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COMMENTARY

NF-κB and the Innate Immune Response in the Respiratory Distress Syndrome of the Newborn

Commentary on the article by Cheah *et al.* on page 616

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The introduction of surfactant therapy for hyaline membrane disease, or respiratory distress syndrome of the newborn (RDS), has been associated with important reductions in mortality and in the duration of assisted ventilation required for most infants (1). For most premature infants, RDS is an acute disease that resolves with minimal long-term sequelae. However, a substantial minority of preterm infants, especially among those born at less than 28 wk of gestation, requires a prolonged period of assisted ventilation or oxygen supplementation, and develop bronchopulmonary dysplasia (BPD). A considerable body of evidence supports the notion that inflammation plays an important role in the pathophysiology of BPD (2,3). A lung inflammatory response leading to BPD can be initiated and promoted by both antenatal and postnatal factors. The most important known trigger of antenatal inflammation is chorioamnionitis, which can be either overt or subclinical. Postnatal factors that promote pulmonary inflammation include exposure to high levels of inspired oxygen, baro and stretch trauma, and infections. How do these inflammatory triggers influence the course of RDS and the development of BPD, and by what mechanism(s) do they mediate their effects? The study of Cheah *et al.* (4) in this issue addresses a mechanism thought to play a central role in many aspects of the inflammatory response.

In their study, Cheah *et al.* examine the activation of NF-κB in inflammatory cells in tracheal aspirates of infants with RDS, and evaluate correlations of NF-κB activation status with risk factors and clinical outcome. NF-κB is a logical choice for investigation, as it is at the center of the signal transduction networks governing the expression of cytokines and chemokines including TNF-alpha, IL-1β, IL-8, and many other inflammatory mediators (5). Cheah *et al.* assessed NF-κB activation using immunofluorescent staining to detect nuclear localization of this molecule. In the unstimulated cell, NF-κB is primarily localized in the cytoplasm, in a complex with its inhibitor IκB. Activation of inflammatory signaling pathways results in the degradation of IκB, which allows NF-κB to move into the nucleus, where it binds to specific promoter sites and stimulates transcription of inflammatory genes. NF-κB activation can be triggered by multiple processes associated with lung injury, including infections, hyperoxia, barotrauma and ventilatory stretch. Cheah *et al.* found that infants with evidence of nuclear localization of NF-κB activation in leukocytes in at least one tracheal aspirate sample required a longer duration of mechanical ventilation than infants in whom all samples were negative, and had higher levels of TNF-α in the tracheal aspirate fluid. NF-κB activation was strongly correlated with a history of chorioamnionitis. However, IL-8 concentrations were not increased, the numbers and proportions of neutrophils and monocytes did not differ in "positive" and "negative" infants, and NF-κB activation status had no predictive value for the subsequent development of BPD.

The failure to predict BPD is surprising, given the large literature documenting associations between increased tracheal aspirate levels of NF-κB-dependent inflammatory factors such as IL-8 with the subsequent development of BPD. The lack of association does not appear to be due to a lack of statistical power, because the proportions of "positive" and "negative" infants who developed BPD were nearly identical (15/31 *versus* 9/20). Does this mean that NF-κB activation is not important in BPD after all? The problem is more likely due to technical limitations. First, the immunofluorescence method used by Cheah *et al.* provides an "all or none" assessment of activation that detects as positive only those cells in which a very substantial fraction of the total cellular pool of NF-κB is located in the nucleus, while NF-κB activation in reality is a dynamic, graded process. Thus, this technique is likely biased toward detection of only the most highly activated cells. Perhaps image-processing techniques could be used to extract a quantitative, and more sensitive, measure of NF-κB translocation. Second, the number of cells available in the tracheal aspirates was small (median of 57 neutrophils and 33 macrophages), with a median of only 16% of the neutrophils and 7%

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of the macrophages positive for NF-κB translocation. The use of different tracheal aspirate collection techniques, or the use of broncho-alveolar lavage samples, might improve the yield of leukocytes and allow a better assessment of their varying degrees of activation.

What mechanisms account for NF-κB activation in infants with RDS? The heterogeneous inflammatory triggers associated with this disease may translate into distinct mechanisms of NF-κB activation. In the study by Cheah *et al.* chorioamnionitis emerged as the key factor associated with NF-κB activation in inflammatory cells in the airway. Microbial pathogens underlying chorioamnionitis may activate NF-κB by several pathways, most notably those coupled to Toll-like receptor (TLR). Secondary activation by inflammatory products including TNF-alpha would also contribute to NF-κB activation in inflammatory infiltrates in the airways. TLRs recognize conserved pathogen associated molecular patterns (PAMPs) shared by large groups of pathogens including lipopolysaccharides (LPS), peptidoglycans, lipoproteins and glycolipids, flagelins, unmethylated CpG dinucleotides (6). Different TLRs recognize distinct microbial products, suggesting that more than one TLR may be involved in NF-kB activation in Chorioamnionitis. Agents associated with overt chorioamnionitis such as *E. coli*, Group B streptococci and *Listeria* monocytogenes activate TLR4, TLR2 and TLR5, respectively. In contrast, *Mycoplasma* agents associated with subclinical chorioamnionitis may activate NF-KB by means of *Mycoplasma* macrophage associated lipoproteins, which engage Toll-like receptor 2 and 6.

There is surprisingly little information, as yet, about the role of TLR pathways in RDS and BPD. The targets of TLR signaling likely include cells of the innate immune system including neutrophils, macrophages and dendritic cells as well as airway epithelial cells and alveolar epithelial type II cells, all of which express various types of TLRs. Further information about the functioning of specific TLR pathways in the preterm infant lung could significantly enhance our understanding of the mechanisms by which chorioamnionitis and other infections contribute to the development of BPD. While the innate immune response of the preterm is considered premature, its activation in preterm infants with RDS appears to be of particular importance. This is intimated by the observation that while colonization with Ureaplasma urealyticum was increased in preterm infants with RDS, chorioamnionitis rather than colonization *per se* emerged as the key factor associated with NF-κB activation in aspirate inflammatory cells. Further information about this response and the pathways involved would be critical in understanding the role of inflammation in general and chorioamnionitis in particular in precipitating RDS.

In addition to TLR and cytokine receptor pathways, NF-κB can also be directly activated by hyperoxia, *via* a redox-dependent mechanism (7). This mechanism is thought to be important in ischemia-reperfusion injury, and could also play a role in RDS/BPD. In this regard, the data of Cheah *et al.* is suggestive, in that an association was noted between the fraction of inspired oxygen (FiO_2) and NF-κB activation in aspirate samples obtained after but not during the first week. One cannot, of course, infer a mechanism or a direct cause and effect relationship from this association.

An important factor that continues to limit our understanding of BPD is the clinical inaccessibility of the distal lung tissue that is the main site of pathology in this disease. Evaluation of tracheal aspirate samples as in the study of Cheah *et al.,* or even bronchoalveolar lavage samples, cannot address NF-κB activation and other changes in airway and alveolar epithelium. NF-κB activation in the lung epithelial cells may well be one of the earliest events in the cascade leading to inflammation and RDS. It would play a pivotal role in setting the stage for the recruitment of inflammatory cells into the airways by virtue of its induction of chemotactic factors such as IL-8. It may also play an important role in initiating tissue injury and remodeling relevant to BPD (8). Some of the same pathways implicated above in mediating NF-κB activation in inflammatory cells are also operative in the airway and alveolar epithelium including TLRs (9–12), hyperoxia (13,14) and chemokines and inflammatory cytokines. The importance of lung epithelial cells in lung inflammatory responses is emphasized by a recent study of mice expressing a dominant negative IκB-α transgene under the control of a surfactant protein C promoter (15), which causes a selective inhibition of NF-κB activation in distal airway and alveolar epithelial cells. These mice had impaired inflammatory responses to inhaled LPS, confirming a physiologic role for respiratory epithelial cells in lung inflammation *in vivo*.

The implications of the study of Cheah *et al.* are broad. Of more immediate applicability is the use of NF-κB activation status in inflammatory cells in tracheal aspirates to stratify risks and long-term outcome in preterm infants with RDS. Therapeutically, If NF-κB is the hub that connects different pathogenic threads in RDS, including injurious local tissue and innate immune responses then interventions aimed at selectively blocking NF-κB activation in the airways may be beneficial. In fact corticosteroids such as dexamethasone are one group of agents that act to suppress NF-κB activation by inducing the NF-κB inhibitor I-κB (16,17), and by direct protein-protein interactions that prevent NF-κB (and other transcription factors such as AP-1) from inducing transcription in a promoter site-specific fashion (18). However, it is now widely appreciated that the short-term benefits seen with the extensive use of dexamethasone for prevention and treatment of BPD were not matched by long-term improvements in pulmonary function, and may have been associated with impaired neurodevelopmental outcome (19–21). Perhaps other NF-κB inhibitors, used in a more selective fashion, could eventually prove to be safe and effective in this disease. However, the experience with dexamethasone emphasizes that the risks and benefits of such agents should be carefully evaluated in both animal and well-controlled clinical studies before they are introduced into clinical practice.

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