Commentary

Regulation of Immune Responses by NF-κB/Rel Transcription Factors

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Within peripheral lymphoid organs, complex interactions occur between lymphocytes and antigen-presenting cells during an immune response to antigen. Although there is considerable recognition of the fact that activation of innate immune signaling pathways is necessary for productive adaptive immune responses to occur, a great deal is still unknown about how this coordination is achieved at a molecular level. NF-κB/Rel transcription factors have been the focus of considerable interest over the past few years, in part because they seem ideally positioned to integrate information from both innate and adaptive immune signaling pathways. Recent develoments with gene-targeted knockout mice indicate that NFκB/Rel transcription factors are critical regulators of immune responses at the level of both antigen-presenting cells and lymphocytes.

NF-κB/Rel transcription factors function as dimers held latently in the the cytoplasm of cells by a family of inhibitor IκB proteins (for reviews see references 1–3). There are five known mammalian NF-κB/Rel proteins: Rel (c-Rel), p65 (RelA), RelB, p50 (NFKB1), and p52 (NFKB2). Both the p105 precursor of p50, and the p100 precursor of p52, possess domains that function as IκBs, and there exist at least five distinct IκB proteins: IκBα, IκBβ, IκBε, IκBγ, and bcl-3.

NF-κB/Rel transcription factors are activated by a surprising variety of different signaling pathways involved in immune function and development. Signaling pathways involved in innate immune responses that activate these factors include a newly identified human homologue of *Drosphila* Toll (4), the cytokines TNF-α and IL-1α, the chemotactic peptide fMet-Leu-Phe (5), as well a variety of different bacterial and viral products (1–3). Signaling pathways involved in adaptive immune responses that activate these factors include key lymphocyte receptor signaling pathways such as antigen receptors on B and T cells, CD28 on T cells, and CD40 on B cells (1–3). These signaling pathways converge on phosphorylation and degradation of IκBs, which unmask a nuclear localization signal that leads to translocation of NF-κB/Rel dimers into the nucleus. Recently, several of the kinases involved in phosphorylation of IκBs have been identified, and studies of these long sought after IκB kinases should provide significant insight into the regulation of activation of NF-κB/Rel transcription factors (6–12).

In this issue of *The Journal of Experimental Medicine*, Franzoso et al. report on the phenotype of knockout mice lacking p52. These mice have impaired splenic marginal zone architecture and defective germinal center formation after immunization with T-dependent antigens. Intriguingly, in contrast to knockout mice of the most closely related p50 family member, defective T-dependent responses in p52 knockout mice appear to result from a defect in accessory cell function, rather than lymphocyte function.

Role in Antigen-presenting Cell Differentiation and Function. Some clues as to the function of different NF-κB/Rel family members in immune function have come from analyses of gene and protein expression in lymphoid organs. Whereas p50, Rel, and RelA are found highly expressed in all hematopoietic-derived cells, including lymphocytes, the expression pattern of p52 and RelB appears to be more restricted (13). In situ analysis of protein expression in human tonsil and lymph nodes reveals that physiologically high levels of nuclear p52 is restricted to accessory cells of the immune system including follicular dendritic cells, dendritic cells, and macrophages in the T cell zone (14). In adult lymphoid tissues, high expression of RelB is limited to dendritic cells found in the periarterial lymphatic sheaths of the spleen, the deep cortex of the lymph nodes, and thymic medulla (15).

Consistent with these localization studies, p52 and RelB appear to play important and distinct roles in antigen-presenting cell function. Based on staining with markers for specific macrophage populations, Franzoso et al. report that p52 knockout mice have altered splenic marginal zone architecture (16). The marginal zone surrounding lymphoid follicles is thought to be an important site for regulation of cell migration during an immune response (17). Metallophilic macrophages, detected with the MOMA-1 antibody, are absent from the inner marginal zones of spleens from p52 knockout mice. This defect appears relatively specific as marginal zone macrophages, detected with the ERTR-9 antibody, are present in the outer marginal zones of these mice. Lymphoid follicles of p52 knockout mice are also abnormal, with depleted and absent B cell follicular areas, and the presence of BM8-staining macrophages that are normally excluded from the white pulp of wild-type mice. Immunization of p52 knockout mice with T-dependent antigens results in an impaired antibody response that is charac-

terized by an inability to form germinal centers and follicular dendritic cell networks. Interestingly, this inability to generate germinal centers is not a lymphocyte-intrinsic property as adoptively transferred p52-deficient lymphocytes were able to form germinal centers.

Several studies implicate RelB as a critical regulator of the differentiation of dendritic cells. Dendritic cells are potent antigen-presenting cells that enter resting tissues as precursors and, after antigenic exposure, differentiate and migrate to draining lymph nodes. In RelB knockout mice, numbers of dendritic cells are severely reduced (18–19). In studies examining RelB activity in immature and differentiated human dendritic cells, immunohistochemical staining demonstrated RelB within differentiated lymph node interdigitating and follicular dendritic cells, but not undifferentiated dendritic cells in normal skin (20). Active nuclear RelB was detected by supershift assay only in differentiated dendritic cells and in activated B cells.

The distinct phenotypes observed in antibody responses of other knockout mice suggest that individual NF-κB/Rel members likely regulate different cellular events that take place during antibody responses. Rel knockout mice, for example, exhibit defective antibody responses to both T-independent and T-dependent antigens (21). In contrast, p50 knockout mice have defective responses to only T cell–dependent antigens but germinal center formation is normal (22), whereas p52 and bcl-3 knockout mice have defective T-dependent responses as well as defective germinal center formation (23, 24). In addition, defective antibody responses in p52 knockout mice are observed only in the absence of immunization with adjuvant, whereas defective antibody responses in Rel and p50 knockout mice are observed in mice immunized with adjuvant. Additional adoptive transfer experiments should help sort out whether the cellular basis for these defects occurs in lymphocytes, antigen-presenting cells, or both.

Role in T Lymphocyte Activations.   Recent work has begun to clarify a role for NF-κB/Rel transcription factors in the regulation of T cell activation. In the absence of exogenous IL-2, activation and subsequent proliferation of T cells is critically dependent on the c-Rel transcription factor. T cells from c-Rel knockout mice are unresponsive to most mitogenic stimuli including concanavalin A, and cross-linking of antigen receptor and CD28 (21). Exogenous IL-2, however, can restore the ability of these c-Rel knockout T cells to proliferate, suggesting a possible role for c-Rel in the regulation of IL-2 transcription. Several groups have recently defined such a role for c-Rel, by demonstrating that stimulation of CD28 on T cells induces the association of c-Rel to a CD28 response element (CD28RE) in the IL-2 promoter (25–27). This CD28RE is a variant NF-κB binding site that was previously defined as critical for transcriptional upregulation of IL-2 by CD28 receptor activation in T cells.

Role in B Lymphocyte Activation and Class Switching.   As with T lymphocytes, analyses of different knockout B cells reveal that NF-κB/Rel factors regulate B cell activation and proliferation by a variety of general B cell activators, including lipopolysacharride, CD40 ligand, and antigen-receptor cross-linking (21, 22, 28, 29). Interestingly, emerging studies also reveal that NF-κB/Rel activation by these same general B cell activators regulates immunoglobulin heavy chain class switching to specific isotypes.

It has been recognized for some time that general B cell activators, in addition to activating B cell differentiation, also participate in regulation of isotype switching. Recent studies from several groups have begun to pinpoint NF-κB/Rel transcription factors as important signaling intermediates in the regulation of isotype switching by general B cell activators. Snapper's group has recently demonstrated through in vitro studies that B cells from Rel and p50 knockout mice have distinct intrinsic defects in their ability to class switch to IgE, IgA, and IgG1 (30–32). Stavnezer's group has identified κB binding sites within the germline C_H promoters regulating class switching to IgG1 and IgE, and has demonstrated distinct regulation of these κB binding sites by different NF-κB/Rel members (33–35). Birshtein's group has also identified κB binding sites within the 3′ IgH enhancers that have been implicated in isotype switch regulation (36–37). Taken together, these studies suggest that distinct NF-κB/Rel dimers may regulate class switching to specific isotypes.

The involvement of NF-κB/Rel factors in isotype switching is intriguing because it suggests several cell-intrinsic mechanisms by which B cells could use the complex regulation of NF-κB/Rel factors to further regulate cell-extrinsic signals. Studies with B cell lines and primary B cells indicate that the composition of NF-κB/Rel dimers found in B cells is variable and reflects both the activation and differentiation state of a B cell, presumably due in part to transcriptional cross-regulation of different family member genes (38–40). Thus, activation of the same extracellular signaling pathways may lead to the translocation of different NF-κB/Rel dimers depending upon the state of the B cell when activated. Since the pool of NF-κB/Rel dimers available for translocation likely reflects information from multiple signaling pathways, use of NF-κB/Rel transcription factors to regulate isotype switching may represent one mechanism by which information from innate and adaptive signaling pathways can be integrated in the response of B cells.

Regulation of Immune Homeostasis.   The importance of NF-κB/Rel transcription factors in immune regulation is highlighted by defects observed in immune homeostasis in knockout mice. Recent studies from Bravo's group suggest a similar regulatory role for both the p105 precursors of p50, and the p100 precursor of p52 (41). In mice lacking both p105 and p50 proteins, no obvious developmental immune defects were observed (22). In contrast, Bravo, R., D. Dambach, E. Claudio, C. Ryan, and H. Ishikawa (personal communication) report that mice lacking the p105 precursor, but still expressing p50, exhibit chronic inflammation. This result suggests an important role in maintaining immune homeostasis for the p105 precursor. Similarly, mice lacking both p100 and p52 proteins reported in this issue by Franzoso et al. do not exhibit an inflammatory phenotype, whereas mice lacking only the p100 precursor

exhibit gastric hyperplasia, enlarged lymph nodes, and enhanced cytokine production by activated T cells (41). These studies suggest an important role in maintaining immune homeostasis for both p105 and p100 precursors. A complex inflammatory phenotype characterized by myeloid hyperplasia and splenomegaly due to extramedullary hemopoiesis has also been reported for RelB knockout mice (19).

Future Prospects. Results from transgenic and knockout mice are beginning to reveal how NF-κB/Rel transcription factors function as critical mediators of immune responses. The complex regulation of this transcription factor family by multiple innate and adaptive signaling pathways within multiple cell lineages, however, represent formidable obstacles to developing a clearer understanding of how these transcription factors function in immune regulation. Although reconstitution and transfer experiments using different knockout models will continue to be crucial in sorting out the individual contributions of different cell lineages to defective responses, it will likely remain a difficult issue to define which NF-κB/Rel dimers regulate specific responses due to the disruption of multiple dimer complexes in individual knockout mice.

The recognition that NF-κB/Rel transcription factors are critical regulators of immune responses at the level of both antigen-presenting cells and lymphocytes poses several intriguing issues for future study: (*a*) Are the defects in germinal center formation and antigen-presenting cells reported for p52 knockout mice related to the defects observed in TNF ligand and receptor knockout mice (16)? (*b*) When and where are NF-κB/Rel transcription factors activated by innate and adaptive immune signaling pathways during an immune response? (*c*) Can information from the activation of multiple signaling pathways be integrated within individual cells through NF-κB/Rel transcription factors?

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