Commentary

An Alternative to Lung Inflammation and Fibrosis

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The literature clearly links canonical activation of nuclear factor κ B (NF- κ B) in immune cells to the pathogenesis of inflammatory diseases.¹ Less well understood is activation of NF- κ B via the alternative/noncanonical pathway, the function of NF- κ B in nonimmune cells, and the NF- κ B-dependent signaling between immune and structural cells within an inflamed tissue. The article by Yang et al, in this issue of *The American Journal of Pathology*, addresses these topics.²

The NF- κ B family of transcription factors, which includes five members (p65 (RelA), c-Rel, RelB, p50/p105, and p52/p100), regulates inflammation, survival, proliferation and other biological processes (see Refs. 1 and 3 for recent reviews). Members of the family bind DNA and regulate transcription as hetero- and homodimers, with p65/p50 heterodimers the most abundant, followed by homodimers of p50 and p65 in relative abundance.⁴ In resting cells, NF- κ B exists in the cytoplasm in an inactive complex bound with any of a number of inhibitor of NF- κ B (I κ B) molecules. Two signaling pathways, canonical and noncanonical, lead to NF- κ B activation and translocation to the nucleus.

The I κ B kinase complex, which consists of two catalytic subunits (IKK α /IKK1 and IKK β /IKK2) and one regulatory subunit (IKK γ /NF- κ B essential modulator), controls NF- κ B activation.^{1,3} In the canonical pathway of NF- κ B activation, IKK β -dependent phosphorylation of I κ B α initiates degradation of the inhibitor and nuclear translocation of the active dimer, frequently p65/p50. The alternative pathway is constitutively active at a low level but can be induced by stimuli associated with organogenesis of lymphoid tissue and lymphocyte development.⁵ Alternative NF- κ B activation proceeds through IKK α -dependent phosphorylation and consequent C-terminal processing of p100 to p52 and, generally, nuclear accumulation of the p52/RelB dimer.^{5,6}

Alternative NF- κ B Activation in Lymphocytes

In their report, Yang et al² selectively activated the alternative pathway in lymphocytes by preparing mice that express mature p52 from a lymphocyte-specific promoter (p52-Tg mice). Because p52 activates expression of its

p100 precursor and p100 also functions as an I κ B,⁷ the authors ensured constitutive activation of p52 in lymphocytes by selectively interbreeding the p52-Tg mice to p100-null mice to yield p52-Tg, p52-Tg/p100^{-/-}, and p100^{-/-} littermates. Activation of NF- κ B in lymphocytes was confirmed by immunoblot analyses of nuclear extracts from p52-Tg/p100^{-/-} thymocytes, which revealed significant increases in p52, as expected, but also elevated levels of the binding partners RelB and c-Rel relative to wild-type littermates. Similar analyses of thymocytes from p52-Tg mice also revealed evidence of NF- κ B activation, but at a more modest level presumably because of the I κ B activity of p100. Despite this similarity, the p52-Tg/p100^{-/-} and p52-Tg mice displayed dramatically different phenotypes. The p52-Tg/p100^{-/-} mice had reduced survival with pronounced lung inflammation, diffuse alveolar damage, and peribronchial fibrosis, whereas p52-Tg and p100^{-/-} littermates developed mild lung inflammation and lived normal life spans. Lymphocytic infiltrates in the lungs of p52-Tg/p100^{-/-} mice consisted predominantly of T cells with activated and memory CD4⁺ T helper (Th) cells, which suggests an ongoing immune response, an observation that emphasizes the role of p100 in limiting the immune response in lymphocytes.² It is possible that the extent of NF- κ B activation in lymphocytes in p52-Tg/p100^{-/-} mice accounts for the obvious phenotypic difference with p52-Tg and p100^{-/-} littermates, but inflammatory signaling in other cell types, immune and nonimmune, should be considered.

Loss of the p100-associated I κ B activity in all cells of p52-Tg/p100^{-/-} mice would likely enhance NF- κ B-dependent transcription, but the corresponding loss of p52 could produce multiple outcomes. Because p52 lacks a transcription activation domain, it can repress transcription on binding DNA as a homodimer or form a heterodimer with a transcription activation domain-containing partner and thereby alter the binding site preference of NF- κ B or activate transcription from NF- κ B sites by recruiting other transcription activation domain-containing partners.³ Consequently, it is impossible to predict the result of p52 overexpression or p100 deletion in a

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given cell type on NF- κ B-dependent transcription within that cell. One might assume, for example, that activation of transcription by p52 overexpression in lymphocytes accounts for the continuous activation of these cells. However, inhibition of NF- κ B prevents leukocyte apoptosis and TGF- β release associated with resolution of inflammation.⁸ In accord with this observation, overexpression of p52 and formation of repressive homodimers could prevent inflammation resolution by prolonging the viability of lymphocytes in 52-Tg/p100^{-/-} mice.

NF- κ B Function in Other Cell Types

Because p100 deletion alone does not produce prominent effects in mice, one must conclude that interactions between the p52-expressing activated lymphocytes and other cells in p52-Tg/p100^{-/-} mice, presumably in the lung, are regulated by p100, but the complexity of transcriptional regulation by p52/p100 cited above will make the dissection of p100-mediated effects difficult. Moreover, identification of the relevant lung cell type(s) that cooperate with the activated lymphocytes to perpetuate the inflammation further complicates the problem. Recent evidence indicates that NF- κ B function within epithelial cells of a tissue prevents destructive inflammation.⁹ Blockade of NF- κ B in keratinocytes or intestinal epithelium elicits inflammation that can be mitigated by disruption of tumor necrosis factor (TNF) receptor 1 signaling.^{10,11} Thus, the antiapoptotic activity of NF- κ B in epithelial cells prevents tissue damage from ongoing inflammation. Indeed, constitutive activation of NF- κ B in the intestinal epithelium via Toll-like receptor 5-dependent recognition of intestinal bacteria may be essential to maintain homeostasis of the intestine.¹² The lung may be resistant to the inflammatory consequences of NF- κ B inhibition in the epithelium,^{13,14} but complete ablation of NF- κ B in lung epithelial cells has not been reported.⁹ p52-Tg/p100^{-/-} mice express elevated levels of TNF- α ,² and it is possible that the absence of p100 sensitizes lung epithelial cells to TNF-induced apoptosis. In this scenario, induction of p100 expression may prolong NF- κ B-dependent transcription by fostering the exchange of p52/RelB heterodimers for p65/p50,¹⁵ thereby protecting epithelial cells from TNF-induced apoptosis by preventing negative feedback mechanisms that target p65-containing complexes.³ Activation of p100 by p52 would provide the autoregulatory means to sustain this protective NF- κ B activity. Hence, lung epithelial cells lacking p52/p100 would undergo TNF-induced apoptosis because of their failure to maintain NF- κ B-induced antiapoptotic activity in the presence of constitutively high levels of TNF- α .

The lack of p52/p100 in other cell types in the lung may contribute to the pathogenesis in other ways. Activated macrophages infiltrate the lungs of p52-Tg/p100^{-/-} mice.² Skin macrophages contribute to inflammation in a model of psoriasis,¹⁶ but in a murine model of colitis, macrophage-derived cytokines provide cytoprotective effects.¹⁷ This latter finding is consistent with the idea that NF- κ B function in infiltrating leukocytes associates with resolution of inflammation.⁸ The lack of p52/p100 in macrophages infiltrating the lungs of 52-Tg/p100^{-/-} mice may cause the cells to

contribute to, rather than to ameliorate, the lung inflammation. Potentially, p52-dependent transcriptional repression of promoters for proinflammatory mediators, eg, TNF- α , may assist in suppression of the inflammatory response.¹⁵ Therefore, the activated p100^{-/-} macrophage would remain so because it lacks p52-mediated transcriptional repression of proinflammatory mediators. In the absence of the three classical I κ Bs, p100 levels become elevated, and the majority of NF- κ B subunits remain cytoplasmic.¹⁸ This I κ B function of p100 that represses classical NF- κ B dimers^{1,7} may also serve to suppress continued macrophage activation. Thus, constitutive p52 expression in lymphocytes coupled with these potential disruptions of NF- κ B function in lung epithelial cells and/or lung macrophages could contribute to the fatal lung inflammation that develops in p52-Tg/p100^{-/-} mice.

Th Immunity in p52-Tg/p100^{-/-} Mice

Characterization of the inflammation that develops in p52-Tg/p100^{-/-} mice indicates a Th1 immune response with high levels of interferon (IFN)- γ and IFN- γ -induced chemokines, including CXCL10, CCL2, CCL3, CCL4, and CCL5.² Consistent with Th1-mediated immunity, mRNA levels for T-bet, a transcription factor that maintains the Th1 phenotype, are elevated in cells infiltrating the lungs of p52-Tg/p100^{-/-} mice.² These observations indicate a role for p52 activation in differentiation of T cells into the Th1 phenotype. In a murine model of asthma, which generally correlates with a Th2-type immune response, IFN- γ modulates inflammation, such as eosinophilia, and production of Th2 cytokines IL-5 and IL-13 and suppresses antigen-presenting cell function.¹⁹ In addition, IFN- γ also suppresses the hallmark Th17 cytokine IL-17, as well as IFN- γ itself, in this model of allergic inflammation.¹⁹ This ability of cytokines of an opposing immune phenotype to reprogram a polarized inflammatory response conceivably could participate in resolution of inflammation. Consistent with this postulate, IFN- γ /IL-13 (Th1/Th2) and IFN- γ /IL-17 (Th1/Th17) ratios are significantly reduced in p52-Tg and p100^{-/-} mice, which display modest inflammation, relative to those in p52-Tg/p100^{-/-} mice, which display unrestrained inflammation.² An inference of this concept is that p52/p100 expression participates in resetting the inflammatory phenotype during the resolution phase.

As expected, the IFN- γ -induced chemokines recruit monocytes and macrophages to the lung, but the consequences of macrophage recruitment appear to be altered in p52-Tg/p100^{-/-} mice. An anti-inflammatory role for NF- κ B in macrophages during the wound healing phase has been described previously.²⁰ In contrast to other leukocytes, macrophages possess long lifetimes. To prevent prolonged inflammation, tissue macrophages must be reprogrammed by their microenvironment to assume an anti-inflammatory purpose and promote wound healing.²⁰ In p52-Tg/p100^{-/-} mice, the absence of p100 has compromised the cues from structural cells to macrophages that promote wound healing or has undermined the reprogramming capacity of the macrophages.

Because of one or both of these defects, an influx of macrophages into the lungs of p52-Tg/p100^{-/-} mice fails to resolve the ongoing inflammation.

The Th1 inflammation is accompanied by peribronchial fibrosis revealed by trichrome staining and accumulation of fibroblasts and myofibroblasts in the perivascular and peribronchial regions of p52-Tg/p100^{-/-} mice.² This finding contrasts with the prevailing view that fibrogenesis generally correlates with a Th2 inflammatory response and that Th1 cytokines, particularly IFN- γ , possess antifibrotic activity.²¹ Moreover, cytokines of the Th1 lineage repress expression of Th2 cytokines and vice versa.²² Accordingly, IFN- γ has recently been tested, albeit unsuccessfully, in a clinical trial as a therapy for idiopathic pulmonary fibrosis.²³ CCL2, CCL3, and their receptors, CCR2 and CCR1, respectively, are elevated in p52-Tg/p100^{-/-} mice,² and these signaling pathways have been associated with fibrogenesis.²¹ Furthermore, the p52-Tg/p100^{-/-} mice display simultaneous increases in IL-13, a cytokine that can cooperate with these chemokines in fibrogenesis.²¹ Although TGF- β -independent mechanisms of fibrosis have been reported, Th2 cytokines cooperate with TGF- β in fibrogenesis by promoting dissociation of active TGF- β from the latency-associated protein.²¹ Whether or not these activities occur in p52-Tg/p100^{-/-} mice has not been addressed. Yang et al² suggest that induction of TNF- α is a major contributor to fibroblast accumulation in p52-Tg/p100^{-/-} mice. Consistent with this view, overexpression of TNF- α in lung epithelial cells promotes fibrosis, TNF- α can promote fibroblast proliferation, and mice lacking TNF receptors resist pulmonary fibrosis induced by a variety of profibrotic exposures.²⁴ However, the functions of TNF- α in apoptosis and inflammation described above will be difficult to dissociate from its fibroproliferative effects.

Lung-Specific Inflammation in p52-Tg/p100^{-/-} Mice

It is puzzling why the p52-Tg/p100^{-/-} mice develop lung-specific inflammation and fibrosis but don't display pathology in other tissues. Yang et al² suggest that the constant exposure of the lungs to antigenic stimuli triggers the immune response and prolongs the inflammation. The authors note that the predominance of activated memory CD4⁺ Th cells in the lungs of p52-Tg/p100^{-/-} mice is consistent with a continuous immune response. Thus, p100 function in lung cells, as discussed above, appears to be critical to restrain lung inflammation.

The large differences in survival of p52-Tg/p100^{-/-} mice (~20% die by 10 weeks, whereas >20% survive >1 year) might reflect differences in environmental exposure that triggers the immune response. Another possibility is that the mixed genetic background (F3 generation of a SJL $\times$ C57BL/6 cross) accounts for this heterogeneity. Correlating strain-specific genetic polymorphisms with survival times would address this possibility.

Conclusions

Yang et al² provide evidence in an animal model that p100 protects against activation of the alternative NF- κ B pathway in lymphocytes, but many questions remain. NF- κ B encompasses five transcription factors that are regulated by a variety of I κ B molecules,¹ and how these members interact in different cells, immune and nonimmune, determines whether a tissue remains in homeostasis.⁹ The ability to alter the activities of these proteins by posttranslational modifications adds to the complexity.⁴ Dissecting the interactions between members of the NF- κ B signaling pathway, establishing the functional consequences of those interactions and determining the relevant cell types, continues to be areas rich for investigation.

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