response, a canonical feature of MBC. The same study showed that MBC also require the BCR and signalling through the associated CD79A subunit, demonstrating that like naïve B cells, the survival of MBC requires signals through both BAFFR and BCR [16••].

In a related study, the survival and function of GC B cells was shown to be BAFFR-independent, as measured by numbers of GC B cells and by production of affinity-matured IgG1 in the serum following immunisation with a T-dependent antigen [17••]. In contrast, the study showed that loss of BAFFR or blockade of BAFF caused a large decrease in the numbers of $IgG1^+$ MBC. This decrease was greater for MBC with unmutated antigen receptors compared to MBC with mutated receptors, although both populations were affected. This indicates that the requirement for BAFF and BAFFR is greater among GC-independent MBC compared to GC-dependent MBC. This conclusion was further supported by experiments showing that increased BAFFR expression or increased BAFFR signalling following loss of TRAF3 resulted in larger numbers of unmutated GC-independent MBC [17••].

Taken together these studies clearly demonstrated that while the survival of GC B cells and plasma cells is BAFF-independent and BAFFR-independent, IgM^+ and $IgG1^+$ MBC require BAFF and BAFFR for their long-term survival (Figure 2). Given the similarity of these survival requirements to those of naïve follicular B cells, MBC may compete with follicular B cells for BAFF. The levels of BAFF are known to be limiting, since overexpression of BAFF leads to an increase in mature B cell numbers [18,19], raising the question of how MBC can survive in competition with much larger numbers of follicular B cells. Indeed, despite this excess competition, MBC are much longer-lived than follicular B cells [20]. One possibility is that MBC respond better to BAFF than follicular B cells, but at least in an *in vitro* survival assay, no difference was seen in BAFF-induced survival between these two B cell subtypes [16••]. Further work is needed to understand how the survival of long-lived MBC is regulated in the face of competition from a large excess of follicular B cells.

In contrast to the results described above on the role for BAFF and BAFFR in MBC survival in mice, data from humans is less clear. Studies of SLE patients treated with Belimumab showed significant decreases in naïve and transitional B cells in the blood, with smaller decreases in plasmablasts and non-switched MBC [21–24]. In contrast, the numbers of switched MBC remained largely unchanged [21–23]. However, one study reported a temporary increase in MBC in the blood eight weeks after treatment [24]; these may be MBC mobilised from tissues. Long-term treatment with Belimumab for seven years, caused a decline in all B cell populations in the blood including MBC [25]. The difference in BAFF requirement for MBC survival between mouse and humans may be a species difference. Alternatively, it may be caused by analysis of different tissues. The human studies only looked in blood, and not in lymphoid tissue where most MBC reside, and numbers of MBC in the blood may not reflect changes in other organs. Further investigations are needed to resolve the basis for these differences.

BAFF-dependent survival of CLL cells

Treatment of CLL has been greatly improved through the use of ibrutinib and idelalisib, small molecule inhibitors of BCR-associated kinases BTK and PI3-kinase δ, respectively, and venetoclax, a BCL*2* inhibitor [26]. However, these inhibitors do not cause complete remission, and some patients can relapse. Since CLL cells express high levels of BAFFR, focus has turned on whether blocking BAFF–BAFFR interactions might improve therapy. Three recent studies have shown that BAFF promotes survival of CLL cells *in vitro*, counteracting the apoptosis-promoting effects of ibrutinib, idelalisib and venetoclax, as well as those of conventional chemotherapeutics fludarabine and bendamustine that inhibit cell division [27••,28•,29••]. Furthermore, addition of VAY-736, an anti-BAFFR antibody, or belimumab, an anti-BAFF antibody, to the *in vitro* cultures blocked the pro-survival function of BAFF. Extending this to *in vivo* experiments, treatment with VAY-736 in combination with ibrutinib resulted in prolonged survival of Eμ-TCL1 mice, a model of CLL [28•]. Surprisingly, the enhanced survival induced by VAY-736 was caused by antibody-dependent cellular cytotoxicity (ADCC) mediated by binding of VAY-736 to BAFFR on the CLL cells, rather than blocking of BAFF binding. Nonetheless, these studies suggest that blocking BAFF–BAFFR interactions in combination with ibrutinib or other small molecule inhibitors is a promising therapeutic strategy for CLL.

Crosstalk between BAFFR and other receptors

Transgenic mice overexpressing BAFF (BAFF-Tg) develop autoimmunity similar to SLE or SS, characterised by production of auto-antibodies [1]. Surprisingly, this autoimmunity is dependent on expression of TACI on the autoreactive B cells [30,31]. In a recent study, the autoreactive B cells in BAFF-Tg mice were shown to derive from TACI-expressing transitional B cells [32••]. Autoimmune pathology depends on the BTK kinase, most likely acting downstream of the BCR, since deletion of the *Btk* gene in BAFF-Tg mice eliminated expression of TACI on transitional B cells and abrogated auto-antibody production. Thus, excessive BAFF–BAFFR signalling in transitional B cells acting via BTK (and possibly

the BCR) results in expression of TACI, and subsequent expansion of autoreactive B cells and generation of autoantibodies.

Crosstalk between BAFFR and the BCR was also reported in CLL cells, whose treatment with BAFF resulted in phosphorylation of the SYK kinase, a critical transducer of BCR signals [27••]. Treatment with SYK inhibitors was shown to block BAFF-induced survival of CLL cells. Mechanistically, the study showed that SYK inhibition blocked BAFF-induced phosphorylation of STAT3 and expression of the anti-apoptotic MCL1 protein. Furthermore, BAFF stimulation of CLL cells was shown to result in association between SYK and TRAF2 and TRAF3, suggesting a possible direct biochemical connection between signalling molecules downstream of BAFFR and BCR. This work indicates that SYK may be another promising therapeutic target in CLL.

Patients with chronic graft versus host disease (cGVHD) show elevated levels of BAFF in the blood [33,34], and a recent report shows that the same is seen in a mouse model of the condition [35]. Here, BAFF was found to cause the accumulation of autoreactive B cells, which contained elevated levels of SYK, potentially because of reduced degradation of the kinase, again supporting a connection between BAFFR and BCR signalling.

BAFF and BAFFR have also been connected to other receptors. Treatment of Burkitt's lymphoma cell lines with BAFF resulted in BAFFR-dependent upregulation of BST2 (CD317), a cell surface protein best known for its role as a virus restriction factor [36]. This study showed that BST2 was required for BAFF-induced activation of canonical NF-κB signalling and increased cell survival, however the mechanism by which BST2 signals is unknown.

B cells have been shown to play important roles during the pathogenesis of multiple sclerosis (MS) an autoimmune condition of the central nervous system (CNS) characterised by destruction of myelin [37]. Interestingly, BAFF has been reported to act as a ligand for the Nogo receptor NgR1 (RTN4R) on neurons [38]. Unexpectedly, a recent study found that B cells in the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, express NgR1 and the related NgR3 receptor [39•]. These NgR1-expressing and NgR3-expressing B cells produced autoantibodies to myelin and treatment of the cells with BAFF induced their proliferation. This response could be blocked by the addition of recombinant BAFFR or NgR1-Fc fusion protein to the medium, indicating that BAFF signalling via both BAFFR and Nogo receptors was required to induce proliferation of these pathogenic B cells. These results imply that BAFFR cooperates with NgR1 and NgR3, but again the biochemical mechanisms underlying this remain unknown.

TRAF2 and TRAF3

In unstimulated B cells, TRAF2 and TRAF3 are complexed with BIRC2 and BIRC3. This TRAF2–TRAF3–BIRC2–BIRC3 complex of E3 ubiquitin ligases ubiquitinates NIK, leading to its degradation. Binding of BAFF to BAFFR results in recruitment of TRAF3 to the cytoplasmic domain of the receptor. This results in redirection of the ubiquitination activity of the TRAF2–TRAF3–BIRC2–BIRC3 complex towards TRAF3 and away from NIK, leading to degradation of TRAF3, stabilisation of NIK and activation of IKK1 and non-canonical NF-κB signalling (Figure 1). Since TRAF3 is the only known signal transducer to bind to the BAFFR, it is of importance to understand the consequences of BAFF-induced loss of TRAF3.

Two studies of TRAF3-deficient B cells have revealed several signalling pathways regulated by TRAF3 other than the non-canonical NF-κB pathway. In the absence of TRAF3, B cells show higher levels of the PIM2 kinase, which in turn leads to increased MYC protein [40•]. This increase in PIM2 is dependent on STAT3 but not on NIK. In a further study from the same group, BCR stimulation was shown to result in association of TRAF3 with the CD79A signalling subunit of the BCR and with the SYK and BTK kinases [41••]. Furthermore, TRAF3-deficient B cells have increased BCR-induced phosphorylation of SYK, BTK, Phospholipase Cγ2, ERK1 and ERK2 and increased BCR-induced intracellular Ca^{2+} flux. Taken together with previously described work in CLL cells showing that BAFF treatment induced association of SYK with TRAF2 and TRAF3 and that SYK was required for BAFF-induced phosphorylation of STAT3 [27••], a plausible mechanism is that by associating with CD79A and SYK, the TRAF2–TRAF3–BIRC2–BIRC3 complex negatively regulates SYK activation. BAFF binding to BAFFR would lead to loss of TRAF3 and hence BCR-dependent activation of SYK and downstream signalling pathways including BTK, ERK1, ERK2, STAT3 and PIM2, contributing to increased B cell survival (Figure 1). This BCR-dependent and SYK-dependent signalling is independent of TRAF3-regulated NIK and non-canonical NF-κB signalling.

In a separate study, BAFF treatment of B cells was shown to lead to increased expression of the NLRP3 inflammasome and of pro-IL1β [42•]. Furthermore, BAFF activates NLRP3 through two mechanisms. BAFFR signalling causes BIRC2-TRAF2 to interact with NLRP3, ASC and pro-caspase-1, leading to caspase-1 activation and IL1β secretion. In addition, BAFFR signalling via SRC-family kinases activates NLRP3 through increased reactive oxygen species (ROS) and increased K^+ efflux.

NF-κB signalling

The importance of non-canonical NF-κB signalling was demonstrated by studies of a novel small molecule inhibitor of NIK (NIK SMI1). NIK SMI1 was shown to inhibit both BAFFR and CD40 signalling in B cells *in vitro* and treatment of mice with NIK SMI1 resulted in a large reduction of mature B cells, similar to effect of blocking BAFF or BAFFR [43••]. Using a mouse model of SLE this study showed that NIK SMI1 reduced the severity of symptoms and prolonged survival, and that it was more effective than blockade of BAFFR, indicating that NIK may be a good therapeutic target for SLE. It is notable that inhibition or genetic deletion of NIK results in substantial loss of mature B cells, in contrast to loss of IKK1 which had no effect [8,44]. This suggests that NIK transduces signals to more than just the IKK1-dependent non-canonical NF-κB pathway. The identity of these other NIK-dependent processes is unknown.

BAFFR signalling also activates the IKK2-dependent canonical NF-κB pathway, although the mechanism by which it does so is unknown [10,11]. The importance of canonical NF-κB signalling for B cell survival had not been tested until recently. Inducible genetic deletion of IKK2 causes a large drop in the number of mature naïve B cells and IgM^+ and $IgG1^+$ MBC demonstrating that canonical NF-κB signalling is essential for B cell survival [16••]. It is unclear, however, whether this IKK2-dependent signalling for survival is downstream of BAFFR or other receptors.

The canonical NF-κB pathway is also important for the BAFF-dependent survival of CLL cells. BAFF stimulation of such cells leads to IKK2-dependent accumulation of the p62 (SQSTM1) adaptor protein via canonical NF-κB signalling. p62 in turn activates mTORC1 and NRF2, a master regulator of the antioxidant response. This improves survival of CLL cells and protects them from ROS-inducing therapeutics such as venetoclax [45••]. These observations again support the potential for combining anti-BAFF antibodies with venetoclax for the treatment of CLL.

BAFF binding to BAFFR on CLL cells, results in increased expression of BIRC3 [46•]. Genetic deletion of BIRC3 resulted in increased p65 and REL (c-Rel) in nucleus, indicating increased canonical NF-κB signalling, and led to increased expression of BCL2. Thus, BIRC3 is a negative regulator of the canonical NF-κB pathway. Interestingly, CLL patients whose leukemic cells have low expression of BIRC3 have poorer prognosis. However, such $BIRC3^{low}$ CLL cells express higher amounts of BCL2 and are more sensitive to venetoclax, a BCL2 inhibitor, indicating that this may be a useful therapeutic agent for CLL patients with low BIRC3 expression.

Concluding remarks

Recent advances have shown that BAFF and BAFFR are essential for the survival of MBC, and that BAFF supports the survival autoimmune B cells as well as neoplastic B cells such as CLL. More details have emerged on how BAFFR cooperates with other receptors, especially the BCR, indicating that TRAF3 may be involved in this receptor crosstalk. Finally, it is clear that both canonical and non-canonical NF-κB signalling pathways are important for the survival of B cells. Despite this progress, much uncertainty remains about the biochemical mechanisms by which BAFFR signals, for example to the BCR and SYK and to the canonical NF-κB signalling pathway.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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Contrary to previous publications, the survival of both IgM^+ and $IgG1^+$ MBC requires BAFF and BAFFR. Furthermore, MBC survival also requires the BCR, CD79A, and IKK2, indicating that signalling from both BAFFR and BCR are essential for MBC survival. See also next publication.

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BAFF binding to BAFFR on CLL cells, results in increased expression of BIRC3, which negatively regulates the canonical NF-κB pathway. CLL patients whose leukemic cells have low expression of BIRC3 have poorer prognosis because increased canonical NF-κB signalling leads to higher expression of BCL2.