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How pre-B cells know when they have it right

Mark Schlissel

Early B cell development faces a critical checkpoint at the pro-B → pre-B transition stage, at which proper assembly and surface expression of an immunoglobulin heavy chain is somehow signaled by the pre-B cell receptor. Triggering of this signal might not require exogenous ligands.

Recombination of variable, diversity and joining regions (V(D)J recombination) affords the immune system the ability to encode an enormous repertoire of antigen receptors with a relatively modest investment of genetic capacity. This reliance on combinatorial diversity introduces problems, however. Developing lymphocytes must have a way to monitor the assembly process to prevent expression of multiple functional receptors in a given cell (allelic exclusion) and to exercise 'quality control' on the proteins produced. Progenitor B cells do so through assembly in the membrane of a 'pre-B cell receptor' (pre-BCR), which is essential for normal development. Whereas the mature BCR initiates signaling through interaction with its cognate antigen, it remains uncertain how the pre-BCR signals. In this issue of *Nature Immunology*, one of the discoverers of the pre-BCR and his colleague present unexpected data showing that conserved pre-BCR components may interact with one another to induce receptor aggregation and signaling without involvement of an exogenous ligand[1].

Rearrangement of the immunoglobulin heavy-chain (H) gene (*Igh)* precedes *Igk* or *Igl* light-chain gene rearrangement, with D_H-to-J_H rearrangement occurring before V_H-to-DJ_H rearrangement. Because of the imprecise nature of the joining step and the addition of N regions to *Igh* coding joints, two of three times V(D)J recombination generates out-of-frame (nonproductive) alleles. In addition, the requirement for two rearrangements to generate a complete heavy-chain variable region exon results in considerable length heterogeneity that is not found in light-chain gene rearrangements (because only a single recombination event is required and because N regions are rare in

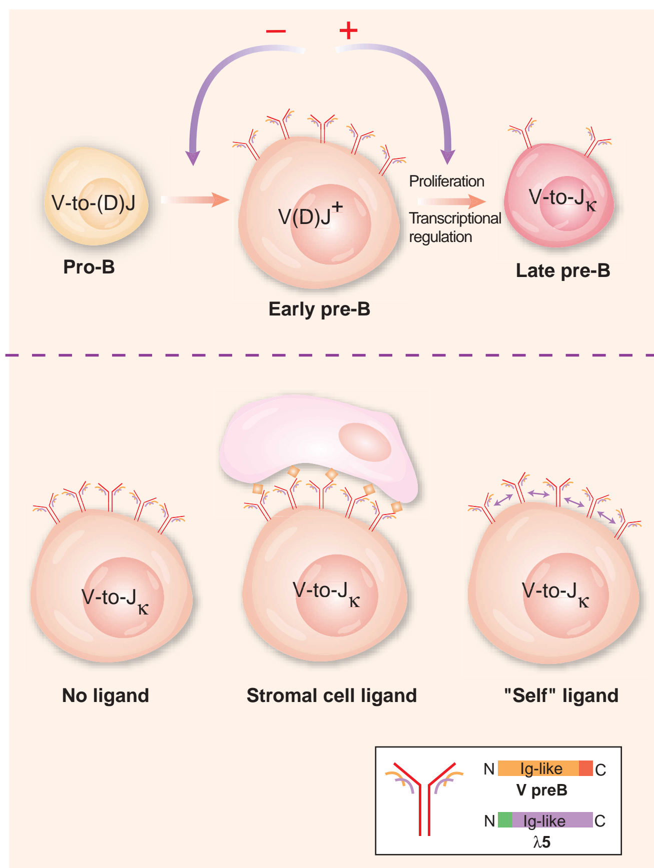

Figure 1 The pre-BCR: signals and possible signaling mechanisms. (**a**) The progression of developing B cells across the pro-B-to-pre-B cell transition. Pro-B cells rearrange gene segments in an attempt to generate an immunoglobulin heavy-chain gene encoding a protein capable of pairing with SLCs to form a pre-BCR. Early pre-B cells undergo multiple rounds of cell division, inactivate further heavy-chain gene rearrangement (–) and activate light chain gene rearrangement (+). (**b**) Models proposed to explain the initiation of the pre-BCR signal upon surface expression of the pre-BCR. Inset, the surrogate light chains consist of Ig-like (orange and purple boxes) and non-Ig like (red and green boxes) domains, which presumably assemble with a heavy chain into an Ig-like structure with a new central region.

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these joints). Therefore, about half of all pro-B cells fail to make μ protein at all, and a fraction of μ proteins might be expected to not associate efficiently with IgL. It is at this stage that the pre-BCR is essential in signaling the successful assembly of a functional μ chain gene.

The pre-BCR consists of a clonally expressed μ chain in a membrane-associated complex with the surrogate light chains (SLCs) VpreB and λ5 and the signaling chains Igα and Igβ. The SLCs were discovered by their homology to conventional λ light chain and their unusual pattern of expression: high in pro-B cells, diminished in pre-B cells and absent in more-mature cells[2]. Targeted disruption of the unique gene encoding λ5 (two genes encode nearly identical VpreB proteins) results in a partial but very important block in development at the pro-B-to-pre-B cell transition[3]. Deletion of the μ membrane exons results in a complete block at this same transition[4], whereas null mutations in genes encoding various downstream signaling molecules also result in partial blocks. Thus, compelling genetic evidence points to the pre-BCR signal as a critical checkpoint in B cell development.

Pre-BCR assembly has a variety of developmental consequences (**Fig. 1**), including activation of the cell cycle leading to several rounds of cell division, transient inactivation of the recombinase, alterations in the expression of a variety of genes, and changes in chromatin structure that lead to retargeting of the recombinase away from *Igh* and toward the light-chain loci, resulting in *Igh* allelic exclusion[5]. Among the genes affected by pre-BCR expression are those encoding VpreB and λ5, resulting in the feedback inhibition of pre-BCR assembly and thus limiting the duration of the pre-BCR signal. It is possible that the disappearance of the pre-BCR serves as a 'molecular clock' that limits the amount of time a pre-B cell has to successfully rearrange and express a light chain gene as part of a proper BCR, as B cells that lack surface receptor undergo apoptosis. Proliferative expansion of populations of cells that successfully produce an appropriate μ protein increases the efficiency of B cell development.

Pre-BCR assembly is known to influence the B cell repertoire in at least one way: some μ proteins complex with the SLCs more readily than others, resulting in the clonal selection of pre-B cells based on the structure of their heavy chains[6]. It is possible that the pre-BCR might also serve to test the specificity of μ proteins through interaction with a positively selecting ligand in the developing microenvironment. Such a ligand might be specific for clonotypic aspects of individual μ proteins. Pre-BCR signaling clonal deletion might occur at this stage based on high-affinity interaction with a self antigen. Alternatively, the pre-BCR may function independently of μ clonotype by interacting with a ligand that binds to either the μ constant domain(s) or to the SLC component of the pre-BCR. Previous evidence has indicated that a stromal cell surface ligand[7], perhaps galectin-1 (ref. 8), might interact with the pre-BCR to trigger its activity (**Fig. 1**). Finally, it has been suggested that the pre-BCR might signal in a ligand-independent way. It is this last assertion that is supported by the new work by Ohnishi and Melchers.

Several experiments already published indicate ligand-independent signaling. First, two groups independently showed that a mutant *Igh* transgene, missing its variable-region exon and different amounts of constant region, can nonetheless move to the pre-B cell surface and signal the pro-B-to-pre-B transition, even in a λ5-deficient genetic background[9,10]. These results were used to argue that the mere presence of the pre-BCR in the unique milieu of the pre-B cell membrane might colocalize a sufficient number of positively acting signaling molecules to result in constitutive activation[5]. Consistent with this idea is the observation that BCR expression on the surface of mature naive B cells is essential for their survival. Thus, the BCR must send a tonic survival signal to the cell in the absence of antigen; perhaps the pre-BCR can signal in this way as well. Second, treatment with antibody to μ or to SLC neither interferes with nor enhances the pro-B-to-pre-B cell transition *in vitro* or *in vivo*. The final piece of evidence in support of ligand independence comes from mutational experiments done on the surrogate-chain component of the pre-T cell receptor (TCR) preTα[11]. Deletion of nearly the entire ecto domains of preTα and TCRβ nonetheless results in a pre-TCR capable of mediating the pro-T to pre-T transition. Thus, by analogy, the pre-BCR may likewise be independent of its ecto domain and therefore be ligand independent. Neither of these sets of experiments could adequately control for the possibility that mutant pre-BCR or pre-TCR complexes might aggregate in the cell membrane, thus signaling in an artefactual way.

The paper in this issue of *Nature Immunology* from Ohnishi and Melchers also supports the idea that the pre-BCR does not require a ligand, but with a twist: the pre-BCR might serve as its own ligand. The C terminus of VpreB and the N terminus of λ5 contain unique sequences rich in evolutionarily conserved charged amino acids. Ohnishi and Melchers generated various λ5 mutants that lack or disrupt this unique N terminus and assayed them for function by retroviral transduction into a λ5-deficient, μ-expressing Abelson virus–transformed pre-B cell line. They found that cells expressing several of these mutant λ5 proteins displayed increased surface expression of the pre-BCR, showed considerably delayed pre-BCR internalization, expressed lower amounts of associated phosphotyrosinated proteins and failed to form high-molecular-weight aggregates, all in contrast to cells expressing wild-type λ5. Each of these characteristics is consistent with what would be expected in the absence of pre-BCR signaling. Identical λ5-deficient cells transduced with wild-type *Igk* acted like cells transduced with genes encoding various mutations of the N-terminal region of λ5. These results show that simply getting a pre-BCR complex to the cell surface may not be enough to trigger signaling. Appropriate pre-BCR signaling may depend on the unique non-Ig-like region of λ5 (and perhaps VpreB). Furthermore, as these experiments used clonal populations of transformed pre-B cells, they show that extrinsic ligand was not required for pre-BCR signaling. The authors suggest that either pre-B cells themselves express a macromolecule that specifically interacts with and cross-links the non-Ig region of SLCs, or that SLCs can directly interact with one another through these non-Ig domains, resulting in aggregation and signaling.

Although previously unknown and unexpected, these data do not represent the final chapter in our understanding of the pre-BCR. First, these studies used exclusively virally transformed pre-B cells, in which signaling is obviously aberrant. Analysis of essential SLC mutants in primary developing B cells using targeted germline mutant mice will be necessary to corroborate these results. Second, the assays used to infer pre-BCR function in this study (aggregation, membrane uptake and protein phosphotyrosination) are all indirect. Mutant λ5 proteins must be assayed for their ability to induce in pre-B cells proliferation, changes in gene expression and alterations in the regulation of V(D)J recombination. Third, this study leaves open the possibility that a cross-linking ligand for the pre-BCR might be expressed by pre-B cells themselves. It will be difficult to disprove this. Finally, these results must be reconciled with the studies involving

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mutant μ chains and mutant TCRβ and preTα chains described above.

The next main frontier to be traversed in attempting to understand pre-BCR function lies downstream of the receptor. Which signaling pathways and effector molecules are responsible for the critical effects of pre-BCR signaling and how do they work? There are already indications that independent pathways mediate different aspects of pre-BCR signaling. Studies taking advantage of targeted null mutants made for other purposes have shown the importance of the B cell costimulatory molecule CD19 and the adapter protein BLNK[12], but many important details remain to be discovered.

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ComPLEXIN new targets for CIITA

Walter Reith

Growing evidence indicates immune and nervous systems use common mechanisms to mediate intercellular communication. Adding to this list is the discovery that dendritic cells modulate T cell interactions through expression of the neuronal receptor plexin-A1, which is regulated by the transcriptional activator CIITA.

Evidence has accumulated over the past 4 years that proteins called semaphorins and their receptors, first identified as molecules providing guidance cues for neuronal axons in developing neural tissues, are essential in the immune system by modulating interactions between T cells and antigen-presenting cells. In this issue of *Nature Immunology*, Wong *et al.*[1] report that the semaphorin receptor plexin-A1 is expressed abundantly in mature dendritic cells (DCs) and that this expression is dependent on the major histocompatibility complex (MHC) class II–specific transactivator CIITA. Furthermore, they document that plexin-A1 expression in DCs is required for optimal stimulation of T cells. These findings not only shed new light on the specificity and function of CIITA but also extend our knowledge of the function of semaphorin receptors in the immune response.

CIITA is a transcription factor that controls most quantitative and qualitative aspects of MHC class II expression[2,3]. It was first identified as a key transactivator of MHC class II genes because it was found to be mutated in certain patients with bare lymphocyte syndrome, a primary immunodeficiency disease resulting from the nearly complete absence of MHC class II expression[2]. Subsequent work demonstrated that CIITA also activates the expression of genes encoding accessory proteins required for MHC class II restricted antigen presentation—namely the invariant chain, HLA-DM and HLA-DO—and contributes to a lesser degree to MHC class I expression[2,3]. It was long believed that CIITA was highly specific for these genes because the clinical and immunological phenotypes of patients with bare lymphocyte syndrome could all be accounted for by the defects in MHC expression[2]. This view was challenged, however, by recent reports indicating that CIITA can modulate the expression of non-MHC genes. A gene expression profiling experiment has indicated that CIITA affects the expression of

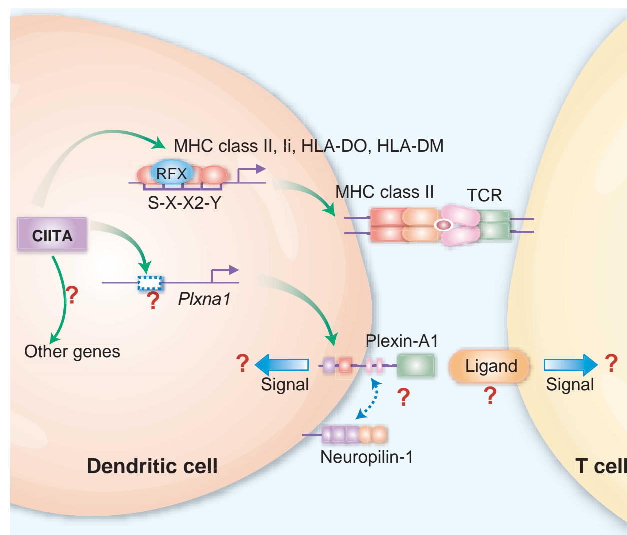

Figure 1 Plexin-A1, a target of CIITA, is linked to DC–T cell interactions. CIITA activates genes involved in MHC class II–mediated antigen presentation by binding to a multiprotein 'enhanceosome' complex assembled on the S-X-X2-Y regulatory modules conserved in their promoters. The MHC class II–specific transcription factor RFX is a key component of the enhanceosome. CIITA also activates, by an as-yet-unknown mechanism, the gene (*Plxna1*) encoding plexin-A1, a neuronal semaphorin receptor contributing to DC-mediated T cell stimulation. Unresolved questions and future directions of research are indicated by question marks. Ii, invariant chain.

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