Crawling with Virus: Translational Insights from a Neonatal Mouse Model on the Pathogenesis of Respiratory Syncytial Virus in Infants

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The infant immune response to respiratory syncytial virus (RSV) remains incompletely understood. Here we review the use of a neonatal mouse model of RSV infection to mimic severe infection in human infants. We describe numerous age-specific responses, organized by cell type, observed in RSV-infected neonatal mice and draw comparisons (when possible) to human infants.

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections in infants, and severe RSV disease during infancy is associated with an increased risk of asthma development. The medical and economic burdens of RSV infection are enormous. In the United States alone, there are 85,000 to 144,000 annual hospitalizations with \$2.6 billion in associated estimated medical cost (1). Despite its continued impact on global health, RSV remains without a vaccine or adequate therapeutic.

While prematurity, immunodeficiency, and congenital heart or chronic lung diseases are risk factors for severe RSV disease, the majority of the infants requiring hospitalization were previously healthy and less than 6 months of age (2), suggesting that age at the time of initial infection is an important predictive factor for disease severity. Therefore, our grasp of severe RSV disease in infants is inevitably tied to our understanding of developmental immunity during the first year of life.

The neonatal mouse model of RSV infection has been an invaluable tool for RSV research (3, 4). Briefly, neonatal mice (≤ 7 days of age) are infected with RSV and then reinfected 4 weeks later as adults. Compared to mice initially infected as adults, these mice develop the characteristic Th2-biased immunopathologies, including exaggerated pulmonary Th2 cells/cytokines, eosinophilia, mucus hyperproduction, and airway hyperreactivity. In recent years, this model spurred several significant advances in research on how age-dependent differences in various immune and nonimmune cells initiate the immunopathogenesis of RSV infection in infants. This article will briefly discuss said advances, organized by the cell types involved in responding to RSV.

EPITHELIAL CELLS

The major cellular target of RSV is airway epithelial cells. In the lower respiratory tract, RSV infects bronchiolar and alveolar (type I and II) epithelial cells (5) and similar cells in mice (6). During infection, the airway epithelium is a major source of the “alarmin” cytokine interleukin-33 (IL-33). Intriguingly, nasal aspirates from infants with severe RSV disease have elevated levels of IL-33 (7, 8), and variants of the IL-33 receptor gene *IL1RL1* are associated with severe RSV disease in human infants (9). Immediately following RSV infection in neonatal mice, a robust IL-33 response is elicited in CD45⁺ EpCam⁺ pneumocytes (7); this type of response to RSV infection does not occur in adult mice, suggesting age-dependent regulation of IL-33 expression in the lung. Furthermore, the se-

verity of RSV infection (i.e., Th2-mediated immunopathology) is dependent on the levels of IL-33 early during infection.

ILC2S

More recent work has provided a role for a previously unexplored area of RSV immunology, group 2 innate lymphoid cells (ILC2s). The receptor of IL-33, ST2, is highly expressed on ILC2s, in addition to Th2 cells, dendritic cells (DCs), eosinophils, and mast cells (10). In addition to strongly promoting Th2 differentiation, IL-33 stimulates ILC2s to produce high levels of IL-13, leading to elevated mucus production and airway dysfunction. Furthermore, IL-33 and ILC2s are required for the immunopathophysiology typically observed in allergic asthma, a disease phenotypically similar to severe RSV in human infants. Interestingly, neonatal mice have higher percentages of pulmonary ILC2s than adult mice do (7). These data demonstrate a mechanistic role for epithelium-derived IL-33 in RSV immunopathogenesis and suggest that IL-33-mediated ILC2 responses are significant drivers of the Th2 immune response.

DENDRITIC CELLS

Dendritic cells are the major antigen-presenting cells of the lungs that orchestrate the adaptive immune response to RSV. Interestingly, neonatal DCs (specifically, CD11b⁺ myeloid DCs [mDCs]) express higher levels of IL-4R α (unpublished data) and IL-13R α 1 (11) than adult mDCs do. This increased IL-4R α expression is associated with a “less mature” phenotype of neonatal CD11b⁺ mDCs, as evidenced by reduced expression of the costimulatory molecules CD80 and CD86.

In addition to IL-4R α and IL-13R α 1, OX40L is expressed on pulmonary CD11b⁺ mDCs in an age-dependent manner (12). The interaction between OX40L (expressed on antigen-presenting cells) and its receptor, OX40 (expressed on CD4⁺ T cells), is

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important for Th2 cell activation and survival and therefore is critical in inducing asthmatic responses in animal models. Neonatal mDCs express more OX40L than their adult counterparts do, and its expression is further induced by RSV infection. When neonatal mice are treated with antibodies against OX40L prior to primary infection, the Th2-biased immunopathologies are also diminished upon reinfection (12).

CD4⁺ T CELLS

In addition to the increased expression of IL-4R α on neonatal DCs, the expression of IL-4R α on CD4⁺ T cells is higher in neonatal mice than in adult mice. Similar age-dependent IL-4R α expression is observed in human infant and adult CD4⁺ T cells, which further increases in response to RSV stimulation (13). The exact effect of elevated IL-4R α levels on CD4⁺ T cells in the infant is unclear, but in mice, increased IL-4R α expression is associated with enhanced proliferation of Th2 cells, resulting a Th2-biased responses during RSV reinfection (4). Further, the absence of IL-4R α specifically on CD4⁺ T cells substantially decreases Th2-biased immunopathologies upon RSV reinfection (13).

OTHER CELLS THAT MAY PLAY A ROLE IN RSV PATHOGENESIS OR PROTECTION

In addition to age-dependent factors that appear to drive Th2-biased immunopathogenesis during RSV infection, other cells and factors specific to infants are likely influencing disease severity. In human infants, RSV severity is associated with increased viral loads in nasal aspirates (14), and plasmacytoid dendritic cells (pDCs) from cord blood mononuclear cells (CBMCs) are less able to induce antiviral type I interferons (IFNs) than pDCs from adult human PBMCs are (15). Consistent with human data, neonatal mice are incapable of inducing a robust type I IFN and pDC response in the lung (16).

The importance of IL17A-expressing gamma delta ($\gamma\delta$) T cells in protecting against neonatal RSV infection has also been demonstrated (17). Neonatal mice do not produce IL-17A during the early responses to RSV, though they have more lung $\gamma\delta$ T cells than adult mice do. This deficit is attributed, in part, to impaired inflammasome activation in neonatal mice, which mirrors data from human studies using $\gamma\delta$ T cells from CBMCs compared to those from adult PBMCs.

CD8⁺ T cells have also been shown to be critical in both RSV clearance and pathogenesis. In fact, neonatal CD8⁺ T cells respond to RSV infection with an epitope hierarchy significantly different from that of adult CD8⁺ T cells (18). This difference is due mainly to the immaturity of neonatal CD103⁺ mDCs (i.e., less expression of CD80 and CD86) (19).

THE OVERALL MODEL AND FUTURE STUDIES

On the basis of all of the existing data, we propose an overall model of how age influences the nonimmune and immune cellular responses to RSV to induce Th2-biased immunopathologies (Fig. 1). In this model, RSV infects airway epithelial cells and induces IL-33. IL-33 binds to ILC2s and induces IL-13. IL-13 then activates and polarizes DCs to induce a Th2-biased response. Alternatively, IL-33 can bind to DCs directly and increases the expression of OX40L, thereby inducing Th2 polarization via OX40-OX40L interaction with Th cells. At each step, there is a developmental check point resulting in the Th2 bias. Neonatal, but not adult, epithelial cells produce excessive IL-33 in response

FIG 1 RSV infection in neonatal mice. Airway epithelial cells (AEC) infected with RSV produce high levels of IL-33, which signals through its receptor ST2 on ILC2s, resulting in increased ILC2 numbers and enhanced production of IL-13. Neonatal DCs express more IL-4R α , IL-13R α 1, and ST2 than adult DCs do. Signaling through the IL-4R α –IL-13R α 1 receptor complex is associated with an enhanced ability of DCs to polarize Th2 responses. Epithelium-derived IL-33, on the other hand, is believed to cause OX40L upregulation on DCs, further enhancing their Th2-polarizing ability and exacerbating RSV immunopathogenesis. This paradigm distinctly occurs in neonatal, but not adult, mice. LN, lymph node.

to RSV infection; ILC2s are present in greater amounts in neonatal lungs and are capable of producing more IL-13 than adult ILC2s do, resulting in a larger amount of IL-13 produced in response to RSV infection. Neonatal DCs express more IL-4R α (one IL-13 receptor component) than adult DCs do. Elevated IL-13 induction early during RSV infection suppresses CD11b⁺ mDC maturation, which favors Th2 development. Further, neonatal Th2 cells express more IL-4R α during RSV infection than their adult counterparts do, and elevated IL-13 enhances their proliferation. All of these factors act in concert and result in Th2-biased immunopathogenesis in neonates but not in adults. How are these factors developmentally regulated? Why do only a portion of young infants develop severe disease during RSV infection? Does any genetic or environmental component influence these aforementioned factors? Can therapeutics or pediatric vaccines be developed by using these factors as targets? The answers to all of these questions are unclear and warrant further investigations.

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