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Signalling crosstalk in B cells: managing worth and need

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Abstract

The B cell receptor (BCR) and the receptor for B cell-activating factor (BAFFR) have complementary roles in B cells: BCR signals provide a cell-intrinsic measure of suitability for negative or positive selection, whereas BAFFR responds to homeostatic demands based on a cell-extrinsic measure of the size of the mature B cell pool. Because continuous signals from both receptors are required for B cell survival, it is probable that there are mechanisms to integrate the selective and homeostatic signals from these receptors. In this Opinion article, I describe recent evidence to indicate that crosstalk between the downstream biochemical pathways of these receptors mediates this interdependence, such that BCR signals generate a limiting substrate for BAFFR signal propagation.

Maintaining the primary lymphocyte pools is a challenge for the immune system: it must balance the need to minimize the risk of developing a lymphocyte repertoire that is autoreactive or of ineffective specificity with the need to preserve sufficient lymphocyte numbers for adequate immunological surveillance. In B cells, these conflicting demands are coordinated by a mutually dependent relationship between the B cell receptor (BCR) and the receptor for B cell-activating factor (BAFFR; also known as BR3 or TNFRSF13C).

BCR-mediated selection controls B cell survival based on intrinsic clonal fitness, such that excessively strong or weak BCR signals identify autoreactive or functionally unfit B cell clonotypes, respectively. Negative selection against autoreactive BCR specificities begins at the immature B cell differentiation stage in the bone marrow and continues during the transitional B cell stages in the periphery (FIG. 1a). In general, the elimination (or negative selection) of B cells at the immature stage in the bone marrow occurs following high avidity BCR interactions with antigen, whereas B cells that undergo sustained BCR–antigen interactions of intermediate avidity persist to the transitional stages in the periphery, but die before they mature^{1–3}. The occurrence of positive selection among B cells has only been recognized more recently, but it is thought to require persistent sub-threshold BCR signalling in both developing and mature B cells^{4–7}. Indeed, B cell differentiation requires BCR signalling from the late pro-B cell stage onwards⁵, and mature B cells require continuous signals (known as tonic signals) through the BCR to survive⁶. Cells that survive negative selection and meet the BCR signalling requirements for positive selection can mature and persist as primary B cell subsets.

Together, these observations led to the idea that BCR signal strength is the sole determinant of B cell survival at the immature and transitional stage checkpoints, as well as for the mature follicular and marginal zone B cells that comprise the pre-immune primary B cell repertoire. However, it is now clear that signals through BAFFR have an equally important role in determining the fate of developing and mature B cells (FIG. 1b). In contrast to the cell-intrinsic measure of B cell fitness provided by BCR signals, BAFFR-mediated survival is linked to the cell-extrinsic parameter of primary B cell population size, such that the amount of available BAFF (also known as BLyS or TNFSF13B) is a measure of unfilled ‘space’ in the B cell

compartment. This is shown by the observations that steady-state numbers of quiescent primary B cells are increased when BAFF levels are increased^{8,9} and, conversely, that B cell numbers are decreased when BAFFR signals are compromised¹⁰ or when BAFF is neutralized *in vivo*^{9,11,12}.

BAFFR is first expressed by B cells during the transitional stages and its expression level increases with further differentiation, such that the highest levels of expression are found in follicular and marginal zone B cells^{13–15}. In keeping with this expression pattern, *in vivo* labelling studies^{10,15,16} showed that BAFF-mediated signals through BAFFR control steady-state B cell numbers by regulating both the proportion of transitional B cells that complete their differentiation and the lifespan of mature primary B cells^{10,15,16}. Whether a B cell can acquire BAFF and transduce BAFFR signals effectively determines the probability of its survival. So, the set point for the size of the primary B cell pool is reached when available BAFF levels become limiting and B cells must compete with one another for BAFF. This competitive relationship was initially revealed through studies using mixed chimeras, in which B cells derived from BAFFR-deficient haematopoietic stem cells had an intrinsic disadvantage in terms of maturation and survival when in competition with BAFFR-sufficient B cells¹⁰. Interestingly, these observations were reminiscent of prior studies showing that BCR specificity and signal strength influence the competitive fitness of B cells^{17–19}, which foreshadowed a connection between the BCR and BAFFR in determining the fate of emerging and mature B cells.

BAFFR and BCR signals are integrated

Importantly, B cells that fail to signal through either the BCR or BAFFR die, indicating that both receptors are necessary but neither is sufficient for B cell survival. For example, conditional deletion of the BCR or its immediate downstream signalling components leads to the death of most B cells^{6,7,20} despite normal levels of BAFF and BAFFR expression. Similarly, mice with mutations in the gene encoding BAFFR²¹ or a deficiency of BAFF, or mice treated with BAFF antagonists, have a marked decrease in B cell number, despite a normal BCR repertoire. These observations are at odds with the notion of a linear pathway between BCR signal strength and the induction of downstream B cell survival mediators. Instead, they indicate that there is a more complex interaction between BCR signal strength, the level of available BAFF and the extent of interclonal competition based on B cell population size. This tripartite relationship was revealed by studies showing that autoreactive B cell clones that usually die at the transitional stage could be rescued by high levels of BAFF^{22–24}. Furthermore, with increased BCR signal strength, the effectiveness of such rescue by excess BAFF decreased²³. So, the range of BCR signal strength that is compatible with B cell survival at the transitional stage checkpoint varies depending on the level of available BAFF (FIG. 1b). B cells that undergo deletion (negative selection) at the immature bone marrow stage, which do not yet express BAFFR, cannot be rescued by excess BAFF²³.

These features of B cell physiology provide a model to study the molecular mechanisms that determine cell fate decisions in response to potentially conflicting demands: the need for appropriate clonotypic fitness, measured in terms of BCR signal strength, and the need for a sufficiently large B cell population, linked to the availability of BAFF. It is possible that there is a direct regulatory relationship between the BCR and BAFFR, but although BCR signals can modulate BAFFR expression levels²⁵, they might not be required for BAFFR expression *per se*. Alternatively, the survival signals directly mediated by each receptor might be additive, but this mechanism does not explain how excess BAFF can rescue B cells that would otherwise be deleted on the basis of high BCR signal strength or why signalling through one receptor cannot overcome blocks in the other receptor signalling pathway.

Recent findings¹³ indicate that crosstalk between the downstream mediators of BCR and BAFFR signals can explain the mutual dependence and cross-regulation of these receptors, as well as the increasing resistance of B cells to BCR-mediated death as the cells mature. In this model, the signals from one receptor (the BCR) generate a rate-limiting substrate that is required for transcriptional regulatory systems that are activated by the other receptor (BAFFR). Such crosstalk might be a general model for how cell fate is determined by balancing intrinsic measures of functional fitness against extrinsic measures of systemic integrity.

BCR signals sustain a BAFFR substrate

The nuclear factor- κ B (NF- κ B) system of transcriptional regulatory proteins is crucial for many downstream events that follow BCR ligation²⁶. This family of transcription factors can be activated by two separate but overlapping pathways²⁷, each of which can generate both negative and positive transcriptional regulators. The canonical (or classical) pathway involves the phosphorylation and degradation of the inhibitor of NF- κ B α (I κ B α) and the subsequent pairing of NF- κ B1 (also known as p50) with one of several partners to form transcriptionally active heterodimers that then translocate to the nucleus to mediate gene transcription. The non-canonical (or alternative) pathway instead involves NF- κ B-inducing kinase (NIK)-initiated processing of p100 to generate NF- κ B2 (also known as p52), leading to the formation and nuclear translocation of transcriptionally active NF- κ B2-RELB heterodimers^{27–29}. Both the canonical and non-canonical NF- κ B pathways are necessary for B cell survival; mice that lack various components of either pathway have similar phenotypes to mice with BCR or BAFFR signalling mutations. For example, deficiencies of components of the canonical NF- κ B1 pathway lead to failures of primary B cell differentiation²⁰. Indeed, BCL-10 (B cell lymphoma 10) deficiency — which prevents NF- κ B1 activation by BCR signalling — results in a marked decrease in the number of mature follicular and marginal zone B cells³⁰ despite normal bone marrow and transitional B cell compartments^{30,31}, a phenotype that is similar to that observed for mice with a mutation in the gene encoding BAFFR^{10,16}. Mutations or deficiencies of components of the non-canonical NF- κ B2 pathway also result in phenotypes that are reminiscent of BAFF and/or BAFFR deficiency^{32,33}. Together, these findings indicate that, analogous to studies directly investigating the BCR or BAFFR, a combination of both NF- κ B pathways is necessary for the full function of either receptor^{33–43}.

Because of their central roles in B cell biology, the signalling cascades downstream of both the BCR and BAFFR have been investigated in great detail. BCR signals are initiated by SRC family kinases, which phosphorylate BCR-associated immunoreceptor tyrosine-based activation motifs (ITAMs)^{44,45}. Subsequent phosphorylation of SYK (spleen tyrosine kinase) initiates signal propagation through multiple pathways, including activation of the phosphoinositide 3-kinase (PI3K) pathway and initiation of the canonical NF- κ B1 pathway. In contrast to the ITAM-initiated signalling of the BCR, the proximal events in BAFFR signalling — as for most tumour necrosis factor (TNF) family members — involve TNF receptor-associated factors (TRAFs). Activated BAFFR interacts with TRAF3⁴⁶, preventing TRAF2–TRAF3 interactions that would otherwise lead to NIK degradation⁴⁷, and thereby allows p100 processing³⁹. Additional analyses in which various downstream components of the BAFFR signalling pathway were genetically removed or replaced^{41–43} showed that although BAFFR ligation mainly results in activation of the non-canonical NF- κ B2 pathway, the canonical NF- κ B1 pathway can also influence BAFFR signalling and B cell survival^{39, 43,48}.

It now seems that molecules generated by BCR signals are directly involved in the BAFFR signalling cascade, thereby mediating the interdependence and cross-modulation of these two receptor signalling pathways (FIG. 2). BAFFR signalling mainly activates the non-canonical NF- κ B2 pathway^{33,49}, and in the absence of other inputs, BAFFR signalling rapidly depletes

the amount of available p100 substrate in this pathway¹³. By contrast, although BCR signalling does not itself lead to activation of the non-canonical NF- κ B2 pathway, p100 is a positively regulated downstream target of BCR-induced gene transcription^{48,50}. So, BCR signals generate p100 but do not promote processing to NF- κ B2, whereas BAFFR signals result in the processing of p100 but quickly exhaust p100 stores. Accordingly, signalling through both the BCR and BAFFR is required for the sustained production and nuclear translocation of NF- κ B2–RELB heterodimers and the subsequent expression of anti-apoptotic genes to allow continued B cell survival. These findings support a model whereby BCR signalling is itself insufficient to ensure B cell viability but enables the sustained delivery of BAFFR-mediated survival signals by replenishing p100 levels.

This model of receptor crosstalk provides a mechanism for several previously unexplained observations. First, it clarifies why continuous signalling through both BAFFR and the BCR is required for transitional and mature B cell survival. Second, it predicts a stoichiometric relationship between three parameters — the level of BAFF, BCR signal strength and cellular BAFFR density — in determining the threshold for transitional B cell selection and persistence in the primary B cell repertoire. This prediction is supported by previous findings showing that the stringency of transitional B cell selection depends on the combination of these three parameters^{22–24}. Finally, the model proposes that acquiring the ability to sustain BCR and BAFFR signalling crosstalk might be a fundamental developmental switch that allows homeostatic demands to modulate the outcome of BCR-mediated cell fate decisions. Indeed, immature B cells undergoing negative selection in the bone marrow cannot be rescued by BAFF, presumably because of their lack of BAFFR expression. At the transitional B cell stages, the levels of BAFFR expression^{13–15} and the ability of BCR signals to sustain p100 production¹³ both increase. This combination of increasing BAFFR signalling capacity and changing BCR signalling properties^{13,50,51} probably underlies the increasing propensity for BAFF-mediated survival and decreasing susceptibility to BCR-driven negative selection that are observed during this developmental stage.

Despite these insights into how selective and homeostatic processes are integrated in B cell development, additional questions remain. First, the extent of the BCR-mediated signalling cascades that mediate BAFFR crosstalk remains unclear. Pharmacological inhibition of the canonical NF- κ B1 pathway blocks p100 generation¹³, which indicates that this part of the BCR signalling pathway has a role in replenishing p100. However, more complex relationships have been reported and will probably be revealed by further investigation. For example, BAFFR signalling can itself result in transient NF- κ B1 activation^{13,39,48}, which brings into question whether the canonical NF- κ B1 pathway by itself is sufficient for sustained p100 generation and indicates that BCR signalling through pathways other than the NF- κ B1 pathway might be involved in the production of p100. Furthermore, constitutively active canonical NF- κ B1 alone can increase B cell numbers in BAFF-deficient mice⁴³. Although this indicates that B cell survival can be extended independently of BAFFR signalling in some circumstances, a BAFF-sufficient background further increased B cell numbers in the presence of constitutively active NF- κ B1, which indicates that the BAFFR and NF- κ B1 signalling pathways are largely non-redundant. In addition, recent findings indicate that the concomitant generation of other NF- κ B components, such as REL (also known as cREL), by BCR signalling could have important roles in determining the outcome of signalling crosstalk⁵⁰. Similarly, additional downstream BCR signalling elements, such as PI3K, could participate in these cross-modulating activities given their known roles in mediating the differentiation of transitional B cells⁵².

In addition to the BCR and BAFFR, the sets of receptors that are involved in such signalling crosstalk might change according to the differentiation stage or type of B cell. Also, some receptors might not require crosstalk for full function, making them particularly potent mediators of B cell survival. For example, CD40 ligation induces both the production and

processing of p100 (REFS^{13,53}), perhaps freeing activated B cells from the need for continued BCR signalling.

A remaining conceptual issue is why both low and high BCR signal strength would lead to B cell death under conditions of limiting BAFFR signals. The stoichiometry and nuclear shuttling of NF- κ B components are complex^{54,55}, which raises the possibility that their ratios — rather than absolute amounts — might be important in determining the end result. For example, if BCR-mediated production of p100 exceeds BAFFR-induced p100 processing, then excess p100 could sequester RELB or block the action of RELA, thereby decreasing the levels of functional NF- κ B heterodimers. Consistent with these general possibilities, mutations in p100 that prevent its processing lead to a super-repressor phenotype in which B cell development is blocked at the transitional stages⁵⁶. Also, increased levels of nuclear p100 are observed after strong BCR signalling¹³.

Concluding remarks

It is tempting to speculate that signalling crosstalk — as described here between the BCR and BAFFR — might be a general mechanism by which the degree of intrinsic functional competence required for cell survival can be modulated by the need to maintain systemic homeostasis. This model proposes that signals induced by a lineage-specific functional molecule (for example, the BCR) will provide limiting components to a survival pathway (for example, the BAFFR pathway), the signalling intensity of which is proportional to ‘space’ in the relevant biological niche. In addition to providing a means to integrate conflicting demands in cell fate decisions, the potential to ‘tune’ levels of acceptable signalling makes this model an attractive concept in lymphocyte biology, albeit with different players in different lymphocyte lineages or subsets. For example, some T cell subsets rely on both the T cell receptor and interleukin-7 or interleukin-15 signals for survival^{57–62}; as the downstream components of these pathways are well characterized, this system would allow the possibility of signalling crosstalk to be investigated further.

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Figure 1. B cell differentiation and selection are controlled by the specificity of the BCR and the availability of BAFF

a. After the expression of a complete B cell receptor (BCR), specificity-based selection of B cells occurs at the immature B cell stage and again after exit from the bone marrow during the transitional B cell stages in peripheral lymphoid tissues. Selective elimination of B cells at the immature stage (negative selection) results from high avidity BCR engagement by the selecting ligand. B cells that undergo sustained, intermediate avidity BCR interactions with antigen persist to the transitional stages but die before they reach the mature stages. Positive selection also occurs, so B cells that fail to reach a minimum BCR signalling threshold undergo apoptosis. B cells in which BCR signalling reaches the minimum threshold necessary for positive

selection but does not reach the high threshold that would result in negative selection survive and enter the mature B cell pool. Expression of the receptor for B cell-activating factor (BAFFR) is first induced at the transitional stages of B cell development and increases as the cells mature. **b.** Signals delivered through BAFFR can modulate the stringency of positive selection of transitional B cells. When the mature B cell pool is full and free BAFF levels are low (left panel), transitional B cells compete for limited free BAFF, so BAFFR occupation on these cells is limited and the range of BCR signal strengths that can promote survival is narrow. When BAFF levels are high (for example, as a result of 'space' in the mature B cell pool) (right panel), the range of BCR signal strengths that can promote B cell survival is broader, because in the presence of excess BAFF, BAFFR signalling on transitional B cells is maximal.

Figure 2. Signalling crosstalk underlies the mutual dependence of the BCR and BAFFR in B cell development and survival

B cell receptor (BCR) signals are initiated by SRC family kinases such as LYN, which phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) on the BCR signalling complex (Igα and Igβ). Phosphorylation of SYK then initiates multiple interacting downstream pathways (for simplicity, not all pathways and components are shown). These include the phosphoinositide 3-kinase (PI3K) pathway, which culminates in the activation of AKT1 and nuclear factor of activated T cells (NFAT), and the activation of Bruton's tyrosine kinase (BTK), which has multiple downstream effects. Among these effects are the induction of phospholipase Cγ2 (PLCγ2) activity, which mediates hydrolysis of

phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) to produce inositol-1,4,5-trisphosphate (InsP₃) and diacylglycerol (DAG), and the activation of protein kinase C β (PKC β), which in turn leads to activation of the canonical nuclear factor- κ B1 (NF- κ B1; also known as p50) pathway through the BCL-10–MALT1–CARMA1 complex. These mediators have multiple downstream effects, including the upregulation of p100 transcription but not p100 processing. By contrast, the B cell-activating factor receptor (BAFFR) preferentially activates the non-canonical NF- κ B2 (also known as p52) pathway by inhibiting interactions between tumour necrosis factor receptor-associated factor 2 (TRAF2) and TRAF3 that otherwise would mediate the degradation of NF- κ B-inducing kinase (NIK). The resulting NIK accumulation leads to increased processing of p100 and the generation of NF- κ B2–RelB heterodimers, which translocate to the nucleus where they upregulate the transcription of anti-apoptotic genes. However, BAFFR signals do not generate additional p100 and they can rapidly exhaust cellular stores of p100. So, only continued signalling through both receptors yields sustained anti-apoptotic gene expression and supports B cell survival. BCL-10, B cell lymphoma 10; BLNK, B cell linker; CARMA1, also known as CARD11 (caspase recruitment domain-containing protein 11); GRB2, growth factor receptor-bound protein 2; IKK, inhibitor of NF- κ B kinase; MALT1, mucosa-associated lymphoid tissue lymphoma translocation gene 1; MCL1, myeloid cell leukaemia sequence 1.