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Signaling by the TNFR superfamily in B-cell biology and disease

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Summary

Members of the tumor necrosis factor receptor superfamily (TNFRSF) participate prominently in B-cell maturation and function. In particular, B-cell activating factor belonging to the TNFR family receptor (BAFF-R), B-cell maturation antigen (BCMA), and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) play critical roles in promoting B-cell survival at distinct stages of development by engaging a proliferation-inducing ligand (APRIL) and/or BAFF. CD40 is also essential for directing the humoral response to T-cell-dependent antigens. Signaling by the TNFRSF is mediated primarily, albeit not exclusively, via the TNFR-associated factor (TRAF) proteins and activation of the canonical and/or noncanonical nuclear factor-κB (NF-κB) pathways. Dysregulated signaling by TNFRSF members can promote B-cell survival and proliferation, causing autoimmunity and neoplasia. In this review, we present a current understanding of the functions of and distinctions between APRIL/BAFF signaling by their respective receptors expressed on particular B-cell subsets. These findings are compared and contrasted with CD40 signaling, which employs similar signaling conduits to achieve distinct cellular outcomes in the context of the germinal center response. We also underscore how new findings and conceptual insights in TNFRSF signaling are facilitating the understanding of B-cell malignancies and autoimmune diseases.

Keywords

BAFF; BCMA; TACI; CD40; NF-κB; B cell

Introduction

Early B-cell development is focused on the successful generation of immature B cells bearing a functional antigen receptor (BCR). Immature B cells egress to the spleen to undergo further maturation through ‘transitional’ T1 and T2 stages prior to entering the pool of mature recirculating B cells (1–4). Although T1 cells undergo apoptosis in response to BCR engagement, they require signaling via the B-cell activating factor belonging to the tumor necrosis factor (TNF) family receptor (BAFF-R, TNFRSF13) to mature to the T2 stage (5). T2 cells are only present in the spleen and reside in the follicles, whereas T1 cells are found in the red pulp and outer periarterial lymphatic sheath (PALS) (3, 6). Organization of the B-cell follicles and apposing T-cell zones is achieved by the secretion of chemokines by distinct stromal cell subsets. Of these subsets, follicular dendritic cells (FDCs) are essential to retain immune complexes and produce B-lymphocyte chemoattractant (BLC/CXCL13). FDC maintenance requires continual membrane expression of lymphotoxin $\alpha_1\beta_2$

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($LT\alpha_1\beta_2$) trimer, as well as TNF secretion by B cells, and LTβR and TNF-R1 expression on FDCs (7). The marginal zone (MZ) demarcates the perimeter of the white pulp of the spleen and contains a subset of B cells that likely arises from the transitional B-cell compartment (8). MZ B cells are strategically located to respond to blood-born antigens and can rapidly differentiate into antibody-producing cells in the red pulp.

Upon antigen encounter, follicular B cells migrate to the border regions of the PALS/cortex to present bound peptide and costimulate T cells. Reciprocal B-cell activation is mediated by engagement of CD40 and provision of cytokine support. CD40-dependent B-cell activation is required to undergo proliferative expansion and differentiation in the germinal center (GC), where somatic mutation and enhanced immunoglobulin class switch recombination (CSR) occur. The architecture of the GC is divided into distinct regions: rapidly dividing B cells or centroblasts in the 'dark zone' of the GC give rise to centrocytes which occupy the 'light zone'. The light zone is thought to be the site of B-cell selection by FDC-bound antigen that is processed and presented by B cells to primed T cells of the follicular helper $CD4^+$ (Tfh) subtype. B-cell maturation in the GC is accompanied by somatic hypermutation of antibody variable region (V) genes, which provides the molecular basis for the production of B cells bearing high-affinity antigen receptors. These B cells are thought to have a competitive advantage when antigen becomes limiting and GC structures atrophy. B cells unable to bind antigen or receive sufficient T-cell help die *in situ* by apoptosis and are cleared by macrophages, whereas antigen-selected B cells that leave the GC become memory B cells or plasmablasts by a process that is not understood. Long-lived plasma cells are actively retained in the bone marrow by responding to stromal derived factor (SDF)/CXCL12 as well as survival factors such as interleukin-6 (IL-6) and BAFF-a proliferation-inducing ligand (APRIL).

Peripheral B-cell maturation, homeostasis, and antigen-dependent differentiation are complex processes occurring in distinct anatomic locations. Nonetheless, steady progress is being made in understanding the molecular cues that govern B-cell fate at each of these distinct stages of differentiation. Members of the TNF receptor superfamily (TNFRSF) perform critical roles in this decision-making process (Fig. 1). As B cells egress from the bone marrow, further maturation into follicular or MZ B cells is dependent upon BAFF [also called B-lymphocyte stimulator (BLyS) or TNF superfamily member 13B (TNFSF13B)]. BAFF is also essential for the homeostasis of naive recirculating B cells and MZ B cells. Downregulation of BAFF-R on plasma cells is coincident with the upregulation of B-cell maturation antigen (BCMA) (TNFRSF5), which can bind BAFF as well as APRIL. Coordination of BAFF and APRIL signaling is also achieved in part by transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) (TNFRSF13b). B-cell compartmentalization and cell-cell interactions in the secondary lymphoid tissues require expression of membrane-bound LTα/β trimers and TNF, whereas T-cell-dependent B-cell differentiation requires engagement of CD40 (TNFRSF5) by CD40L on activated $CD4^+$ T cells. CD30 (TNFRSF8) is expressed on activated B cells and has been found to be required for efficient memory B-cell generation. CD27 is also implicated in B-cell memory; however, significant species-specific differences between human and mouse CD27 have complicated clear functional assignment.

In this review, we focus on the distinctive biology and signal transduction of CD40 and BAFF-R/BCMA/TACI and elaborate on how this can be applied towards understanding the molecular basis of B-cell-associated autoimmune diseases and malignancies.

Canonical and non-canonical pathways of NF-κB signaling

Activation of NF-κB is fundamental to signal transduction by members of the TNFRSF (Fig. 2). NFκB represents a family of transcription factors activated by a diverse array of proinflammatory cytokines, pathogen-associated molecular patterns (PAMPs), cell-bound ligands, antigens, and physical stresses. Expression of NF-κB target genes is essential for mounting innate immune responses to infectious microorganisms but is also important for the proper development and cellular compartmentalization of secondary lymphoid organs necessary to orchestrate an adaptive immune response. In mammals, the NF-κB family consists of five members [RelA(p65), RelB, c-Rel, p50(NF-κB1) and p52(NF-κB2)] that utilize Rel homology domains to form an array of 15 hetero- and homo-dimeric transcriptional regulators. In addition to transcriptional control of NF-κB expression, a number of studies have shown that NF-κB function can be altered by post-translational phosphorylation or acetylation (9, 10). Most notably, NF-κB activity is modulated by inhibitor of κB (IκB) proteins, which can be classified into three functional groups: the typical IκB proteins (IκBα, IκBβ, and IκBε), the precursor proteins (p100 and p105), and the atypical IκB proteins (IκBζ, BCL-3, and IκBNS) (11). IκBα is the prototypic member of the family and retains NF-κB dimers inactive in the cytoplasm. Upon stimulation, IκBα undergoes ubiquitin-mediated proteasomal degradation, releasing bound NF-κB dimers to translocate freely to the nucleus to drive gene expression, including that of IκBα itself. Thus, repression of NF-κB by IκBα is associated with a feedback mechanism of inherent periodicity. Supported by early studies of knockin mice showing functional redundancy between IκBα and IκBβ (12), the other typical IκB proteins were thought to function in a highly similar manner to IκBα. However, recent characterization of IκBβ$^{-/-}$ mice revealed both negative and positive roles for IκBβ in regulating gene expression (13, 14). The latter case is represented by the activity of a hypophosphorylated form of IκBβ that binds p65:c-Rel heterodimers to promote expression of the *Tnfα* and *Il1β* gene (13, 14). The atypical IκB proteins are also known to both repress and enhance gene expression in a gene-specific manner by modulating the binding activity of particular NF-κB dimers. Lastly, the p100 and p105 precursor proteins share homology with IκB proteins in the C-terminal portion and sequester NF-κB proteins in the cytoplasm (9). Cleavage of p100 or p105 results in the generation of p52 and p50, respectively, and the loss of inhibitory activity. Thus, multiple levels of regulation combine to govern the strength, specificity, and duration of NF-κB signaling.

Given that there are multiple NF-κB members and inhibitors with distinct functional properties and expression patterns, it is not a simple task to predict stimulus-specific transcriptional readouts. Interpreting this complexity has been the goal of recent efforts in mathematical modeling and systems biology (15). Original work in fibroblasts demonstrated that TNF-induced gene activation could be modeled in a predictive fashion by understanding the tempo and oscillations of IκBα, β, γ expression (16). Current efforts in the field are targeting multiple ligands and receptors in primary cells in the context of discerning cell fate decisions. Applied to B cells, such studies could provide considerable insight into how extracellular signals are integrated in a stage-specific manner.

IκB degradation depends on site-specific serine phosphorylation by the IκB kinase (IKK) complex, which triggers IκB poly-ubiquitination and proteasome-mediated destruction (17). The IKK complex consists of the catalytic subunits IKK1 and IKK2 as well as the NF-κB essential modulator (NEMO), which is unrelated in sequence and performs a regulatory role (17). Despite significant sequence similarity, examination of *Ikk1*-, *Ikk2*- and *NEMO*-deficient mice revealed that activation of NF-κB by most pro-inflammatory stimuli and PAMPs does not require IKK1 but is dependent on IKK2 and NEMO function. *Ikk2*$^{-/-}$ mice die in mid-gestation due to extensive apoptosis of fetal hepatocytes culminating in liver

failure (28, 30, 35). This phenotype is shared by *Rela*$^{-/-}$ mice and is attributed to TNF*α*-induced programmed cell death, as compound mutants defective in either *Ikk*2 or *Rela*, and either *Tnfr1* or *Tnfα* genes are rescued from embryonic lethality (18–20). Regulation of RelA (p65)-containing dimers is achieved primarily by the typical IκB proteins, resulting in rapid and dynamic control of gene expression. IKK2 activity is thus necessary for activation of innate immune responses and protection from apoptosis, consistent with the 'classical' role of NF-κB. Thus, this pathway, which is largely but not exclusively attributed to gene induction by RelA (p65)-containing dimers has been termed the 'classical' or 'canonical' pathway (Fig. 2).

Although IKK1 inactivation also results in embryonic lethality, epidermal and skeletal defects rather than impaired classical (p50/RelA(p65)) NF-κB activity account for the severity of this mutation (21). Studies of induced p100 processing revealed that cleavage of p100 to release p52 is dependent upon NF-κB-inducing kinase (NIK), which acts by phosphorylating IKK1 (22). Indeed, mutation of the two NIK phosphorylation sites (serines 176 and 180) to alanines in the activation loop of IKK1 (IKK1^{AA}) or ablation of IKK1 expression both result in impaired p100 cleavage (23). p100 processing into p52 and the formation of p52/RelB complexes is the biochemical signature of the 'non-canonical' NF-κB pathway, which requires new synthesis of p100 and RelB to form p52:RelB dimers which are not subject to IκB control (Fig. 2). For these reasons, induction of the non-canonical pathway is slow and sustained. However, there is also crosstalk between the classical and alternative pathways, since activation of the classical pathway stimulates expression of p100 and RelB (24, 25). Thus, the canonical and non-canonical NF-κB signaling pathways have distinctive properties that dictate the tempo and specificity of gene expression.

NF-κB function and B-cell differentiation

NF-κB activity has been associated with B-cell proliferation, survival, differentiation, and antibody production (26). Targeted deletion of individual Rel/NF-κB components as well as subunits of the IKK complex have revealed both the selectivity and complexity of NF-κB function in the B-cell lineage (Fig. 1). Early B-cell development in the bone marrow is not strongly affected by impaired NF-κB signaling (26); however, inactivation of IKK1 or IKK2 results in profound but distinct reductions in peripheral B cells. B cells lacking IKK1 do not mature through the transitional B-cell stage to become mature B cells (27), whereas conditional inactivation of *Ikk2* or *Nemo* results in a general defect in peripheral B-cell survival (28, 29). Targeted deletion of *Nfkb1* results in a selective impairment in MZ B-cell formation, while *Nfkb*$^{-/-}$ mice have a dramatic B-cell-extrinsic defect in GC formation (21). Prompted by the unexpected embryonic lethality of *Ikk1*$^{-/-}$ mice, subsequent studies have revealed that IKK1 likely possesses important functions in addition to its unique ability to induce the non-canonical pathway of NF-κB activation. Some of these functions involve phosphorylation of critical substrates outside the realm of NF-κB signaling (22). We reported that IKK1 serves an intrinsic function in follicular dendritic cells (FDCs) (30) and is also important in B cells for GC formation (31).

BAFF and APRIL signaling and function

BAFF and APRIL exhibit structural similarity and overlapping yet distinct receptor binding specificity (Fig. 3). Both BAFF and APRIL bind BCMA, but the APRIL:BCMA interaction is of higher affinity (32, 33). Conversely, BAFF-R binds BAFF exclusively with high affinity (32). The negative regulator TACI binds to both BAFF and APRIL with similar affinity (33, 34). APRIL also has the capacity to bind heparin sulphate proteoglycans (HSPGs), which may help to retain or focus binding to BCMA/TACI akin to a co-receptor

function and also to promote multimerization (35). As is the case for other members of the TNF family, BAFF is produced in both membrane and soluble forms. On the cell surface, BAFF is cleaved in a largely furin-dependent manner to release a trimer that appears to be the primary bioactive form of BAFF (36). Although BAFF and APRIL are secreted as trimers (36, 37), crystallography studies revealed that BAFF trimers can be ordered into 60-mer arrays (37, 38). These multimers are found in the serum and effectively engage TACI to an extent that is not achieved with BAFF trimers (39). Thus, binding of APRIL or BAFF to TACI is aided by distinct mechanisms.

BAFF-R and B-cell fate

BAFF is the most critical soluble factor for peripheral B-cell maturation and survival (40, 41). BAFF is fairly widely expressed by various cell types including macrophages, dendritic cells, and neutrophils and even in an autocrine fashion (42). Nonetheless, the bioavailability of BAFF is limiting and serves as a competitive driver of B-cell homeostasis. Moreover, there is a need for regulating BAFF production, as overexpression promotes autoimmunity and B-cell transformation (43, 44). Studies of radiation chimeras demonstrated that stromal cells, including FDCs, provide sufficient BAFF to sustain B-cell maturation and survival (45). Early appreciation for the importance of BAFF-R and its ligand to peripheral B-cell maturation and survival came from the phenotypic similarity of mice lacking BAFF or BAFF-R (5, 46, 47). The latter defect was originally characterized in AsWyn/J mice, which were found to bear a transposon insertion that resulted in the replacement of the 8 C-terminal amino acids (48, 49). Studies of AsWyn/J and gene-targeted *Baff-r*$^{-/-}$ animals revealed a similar phenotype of a peripheral B-cell block (46, 47, 50, 51) (Fig. 1).

The onset of BAFF-R expression occurs in the newly formed B cells poised to egress from the bone marrow (52, 53) (Fig. 1), indicating that early B-cell differentiation events associated with pre-B-cell expansion and the generation of surface IgM^{+} cells is not influenced by the pro-survival effects of BAFF stimulation. BAFF-R expression is upregulated as transitional B cells mature to become follicular or MZ B cells (52–54). Of note, BCR signaling promotes *Baff-r* expression and (55, 56), in turn, BAFF signaling upregulates CD21 and CD23 expression (57). Consistent with the pattern of BAFF-R upregulation, BAFF or BAFF-R deficiency imposes a block at the T1 → T2 maturation step due to failed survival (5, 46, 47). Thus, follicular and MZ B cells are reduced > 90% and do not recover with age. Interestingly, B-1 cell formation is unaffected by impaired BAFF signaling, raising the possibility that elevated BCR signaling in these cells or other microenvironmental factors in the pleural cavity where B1 B cells reside may provide for BAFF-independent survival. Provision of a survival signal in the form of forced Bcl-2 expression rescues the transitional B-cell block, leading to the generation of follicular B cells (51, 58, 59); however, MZ B-cell formation remains impaired (58, 59), indicating that BAFF-R engagement also imparts essential differentiative signals.

Given the early and dramatic impact of impaired BAFF-R signaling, blocking antibodies or receptor fusion proteins have been employed to acutely deplete BAFF to observe consequent effects on naive recirculating and antigen-experienced B cells. Similar to transitional B cells, BAFF depletion leads to the rapid loss of follicular B cells by apoptotic death (60). The role of BAFF/BAFF-R signaling in GC formation is more complex (61). Despite the paucity of mature B cells in mice lacking BAFF or BAFF-R, GCs are formed and some IgG is produced (46, 62, 63); however, the GC response is transient with impaired proliferation and an associated failure to form mature FDC networks (58, 62, 63). The latter defect may reflect failed selection of GC B cells by FDC-bound antigen (58, 63); however, this possibility has not been directly tested by transfer of immune complexed antigen. In addition, BAFF-dependent signaling is likely more stringent in the GC due to reduced

expression of BAFF-R on GC B cells (64). An additional consideration is that the immature (T1) B cells that remain in the absence of BAFF may not efficiently express $LT\alpha_1\beta_2$, which is required for FDC formation and the GC response (7). As in the case of MZ B-cell formation, ectopic expression of Bcl-2 does not rescue the GC response, resulting in the accumulation of B cells bearing an immature phenotype and disrupted follicular architecture (58). Upon leaving the GC, memory B cells become independent of BAFF signaling (65, 66). While long-lived plasma cells in the bone marrow can respond productively to BAFF stimulation, APRIL and BAFF serve redundant roles in promoting plasma cell longevity (60, 65). Thus, while BAFF signaling is critical for the survival of transitional and mature recirculating B cells, it is also required to promote MZ and GC B-cell differentiation via yet to be determined molecular mechanisms.

BAFF has been characterized chiefly as a pro-survival factor (67). The critical targets of BAFF-dependent survival have yet to be clearly assigned but likely result from a favorable balance of upregulated expression of Bcl-2 family members (Bcl-2, Bcl-x_L, Bcl-w, Mcl-1, and A1) and downregulated expression of pro-apoptotic BH3-only proteins (Bid, Bad, Bik, Bim, Bmf, Hrk/DP5, Noxa, and Puma), thus preventing Bak/Bax-dependent apoptosis (68). Although Bim is the better characterized BH3 family member, governing peripheral B-cell survival, inactivation of Bim or Bmf results in dramatic B-cell hyperplasia and lymphadenopathy (69–71). As in the case of forced Bcl-2 expression (51, 58, 59), inactivation of Bmf or Bim does not result in an expanded MZ B-cell compartment (69, 71). Interestingly, binding specificity may underlie distinctions and overlaps in Bmf/Bim function, as Bim binds tightly to all Bcl-2 family members (72), whereas Bmf only binds Bcl-2, Bcl-x_L, and Bcl-w (73). In addition to neutralizing pro-survival functions, the BH3-only proteins may also directly activate Bak/Bax (68). BAFF-R engagement causes a reduction in Bim expression (74), but it is not known if this effect applies to other BH3-only proteins.

BAFF induces the transcription of the pro-survival factors, *A1*, *Bcl-x_L* and *Pim2* (54, 75, 76). Consistent with the role of BAFF in the generation of T2 B cells, early studies of *Bcl-$x_L^{-/-}$* mice revealed a reduced percentage of IgM^+IgD^- B cells (77). However, the B cells that overcome this bottleneck exhibit normal survival as mature recirculating cells (77). While *Pim2*$^{-/-}$ and *NF-kB2*$^{-/-}$ B cells show similar defects in BAFF-mediated survival *in vitro* (76), inactivation of all three *Pim* genes results in only a subtle defect in peripheral B cells in younger mice (78). Induction of A1 transcription by BAFF is not supported by increased protein expression (75). Moreover, A1 represents a quartet of highly similar genes, of which A1a has been shown to be dispensable for BAFF-mediated survival, suggesting that A1 induction by BAFF may not be critical (75). Mcl-1 expression has also been linked to BAFF signaling (79, 80) and has recently been shown to be essential for GC and, to a lesser extent, follicular B-cell survival (81). By contrast, loss of Bcl-xL is inconsequential for GC B-cell differentiation and survival (81). These studies extend original findings that early B-cell development is dependent upon Mcl-1, likely downstream of IL-7R signaling (82). Mcl-1 is known to be an extremely labile protein and thus it will be important to elucidate transcriptional versus post-translational control of Mcl-1 in B cells. We posit that BAFF-dependent survival of T2, follicular, and GC B cells likely involves distinct pathways of regulation affecting both pro-apoptotic and pro-survival factors. Based on existing data, augmented expression of Bcl-xL, Bcl-2, and Mcl-1 by BAFF would be most crucial for transitional, follicular and GC B cells, respectively.

In addition to intrinsic differences impacting B-cell fate decisions at different stages of development, extrinsic factors dictated by the microenvironment also have a strong influence on peripheral B-cell maturation (83). In addition, some of these signals have a sustained impact, meaning that B cells present in one location are conditioned by signals

received in the prior microenvironment. For example, it has been shown that the impact of the bone marrow versus splenic microenvironments has a strong influence on the susceptibility of newly formed B cells to undergo BCR-mediated apoptosis (84). Profiling of expressed genes by microarray analysis has provided some insight into the genetic basis for the differential responsiveness of distinct B-cell subsets. In the case of transitional B cells, upregulation of BAFF-R demarcates the release of B cells from the influence of the bone marrow environment and continued maturation in the spleen. Recent studies have shown that transitional B cells enter the splenic red pulp bearing the phenotype of 'T0' cells that require Rac-dependent signals to mature further into T1 and T2 cells, which is coincident with entry into the white pulp and follicle (85). Survival and migration of T0/T1 cells is associated with intact chemokine receptor signaling prior to entering the follicle where ample sources of BAFF are provided by myeloid cells and FDCs. It is not known if BAFF is abundant in the red pulp and thus, by extension, available for T0/T1 cell maturation. The intact T1 compartment in BAFF/BAFF-R-deficient mice is consistent with this association of transitional B-cell maturation and splenic microenvironments.

BAFF-R signaling

Clearly BAFF is essential for the survival of newly formed and recirculating B cells, but it also promotes cell growth, proliferation, and differentiation in some contexts. Therefore, studies of the signal transduction mechanisms emanating from BAFF-R need to explain these cellular processes in molecular terms (Fig. 4). Membrane proximal signaling by BAFF-R has been attributed to the TRAF molecules, which bind directly or via adapter molecules to intracellular domains of TNFRSF members (86, 87). The N-terminal regions of the TRAFs (except TRAF1) include a RING domain that mediates E3 ligase activity for K63-linked ubiquitin modifications to modulate protein function (88). In addition, some of the TRAFs bind the RING-containing cellular inhibitor of apoptosis 1 (cIAP1) and cIAP2 proteins to mediate protein degradation by K48-linked ubiquitin modifications (88). The TRAF C-terminal portion mediates protein-protein interactions with surface receptors and other TRAF proteins to form hetero- and homo-dimers (87, 88). Thus, receptor-specific functions are largely attributed to the nature and kinetics of TRAF protein oligomers and their binding partners. Although only TRAF3 binds BAFF-R directly (89, 90), signaling occurs via the concerted actions of TRAF2 and TRAF3, which negatively regulate the receptor (86). Thus, mice lacking TRAF2 or TRAF3 exhibit a phenotype consistent with BAFF transgenic mice and can persist *in vitro* in the absence of survival factors as well as *in vivo* in the absence of BAFF (91–94). Of note, inactivation of TRAF3 also allowed for the formation and maintenance of the MZ B-cell compartment (93, 94), indicating that both BAFF-dependent survival and differentiation signals are dependent upon TRAF2/TRAF3.

The biochemical interplay of TRAF2/TRAF3 and associated molecules is coming into focus. In early work distinguishing the canonical and non-canonical NF-κB pathways, it was observed that BAFF-R engagement efficiently induced the cleavage of p100 into p52 in an enzymatic cascade that required the partnership of NIK acting on IKK1 (95–97). It is known that NIK stability is TRAF3-dependent (98); however, the relationship of TRAF2, TRAF3, and the cIAPs has only recently been clarified (99, 100) (Fig. 4). In unstimulated B cells, TRAF2 recruits cIAP1/cIAP2 and facilitates binding of cytosolic TRAF3 to NIK, mediating TRAF3 ubiquitination and degradation (99, 100). BAFF-R engagement recruits the NIK/TRAF2/TRAF3/cIAP1/2 complex to the receptor, inducing the ability of TRAF2 to ubiquitylate cIAP1/2 by K63 modification. This event redirects cIAP1/2 to ubiquitylate TRAF3 in a K48-specific manner, resulting in its degradation and allowing newly formed NIK to persist and activate IKK1. Modeling on recent findings on LT-βR signaling (101), BAFF-R engagement and binding to TRAF3 may also displace NIK and prevent its degradation. Supporting the important yet redundant roles of cIAP1/2 in BAFF-R signaling

is the recent description of mice lacking cIAP1 and or cIAP2 (102). While inactivation of either cIAP1 or cIAP2 does not result in a discernable phenotype, B cells in mice lacking both enzymes accumulate due to sustained NIK expression (88, 102). Moreover, a knockin mutation inactivating the E3 ligase activity of cIAP2 confers a dominant negative effect on cIAP1, resulting in NIK stabilization (103). These phenotypes are consistent with earlier reports of gene targeted mice expressing a mutated NIK molecule that cannot interact with TRAF3, leading to its accumulation and constitutive activation of the non-canonical NF-κB pathway (104). Interestingly, recent evidence suggests that these feed forward mechanisms are countered by phosphorylation of NIK by IKK1, leading to NIK instability (105).

Notably, while NIK$^{-/-}$ and IKK1^{AA} (see above) mice both present strong impairments in p100 processing and associated defects in GC formation (31, 106), defects in the follicular and MZ B-cell compartments in these mice are less severe than those observed in *Baff*/*Baff-R*$^{-/-}$ or *Ikk1*$^{-/-}$ mice (24, 106, 107). By contrast, *Ikk1*$^{-/-}$ mice present a block at the transitional B-cell stage (27), consistent with impaired BAFF-R signaling. Dual elimination of *Nf-κB1/Nf-κB2* also presents an early peripheral B-cell block at the transitional stage (95). Several explanations may collectively explain these related yet distinct phenotypes. In *Ikk1*^{AA} mice, there is residual cleavage of p100 into p52 (96). Given that the IKK1^{AA} molecule cannot be activated by NIK (or another kinase) at the critical residues in the activation loop, it is possible that basal activity of IKK1 is important. In addition, there is substantial evidence that IKK1 phosphorylates substrates that are independent of NF-κB activation, or could provide a scaffolding role (22). Lastly, IKK1 can contribute to the canonical IKK2/Nemo pathway, imparting important survival signals (108, 109).

BAFF-R function has been closely linked to the negative regulation of PKCδ (110). Indeed, B cells lacking PKCδ can survive in the absence of BAFF and are less susceptible to tolerogenic signaling, resulting in the generation of autoantibodies (111, 112). PKCδ is a target for caspase 3 cleavage, which generates a constitutively active form of the kinase that is active in the nucleus (113). In mice expressing constitutively active IKK2 or lacking TRAF3 in the B lineage, increased survival has been linked to enhanced nuclear exclusion of PKCδ (94, 114). Thus, sustained activation of the canonical or non-canonical NF-κB pathways may converge on PKCδ regulation. Despite the prominence of PKCδ in BAFF signaling and autoimmunity, nuclear substrates for the pro-apoptotic function of PKCδ have not been identified.

BAFF-R signaling and the PI3K pathway

For good reason, the majority of studies on BAFF-R function have focused on signaling via the TRAF/IKK/NF-κB pathway. However, additional pathways may also contribute to augment or to complement NF-κB activation. Most notably, perhaps, is the phosphatidylinositol (PtdIns) 3-kinase (PI3K) pathway (115), which has recently been implicated in BAFF-R function (Fig. 4). The class IA PI3Ks consist of three catalytic isoforms (p110α, β, and δ) that form heterodimers with adapter subunits (p85α, p55α, p50α, p85β and p55γ), which regulate location and enzymatic activity (116). In B-cell receptor signaling, p110 can act on membrane substrates via p85-mediated recruitment to the transmembrane adaptor CD19 as well as cytosolic BCAP. As PI3K is known to promote cell survival and proliferation, its activity must be tightly regulated to prevent cell transformation. Thus, in resting cells PI3K lipid products are present in low abundance but are rapidly induced in response to various stimuli. PtdIns(3,4,5)P_3 and, perhaps, PtdIns(3,4)P_2 are substrates for the phosphoinositide 3-phosphatase PTEN, which has emerged as the key functional antagonist to PI3K (117). The generation of PtdIns(3,4,5)P_3 induces several downstream pathways promoting cell growth, proliferation, survival, and differentiation.

Using Akt phosphorylation as a surrogate read out, it has been observed that BAFF induces PI3K activity with both rapid and delayed kinetics (118, 119). The significance of this induction is supported by findings showing that p110δ-deficient B cells exhibit impaired responses to BAFF-induced survival (120). How PI3K is recruited to BAFF-R is unclear, but it may utilize components of the BCR complex, particularly CD19 (A. Miletic *et al*., unpublished data) or via the TRAF proteins. With respect to the latter, induction of Akt activity by OX40 has been shown to be dependent upon TRAF2 (121). In terms of downstream effector pathways (Fig. 4), BAFF engagement activates Btk and PKCβ as well as Akt to promote ribosome biogenesis and increased metabolic activity to prime B cells for antigen-induced proliferation (118, 122, 123). In addition, BAFF promotes the upregulation of the pro-survival factor Mcl-1 (79, 80), which may be due to Akt-dependent inactivation of GSK3α/β (124). Akt inactivates Foxo1 as well (125), preventing it from transcribing pro-apoptotic genes. Accordingly, we and others have shown that peripheral B cells accumulate in the absence of Foxo1 (126, 127).

The biochemical events occurring downstream of BAFF-R engagement suggest some important signaling nodes and specificity in the NF-κB and PI3K pathways (Fig. 4). Membrane recruitment of PI3K by the adapter proteins CD19 and BCAP initiates PI3K activity and the generation of PtdIns(3,4,5)P_3, which in turn recruits Btk and PLCγ2 via the binding of their pleckstrin homology domains. Btk activates PLCγ2, promoting the generation of DAG and the release of intracellular Ca^{++} which combine to activate PKCβ (128). PKCβ activation is critical for BCR-induced activation of the canonical NF-κB pathway by the Carma/Bcl10/Malt1 (CBM) complex and thus can be facilitated by BAFF-R signaling (see below). In addition, PI3K activation can also promote c-Rel-dependent upregulation of *Baff-r* (129), creating an additional positive feedback loop. While the NF-κB pathway can drive the expression of A1 and Bcl-x [and perhaps Bcl-2 (130)], Mcl-1 has not been identified as an NF-κB target. Thus, Mcl-1 expression may be regulated primarily in a post-translational manner that requires PI3K signaling. Notably, several of the BH3-only family members are inhibited by the PI3K family. Bad is sequestered and degraded following phosphorylation by Akt (131), and Bim as well as Puma are reported targets of the Foxo factors (132, 133).

Synergistic signaling by BAFF and the BCR

Signaling by the BAFF-R and BCR represents the two essential stimuli necessary for B-cell homeostasis and has been the topic of several recent reviews (107, 122, 134). Similar to the outcome of BAFF depletion, elimination of the BCR leads to rapid apoptosis (135). Interestingly, survival of these ‘BCR-less’ B cells is achieved by expression of a constitutively active form of PI3K but not by constitutively active IKK2 (127). Similarly, Foxo1-deficient pre-B cells survive in the periphery in the absence of a pre-BCR or Ig-α/Ig-β (H. Dengler *et al*., unpublished data). Synergy between the BCR and BAFF-R are not limited to survival. While BAFF stimulation promotes cell cycle entry, a failure to upregulate cyclin E and downregulate the cell cycle inhibitor $p27^{Kip1}$ prevents progression to S phase (136). This block is relieved by BCR engagement, leading to augmented clonal expansion. *In vivo* depletion of BAFF reduces homeostatic proliferation of a subset of T2 B cells expressing intermediate levels of CD21 and the highest density of BAFF-R (52). These findings suggest that homeostatic proliferation is aided by BCR/BAFF-R costimulation. Consistent with the identification of $p27^{Kip1}$ as a Foxo target (137), we reported that BAFF has a mitogenic effect on PTEN-deficient cells and may contribute to the oncogenic effect of sustained PI3K signaling in B cells (138).

Elucidating the complementary and synergistic contributions of the BAFF-R and BCR signaling pathways is a priority for understanding peripheral B-cell maintenance and how

disruption of homeostatic signals may promote transformation. Signal transduction by the BCR on mature naive recirculating B cell is achieved by the associated Ig-α/Ig-β heterodimer. In addition, the strength and quality of signaling is modulated both negatively and positively by associated co-receptors of which CD19 factors most prominently. Activation of canonical NF-κB signaling is mediated primarily by the CBM complex following activation of upstream kinases (Btk, Syk, and Src family kinases) (128). In T cells, activation of PDK1 by PI3K is associated with promoting the assembly of the CBM complex and the activation of PKCθ (139, 140). In this context, PDK1 is required for T-cell activation by TCR/CD28 costimulation but is dispensable for the survival of resting T cells (140). In B cells, PI3K signaling promotes the activation of PKCβ, which subsequently phosphorylates CARMA1 to promote canonical NF-κB activation via the CBM complex and phosphorylation of IKK2 by the TAB/TAK complex (141, 142). The role of PDK1 and the CBM complex in B cells has not been investigated. However, it is known that B cells lacking the protease Malt1 exhibit a survival defect associate with reduced c-Rel expression (143). As Malt1 activation has also been functionally associated with BAFF signaling (144), its activation and cleavage of downstream substrates such as the pro-apoptotic factor A20 may contribute to synergistic survival signaling by the BCR and BAFF-R.

The canonical NF-κB pathway has been shown to prime the non-canonical pathway by driving the expression of p100 (145), including recent studies showing that the BCR induces p100 to facilitate BAFF-R signaling (53) (Fig. 4). p100 was initially recognized as an inhibitor of RelB, which is relieved by cleavage into p52. However, p100 has recently been shown to aggregate and act as an inhibitor of p50:p65 (146). As such, induction of high levels of p100 can be repressive and cleavage allows for the formation of p52 as well as the release of this repression (15). In support of this view, mice expressing a mutant p100 protein that cannot be cleaved into p52 exhibit failed activation of the non-canonical pathway as well as retention of RelA in the cytoplasm (147). Thus, while induction of p100 by the BCR can augment BAFF-R signaling by providing more substrate for the generation of p52 and formation of p52/RelB heterodimers, overproduction in the absence of NIK/IKK1 signaling can negatively feed back on the canonical pathway. This feedback is an important consideration in systems where the interpretation of forced expression of p100 is limited to augmented activation of the non-canonical pathway.

BAFF-R signaling and disease

Early studies of BAFF function revealed that overexpression of BAFF in mice caused overt autoimmunity with the development of disease conditions bearing similarity to systemic lupus erythematosus (SLE) and Sjögren's syndrome (34, 91, 148). Moreover, patients with SLE, Sjögren's syndrome, or rheumatoid arthritis (RA) showed elevated BAFF levels in serum or synovial fluid (149–152). The potential sources of BAFF in patients with autoimmune disorders are incredibly diverse and include fibroblast-like synoviocytes in the synovium of patients with RA, epithelial cells in tonsils and airways, salivary gland epithelial cells in patients with Sjögren's syndrome, and astrocytes in with primary central nervous system lymphomas or multiple sclerosis patients (60). Consistent with the co-contributing roles of the BCR and BAFF-R, studies of B-cell tolerance in mouse transgenic models revealed that elevation of serum BAFF concentration caused a breach of negative selection, leading to the survival and differentiation of autoreactive B cells (153–155). In addition to providing an escape mechanism for newly formed self-reactive B cells, BAFF promotes the retention and survival of autoreactive B cells in the MZ (76, 153, 154). Moreover, in mature B cells, BAFF is capable of inducing immunoglobulin class switch recombination (156), indicating that in the case of autoreactive B cells, the generation of pathogenic antibody of the IgG and IgA subtypes can occur in the absence of T-cell help. Thus, the amount and source of BAFF production can impact the generation of self-reactive

B cells as well as their potential to differentiate into pathogenic autoantibody-producing cells. For these reasons, BAFF depletion was recognized early on as a therapeutic approach of great potential (157). That potential was realized with the recent FDA approval of the human antibody belimumab for the treatment of SLE (158). Nonetheless, the efficacy of belimumab is limited, perhaps owing to the persistence of plasma cells responding to APRIL (see below). If this is the case, then treatment with atacicept, a TACI-Fc fusion, may prove to be more efficacious. Moreover, maintenance of memory B cells does not require BAFF, and thus their subsequent differentiation into plasma cells should not be hindered.

The ability of BAFF to promote B-cell survival underscores its potential to promote transformation in situations where BAFF-R engagement or downstream signaling components is amplified. Indeed, dysregulated BAFF expression by transgenic overexpression promotes B-cell non-Hodgkin's lymphoma (B-NHL)-like disease when crossed onto a *TNFα*$^{-/-}$ background (159). Unlike normal B-cell counterparts, most B-NHL types express BAFF in an autocrine fashion, and elevated serum BAFF has been noted in these patients (160–162). Given that BAFF can be produced in an autocrine fashion, studies have shown that *in vitro* survival of malignant B cells is curtailed in the presence of BAFF/APRIL-depleting reagents (163–165). *In vivo* studies of the impact of BAFF depletion on lymphoma progression are limited. However, we found that primary B cells tumors arising from the co-inactivation of *Pten* and *Ship* can progress in the absence of BAFF (138). Thus, based on murine models, one may predict that malignant B cells displaying constitutive activation of canonical or non-canonical NF-κB or PI3K may not be responsive to anti-BAFF treatment. In addition to altered production of BAFF, gain-of-function mutations encoding the cytoplasmic tail of the BAFF-R have also been reported (166). In these mutants, increased TRAF2, TRAF3, and, interestingly, TRAF6 were found to be associated with the BAFF-R. TRAF6 association is a novel finding and may contribute to activation of the canonical NF-κB pathway, as in the case of CD40 signaling (see below). In addition to BAFF-R, loss-of-function mutations in cIAP1/cIAP2, TRAF2, and TRAF3 have also been observed in B-cell malignancies (167). As these mutations presumably would only promote survival, additional driver mutations are likely involved to promote proliferation in more aggressive B-cell malignancies.

BCMA receptor

Although BCMA was identified prior to BAFF-R as a receptor for BAFF (5, 168) (Fig. 3), its expression in the B lineage is restricted to GC B cells, memory B cells (in human), and plasma cells (166, 169, 170). *Bcma*$^{-/-}$ mice exhibit normal B-cell development and humoral responses to T-cell-dependent and –independent antigens in the short-term as well as memory responses (171). However, there is a sharp reduction in the long-lived IgG-producing plasma cells in the bone marrow in *Bcma*$^{-/-}$ mice (172). Additional *in vitro* studies showed that BAFF can promote plasma cell survival in synergy with IL-6. Recent studies have also shown that eosinophils provide critical support for plasma cell retention and survival in the bone marrow through the production of IL-6 and APRIL (173). Additional bone marrow cell types including the stroma, macrophages, and osteoclasts can also produce BAFF and APRIL (174). BCMA is a major receptor for BAFF/APRIL engagement on bone marrow plasma cells, although TACI is also expressed on these cells. Consistently, the survival of long-lived plasma cells does not require BAFF but is dependent upon APRIL (174, 175). Binding of APRIL to BCMA is aided by the expression of HSPG-modified receptors, particularly CD138 (Syndecan-1), on plasma cells, and perhaps expression of HPSG-modified receptors on other resident bone marrow cells to promote retention and multimerization of APRIL (35, 175, 176) (Fig. 3). Early studies showed that overexpression of BCMA in human embryonic kidney 293 (HEK 293) cells activated canonical NF-κB signaling, and co-immunoprecipitation studies indicated that BCMA could

interact with NIK and the IKK complex (33, 177, 178). We have previously demonstrated that IKK1 plays a crucial role in plasma cell maintenance, with the *Ikk1*$^{AA/AA}$ plasma cells showing impaired survival (31). However, it is unclear how BCMA engagement employs the TRAF proteins and downstream activation of the canonical and non-canonical NF-κB pathways (177, 178). Thus, much remains to be known regarding BCMA signaling by APRIL/BAFF in terminally differentiated plasma cells.

Despite the paucity of knowledge of BCMA signaling on normal plasma cells, recent findings in multiple myeloma (MM) cells have established the importance of gain-of-function mutations in both canonical and non-canonical NF-κB activation in this of this disease (179, 180). These mutations include activation of NFKB1, NFKB2, NIK, CD40, and TACI, as well as inactivation of TRAF2, TRAF3, cIAP1/cIAP2, or CYLD. Inactivation of *Traf3* represents one of the most common mutations in MM (179, 180), leading to dysregulation and sustained activation of both NF-κB pathways via the sustained presence of NIK and its constitutive activity (88). Interestingly, in some of these cases, NIK expression was found to be essential for propagation of the MM cells, whereas IKK1 was dispensable (179). Moreover, constitutive activity of NIK or IKK2 in MM cell lines produced similar patterns of gene induction (181). These findings are consistent with a role for NIK in promoting activation via the canonical NF-κB pathway (108, 109). MM cells variably express BAFF-R, TACI and BCMA, and are a source of autocrine BAFF (182). It appears that MM cells bearing NF-κB mutations become independent of APRIL/BAFF for retention and expansion in the bone marrow, thus enabling the dissemination and propagation of tumor cells. Treatment of these malignancies would require targeting of both NF-κB pathways, perhaps through NIK. On the other hand, approaches to disrupt growth factor support in the bone marrow microenvironment may not be effective in MM. That said, nonmalignant monoclonal gammopathy of undetermined significance (MGUS) tumors that give rise to MM may be more responsive to BAFF/APRIL depletion.

TACI receptor

Transmembrane activator and CAML (calcium-modulator and cyclophilin ligand) interactor (TACI) binds both APRIL and BAFF and is expressed on mature B cells and plasma cells (183) (Fig. 3). BAFF and APRIL will only engage TACI when presented in an oligomeric or membrane-bound form (39). Multimeric BAFF will interact with both TACI and BAFF-R on resting, MZ, and GC B cells, whereas multimeric APRIL can bind both BCMA and TACI on plasma cells (Fig. 3). TACI is upregulated on activated B cells, particularly in response to TLR ligands (184). Despite its discovery as a CAML-associated protein (185), the functional significance of this interaction is unclear. TACI also has the capacity to bind TRAF2, TRAF5, and TRAF6 (164, 186), but the regulation of signal transduction by TACI is not well characterized. Only recently has it been determined that TACI can activate NF-κB in a MyD88/IRAK4-dependent manner akin to TLR signaling (187). MyD88 and TRAF2 bind to the same region of TACI and act cooperatively to activate NF-κB (187). BCMA, BAFF-R, and CD40 do not share the ability to bind MyD88.

Approximately 10% of patients with combined variable immune deficiency (CVID) have mutations in TACI and present a lymphoproliferative disease and defects in IgA production (188–190). CVID is an antibody deficiency syndrome diagnosed on the basis of an impaired ability to produce antigen-specific antibody, including IgM and the switched isotypes (188). Mutations in *TACI* occur throughout the gene in CVID patients, but the most common are autosomal dominant mutations C104R and A181E, affecting the ligand-binding and transmembrane domains, respectively (191–193). The C104R mutation disrupts BAFF/APRIL binding and can have a dominant negative or a haploinsufficiency effect on the TACI complex as composed of mixed molecules, whereas the A181E mutation is thought to

cause a conformational defect (188). The murine equivalents of A181E and C104R (A144E and C76R, respectively) have both been recently examined in a transgenic model bred onto the TACI$^{-/-}$ background (193, 194). Both strains reveal defects in IgG1 and IgA production and responses to TI-2 antigens but not B-cell homeostasis (193, 194). One explanation for the latter observation is that the absence of soluble or membrane-bound TACI may provide for more BAFF available to bind BAFF-R and thus promote B-cell longevity. This interpretation implies that TACI may not possess an intrinsic negative regulatory function.

The CVID syndrome is recapitulated in *TACI*$^{-/-}$ mice, which also exhibit a general defect in IgA production and responding to polymeric T-cell-independent type 2 (TI-2) antigens, and show an accumulation of peripheral B cells (195–198). *TACI*$^{-/-}$ B cells appear to be activated normally in response to TI-2 antigens but display delayed cell cycle progression and differentiation into plasmablasts (199). Both BAFF and APRIL can induce CSR, which in the case of mucosa-associated IgA production may involve APRIL/BAFF production by multiple cell types (156, 198, 200, 201). Defects in IgA production were noted in one line of *APRIL*$^{-/-}$ mice (202) but not another (203), perhaps reflecting strain differences as well as the contribution of other environmental stimuli and redundancy with BAFF. The CSR defect is intrinsic to the loss of the TACI receptor, since *TACI*$^{-/-}$ B cells but not *BCMA*$^{-/-}$ or A/WySnJ B cells fail to induce transcription of the IgA locus upon treatment with APRIL or BAFF (198, 201). Interestingly, TACI was recently shown to induce *Aicda* and CSR through the recruitment of MyD88 in both mouse and human (187). As B cells continue to differentiate into long-lived plasma cells following CSR, they remain dependent on APRIL and BAFF support, but these survival signals are delivered by TACI and BCMA (65, 174).

TACI shows varied expression on MM cells, which utilize BAFF and APRIL provided by multiple cell types in the bone marrow to promote survival and proliferation (169, 204). The level of TACI expression is correlated with maturation profile in that TACI^{lo} versus TACI^{hi} MM cells have gene expression profiles resembling plasmablasts versus bone marrow plasma cells, respectively (204). MM cases bearing the TACI^{lo} phenotype are associated with a worse prognosis, perhaps owing to a more aggressive and less growth factor-dependent profile for this subset. Thus, this information may be useful in stratifying the disease and determining susceptibility to BAFF/APRIL-depleting biologics or small molecule inhibitors. Recent studies have shown that APRIL promotes cell cycle progress via upregulation of the D-type cyclins in follicular lymphoma as well as MM cells (33, 205). Interestingly, in the former case, this effect was shown to be mediated by PI3K/mTOR signaling (33). Thus, as in the case of BAFF-R signaling, the PI3K pathway may serve important functions in synergy with NF-κB signaling. Lastly, it is important to recognize that the lymphoproliferative disease that develops in some CVID patients bearing TACI mutations can be a precursor to transformation (183, 190, 195, 206).

CD40 receptor

The CD40/CD40L axis is central to T-cell-dependent antibody responses and underlies genetic defects that present as hyper-IgM syndrome (207–210). Thus, the primary functions of CD40 are to promote B-cell survival, costimulate proliferation, enhance T-cell collaboration, and enable CSR (211). In the absence of CD40, GC formation is averted and antibody responses are largely limited to low affinity IgM elicited in a T-cell-independent manner (212, 213). As discussed elsewhere (211), CD40 also contributes important functions for other hematopoietic and non-hematopoietic cells. During an immune response, activated CD4^{+} T cells transiently upregulate CD40L to engage CD40 on B cells that are directed to the T-B border following BCR stimulation (214). Activation through the BCR as well as CD40 promotes the antigen presentation capabilities of the B cell through upregulation of MHC class II and member of the B-7 family of costimulatory molecules.

CD40 signaling is required for the extrafollicular response characterized by the generation of foci of proliferating plasmablasts producing antigen-specific IgM as well as the switched isotypes (215). In addition, some of the clonal descendents of the activated B cells will be directed towards the GC pathway. There is evidence that the strength of CD40 signaling may influence differentiation along the plasma cell versus GC B-cell pathway (216). Recent studies suggest that these early events may take place in the perifollicular regions where B-cell migration is governed by the newly characterized EBI2 receptor and oxysterols ligands (217–221). EBI2 is upregulated upon BCR and CD40 engagement and is required for the extrafollicular plasmablast response but also needs to be downregulated to allow for GC B-cell differentiation (86, 220, 221). Continued CD40 signaling is required during the GC reaction as blocking anti-CD40 antibodies cause the rapid dissolution of the GC (222). In this context, CD40 stimulation is provided by co-resident Tfh cells expressing inducible costimulator (ICOS) (223). Interestingly, downregulation of ICOS-L by activating stimuli such as the BCR and lipopolysaccharide (LPS) is prevented by CD40 engagement and promotes the sustained expression of ICOS-L on the B cell. Thus, CD40 signaling is vital for initiating and sustaining the humoral response.

CD40 signaling occurs through the recruitment of TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6. TRAF2 and TRAF3 bind to the same region, whereas TRAF6 binds to a distinct region; TRAF1 and TRAF5 associations with CD40 are indirect (87, 224). TRAF6 activates TAK1 to promote NF-κB signaling via the canonical pathway (Fig. 5). CD40-induced activation of TRAF2 activates the cIAP proteins to ubiquitylate TRAF3, promoting its degradation and releasing MEKK1 for downstream signaling (225). As in the case of BAFF-R signaling, CD40-induced degradation of TRAF3 also leads to stable expression of autocatalytic NIK (Fig. 3). Thus, CD40 signals via the canonical and noncanonical NF-κB pathways (226). Despite dramatic B-cell accumulation due to BAFF-R-independent survival, the loss of TRAF2 or cIAP1/cIAP2, but not TRAF3, results in a failure to form GCs due to impaired activation of the MAPK pathways that are required for GC B-cell expansion and survival (92, 102, 227). These TRAF2-dependent effects appear to be independent of TRAF3 as well as TRAF6 function (227). Correspondingly, the recent generation of B-cell specific TRAF6-deficient mice reveals defects in B-cell survival associated with impaired activation of the canonical pathway, but GC formation remains intact (228). Generation of antibody-producing cells and, curiously, B-1a cells are also reduced in these mice (228). It is unclear how the noncanonical pathway is contributing to GC formation downstream of CD40. B cells lacking p53 or RelB are capable of undergoing GC formation (229, 230), and follicular B cells from $IKK1^{AA}$ mice respond normally to CD40-mediated proliferation, survival, and CSR (31). Moreover, the constitutive activity of NIK in the absence of cIAP1/cIAP2 is not sufficient to sustain GC B-cell differentiation, consistent with the observation that overexpression of BAFF does not rescue GC development on the CD40 *null* background (76). Thus, the failure of $IKK1^{AA}$ B cells to undergo GC B-cell differentiation suggests a novel activity independent of CD40 and NF-κB.

Perspective

Twenty-five years has passed since two seminal findings in immunology: (i) the identification of a membrane protein by Clark and Ledbetter that would later become known as CD40 as an important cognate factor for B-T collaboration (231), and (ii) the seemingly unrelated contemporaneous identification by Sen and Baltimore of a nuclear factor that bound the immunoglobulin κ enhancer (NF-κB) (232). We now know that the functions of CD40 and NF-κB signaling are intimately linked. NF-κB is now recognized as a pivotal factor in inflammation and development as well as disease. Regulation of NF-κB function continues to be a fascinating and complex area of investigation, and CD40 remains the

essential gatekeeper eliciting T-cell help and ultimately determining the quality of the antibody response. Looking ahead, challenges remain in understanding the molecular basis of APRIL/BAFF signaling by their respective receptors expressed at distinct stages of B-cell development. These studies need to address the microenvironmental influences (primary/secondary lymphoid tissues and mucosa) that combine to regulate the impact of BAFF/APRIL and CD40 signaling. Such studies will require new approaches to understand combinatorial signaling on scarce B-cell subsets such as GC B cells and plasma cells. An evolution of our understanding of these processes at the molecular level will provide new insights into the use of biologics and small molecules to manipulate signaling by TNFRSF members in the context of autoimmunity and cancer.

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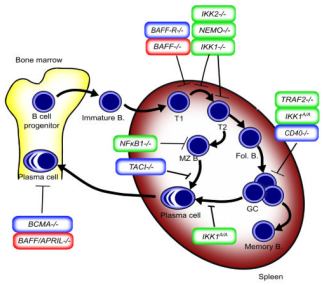

Fig. 1. Role of signaling mediated by different members of the TNF receptor family during B-cell maturation and differentiation

B cells develop in the bone marrow and migrate to the periphery. After stimulation, mature B cells differentiate into germinal center B cells, memory B cells, and plasma cells. Some plasma cells re-enter the bone marrow where they find the microenvironment supporting their longterm survival. The figure shows stages of B-cell maturation and differentiation affected by the mutation or loss of different TNF receptors (in blue rectangles) their ligands (in red rectangles) or members of the NFκB pathway (in green rectangles).

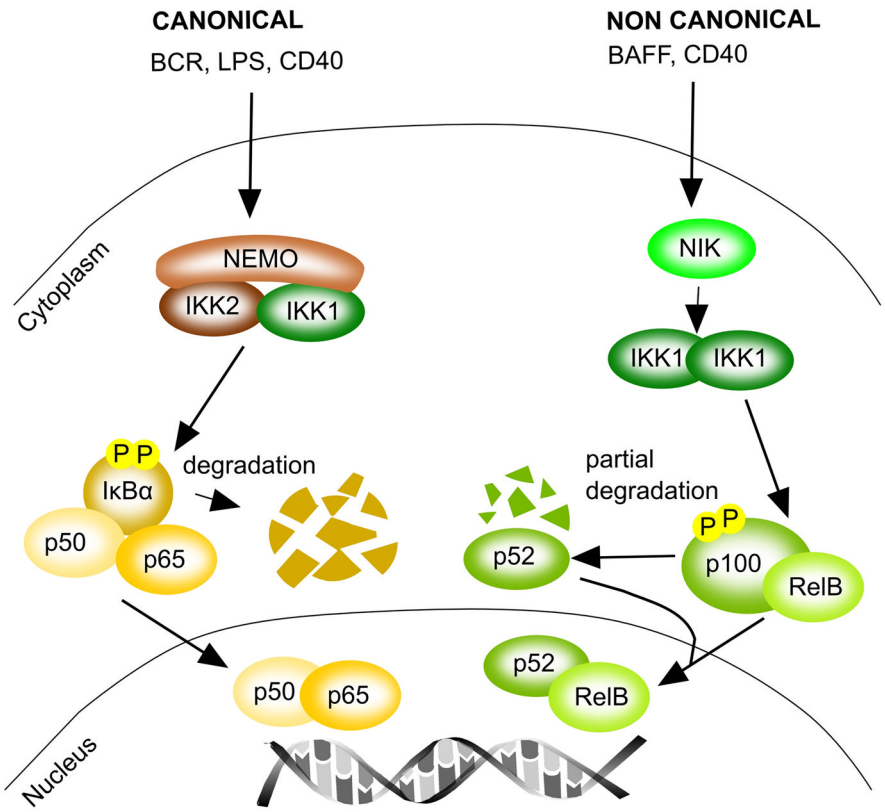

Fig. 2. Model depicting the canonical and non-canonical NFκB pathway

Activation of the IKK complex in the canonical pathway leads to the phosphorylation and ubiqutination of IκB, which is subsequently cleaved in the proteasome. The freed NFκB dimers are now able to enter the nucleus. NIK mediated IKK1 phosphorylation leads to the activation of the non-canonical pathway which is characterized by the cleavage of p100 and release of the NFκB heterodimer p52/RelB.

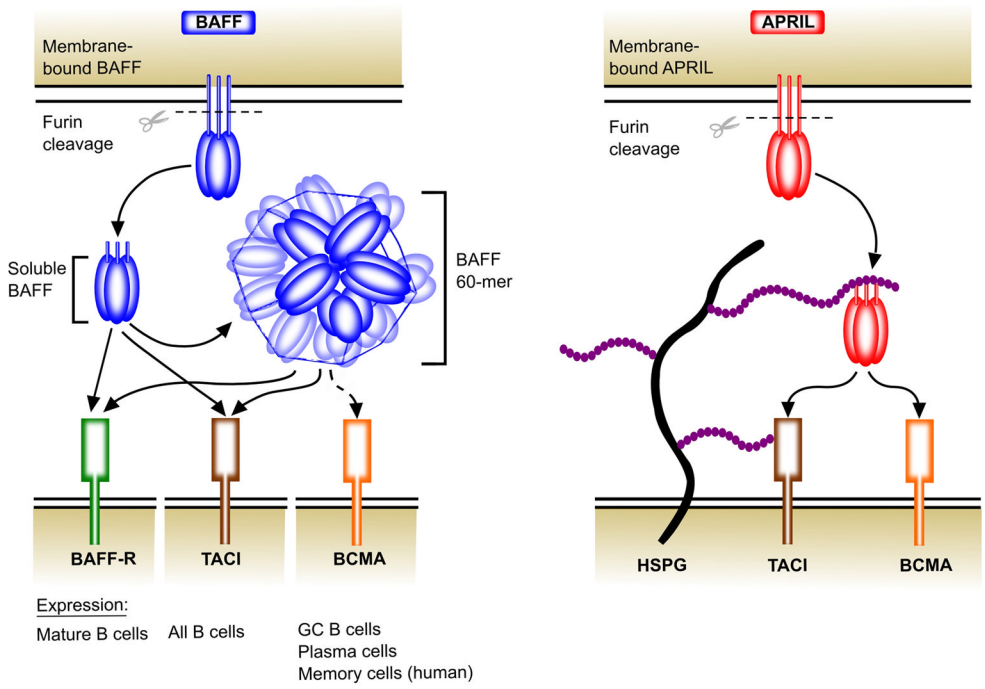

Fig. 3. BAFF, APRIL, and their receptors

Schematic of the binding of the various forms of B-cell activating factor belonging to the TNF family (BAFF) and APRIL (a proliferation-inducing ligand), to their receptors, BAFF receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen (BCMA). (A) BAFF is synthesized as a membrane-bound protein that can be released as a soluble cytokine via proteolytic cleavage by furin proteases. Soluble BAFF exists in two forms: as a homotrimer or as an assembly of 20 trimers that form a 60-mer. The BAFF trimer can bind BAFF-R, APRIL, and BCMA while oligomeric 60-mer BAFF can bind to BAFF-R, TACI, and also with low affinity to BCMA. (B) APRIL is also synthesized as a membrane-bound protein that can be released as a soluble cytokine by furin protease cleavage and forms soluble trimers. APRIL binds to sulphated side chains of heparan sulphate proteoglycan (HSPG) at a site distinct from the site it uses to bind to TACI and BCMA.

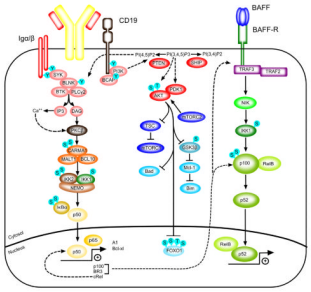

Fig. 4. Signaling and crosstalk between the BCR and BAFF-R

Signaling via the BCR and BAFF-R are both critical for B-cell maturity and homeostasis. Phosphorylation of tyrosine residues in the immunoreceoptor tyrosine activation motifs (ITAMs) of the cytoplasmic tails of BCR-associated Igα and Igβ chains initiates BCR-proximal signaling and the formation of an active signalosome. Phosphorylation of ITAM tyrosines by Src family kinases leads to the recruitment and activation of the kinase Syk and the Tec family kinase BTK, as well as phosphorylation and recruitment of the adaptor BLNK and the lipase PLCγ2. The assembly of an active BCR-proximal signaling complex ultimately leads to activation of MAPKs (not pictured) and canonical NFκB signaling via PKCβ and the CARMA1-MALT1-BCL10 complex which functions to activate IKK1/IKK2. Activation of the IKK1-IKK2-NEMO complex results in phosphorylation of IκBα, marking it for ubiquitination and proteosomal degradation. Activation of canonical NFκB signaling by the BCR leads to induction of transcription of p100, BAFF-R, and cRel (among others), which are critical for BAFF-R signaling. The CD19 coreceptor molecule promotes activation of the PI3K pathway leading to activation of AKT. AKT activation promotes B-cell survival and metabolism through the inhibition of FOXO1, upregulation of MCL-1 and inhibition of BIM, as well as activation of the mTORC1 complex. BAFF-R signaling also activates PI3K (via an unknown mechanism), as well as non-canonical NFκB signaling. Following BAFF engagement of the BAFF-R, TRAF2 and TRAF3 are recruited leading to the release of NIK, which phosphorylates IKK1 leading to p100 processing to p52 and activation of non-canonical NFκB.

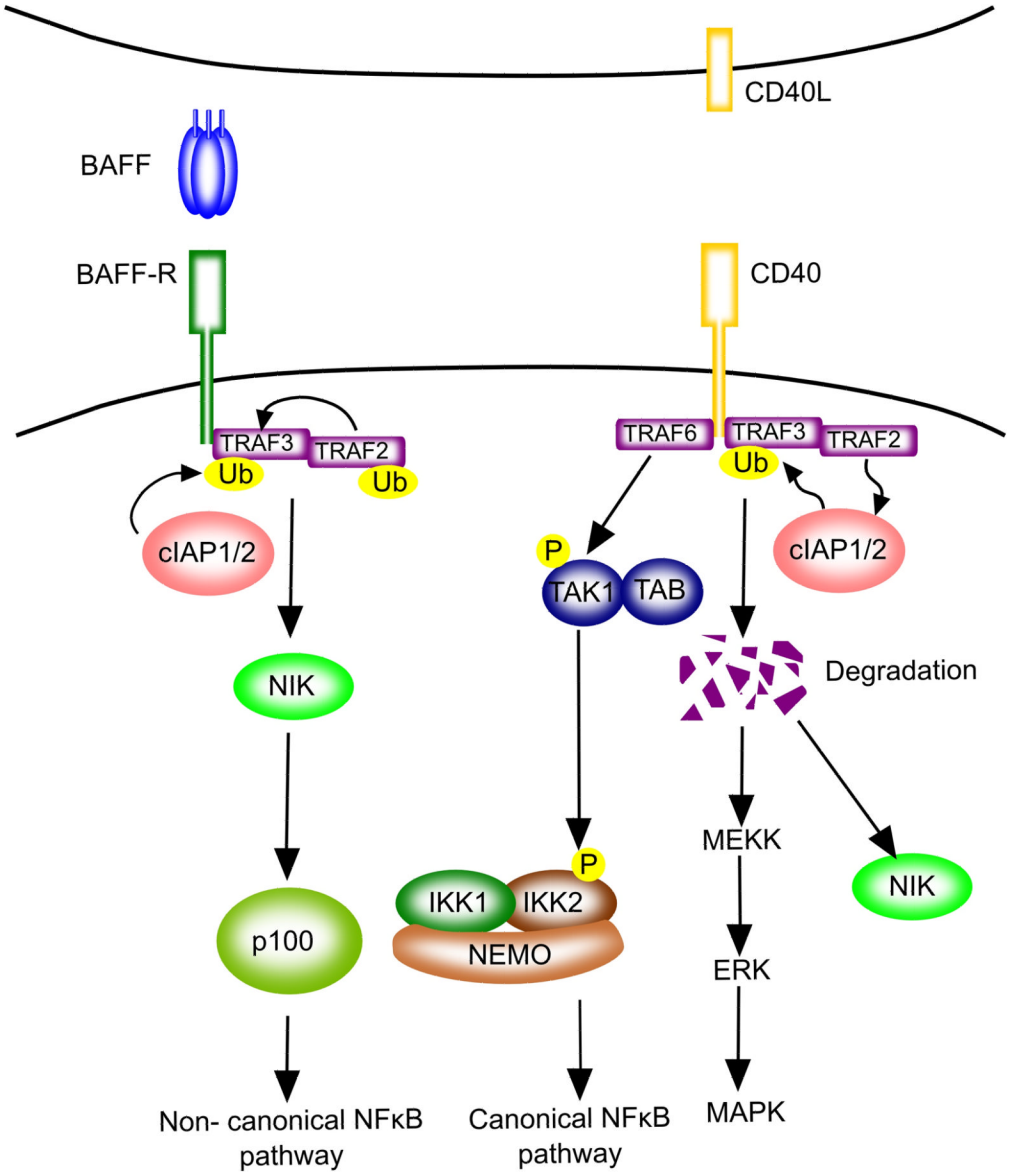

Fig. 5. Signaling differences and similarities between CD40 and BAFF-R

Both BAFF-R and CD40 signaling utilize TRAF molecules to promote activation of NFκB. In the case of BAFF-R, direct binding of TRAF3 initiates a cascade resulting in release of NIK and downstream activation of non-canonical NFκB. In this regard, recruitment of a complex containing TRAF3- TRAF2-cIAP1/2-NIK leads to TRAF3 degradation via TRAF2-activated cIAP1/2. Alternatively, NIK can be released from the TRAF3-TRAF2-cIAP1/2-NIK complex via displacement of cAP1/2 and NIK from TRAF2 and TRAF3 which are then degraded. NIK then functions to activate IKK2 resulting in non-canonical NFκB signaling. Upon CD40L engagement of CD40, TRAF2 activation of cIAP can not only lead to activation of NIK but also of MEKK, promoting downstream MAPK signaling. In addition, TRAF6 recruitment results in the activation of the TAB1-TAK complex, which is also responsible for activation of the canonical NFκB pathway.