CHAPTER 2

B-Cell Receptor

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Abstract

The subunit structure of the B-cell antigen receptor (BCR) and its associated compartmentalization of function confer enormous flexibility for generating signals and directing these toward specific and divergent cell fate decisions. Like all the multichain immune recognition receptors discussed in this volume, assembly of these multi-unit complexes sets these receptors apart from almost all other cell surface signal transduction proteins and affords them the ability to participate in almost all of the diverse aspects of, in this case, B-cell biology. We discuss here the structural aspects of the BCR and its associated coreceptors and relate these mechanistically to how BCR signaling can be directed towards specific fate decisions. By doing so, the BCR plays a pivotal role in ensuring the effective and appropriate B-cell response to antigen.

Introduction

Antigen receptors on B- and T-cells exhibit enormous flexibility with regards to not only the diversity of ligands (antigens) that they can engage but also their ability to trigger signals that result in very different cellular fates. This flexibility is possible in part by separating ligand-binding components from signal transduction units. This separation of functions allows the former to be genetically diversified in order to confer on each developing B-cell a unique specificity without affecting the signals generated following ligand engagement. The latter are generated by invariable protein units that associate noncovalently with the ligand binding unit. This multi-unit design (termed through out this book as multichain immune recognition receptors, MIRRs) is common to receptors involved with triggering responses in cells of the immune system.

Once ligand facilitates oligomerization of BCR units, the signals generated lead to remarkably different responses dependent upon the maturation or developmental stage at which the B-cell resides when antigen is introduced. These responses can range from rapid induction of apoptotic programs to initiation of strong survival and activation programs. From an immune system perspective, the choice between these cell fates determines whether the ligand engaged B-cell (and its unique specificity) will be eliminated from the immune system repertoire of reactive B-cells. On the other hand, positive signaling initiates processes that will ultimately result in generation of antibody-secreting cells, cell division and clonal expansion of B-cells with identical specificity and the development of memory B-cells. In part, this decision is guided by the precise plasma membrane lipid environment to which the oligomerized BCR complexes are localized.

Finally, it has been very recently appreciated that BCR complexes can generate biologically meaningful signals even in the absence of ligand. These signals, termed tonic signals, may play a role in driving early B-cell development and later in peripheral survival of B-cells by selecting for

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cells with functional BCR and eliminating those that fail to assemble BCR properly or later lose its expression at the cell surface.

Each of these characteristics of the BCR will be discussed in this chapter. In addition, where appropriate we will point out challenges that remain in order for us to fully understand in mechanistic terms how BCR signals are initiated, regulated and directed to trigger specific B-cell fate decisions.

Structure of the BCR

The B-cell antigen receptor (BCR) provides B-cells with the capacity for antigen recognition, thereby allowing them to engage potentially dangerous pathogens and is directly responsible for generating signals that result in an appropriate antigen-induced B-cell response. The BCR is a transmembrane protein complex comprised of multiple subunits, including an Immunoglobulin Heavy Chain (IgHC) covalently linked by disulfide bonds to an Ig Light Chain (IgLC). The amino-terminal region of the IgHC and IgLC is characterized by extensive sequence variability brought about by gene recombination, nucleotide addition and somatic hypermutation. It is this region, termed the complementarity-determining region (CDR) that is responsible for the clonal specificity of antigen recognition by the receptor as well as for the affinity of ligand binding. In addition to the ligand binding components, the BCR contains Ig alpha (Igα) and Ig beta (Igβ), the subunits responsible for signal transduction. A single surface BCR unit contains two IgHC, two IgLC, one Igα and one Igβ.[1] The carboxy-terminal invariable portion of the IgHC is termed the constant region and it is this region that defines the 5 different isotypes of BCR, IgM, IgD, IgG, IgA and IgE (see Fig. 1). IgM and IgD are expressed by newly generated and naive B-cells whereas the other isotypes are typically expressed only on activated or memory B-cells.

Efficient surface expression of the BCR complex requires the concomitant expression of each of the individual components. In part, this appears due to the unusual nature of the transmembrane domains of IgHC, Igα and Igβ. Transmembrane domains are generally comprised of neutral and nonpolar amino acids, allowing them to fold into alpha helices that are thermodynamically stable within the hydrophobic environment of the plasma membrane. However, the heavy chain of IgM (Igμ) contains 9 polar amino acids in its transmembrane domain, while the heavy chain of IgD (Igδ) contains 7 and Igα and Igβ each contain 3.[2,3] Noncovalent interactions between the transmembrane domains of IgHC and Igα/β are postulated to shield the polar amino acids from the hydrophobic lipid environment, thereby allowing the BCR complex to exist stably in the plasma membrane.[4,5]

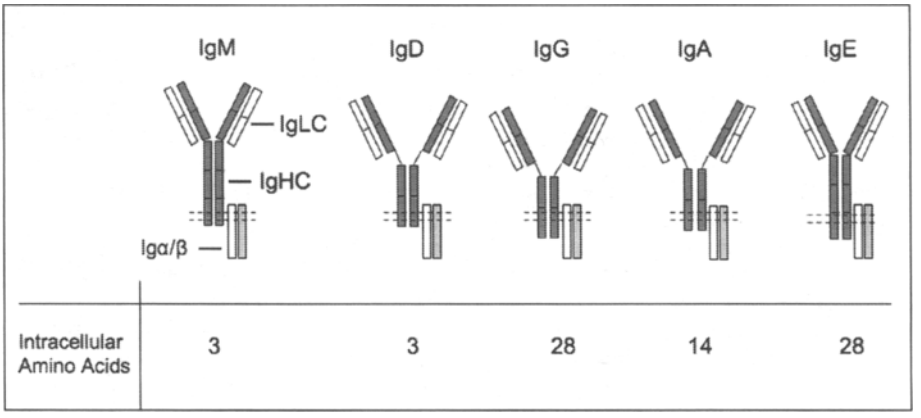

Figure 1. Structure of the monomers of the 5 isotypes of BCR. Each isotype is paired with an Igα/β heterodimer. The isotypes differ in the number of immunoglobulin domains as well as the number of intracellular amino acids.

Once at the cell surface, the BCR complex is capable of transducing signals in response to ligand (antigen)-induced aggregation. The relative paucity of amino acids in the cytoplasmic portion of IgHC (e.g., Igμ and Igδ each contain only 3 amino acids)[6] led to the early hypothesis that the IgHC alone would be incapable of signal transduction. This hypothesis was confirmed in a series of studies in which IgHC was expressed on the surface of B-cells in the absence of the Igα/β heterodimer. Expression of the IgHC alone in this case occurred following mutation of a YS to VV sequence in the transmembrane domain of Igμ[7] In the absence of Igα/β heterodimer association, BCR complexes containing the mutated Igμ were unable to transduce signals, indicating that IgHC-associated Igα/β heterodimers directly participated in BCR-induced signal transduction. The subsequent identification by Reth and colleagues of a signaling motif, termed an Immunoreceptor Tyrosine-Based Activation Motif (ITAM), in both Igα and Igβ supported the idea that the Igα/β heterodimer mediates BCR signaling.[8] The canonical ITAM is Yxx(L/I) $x_{6\text{-}8}$Yxx(L/I) and ITAM motifs are ubiquitously expressed in the MIRR signal transduction components.[9] As described further below, phosphorylation of tyrosines embedded within the Igα/β ITAMs plays a critical role in initiating BCR-induced signal transduction.[10]

While it is quite clear that Igα/β heterodimers play a critical role in initiating and propagating BCR-induced signals, it has also been postulated that higher order complexes may regulate BCR-induced signal transduction. In particular, while it is known that monomers of surface BCR have a stoichiometry of 2 IgHC/LC to 1 Igα/Igβ heterodimer, one study has shown that several of these units can be grouped together to form an oligomeric complex on the surface of resting B-cells.[1] Based on these data, it was postulated that disruption of preformed oligomeric complexes by ligand binding would promote BCR-induced signal transduction. However, the existence of preformed oligomeric complexes was not observed in fluorescence resonance energy transfer (FRET) analysis in which it was demonstrated that the BCR existed as a monomer on the surface of resting B-cells.[11] Thus, in the absence of aggregation by multimeric ligand, the role that higher order BCR structures play in BCR-induced signal transduction remains an issue warranting further investigation.

B-Cell Development

B-cell development occurs in a stepwise manner and the developmental stages are defined by the ordered and sequential assembly of the preBCR followed by the BCR (see Fig. 2). At each stage, the functionality of the receptor complex is assessed and only those B-cells that express signaling competent receptors are allowed to continue development.[12] Indeed, genetic alterations that abrogate the surface expression or signal transduction capacity of any of the PreBCR/BCR components block B-cell development.[13]

To a large extent, expression of the IgHC and IgLC is regulated by a process known as V(D)J recombination. During V(D)J recombination, gene segments known as the variable (V), diversity (D) and joining (J) segments within the IgHC and IgLC genes are brought together to create the mature ligand-binding form of the protein. During the initial stages of B-cell development, recombination of the IgHC and IgLC genes has not yet occurred. However, proB-cells, the first cells committed to the B-cell lineage, do express an Igα and Igβ-containing complex known as the proBCR on their surface.[14] In the proBCR, Igα and Igβ are associated with the endoplasmic reticulum (ER) chaperone calnexin, presumably to mask the polar amino acids found within their transmembrane domains.

As development progresses, the IgHC is the first to recombine. Expression of the mature form of the IgHC marks the transition to the preB-cell stage. However, as IgLC gene recombination has not yet occurred, preB-cells do not express IgLC. Instead, preB-cells express a surrogate light chain (SLC) composed of VpreB and Lamba5 (λ5) which together with IgHC, Igα and Igβ comprise the preBCR.[15] Expression of SLC is essential to get the IgHC to the cell surface as it masks ER retention signals located at the N-terminal portion of the IgHC that retain unassociated IgHC within the ER.[16-18]

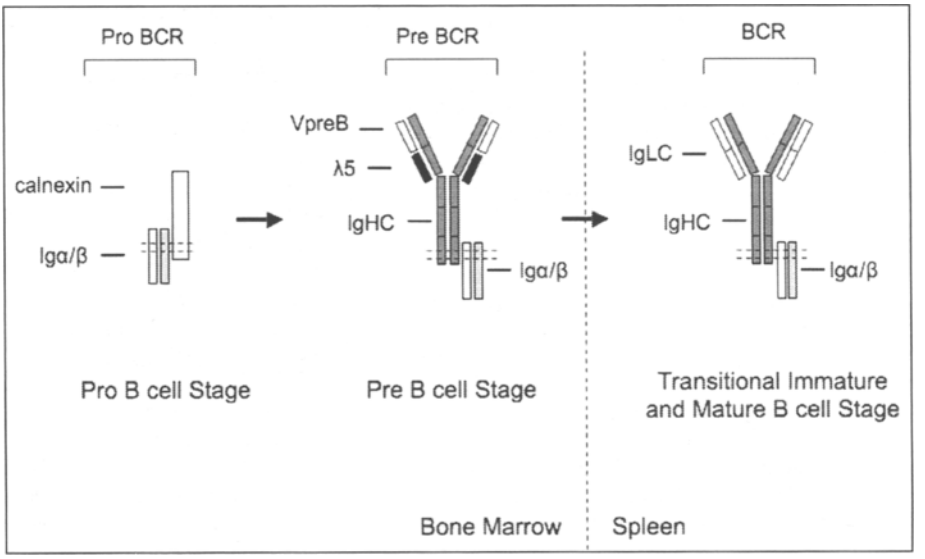

Figure 2. Structure of the BCR during B-cell development. As B-cells develop, the BCR undergoes series of changes in its structure and the subunits expressed. At the proB-cell stage, the proBCR contains an Igα/Igβ heterodimer associated with calnexin. The preB-cell expresses the preBCR composed of IgHC associated with the SLC (VpreB and λ5) and a Igα/Igβ heterodimer. The BCR expressed on transitional immature and mature B-cells contains two IgHC, two IgLC and the Igα/Igβ heterodimer. ProB and preB-cells are localized in the bone marrow, while transitional immature and mature B-cells are localized in the spleen.

Following recombination of the IgLC, the mature form of the BCR can now be assembled and it is the expression of the ligand-binding form of the BCR that marks the transition to the immature B-cell stage. This newly acquired capacity for antigenic recognition provides the first opportunity for selection against cells reactive to endogenous or "self" antigens, a process known as negative selection.[19] Non-autoreactive immature B-cells that have evaded negative selection emerge in the periphery and are known as transitional B-cells. Transitional B-cells are considered direct precursors to mature follicular B-cells.[20]

On mature B-cells the BCR serves two functions. The first is to maintain the survival of BCR-expressing B-cells by generating signals in the apparent absence of a requirement for antigen binding. Evidence for this function comes from studies where conditional ablation of components of the BCR signaling complex at the mature B-cell stage results in apoptosis and loss of the peripheral B-cell population,[21,22] suggesting that ligand-independent signaling is required for the survival of mature B-cells (discussed further below). The second function is to facilitate antigen-specific responsiveness by the mature B-cell. In this case, ligand-induced aggregation of the BCR on mature B-cells elicits effector functions required for an effective humoral response such as proliferation, increased antigen presentation and upregulation of costimulatory molecules required to elicit cognate T cell help. Thus, in the absence of ligand-induced aggregation, low-level BCR-induced signal transduction is sufficient to maintain B-cell survival but is insufficient to fully activate the B-cell, whereas aggregation by multimeric ligand elicits B-cell effector function.

Molecular Aspects of Ligand-Induced BCR Signal Transduction

The tyrosines embedded in the ITAMs of Igα and Igβ are critical for preBCR/BCR-induced signaling function. As some evidence indicates that Src family kinases are constitutively associated with the BCR in resting B-cells[23,24] they are generally thought to initiate BCR-induced signaling.

Following ligand-induced aggregation, Src kinases phosphorylate tyrosines within the ITAMs of Igα and Igβ, with preference for the proximal tyrosine due to the presence of an isoleucine or leucine at the -1 position.[25] This initial phosphorylation creates sites for proteins with Src homology-2 (SH2) domains (highly conserved phosphotyrosine-binding domains) to dock. Following this initial signal amplification, dually phosphorylated ITAMs recruit and activate Syk family kinases by virtue of their tandem SH2 domains, leading to their allosteric activation and engaging a positive feedback loop that further amplifies BCR-induced signal transduction.[26] Indeed, Syk activation is considered essential for optimal BCR-induced signal transduction as SH2 domain mutations that block recruitment to the BCR and mutations in its autophosphorylation site that block function or genetic ablation compromise BCR-induced signaling.[27,28]

Syk activation is responsible for the recruitment and phosphorylation of adaptor molecules that serve as scaffolds to recruit downstream effector molecules through SH2 and phosphotyrosine-binding (PTB) domain binding. These events allow for the generation of a stabilized signalosome to engage second messenger pathways that direct changes in gene expression that ultimately dictate the cellular response. A key component of this complex is the adapter protein SH2 domain containing leukocyte protein of 65 kDa (SLP-65), also known as BLNK. The SH2 domains on SLP-65 bind to phosphorylated tyrosines and recruit SLP-65 to the BCR. While the ITAM-embedded tyrosines appear necessary for Syk recruitment, early studies in cell lines suggest that SLP-65 is recruited to the complex via non-ITAM-associated phosphotyrosines in Igα.[29,30] In more recent "knock-in" studies, generation of genetically altered mice in which the non-ITAM tyrosines (tyr-204 and tyr-176) of Igα were mutated to phenylalanine revealed normal recruitment and activation of Syk to the BCR signalosome, but reduced SLP-65 recruitment (most notable in the tyr-204 mutant) following BCR engagement.[31] While the inability to recruit SLP-65 was associated with reduced IkBα degradation, impaired JNK and ERK phosphorylation and a relative inability to enter the cell cycle, other aspects of BCR signaling (Syk, PLCγ2 and Btk phosphorylation) were unimpaired. Furthermore, the deficiencies in BCR-induced signal transduction observed in the $Ig\alpha^{tyr\text{-}204\text{-}phe/tyr\text{-}204\text{-}phe}$ mice were not as severe as those displayed by SLP-65-deficient B-cells, suggesting that SLP-65 can function in a tyr-204-independent manner, perhaps by binding to the adaptor linker for activation of B-cells (LAB).[32] However, based on these studies, it is likely that non-ITAM-dependent phosphotyrosine-mediated recruitment of SLP-65 to the BCR signalosome plays a critical role in the induction of multiple elements required for optimal functional responses of B-cells.

Finally, for B-cell effector function to be elicited, BCR-induced signals must be transduced for 20-24 hours.[33] However, the BCR internalizes within minutes of antigen encounter so that bound antigen can be delivered to endosomal compartments for processing and presentation to major histocompatibility complex (MHC) class II-restricted CD4 T cells. It is not clear how signal transduction is maintained in the face of concomitant internalization. However, recent studies propose mechanisms that may help to resolve this paradox. The first model proposes that when IgHC:antigen is internalized, Igα/β heterodimers remain on the cell surface, maintaining the signalosome and thereby allowing for continued signal propagation. This hypothesis is supported by data obtained using a variety of approaches indicating that the IgHC is physically separated from Igα and Igβ following ligand-induced aggregation.[34-36] However, it is not immediately apparent how a receptor complex that requires its components to associate during transport to the cell surface can dissociate following ligand binding, particularly as it is likely that the polar amino acids in the transmembrane domains will now be exposed. While it was observed that in some circumstances Igα and Igβ may associate with MHC class II molecules following their dissociation from IgHC,[37] Igβ-containing complexes can still be observed on the cell surface after IgHC internalization in cells devoid of MHC class II.[34] Alternatively, it has been proposed that while the vast majority of the receptor complexes are indeed internalized following ligand binding, the small subset of receptors that is inducibly phosphorylated is selectively retained at the cell surface to propagate BCR-induced signals.[38] In this model, each receptor undergoes one of two mutually exclusive fates that is determined by its phosphorylation status. Clearly further analysis

of the mechanisms involved in the endocytosis of the BCR complex following ligand binding is required to provide greater insight into the interrelationships between receptor internalization and BCR-induced signal transduction.

Membrane Compartmentalization of the BCR

Recent evidence suggests that the spatial organization of the BCR, its signaling effector molecules and positive and negative coregulatory molecules plays a critical role in the initiation and regulation of BCR-induced signal transduction.[39] In part, this spatial organization is postulated to occur by the association of ligand-aggregated BCR into lipid-ordered, cholesterol- and sphingolipid-rich plasma membrane microdomains commonly referred to as lipid rafts.[40] The plasma membrane of the cell is composed of lipid-disordered and lipid-ordered regions. In lipid-ordered domains, the acyl chains of the sphingolipids are saturated, promoting a rigid organization mediated by van der Waals interactions between adjacent acyl chains. Cholesterol further stiffens these domains by packing between the saturated acyl chains of the sphingolipids. The association of cholesterol and the sphingolipids of the lipid-ordered domain makes them resistant to solubilization by non-ionic detergents such as Triton X-100 and confers upon them increased buoyancy in discontinuous sucrose gradients. Based on the latter characteristic, the lipid-ordered domain is referred to as the lipid raft fraction.[41]

Lipid rafts are hypothesized to concentrate proteins necessary for the propagation of signals and exclude proteins and receptors that attenuate signals. In this regard, they are often referred to as platforms for signal transduction. Recent studies on the structure of lipid rafts suggest that in resting cells lipid rafts are small (diameters ranging from 4 to 200 nm) but coalesce into larger structures when cell surface receptors are engaged.[42] While the BCR is predominantly detected in the lipid-disordered, detergent soluble domains of the plasma membrane in mature resting B-cells, it is unclear at the present time if the BCR is located in the nonraft fraction or in submicroscopic lipid raft structures (described above) that are too small to be isolated by sucrose density centrifugation. Regardless, multiple studies suggest that BCR cross-linking in mature B-cells promotes stable association of the BCR with the lipid raft domain. This association is thought to enhance BCR-induced signal transduction as the Src kinase Lyn preferentially localizes to lipid rafts due to the addition of myristoyl and palmitoyl posttranslational modifications.[43] As described further in the section below, there is also evidence that negative coregulatory transmembrane proteins such as CD45 and CD22 that down-modulate signal transduction through the Igα/β-organized signalosome are selectively excluded from lipid rafts. Thus, association of the BCR with lipid rafts promotes sustained signal transduction by providing a microenvironment rich in signal effector molecules and devoid of negative coregulatory molecules.

As lipid rafts directly influence the ability of the BCR to interact with signal transduction effector molecules, their presence can affect BCR-induced signal transduction in both a quantitative and qualitative manner. The influence of lipid rafts on BCR-induced signal transduction seems most apparent when evaluating the differential response of transitional and mature B-cells to BCR crosslinking. While mature B-cells proliferate and upregulate costimulatory molecules involved in cognate T cell interactions, transitional immature B-cells fail to sustain BCR triggered pathways that have been linked to survival and proliferation and instead undergo BCR-induced apoptosis.[44,45] Specifically, transitional B-cells manifest an impaired ability to signal through the PLCγ/PKCβ/NF-κB/*c-myc* pathway.[46,47] The inability of transitional B-cells to sustain these pathways is associated with a relative inability to detectably colocalize their BCR with cholesterol-enriched lipid rafts following BCR cross-linking.[47,48] As transitional B-cells maintain distinctly lower levels of membrane-associated cholesterol than do follicular mature B-cells, it has been proposed that the differential ability of transitional B-cells and mature B-cells to colocalize their BCRs with cholesterol-enriched lipid rafts following BCR engagement is due to developmentally regulated differences in membrane-associated unesterified cholesterol levels. In support of this idea, increasing the membrane-associated cholesterol levels of transitional B-cells to levels equivalent to that of follicular mature B-cells results in colocalization of the BCR into cholesterol enriched domains

and enhances signal transduction through the PLCγ2/PKCβ/NFκB/*c-myc* pathway to an extent that resembles that of the mature B-cell.[47] These results argue that the relative ability of the BCR to associate with lipid rafts has profound effects on BCR-induced signaling and a significant impact on the physiologic response of the cell.

Balance between Positive and Negative Regulators of BCR Signaling

BCR-induced signal transduction is subject to both positive and negative regulation and the spatial repositioning of BCR relative to either negative coregulators or effector molecules plays a critical role in the determining the final B-cell response. For example, the transmembrane protein CD22 is a negative regulator of BCR signaling and contains an Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM). Stochastic activation of Src kinases constitutively associated with the BCR results in phosphorylation of the tyrosines embedded in the ITIM of CD22. These phosphotyrosines subsequently recruit the tyrosine phosphatase SHP-1 via its SH2 domain. SHP-1 attenuates BCR-induced signals by dephosphorylating the Igα/β heterodimer, thereby dissociating the BCR signalosome.[49] As noted above, ligand-induced aggregation of the BCR promotes its association with lipid rafts and this translocation spatially segregates it from CD22.[40] Such spatial segregation potentially allows for more prolonged BCR-induced signaling events. The importance of both CD22 and SHP-1 as negative regulators in B-cell signaling is underscored by the observation that B-cells deficient in either of these proteins are constitutively hyperactive.[50]

Alternatively, coregulatory molecules can affect BCR-induced signal transduction by promoting colocalization with downstream effector molecules. For example, the transmembrane protein CD19 is a positive regulator of BCR signaling and cocrosslinking of the BCR with CD19 lowers the threshold for BCR activation.[51] One mechanism by which CD19 has been postulated to mediate this effect is by reducing BCR internalization, thereby prolonging the residency of the BCR in lipid rafts.[52] The persistence of BCR in lipid rafts at the cell surface correlates with prolonged signaling as revealed by the extended duration of activated signaling molecules in the lipid raft fractions.[52,53]

Finally, recent studies have shown that cross-linking of the BCR results in a Ca^{2+}-dependent generation of reactive oxygen intermediates (ROIs).[54] ROIs such as hydrogen peroxide have the potential to oxidize a conserved cysteine residue within the catalytic active site of tyrosine phosphatases, such as SHP-1 and CD45, rendering them inactive. Only BCR-proximal phosphatases were inactivated and it was proposed that a short pulse of localized phosphatase inactivation favored the initiation of BCR-induced signal transduction. While the mechanism by which H_2O_2 is produced following BCR ligation has not yet been established, it likely involves a plasma membrane-associated NADPH oxidase.[55] As NADPH oxidase is localized to lipid rafts in neutrophils,[56] it is reasonable to predict that BCR translocation into rafts enhances BCR-induced signal transduction by providing an ROI-enriched environment in which inhibitory phosphatases will be inactivated.

Ligand-Independent BCR-Induced Tonic Signaling

While signal transduction through the BCR is generally thought to be initiated by ligand-induced aggregation, the BCR can also transduce tonic signals in a ligand-independent manner. The existence of ligand-independent BCR-mediated tonic signal transduction was originally suggested in studies in which BCR-expressing cells were treated with the tyrosine phosphatase inhibitor pervanadate.[57] In the absence of any known ligand, induction of a pervanadate-induced phosphotyrosine "footprint" that resembled that induced by BCR-crosslinking suggested that, by inhibiting phosphatases, low-level ligand-independent BCR-induced tyrosine kinase activity can be revealed. More recent studies suggest that tonic signaling through both the BCR and preBCR provide functionally relevant signals for mature B-cell survival and developmental progression, respectively. For example, despite any obvious requirement for antigen, conditional deletion of the BCR signaling complex in mature B-cells results in a rapid loss of the peripheral B-cell compartment, suggesting a requirement for continued BCR signaling for mature B-cell survival.[21,22] In less mature

B-cell populations, tonic BCR-induced signaling is thought to maintain developmental progression by preventing back-differentiation.[58] Finally, the inability of preBCR to bind conventional antigen (due to the lack of IgLC expression) suggested that preBCR-induced signal transduction driving B-cell development occurred in a ligand-independent fashion.[39] A direct evaluation of the ligand-independence for preBCR function was assessed in studies in which a chimeric protein consisting of the cytoplasmic regions Igα and Igβ was stargeted to the plasma membrane using the membrane-targeting sequence of Lck that contains both myristoylation and palmitoylation sites. Expression of this protein, which lacked ectodomains, was sufficient to generate signals for IgH allelic exclusion, progression through the pro-B-preB-cell checkpoint, IL-7-dependent expansion and IgLC gene recombination both in vitro and in vivo.[59-61] Together with earlier studies using amino-terminal truncated IgHC proteins,[62] these studies establish the existence of biologically relevant ligand-independent tonic signals by Igα/Igβ-containing complexes.

Conclusion

While much is known about the biochemical events that are initiated following activation of B-cells with multimeric ligand, the molecular events that trigger these signal transduction pathways remain more enigmatic. In particular, it is quite clear that spatial proximity with signal transduction effector molecules following association with lipid rafts, the balance of positive and negative coregulatory molecules and the oligomeric status of the BCR all play an essential role in determining the final outcome of BCR-induced signal transduction. The further identification and functional evaluation of molecular interactions responsible for these effects will be greatly enhanced by new, more quantitative techniques such as FRET, two photon microscopy and Total Internal Reflection Fluorescence (TIRF). In addition, such techniques may provide insight into the mechanisms that permit sustained BCR-induced signal transduction under conditions where the vast majority of the receptor is internalized.

Finally, while signal transduction through MIRRs has traditionally been considered ligand-induced, it is becoming apparent that several of these receptors are able to signal in a ligand-independent tonic manner.[39] Identifying the molecular events that distinguish tonic ligand-independent from multimeric ligand-induced signal transduction may well aid in designing therapeutic treatments to alleviate autoimmune disease.

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