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NF-κB Comes Home

NF-κB was discovered because of its binding to the *Igκ* locus intronic enhancer, but deletion of its binding site does not appear to affect V-to-Jκ rearrangement. New work by Verkoczy et al. (2005) in this issue of *Immunity* suggests that NF-κB regulates *Igκ* rearrangement after all, by activating *RAG* expression during receptor editing.

Although V(D)J recombination allows for the formation of a near-limitless repertoire of antigen receptor molecules, it frequently generates receptors with self-specificity (Wardemann et al., 2003). The potential for self-reactivity is tested at the immature (IgM^+IgD^-) stage of bone marrow B cell development. Antigen encounter at this stage leads to rapid removal of the offending receptor from the cell surface and continued *RAG* gene expression and Ig light-chain gene rearrangement until an innocuous receptor capable of promoting development is made (Nemazee and Weigert, 2000). Cells that fail to produce a "good" receptor are either deleted or anergized. This editing process ensures that self-reactive B cells do not survive to maturity, thus preventing autoimmunity. Analysis of various knockout and BCR transgenic mice have revealed the importance of the B cell receptor signaling complex and signal strength in mediating developmental progression, the induction of editing, or cell death (Diamant et al., 2005). Both strong signaling in response to BCR crosslinking or weak signaling due to poor heavy- and light-chain pairing and reduced BCR cell surface expression activate receptor editing. Downstream effectors of immature BCR signaling—namely the transcription factors involved in continued *RAG* expression and gene rearrangement—remain largely unknown, however. Now, in this issue of *Immunity*, Verkoczy et al. (2005) show that NF-κB proteins are involved in upregulation of *RAG1* and *RAG2* transcription in cells undergoing receptor editing.

Direct involvement of NF-κB in regulating V(D)J recombination completes an arc of investigation begun nearly 20 years ago. NF-κB was first discovered based on its ability to bind to κB sites within the *Igκ* intronic enhancer (Sen and Baltimore, 1986). Subsequently, NF-κB's involvement in gene regulation was shown to be much more ubiquitous, playing key roles in developmental, proinflammatory, and cell survival pathways in most metazoan organisms. NF-κB homo- or heterodimers made up p50, p52, p65 (RelA), RelB, or c-Rel are retained in the cytoplasm by association with inhibitor of NF-κB (IκB) proteins (Hayden and Ghosh, 2004). Phosphorylation of IκB by IκB kinase (IKK) results in its degradation and translocation of NF-κB into the nucleus, where it binds to cognate κB sites within the enhancers or promoters of target genes, thereby affecting transcription. Previous work related to NF-κB in the immune system has focused on its role in innate immunity and antigen-receptor signaling. The new work brings NF-κB back into the realm of early lymphoid development and antigen receptor gene assembly. Verkoczy et al. (2005) found that the increase in *RAG* mRNA levels seen in immature B cells after BCR crosslinking was due to an increased rate of transcription rather than alterations in mRNA processing or stability. Moreover, when immature cells were treated with the protein synthesis inhibitor cyclohexamide, they found a similar increase in *RAG* mRNA levels. This pointed toward a short-lived inhibitory protein as a control point in this pathway. IκB, the protein that retains NF-κB complexes in the cytoplasm, is known to have an extremely short half-life and is regulated mainly at the level of protein stability. Treatment of cells with various inhibitors of NF-κB activation blocked cyclohexamide or anti-BCR-induced *RAG* mRNA upregulation, thus confirming the role of NF-κB. EMSA analysis of κB binding proteins in nuclear extracts showed that increased *RAG* transcription was correlated with higher nuclear levels of p65 and c-Rel relative to p50, and chromatin immunoprecipitation studies demonstrated direct binding of NF-κB complexes to *RAG* locus enhancers. Because p65 and c-Rel contain transactivation domains, whereas p50 does not, the authors contend the system operates by replacing inhibitory p50 homodimers with complexes containing p65 or c-Rel. Accordingly, they found that immature B cells from $p50^{-/-}$ mice have a higher basal level of *RAG* mRNA and a hyperediting phenotype, with increased levels of both κ light chain and κ RS rearrangement.

At a superficial level, these data seem in conflict with new evidence showing that loss of tonic BCR signaling at the immature stage leads to upregulation of *RAG* gene expression and receptor editing along with other transcriptional changes consistent with de-differentiation to a pre-B cell like state (Tze et al., 2005). Interestingly, that report also shows that transcript levels of *IκBα*, a known direct target of NF-κB, increases upon loss of BCR signaling. How is it that both BCR crosslinking, which activates signaling cascades, and loss of tonic BCR signaling result in increased editing? One possible explanation is that antigen encounter by immature B cells causes rapid receptor internalization and subsequent loss of tonic BCR signaling, and this in turn activates NF-κB nuclear translocation or causes a change in the subunit composition of nuclear NF-κB complexes. This would also explain why mutations that decrease BCR signal strength increase receptor editing (Diamant et al., 2005). The exact pathway by which receptor engagement and internalization might lead to NF-κB activation in immature B cells remains to be discovered. Because NF-κB is also downstream of BCR signaling in mature B cells, other transcription factors must become limiting for *RAG* transcription in later

stages of development or repressors must prevent *RAG* locus transcription in these cells.

Another important result from the Verkoczy paper is that the RAGs appear to be limiting for receptor editing. In a transgenic anti-self BCR system, *RAG1*$^{+/-}$ mice showed significantly reduced editing compared to *RAG1*$^{+/+}$ mice. This indicates that the *Ig*κ and possibly *Ig*λ loci are constitutively accessible to the recombination machinery in immature B cells, thereby making RAGs limiting in the editing reaction. Indeed a recent publication from the same laboratory reaches an identical conclusion in mice with a polyclonal population of B cells (Aït-Azzouzene et al., 2005). How allelic exclusion is enforced in a system with such a high rate of recombination remains a mystery.

NF-κB was discovered because of its ability to bind to the κ intronic enhancer, and based on correlations between its activation and increased V-to-Jκ rearrangement in transformed pre-B cell lines, it was initially thought to be a controlling factor in recombination of that locus (Schlissel and Baltimore, 1989). A recent report has shown that mutation of the κB element within the enhancer appears to have no effect on κ locus recombination (although that report did not assess the necessity of the κB binding site in an editing context) (Inlay et al., 2004). Yet as Verkoczy et al. (2005) demonstrate, it was premature to rule out NF-κB as a critical regulator of light-chain gene rearrangement.

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