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# Transcriptional and metabolic pre-B cell receptor-mediated checkpoints: Implications for autoimmune diseases

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ABSTRACT

At the pre-B cell stage of lymphocyte development, immunoglobulin light-chains are not yet produced, and heavy-chains are covalently linked to surrogate light-chains composed of VpreB and λ5 to form the pre-B cell receptor (pre-BCR) in a non-covalent association with signal-transducing modules. Even tough the pre-BCR does not have the potential to bind conventional antigens, accumulating evidence indicates that pre-BCR-mediated checkpoints are important both for negative and positive selection of self-reactivity, and that defects in these regulatory nodes may be associated with autoimmune disease. Thus, the transcription factor BACH2, which represents a susceptibility locus for rheumatoid arthritis, has recently emerged as a crucial mediator of negative selection at a pre-BCR checkpoint. The lysosome-associated protein LAPTM5, which is highly expressed in an animal model of Sjögren's syndrome, plays a role in down-modulation of the pre-BCR. Studies of copy number variation in rheumatoid arthritis suggest that a reduced dosage of the *VPREB1* gene is involved in disease pathogenesis. Notably, animal models of autoimmune disease exhibit defects in pre-B to naïve B cell checkpoints. Administration of a pre-BCR ligand, which also plays a role in anergy both in human and murine B lymphocytes, ameliorates disease in experimental models of autoimmunity. Further investigation is required to gain a better insight into the molecular mechanisms of pre-BCR-mediated checkpoints and to determine their relevance to autoimmune diseases.



## 1. Introduction

In the adult bone marrow (BM), B lymphocytes develop from pluripotent hematopoietic stem cells through an ordered process that involves differential expression of cell-surface markers, sequential rearrangement of immunoglobulin (Ig) heavy (H) and light chain (L) gene loci, and expression of stage-specific genes (Melchers, 2005; Sakaguchi and Melchers, 1986). At the progenitor (pro-)/precursor (pre-) BI cell stage, the cells have initiated expression of the Ig surrogate L-chain (SLC) consisting of the VpreB and λ5 chains. At the pre-BII stage, they express the pre-B cell receptor (pre-BCR) composed of the newly rearranged and synthesized H-chain and the SLC on the cell surface. Importantly, not all rearranged H-chains can pair with the SLC to form a pre-BCR. In mice, only half of the in-frame rearranged H-chains pair productively with SLCs (ten Boekel et al., 1997), and only B cells that express an H-chain capable of pairing with the SLC undergo clonal expansion (Hess et al., 2001). After expansion of pre-BII cells, expression of the SLC vanishes as pre-B cells develop into immature B cells that express IgM on the surface as the BCR. Then, cells exit the BM and migrate to peripheral lymphoid organs where they mature and take part in humoral immune responses. Several observations indicate that pre-BCR-mediated checkpoints are important both for negative and positive selection of self-reactivity. This review will discuss the potential implications of these checkpoints to our understanding of autoimmunity (Fig. 1).

## 2. Transcriptional regulation of pre-B cells and autoimmunity

In the mouse, transgenic expression of SLC components throughout B cell development does not alter pre-B cell proliferation and differentiation, results in deletion of immature B cells, and induces constitutive BCR signaling and activation of mature B cells (van Loo et al., 2007). Notably, the presence of SLC induces a significant amount of secondary rearrangements of endogenous Ig L-chain loci (van Loo et al., 2007); and silencing of SLC genes does not seem to be essential for the limitation of pre-B cell proliferation, but is required for the prevention of constitutive B cell activation (Melchers, 2005). The signals that regulate pre-B cell expansion

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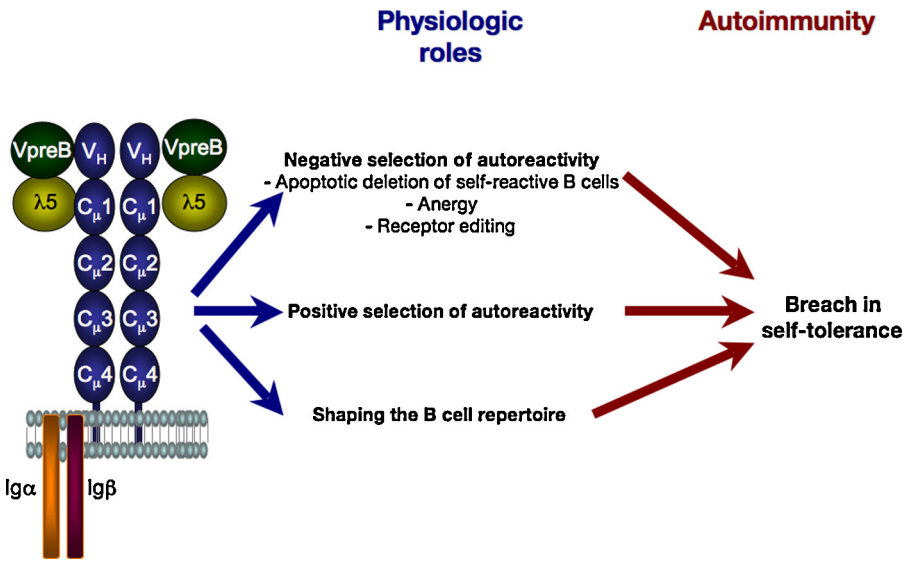


**Fig. 1.** *Pre-B cell receptor-mediated checkpoints and autoimmunity.* Shown is a schematic view of the structure of the pre-B receptor, and its major potential functions in regard to immune tolerance to self.

are not completely understood. Primary pre-B cells were shown to be subject to a broad feedback inhibition of pre-BCR signaling components (Hauser et al., 2013). In addition, Interferon regulatory factors (IRFs) also seem to play a role. IRF4 and 8 are structurally related, hematopoietic cell-specific transcription factors that cooperatively regulate the differentiation of B cells. A series of studies have established an additional role for IRF4 and 8 as essential regulators of B cell development. They have been shown to be critical for pre-B cell development, receptor editing, germinal center reaction, and plasma cell differentiation (Lu et al., 2003). For example, B cell development is almost completely blocked at the large pre-B cell stage in IRF4 and 8 compound mutant mice (*Irf4,8*$^{-/-}$). Moreover, IRF4 and IRF8 were found to be sufficient individually to rescue the development of *Irf4,8*$^{-/-}$ pre-B cells, confirming that they function redundantly in pre-B cell development (Ma et al., 2006). Interestingly, *Irf4,8*$^{-/-}$ pre-B cells are hyperproliferative, suggesting that IRF4 and 8 negatively regulate pre-B cell proliferation. They are critical not only for L-chain rearrangements, but also for limiting pre-B cell expansion.

Analysis of a heterozygous mutant mouse, in which the *NF-kappaB-dependent I-kappa-b-alpha* gene was replaced with a *Lacz* reporter complementary DNA, allowed detection of a subpopulation of pre-B cells that contain active nuclear NF-κB and express increased levels of various markers of receptor editing (Cadera et al., 2009). Intestingly, *Irf4* transcripts were up-regulated in the pre-B cells of the mutant mice. Since IRF4 is a target of NF-κB and is required for receptor editing, it is possible that NF-κB could act through IRF4 to regulate receptor editing. The correlation between NF-κB and IRF4 activity, and receptor editing may indicate a functional role for these transcription factors in self-tolerance. As regards *Irf8*, mice deficient in this transcription factor, both germline and conditional knockout, produced anti-dsDNA antibodies, and B cell anergy was breached in the *Irf8*-deficient mice (Pathak et al., 2013). However, the effects on pre-B cells were not determined. Since several observations showed that receptor editing and L-chain gene rearrangements are defective in B cells of patients with autoimmune disease (Radic and Zouali, 1996; Zouali, 2008), it will be of interest to probe the contribution of IRF4 and 8 to receptor editing in autoimmunity.

It has been estimated that the murine BM produces approximately $10^6$ pre-B cells daily (Osmond, 1991), most of which will die unless they undergo productive $V_H$-$DJ_H$ gene rearrangements followed by pre-BCR signaling and transition into the long-lived peripheral B cell pool (Melchers, 2005). Fidelity of the B cell repertoire is maintained by negative selection of pre-B cells with nonfunctional Ig H-chains that precedes the survival of those expressing functional rearrangements. Pre-B cells that have productively rearranged $V_H$-$DJ_H$ gene segments and emerged from the pre-BCR checkpoint are rescued by BCL6, a transcriptional repressor that acts as a crucial survival factor (Nahar et al., 2011). In addition, BACH2 has recently emerged as a crucial mediator of negative selection at the pre-BCR checkpoint (Swaminathan et al., 2013). In normal pre-B cells, BACH2-mediated activation of p53 is opposed by BCL6, which is a potent transcriptional repressor of p53. While the role of BACH2 in B cell tolerance remains to be investigated, a combined meta-analysis of 17,581 cases and 20,160 controls provided convincing evidence that *BACH2* represents a susceptibility locus for rheumatoid arthritis (McAllister et al., 2013).

## 3. A pre-B cell metabolic checkpoint

Normally, potentially autoreactive lymphocytes are removed by apoptosis during development and after completion of an immune response. In autoimmune diseases, however, there is abnormal lymphocyte activation and cell death, and defective activation-induced cell death may be responsible for persistence of autoreactive cells. Importantly, both cell proliferation and apoptosis are energy-dependent processes. Recently, a phenotype-driven "reverse-genetic", recessive ethylnitrosourea mutagenesis strategy was used in mice to discover genes involved in immune cell development and function (Park et al., 2012). By genome scanning, a B cell immunodeficiency phenotype was mapped to a non-coding deletion in the *Fnip1* gene, which encodes the 160-kDa protein folliculin interacting protein-1 (FNIP1). *Fnip1* germ line targeting led to a marked pro-B cell arrest, which cannot be rescued by rapamycin treatment and is thus mTOR independent (Baba et al., 2012). Transcriptome analyses of *Fnip1*$^{-/-}$ pro-B cells revealed compromised expression of key genes expressed in B cells, including *VpreB1*/*VpreB2*, λ5, and *Rag1*/*Rag2*. The block is driven by caspase activation and intrinsic cell death, and expression of the BCL2 anti-apoptotic transgene promotes normal numbers of peripheral B cells in the knockout mice.

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