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REVIEW ARTICLE

B-cell antigen-receptor signalling in lymphocyte development

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SUMMARY

Signalling through the B-cell antigen receptor (BCR) is required throughout B-cell development and peripheral maturation. Targeted disruption of BCR components or downstream effectors indicates that specific signalling mechanisms are preferentially required for central B-cell development, peripheral maturation and repertoire selection. Additionally, the avidity and the context in which antigen is encountered determine both cell fate and differentiation in the periphery. Although the signalling and receptor components required at each stage have been largely elucidated, the molecular mechanisms through which specific signalling are evoked at each stage are still obscure. In particular, it is not known how the pre-BCR initiates the signals required for normal development or how immature B cells regulate the signalling pathways that determine cell fate. In this review, we will summarize the recent studies that have defined the molecules required for B-cell development and maturation as well as the theories on how signals may be regulated at each stage.

INTRODUCTION

First identified in 1970,[1] the B-cell antigen receptor (BCR) is a multimeric complex consisting of an antigen-recognition structure and a membrane-bound immunoglobulin (mIg), associated non-covalently with a heterodimer of Igα and Igβ (Fig. 1). Except for immunoglobulin G (IgG), the cytoplasmic tails of the five types of mIg are all extremely short and lack signalling capacity.[2–4] The 28 amino acid cytoplasmic tail of IgG does not have independent signalling capacity, but may serve to enhance peripheral immune responses.[5,6] Signalling through the BCR is mediated by Igα and Igβ. Each mIg associates with a single Igα/Igβ heterodimer, and is, in turn, associated on the cell surface with several other mIg–Igα/Igβ complexes.[7] Igα and Igβ each contain a large disulphide-linked extracellular domain (114 amino acids for murine Igα and 132 amino acids for murine Igβ), a transmembrane region and a cytoplasmic tail. Within each extracellular region are an immunoglobulin domain and a membrane-proximal stalk. The latter contains the cysteines that form the heterodimer-stabilizing disulphide bond. In addition, the extracellular region of Igβ contains a highly conserved N-terminal domain of 17 amino acids, the function of which is unknown. The transmembrane regions of Igα and Igβ are unremarkable, except for a polar patch in Igβ that probably associates with the transmembrane domain of the heavy chain.[2,8] Although interactions in the transmembrane domains are dominant for most isotypes, including immunoglobulin D (IgD), other lower-affinity extracellular interactions may stabilize receptor complexes containing immunoglobulin M (IgM).[9–11] The cytoplasmic tails of Igα and Igβ consist of 61 and 48 amino acids, respectively.[12] Although these domains do not have any predicted secondary structure, they contain specific features that are required for initiating intracellular signalling pathways.

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Abbreviations: BAFF, B-cell activating factor; BCR, B-cell receptor; BLNK, B-cell linker protein; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; mIg, membrane-bound immunoglobulin; MZ, marginal zone; NF-κB, nuclear factor κB; PLCγ2, phospholipase C γ2; RAG, recombinase-activating gene; SFTK, Src family tyrosine kinases; TCR, T-cell receptor.

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INITIATION OF RECEPTOR SIGNALLING

The signalling capacities of both Igα and Igβ are dependent upon a specific motif, found within each cytosolic tail, known as the immunoreceptor tyrosine-based activation motif (ITAM). Described by Reth in 1989,[13,14] the core of this motif (D/E(X)$_7$D/EXX**Y**XXI/L(X)$_7$**Y**XX I/L) comprises two tyrosine residues separated by 11 residues, each followed by leucine or isoleucine at the +3 position. Other receptors involved in

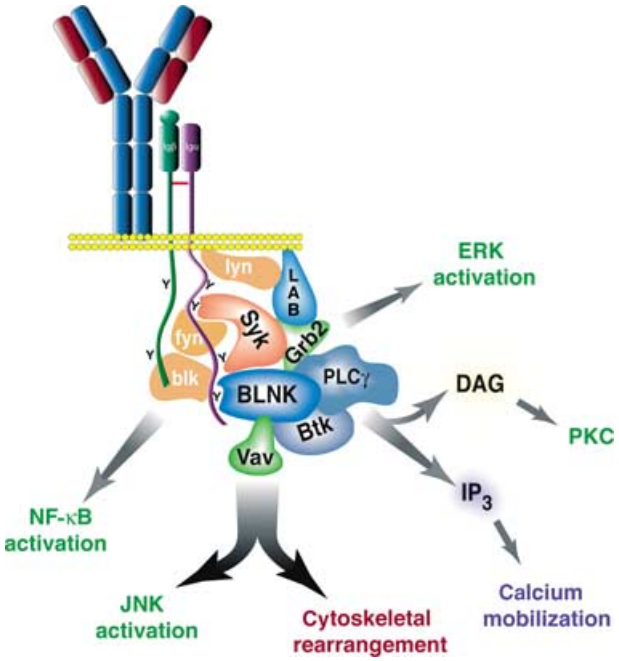

Figure 1. Proximal B-cell receptor-mediated signalling pathways. After binding to antigen, the immunoglobulin (Ig)α and Igβ cytoplasmic tails are phosphorylated on the immunoreceptor tyrosine-based activation motif (ITAM) tyrosines by Src-family tyrosine kinases (SFTKs) and/or Syk. Syk then binds to the Igα ITAM, and the B-cell linker protein (BLNK) binds to tyrosine 204 of Igα. This activates multiple signalling pathways, including: Btk, which activates phospholipase C (PLC)γ2 and leads to calcium flux (blue) and protein kinase C (PKC) activation (green); Grb2, which activates the Ras/Raf/mitogen-activated protein kinase (MEK) extracellular signal-regulated kinase (ERK) pathway (green); and Vav, which activates the Rac/Rho/Cdc42 pathway and results both in cytoskeletal rearrangement (maroon) and c-Jun N-terminal protein kinase (JNK) activation (green). The SFTKs themselves activate nuclear factor-κB (NF-κB) (green).

antigen responses, including the T-cell receptor (TCR) and many Fc receptors, also contain ITAMs.[15,16] Mutational analysis has illustrated that the tyrosines, the 11 amino acid spacer between them[17] and the +3 isoleucine/leucine residues,[17,18] are all required for proper initiation of BCR-mediated signalling pathways.

Significant effort has been spent on determining how receptor aggregation induces phosphorylation of the ITAM tyrosines. The resting BCR is assembled with Src family tyrosine kinases (SFTKs), such as Blk, Lyn and Fyn, which become activated following receptor ligation.[19] The tyrosine kinase Syk can also be detected in the resting receptor complex. The association of these tyrosine kinases with the receptor is mediated by unique tyrosine-independent motifs embedded within the cytosolic tail of Igα.[20–22] These embedded motifs, in part, determine the signalling capacity of each chain and contribute to the preferred role of Igα as the primary activator of tyrosine kinases.[17,23,24] *In vitro* experiments, and reconstitutions in non-immune cells,[25] indicated that Src kinases were the primary mediator of ITAM phosphorylation. However, recent studies have questioned this model. Stimulation of SFTK-deficient *Drosophila* cells reconstituted with Igα, Igβ and the non-Src family tyrosine kinase Syk (see below) resulted in tyrosine phosphorylation of Igα and Igβ,[26] indicating that Syk may be a primary kinase. This conclusion is supported by studies of pro-B cells from mice lacking the Src-family kinases Lyn, Fyn and Blk, in which receptor cross-linking induces robust phosphorylation of Igα and Igβ.[27]

Tyrosine phosphorylation of the ITAM tyrosines enhances the recruitment and activation of Syk, which is the principal kinase that drives many signalling pathways, including the activation of phospholipase C γ2 (PLCγ2) and Ras. Syk is required for normal B-cell development. However, not all signalling pathways are dependent upon Syk, as the activation of nuclear factor κB (NF-κB) appears to be directly dependent on the activation of one or more Src-family kinases.[27] These data indicate that the BCR independently activates both Syk and the Src-family kinases to initiate complementary downstream signalling pathways.

Coupling of receptor-associated kinases to downstream pathways is affected through a series of linker molecules, the most important of which is the B-cell adapter molecule BLNK (also referred to as SLP-65 or BASH). BLNK is a B-lymphocyte-specific 65 000 molecular weight analogue of the T-cell linker molecule SLP-76; it lacks intrinsic enzymatic activity yet contains several functional domains, including an SH2 domain, proline-rich domains and several potential tyrosine-phosphorylation sites. Recent evidence indicates that the SH2 domain of BLNK binds directly to a single, unique non-ITAM-phosphorylated tyrosine in the cytosolic tail of Igα.[28,29] BLNK recruitment is required *in vitro* for coupling Igα to distal pathways, such as PLCγ2,[28] and is important *in vivo* for pre-B-cell development, Dμ counterselection, tumour suppression, and peripheral selection[30] (L. D. Wang, manuscript in preparation). Phosphorylation of the non-ITAM tyrosine and the recruitment of BLNK occur contemporaneously with phosphorylation of the ITAM tyrosines and the enhanced recruitment of Syk.[28] Therefore, it is probable that BLNK recruitment to Igα is important in initiating signal activation.

BLNK interacts directly with PLCγ2 as well as with Btk,[31–33] a tyrosine kinase required for normal PLCγ2 activation. It is probable that BLNK co-ordinates the approximation of both Syk and Btk with PLCγ2 to permit rapid and efficient activation.[34] BLNK also recruits the Rho-family GTPase, Vav, and the linker protein, Nck,[35–40] both of which are important in cytoskeleton remodelling. Recruitment of these molecules to the BCR brings them into close proximity with receptor-associated kinases necessary for their activation. BLNK is also constitutively associated with the linker molecule Grb2, coupling the BCR to the Ras pathway,[33,38] as well as the negative regulatory co-receptor CD72 and the PTPase SHP-1.[41] These results indicate that sequential protein–protein interactions nucleate a signalsome at the antigen receptor.

BLNK, like SLP-76, has been shown to interact indirectly with a lipid-raft associated linker, the linker of activated B cells (LAB), which may serve to recruit activated substrates into lipid rafts and augment distal signalling pathways.[42] However, as with the TCR, it is unlikely that localization of substrates within lipid rafts is required for signal initiation in peripheral B cells.

For example, the ligated receptor localizes to lipid rafts irrespective of receptor kinase activation (M. R. Clark, unpublished observation).[43] However, as discussed below, lipid rafts may have an important function in initiating the pre-B-cell signals required for B-cell development, and may have additional roles in propagating signals.

Assembly of the signalsome on the cytosolic tail of Igα is dependent upon the phosphorylation status of the ITAM and non-ITAM tyrosines. From studies of receptor complexes containing just Igα or Igβ, it is apparent that Igβ can function as an amplifer of total Igα phosphorylation, serving to increase receptor sensitivity and lower receptor threshold.[44] A similar function has been ascribed to the β chain of the Fcε receptor.[45] Conversely, the C-terminal tyrosine in the Igα ITAM is negatively regulated by serine/threonine phosphorylation.[46] As observed in growth factor receptor systems, serine/threonine kinases may provide feedback mechanisms to extinguish receptor signals and raise receptor threshold.

Signalling through the B-cell receptor is an intricate and complex process that has been predominantly studied in mature or activated B-cell lines. However, it is clear that BCR signalling is responsible for a wide variety of physiologically distinct responses through the course of B-cell development, implying that discrete signalling pathway subsets are developmentally co-ordinated as cells mature. Although the regulatory mechanisms that allow this co-ordination to occur are just starting to be elucidated, many of the developmental end-points are well characterized.

B-CELL DEVELOPMENT

Pro-B cells

Pro-B cells represent the first irrevocably committed B-cell precursors,[47,48] which can be distinguished from pre-pro-B cells by surface expression of CD19 and increased expression of HSA[49] (Fig. 2). In these cells, the Igα–Igβ heterodimer is expressed on the cell surface in association with calnexin and perhaps other chaperone molecules.[50] Rearrangement of the BCR heavy-chain locus (see below) is initiated during the pro-B-cell stage. However, the pre-BCR is not required for lineage commitment and the initiation of recombination. Rather, this is dependent upon the intrinsic expression of the E2A family transcription factors E12 and E47,[51] and the transcription factor EBF,[52] which have been shown to up-regulate expression of the B-cell-specific genes λ5, VpreB, Igα and Igβ, as well as the lymphoid-specific recombinase-activating genes RAG-1 and RAG-2, and the B-cell-specific transcription factor Pax5, or BSAP.[53–55] Lineage commitment is enforced at the pro-B-cell stage by Pax5, which both activates B-cell-specific genes (including BLNK, CD19 and Igα) and represses the expression of non-B-lineage genes (including Notch1).[47,56,57]

Early B-cell development is not entirely intrinsic, as signalling through the interleukin-7 receptor (IL-7R) is required to generate $CD19^+$ $CD43^+$ $B220^+$ pro-B cells.[58] Interleukin-7 (IL-7) signalling also induces pro-B cells to proliferate and expand, and has been shown to up-regulate expression of CD19 and Pax5.[59,60]

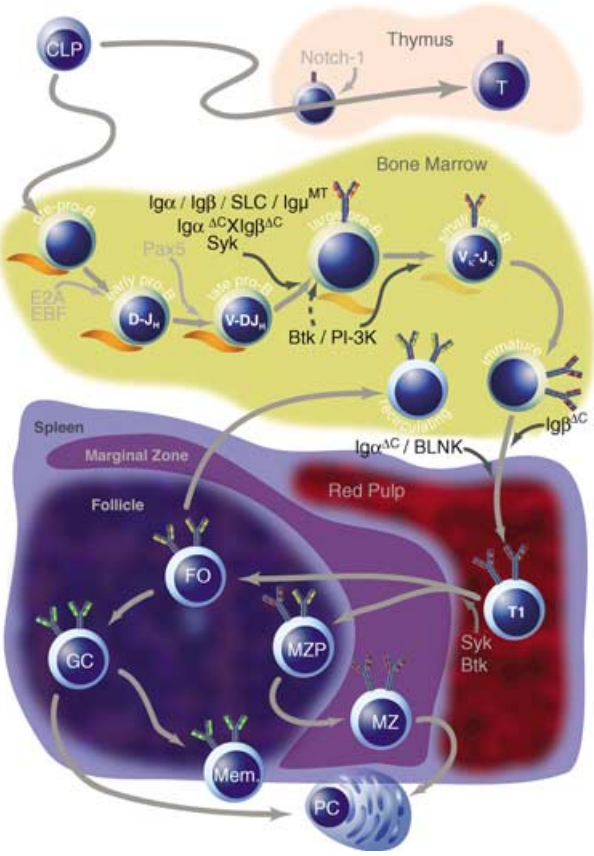

Figure 2. Overview of B-cell development in postnatal mammalian life. Common lymphoid progenitors (CLPs) can migrate to the thymus where they become T cells in response to signalling through Notch-1, or they can remain in the bone marrow where they enter the B lineage as pre-pro-B cells. Possibly in response to Notch-1 down-regulation by Pax5, transcription factors EBF and E2A are up-regulated to initiate a B-cell-specific programme of gene transcription. D_H–J_H rearrangements occur at the early pro-B stage, and subsequent V–DJ rearrangements commence at the late pro-B stage. These stages require direct contact with bone marrow stromal cells, depicted in orange. Signalling molecules, whose absence results in a developmental block, are listed in black; dashed arrows indicate incomplete impairment. Appropriate signalling through the pre-B-cell receptor (BCR) (the surrogate light chain is depicted in orange) mediates heavy-chain allelic exclusion and induces several rounds of proliferation. Subsequently, at the small pre-B stage, light-chain rearrangements begin. Successfully rearranged heavy and light chains appear on the cell surface at the immature B-cell stage, after which B cells emigrate to the periphery. Newly immigrated transitional immature B cells (T1) probably undergo further differentiation into T2 and possibly T3 transitional immature B cells, although this is controversial. Eventually, transitional cells enter splenic follicles, where they differentiate further along the marginal zone (MZP, MZ) or follicular (FO) pathways, probably in response to the strength of signals received through the BCR. Follicular cells differentiate either into plasma cells (PCs) or germinal centre B cells (GC) in response to primary antigen challenge, whereupon they undergo somatic hypermutation, affinity maturation and class switch before they differentiate into memory cells. Marginal zone cells, which leave the splenic follicle to reside in the marginal zone, are also capable of differentiating into plasma cells in response to primary antigen exposure.

The development of pre-B cells

Surface expression of a signalling-competent pre-BCR, containing an in-frame V–DJ rearrangement of the heavy chain, allows progression from the pro-B-cell to pre-B-cell stage; this is the earliest stage at which BCR signalling is required. Targeted gene mutations that eliminate surface expression of the pre-BCR result in developmental arrest at the pro- to pre-B-cell checkpoint. These include mutations in pre-BCR components, such as membrane-anchored heavy chain (μMT), λ5, VpreB, Igα and Igβ.[61–64] Appropriate pre-BCR signalling results in allelic exclusion at the heavy-chain locus[65,66] and also leads to changes in the phenotype of developing B cells (Fig. 2); cells become larger as they undergo a proliferative burst of two to five cycles[67] and become more IL-7 responsive.[68] After proliferation, cells enter the small pre-B stage, where they down-regulate HSA, CD43 and IL-7R, becoming IL-7 unresponsive. They then begin the process of light chain rearrangement, first at the kappa locus and then at the lambda locus.[69,70]

Although it is clear that a signal must be transduced through the pre-BCR in order for development to progress, the mechanisms responsible for initiating this signal are unclear. It has been proposed that simple assembly of the resting BCR complex is sufficient to mediate development. This model is supported by studies in which retroviral expression of a construct encoding the myristylation domain of Lck and the cytoplasmic domains of Igα and Igβ in $RAG^{-/-}$ pro-B cells is able to rescue progression past the pre-B stage.[71] This model is also consistent with studies in which mice bearing a truncated μ chain that cannot associate with surrogate light chains, or a μ chain in which a majority of the extracellular region has been replaced with an irrelevant protein (CD8), are still competent to mediate later stages of B-cell development.[72,73] In apparent contrast to these findings, a recent study suggests that a surrogate light chain is necessary for pre-BCR aggregation and signal initiation.[74] Therefore, the mechanisms by which the pre-BCR mediates B-cell development are still unclear.

Even if surface expression is all that is required, it is probable that some aggregation of the receptors occurs at the cell surface. This could occur spontaneously, as has been hypothesized in the mature BCR,[7] or through localization in cholesterol- and sphingolipid-rich lipid microdomains. In human pre-B-cell lines, 20–30% of resting pre-B-cell receptors accumulate in lipid rafts, whereas in mature B-cell lines, the BCR is excluded from the lipid rafts.[75] Lipid rafts create microenvironments of skewed signalling molecule composition that may promote or prevent the activation of various proximal cascades.[43]

Some studies argue that pre-B-cell development is mediated by a selecting ligand in the bone marrow that aggregates the pre-B-cell receptor. Ligation of the Igα–Igβ heterodimer on pro-B cells using antibodies to the extracellular domain of Igβ induces cells to mature to the pre-B stage.[50] Potential stromal ligands for the BCR,[76] including galectin, have been postulated as a potential ligand for the human pre-B-cell receptor.[77] However, the contribution of these ligands to selecting B-cell progenitors expressing competent pre-BCRs is unclear.

Beyond demonstrating a global requirement for the pre-BCR, several *in vivo* genetic studies over the last few years have revealed a requirement for specific functional domains of the pre-BCR in the development of pre-B cells. Deletion of the cytosolic tails of both Igα and Igβ ($Igα^{Δc/Δc}$, $Igβ^{Δc/Δc}$) completely prevents the development of pre-B cells, despite normal surface expression of the pre-BCR.[78] Mice bearing the same truncation in Igβ and a specific mutation in the ITAM tyrosines of Igα ($Igα^{FF/FF}$, $Igβ^{Δc/Δc}$) have an identical developmental deficit, indicating that the non-ITAM portions of Igα are insufficient to mediate development.[79] Interestingly, truncation of the tail of Igβ alone ($Igβ^{Δc/Δc}$) does not affect the development of pre-B cells,[78] while deletion of the cytoplasmic tail of Igα alone ($Igα^{Δc/Δc}$) results in an incomplete block.[80] These results suggest that, during early development, Igβ is redundant to Igα, but the reverse is not true. Surprisingly, the ITAM of Igα is not required, as mice expressing $Igα^{FF/FF}$ have no early defect. The non-ITAM tyrosines may be important in early development, as mutations in molecules that are recruited to this motif, such as BLNK[81,82] and Btk,[83] result in a developmental block similar to that of $Igα^{Δc/Δc}$. The role of the Igα non-ITAM tyrosines has not been directly addressed *in vivo*.

Deletion of Syk[84] also results in a partial block in pre-B-cell development, although the severity of this block is somewhat ameliorated by expression of ZAP-70.[85] Likewise, the deletion of individual Src-family kinases has no significant impact on pre-B-cell development, while depletion of the three principal Src-family kinases expressed in B cells (Lyn, Blk and Fyn) results in a complete block in pre-B-cell development.[27] These data indicate that the pathways requisite for B-cell development utilize both Syk and the Src-family of tyrosine kinases.

Analysis of BLNK-deficient mice suggests that the development of pre-B cells may be mediated by the co-ordination of two or more proximal signalling pathways, each with a distinct function. Deletion of BLNK, like the deletion of other signalling molecules in the pre-BCR pathway, leads to a significant block in pre-B-cell development.[81,82] However, not all aspects of B-cell development are equally affected. Deletion of BLNK inhibits the down-regulation of RAG, λ5, IL-7R and CD25 that normally occurs when pre-B cells mature.[30] The pre-B-cell proliferative burst is also inhibited. Conversely, ectopic expression of BLNK in $Pax5^{-/-}$ $μ^{+}$ cells, which are deficient in BLNK and arrest at a pre-B-like stage, rescues some aspects of pre-B-cell receptor signalling and initiates a proliferative and developmental programme.[57] *In vitro*, pre-B cells from $BLNK^{-/-}$ mice proliferate robustly in response to IL-7, indicating that the defect is proliferation is specific to the BCR.[75] In contrast, heavy-chain allelic exclusion in $BLNK^{-/-}$ mice is intact.[30] These findings imply that both BLNK-dependent and BLNK-independent pathways contribute to pre-B-cell development.

Dμ expression and signalling through Dμ

The pre-B-cell receptor is assembled with a V–DJ rearranged heavy chain. If DJ_H rearrangement occurs in reading frame 2, a protein termed Dμ can be translated.[86] Signalling through Dμ leads to cellular deletion[87] in a Syk- and BLNK-dependent manner.[30,84] Because Dμ and pre-BCR signalling effect contrasting outcomes, it seems reasonable to conclude that they

initiate qualitatively different signalling cascades. Indeed, examination of the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway has demonstrated that activated Raf partially rescues development in $RAG^{-/-}$ mice,[88] suggesting that Dμ activates a subset of Ras-dependent signalling pathways.

THE DEVELOPMENT OF IMMATURE B CELLS

Upon light-chain rearrangement, heavy and light chain are co-expressed on the cell surface, in association with Igα and Igβ, to form an antigen-specific surface receptor (Fig. 2). These IgM^+ IgD^- immature B cells undergo receptor-mediated negative selection, a process whereby autoreactive B-cell receptors are culled from the immune repertoire. Very few (≈20%) immature B cells survive this process; those that are not negatively selected leave the bone marrow and emigrate initially to the splenic red pulp, where they are known as transitional B cells.

There are three known mechanisms of negative selection: deletion; anergy; and receptor editing (Fig. 3). Deletion in response to high-avidity ligands has been demonstrated *in vivo* in a number of BCR transgenic systems.[89–91] Isolated immature B cells are also deleted *in vitro* when cultured with anti-BCR antibodies.[92–94] In contrast, immature antigen-specific B cells, which encounter lower-avidity ligands, enter a state of anergy in which they are unresponsive to further antigen stimulation.[95,96] These cells down-regulate surface IgM expression, are short-lived, and exhibit a characteristic signalling signature[97,98] and pattern of gene expression.[99] Self-reactive immature B cells can also induce secondary immunoglobulin gene rearrangements[100–103] to replace their autoreactive receptors. Once this process of receptor editing is initiated, it presumably continues until a non-autoreactive receptor has been generated or, failing that, the cell is deleted.

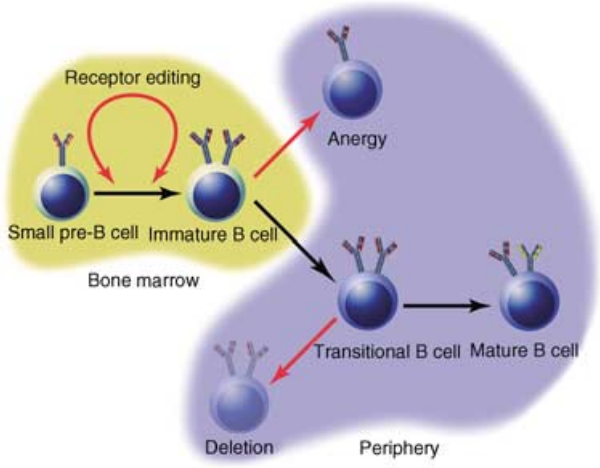

Figure 3. Mechanisms of negative selection during B-cell development. There are three described mechanisms whereby developing B cells can escape an autoreactive fate; all of these are mediated by signals transduced through the B-cell receptor (BCR) in response to BCR ligation and are indicated by red arrows. Which outcome results is largely a function of BCR signalling intensity, developmental stage and environmental milieu. Receptor editing is postulated to be a result either of retarded progression from the pre-B to immature B-cell stage, or of back-differentiation from the immature to pre-B-cell stage. Anergy is induced in immature bone marrow B cells as a response to receptor ligation, whereas deletion is induced in transitional B cells in response to the same stimulus.

Which of these three tolerance mechanisms is invoked depends on a myriad of factors, including receptor affinity, receptor expression level, developmental stage and site of encounter. As described above, ligand avidity is a primary determinant of whether a B cell is deleted or becomes anergic.[91,95,96] The stage of maturation within the immature B-cell pool might also determine how a B cell responds to antigen. *In vitro*, ligation of the BCR on newly generated immature B cells, which are IgM^{lo}, results in receptor editing, while ligating the receptor on more developed IgM^{hi} B cells induces apoptosis.[104] The location of receptor engagement may also determine cell fate; ligation of the immature BCR in a bone marrow environment results in receptor editing, whereas ligation in a splenic environment induces deletion. This difference was found to be caused by the protective effect of an unidentified Thy-1^{dull} cell type in the bone marrow[105] and was similar to the effect of introducing a bcl-2 or bcl-x_L transgene into immature B cells.[106,107] Finally, transport of the ligated BCR into lipid rafts may determine the biological outcome of receptor signalling.[108,109]

SIGNALLING THROUGH THE IMMATURE B-CELL RECEPTOR

The replacement of surrogate light chains by Igκ or Igλ is required for the development of immature B cells.[110] Truncation of the cytoplasmic tail of Igα ($Ig\alpha^{\Delta c/\Delta c}$) causes a dramatic decrease in the numbers of immature B cells,[80] highlighting the importance of BCR signalling at this stage. A similar truncation in Igβ ($Ig\beta^{\Delta c/\Delta c}$) has no effect on the generation of immature B cells,[78] which implies that, as at the pro-B to pre-B transition, the functions of Igβ are redundant of Igα at this stage. Furthermore, truncation of the Igα cytosolic tail ($Ig\alpha^{\Delta c/\Delta c}$) inhibits the generation of immature B cells to a greater degree than pre-B cells. This suggests that as B-cell progenitors progress through development, successively higher signalling capacities are required.

Although receptor editing occurs in the earliest populations of immature B cells, it is still unclear how the BCR initiates editing. One interesting hypothesis proposes that receptor editing is triggered by the absence of basal BCR signalling that occurs following ligand-induced downmodulation of the BCR. In this model, the lack of BCR signalling would allow immature B cells to back-differentate to an earlier stage in which RAG and other recombinase machinery genes are expressed. This hypothesis is based on the observation that conditional deletion of the BCR in immature B cells initiates new light-chain rearrangements (L. E. Tze *et al.*, submitted) and the re-expression of pre-B-cell markers.[111] Alternatively, editing may result from a BCR-dependent transient arrest in development at the pre-B/immature B-cell transition, a stage at which RAG is still expressed.[112] This model is consistent with observations that once RAG expression has been turned off in splenic B cells, it cannot be reintroduced.[113]

Anergy, the outcome of low-affinity receptor crosslinking, results from the activation of calcineurin and extracellular signal-regulated kinase (ERK) signalling pathways and translocation of nuclear factor of activated T cells (NFAT) to the nucleus without the activation of NF-κB.[114] This suggests that anergy may arise from the activation of a subset of the signalling pathways activated by the BCR. However, it is possible that anergy involves specific signalling effectors not requisite for normal activation. For example, B cells deficient in protein kinase Cδ, which have autoimmune nephritis, become activated in response to anergizing ligands.[115]

There are several possibilities for how the BCR on immature B cells may discriminate between activating and anergizing ligands. As recently demonstrated, one possibility is that anergizing ligands preferentially destabilize the BCR complex.[116] Lowering the stoichiometry of mIg with Igα/Igβ may either play a role in initiating anergizing signals or in maintaining unresponsiveness to subsequent ligands. Anergy might also arise from a failure to phosphorylate fully the Igα ITAM tyrosines,[20] leading to incomplete or abbreviated activation of Syk and other downstream effectors. It has also been proposed that anergy is maintained through chronic engagement of the BCR with low-avidity ligands (J. Cambier, personal communication).

TRANSITIONAL B CELLS AND SIGNALLING THROUGH THEIR BCR

Bone marrow immature B cells progress to the periphery, where they continue to mature. These transitional cells can be subdivided on the basis of surface-marker expression into T1, T2 and, possibly, T3 populations. Most investigators agree that T1 cells are $CD23^{-}$ IgM^{+} $IgD^{lo/-}$, whereas T2 cells are $CD23^{+}$ IgM^{+} $IgD^{lo/+}$. However, there is controversy concerning the functional significance of each population and the efficiency with which cells transit through each stage. Bromodeoxyuridine (BrdU) studies investigating the entry of cells into the T1, T2 and T3 populations indicate a 40–50% cell loss at the T1 to T2 transition.[117] Other investigators contend that this can be accounted for by migration to other lymphoid organs and unexamined differentiative events.[118] Regardless of the precise nomenclature, it is generally accepted that T1 cells migrate from the red pulp to the lymphoid follicles in the spleen, where they up-regulate IgD and CD23 as they mature.

Signalling through the BCR is important for B-cell maturation. Mice deficient in Btk have a profound block at, or immediately after, the T2 stage.[117] As mentioned above, deletion of Syk or B-cell linker protein (BLNK), or truncation of the Igα or Igβ cytoplasmic tail, also impairs B-cell maturation (Fig. 2). The few $Syk^{-/-}$ cells that progress to the immature B-cell stage migrate to the splenic red pulp, but fail to enter B-cell follicles.[119] Similarly, BLNK ablation allows transitional immature B cells to develop, but they fail to mature further.[120] Truncation of the Igα cytoplasmic tail impairs pre-B-cell development; however, there is a much more severe block in export of bone marrow immature B cells to the periphery.[121] Truncation of the Igβ tail, in contrast, allows the development of bone marrow immature B cells, but not their subsequent development to transitional immature B cells.[78] The requirements for different signalling molecules or domains at each stage of B-cell maturation indicates that the BCR may be tested at each stage for a different functional capacity.

Aside from the BCR, it is becoming clear that the novel tumour necrosis factor (TNF) family member, B-cell activating factor (BAFF), is extremely important at the immature B-cell stage. BAFF-deficient B cells are developmentally arrested at the T1 stage.[122] This is probably the result of a defect in survival rather than differentiation, however, as T2 cells die *in vitro* in the absence of BAFF signals[123] and BAFF has been shown to stimulate NF-κB activation through a non-classical pathway[124,125] and to modulate expression of bcl-2 family members.[126,127]

B-cell maturation and differentation in the periphery

As B cells mature, they down-regulate AA4·1 and start to express the δ heavy chain. Unlike other isotypes, IgD is expressed as a result of RNA, not DNA, splicing. This preserves the μ locus and allows mature B cells to co-express IgM and IgD. Some of these IgM^{hi} IgD^{hi} cells express intermediate levels of CD21, acquire the ability to recirculate, down-regulate IgM and become follicular/recirculating (IgM^{lo} IgD^{hi} $CD23^{+}$) B cells. Follicular B cells are considered to be classical B2 cells and respond to T-dependent antigens, undergo germinal centre reactions, and give rise to memory cells. Other transitional cells express high levels of CD21 and give rise to marginal zone (MZ; IgM^{hi} $CD21^{hi}$ $CD23^{-}$ IgD^{lo}) B cells.[128] MZ cells, which may also develop from follicular B cells,[129] are long-lived and have a partially activated phenotype. They have some features in common with B1 cells in that they respond primarily to T-independent antigen and probably do not give rise to memory cells. However, they are $CD5^{-}$. Additionally, the V_H segments of their antigen receptors are largely germline in sequence[130] and, despite their short CDR3 regions, some of these cells bear autoreactive receptors.[131]

SIGNALLING THROUGH THE MATURE B-CELL RECEPTOR

The necessity of BCR signalling for the survival of mature peripheral B cells was demonstrated in an elegant set of experiments wherein the BCR was conditionally deleted following treatment with interferon-γ (IFN-γ).[132] Deprived of BCR signals, peripheral B cells quickly died, conclusively demonstrating that at least a basal level of signalling is required to keep mature B cells alive. Whether BCR ligation is needed for peripheral B-cell maintenance is unknown. However, the magnitude of ligand-mediated BCR signalling influences the development of transitional cells into either MZ or follicular B cells; weak signals skew development towards MZ cells and strong ones induce follicular B-cell development.[133] It has also been suggested that MZ cells require antigen-driven positive selection for development.[134]

Relatively little is known about the mechanisms underlying MZ B-cell development. The transcription factors NF-κB and c-Rel are important;[135] deletion of the p50 subunit completely abrogates MZ-cell development, whereas cells lacking the p65 subunit or c-Rel develop into MZ cells at greatly reduced efficiency. Deletion of proline-rich tyrosine kinase 2 (Pyk2),

a non-receptor tyrosine kinas, downstream of chemokine, cytokine and integrin receptors, also results in a complete loss of the MZ subtype.[136] This suggests that factors distinct from BCR signalling may influence MZ B-cell development.

CONCLUSION

The primary purpose of B-cell development is to establish a diverse population of peripheral B cells that is both self-tolerant and reactive to foreign antigens. To ensure that these conditions are met, B-cell development is determined by the structural features and signalling capacities of the B-cell antigen receptor. Some BCR signals, such as those initiated by Syk and the cytoplasmic tail of Igα, are required for both B-cell development and peripheral maturation. Other signalling functions, such as those mediated by Btk and the cytosolic tail of Igβ, are required only at selected checkpoints. These stage-specific requirements reflect underlying differences in how the BCR signals in development and in the periphery, to decide cell fate.

Although BCR-mediated signals are the primary determinants of B-cell fate, environmental factors such as IL-7 and BAFF are also important. Understanding the molecular mechanisms by which the BCR determines cell fate, and how these decisions are influenced by the environments in which they take place, is one of the main challenges in B-lymphocyte biology.

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