Commentary

Interactions between RSV Infection, Asthma, and Atopy: Unraveling the Complexities

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In this issue, Culley et al. (1) present findings from an infant mouse model of respiratory syncitial virus (RSV) infection, which provides a new perspective on the pathogenesis of RSV-mediated lung disease in early life. RSV is one of the most common respiratory pathogens encountered in pediatric practice, and virtually all children experience one or more infectious episodes by the age of 2 yr (2). The initial focus of the infection is the nasopharyngeal epithelium, and in the majority of cases it remains localized to this area and the symptomatology is restricted to a runny nose. In a subset of subjects, however, the virus spreads to the lower respiratory tract, resulting in a wheezing illness or acute bronchiolitis that requires hospitalization. Between 50 and 90% of hospitalizations for bronchiolitis amongst children in the U.S. are directly attributable to RSV and substantial increases have been documented recently in admissions in North America (2), paralleling the spiralling increase which is occurring in asthma in pediatric and adult populations throughout the developed world. A possible causal link between RSV-induced wheeze in infancy and later development of persistent asthma has been widely debated in this field, and elucidation of the factor(s) responsible for susceptibility to these apparently related diseases is considered a matter of urgent priority in pulmonary medicine. However, the precise nature of this linkage is increasingly controversial, as discussed below.

Immunopathogenesis of RSV Infection. A typical pattern of inflammation accompanies RSV bronchiolitis, which includes epithelial sloughing in the small airways accompanied by hypersecretion of mucus and edema, resulting in extensive hyperinflation, airflow obstruction, and cough and wheeze (2). Studies in infected adults indicate that tissue repair is apparent within days after the onset of symptoms, but restoration of ciliated airway epithelial cells takes several weeks, a period during which symptoms of cough, wheeze, and altered lung function commonly persist (3). It is noteworthy, however, that virus elimination and tissue recovery is not accompanied by prolonged resistance to RSV, as rapid reinfection is common amongst both infants and adults (2).

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Studies on nasal secretion in children with RSV suggest that both eosinophils and neutrophils contribute to tissue damage during infection (4, 5), and the participation of these cells in the host response appears due in part to virus-induced secretion of chemokines from infected airway epithelial cells (6). However, studies in animal model systems point also to a key role for T cells in the immunopathogenesis of RSV-induced airways inflammation. In particular, the Balb/C mouse model has provided valuable insight into potential mechanisms by which $CD4^+$ T cells responding to RSV-specific antigens may elicit Th2-polarized responses, which are rarely encountered in viral infections. Using recombinant vaccinia viruses expressing individual RSV proteins to prime mice before infection, Culley and colleagues (7) demonstrated that the responses of $CD4^+$ T cells from infected animals which were directed against the surface G-protein of RSV were strongly Th2 polarized, and elicited prominent pulmonary eosinophilia. These responses involved a dominant population of Vβ-14 T cells specific for the 183–197 epitope of the G-protein and were restricted to certain strains of mice (7, 8), highlighting the importance of background genetics.

Further studies in the Balb/C model have implicated a population of IFN-γ–secreting $CD8^+$ T cells (9) which potentially may attenuate the pathogenic Th2 component of the host response to the G-protein. It is of interest to note in this context a recent report demonstrating direct RSV-mediated inhibition of expression of effector activity by activated RSV-specific $CD8^+$ T cells, via interference with TCR-mediated signaling (10). Effector activity included secretion of IFN-γ, and this mechanism thus provides a potential route for subversion of the Th2 inhibitory effects of the $CD8^+$ T cell population described previously (10). It additionally provides a partial explanation for the short-lived nature of postinfection resistance to RSV via impairment of $CD8^+$ T cell memory development.

The central importance of cellular (as opposed to humoral) immune memory in resistance to RSV infection may also be inferred from the observation that the highest incidence of severe RSV disease occurs in early infancy, during which titres of maternal antibody are highest. Moreover, infected children with impaired cellular immune function show prolonged shedding of RSV and high susceptibility to development of RSV pneumonia (11). However, the potentially pathogenic nature

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of RSV-specific cellular immunity was tragically revealed in trials with formalin-inactivated RSV vaccine in the 1960s in which 80% of vaccinees required hospitalisation post infection, and two deaths ensued, with microscopic evidence of intense infiltrates of mononuclear cells, neutrophils, and eosinophils in the lungs (12).

Epidemiological Findings: The Link with Atopy and Asthma. A variety of epidemiological studies, both cross-sectional and prospective, suggest possible linkages between RSV infection in early childhood and subsequent manifestations of atopy and persistent asthma. Prototypical of these, Sigurs et al. (13) followed a population of children after hospitalization for RSV bronchiolitis during infancy, together with matched uninfected controls, and reported a 3.5-fold excess in allergy and a >10-fold excess of asthma in the postinfected population 3 yr later. Of particular interest was the observation that risk for asthma development after RSV infection was much higher in children with a positive family history (and hence at high genetic risk) of allergy (13). More recent prospective cohort studies from Stein and colleagues (14) indicate that the increased risk for asthma after RSV infection in infancy persists for 10–11 yr.

In relation to the mechanism(s) underlying the association between early RSV infection and subsequent development of asthma, one of the prevailing theories posits indirect stimulation of the disease process via promotion of sensitization to inhalant allergens, as a result of the intrinsically "Th2-trophic" effects of the virus (7). Recent studies (for a review, see reference 15) indicate that development of long-term T cell memory specific for these allergens typically occurs during early childhood, bracketing the period during which RSV infection is most frequent. This raises the possibility that bystander stimulation of ongoing T cell responses to inhalant allergens by intermittent RSV infections, through the triggering of local Th2 cytokine production in the airway mucosa (7, 8), may tip the balance toward consolidation of Th2-polarized memory against the allergens, thus setting the scene for subsequent atopic asthma. While there is some evidence for promotion of sensitization to allergens after RSV infection from small in vitro studies on T cell function in PBMCs from children, results from large scale epidemiological studies have not confirmed these findings, and instead argue that the "asthma-promoting" effects of RSV infection in childhood are independent of atopy (14). It should be emphasized, however, that the association between atopy and asthma has been a consistent finding over a wide range of studies, and moreover that the strongest asthma-promoting effects of atopy are observed in association with early onset of sensitization to inhalant allergens (for a review, see reference 15).

Similar conclusions follow from other large prospective cohort studies that have documented episodes of infection-associated wheezing bronchiolitis in infancy without identification of relevant pathogens, and have correlated the frequency of these episodes with risk for subsequent development of persistent asthma. In one such prospective study from our center on 2,602 children, ≥2 episodes of wheezing lower respiratory illness during the first year of life increased the risk for current asthma at 6 yr of age fourfold in nonatopics and ninefold in atopics, but did not increase risk for atopy per se (16). In our view these findings suggest that the effects of severe respiratory viral infections and atopy upon risk for asthma are mediated via independent causal pathways, and further that these effects can interact in a synergistic fashion in driving asthma development (further discussion below).

It is additionally noteworthy that infections with other viruses, in particular rhinoviruses, have been identified as major triggers of asthma exacerbations in school children with established (usually atopic) asthma (17). Furthermore, respiratory infection with viruses other than RSV is recognized as a major aetiologic factor in nonatopic (or intrinsic) asthma in adults, and this form of asthma also manifests a Th2-like "signature" in the form of eosinophil involvement at lesional sites in the airway mucosa (18).

In summary, it appears that while a strong case can be made for early RSV infection as a risk factor for asthma development in childhood, particularly in combination with atopy, current perceptions of the underlying mechanisms, which emphasize the importance of the unique Th2-trophic properties of this virus, do not in our view provide a satisfactory explanation for many of the available findings. However, recent studies focusing on the late stage development of innate and immune function in the immediate postnatal period, including the report in this issue of *The Journal of Experimental Medicine* by Culley et al. (1), shed fresh light on this complex problem.

Ontogeny of Immune Function: Kinetics of Postnatal Maturation of Immune Competence as a Determinant of Disease Susceptibility during Early Life. It is now recognized that immune function(s) in the fetal compartment are attenuated relative to postnatal life, and that the attenuation is most marked with respect to capacity to generate Th1 cytokines, which are highly toxic to the placenta (19). This Th2 "skew" is maintained via a series of multilayered control mechanisms operative both within the innate immune system (in particular via preferential down-regulation of IL-12p35 gene expression in neonatal dendritic cells, reference 20) and in the T cell system (e.g., CpG hypermethylation in the IFN-γ promoter in neonatal $CD4^+$ $CD45RA^+$ T cells (21).

It is additionally clear that the principal trigger(s) for late-stage functional maturation of the immune system are microbially derived molecules not normally encountered in fetal life, which signal via specific TOLL receptors and CD-14. However, it is also evident that the rate at which the immune system in newborns progresses from the Th2-skewed state characteristic of the fetal compartment, to the more balanced (by comparison more Th1-polarized) adult-equivalent state, is highly variable within the human population, particularly with respect to capacity to produce Th1-associated cytokines such as IFN-γ (22) and also IL-12 (23).

Studies from our group and others (for a review, see reference 15, 16) have demonstrated that atopic family history positive (AFH^+) children at high genetic risk of atopy and asthma exhibit slower rates of postnatal maturation of Th1 competence than the population at large. The genetic mechanisms underlying these variations in Th1 functional capacity amongst children are incompletely understood, but appear to involve

inter alia polymorphisms in CD-14 (24) and also IL-12 (25), the former being associated with severity of atopy, and the latter with severity of asthma.

It is widely acknowledged in the epidemiological literature that early postnatal life represents a period of heightened risk for infectious disease, and also for allergic sensitization, and the accumulating evidence strongly suggests that a generalized maturational deficit in Th1 function underlies this susceptible state. The findings indicating that this deficit is further exaggerated in children genetically at high risk of atopy (15, 16) provide a plausible explanation for their increased propensity to default to the Th2 pathway during development of Th memory against aeroallergens. It also explains the increased Th2 polarity of their responses to vaccine antigens (16, 22).

Given the importance of cellular immunity in limiting RSV shedding during early infection (11), it is highly likely that reduced Th1 function during this life phase plays a central role in susceptibility to bronchiolitis after RSV infection. This possibility is supported by indirect evidence from several studies suggesting an association between this syndrome and attenuated capacity to mount systemic IFN-γ and IL-12 responses (for a review, see reference 15), and in particular by the recent finding that the most severe manifestation of acute RSV bronchiolitis in infants are associated with markedly reduced local production of IFN-γ in airway secretions (26).

In this issue, Culley and colleagues (1) provide compelling new evidence which further emphasizes the importance of issues related to postnatal development of immune function as determinants of susceptibility to RSV bronchiolitis, and ultimately atopy and asthma. They demonstrate that in the Balb/C mouse model, the timing of first infection with RSV determines the polarity of RSV-specific immunological memory which develops, and this in turn influences the severity and nature of the disease after subsequent reinfections. A plausible interpretation of their findings is that if animals are initially infected during early infancy while their capacity to express Th1 immunity is developmentally constrained, they generate Th2-polarized RSV-specific immunological memory, which upon reinfection as adults is expressed in the form of intense infiltrates of eosinophils and IL-4–secreting $CD4^+$ Th2 cells in the lung. In contrast, if initial infection is delayed for several weeks thus enabling maturation of immune function, the ensuing $CD4^+$ Th-memory which develops is Th1-polarized, and both primary (and subsequently secondary) immune responses to the infection lack the pathogenic component of lung eosinophilia. As an aside, these findings raise considerable questions about the commonly used animal models of allergic inflammation, which feature sensitization and challenge of adult mice with mature immune systems, whereas in the human situation initial allergen exposure occurs in early life.

Culley's findings, if directly applicable to human infants, have important theoretical implications for the design of future treatment strategies for acute RSV bronchiolitis, and also for development of prophylactic approaches which aim the break the nexus between infantile bronchiolitis and subsequent asthma. In the scenario depicted in Fig. 1, the sequelae of RSV infection during infancy are determined by the level of maturation/competence of the immune system at the time of first infection (1). Thus, if initial RSV infection occurs beyond infancy at a time when postnatal maturation of adaptive immune function(s) is well progressed, Th1-polarized host defense mechanisms will be triggered, leading to rapid resolution of the primary infection, and accelerated protection against secondary infection via generation of Th1-polarized memory. In contrast, infection occurring early in infancy (<1 yr of age) when the immune system is still in the fetal-like, Th2-skewed state, will have a high likelihood of triggering Th2-polarized primary immunity, with attendant risk of spread to the lower respiratory tract and development of acute wheeze. Primary RSV infection at this early age also carries the additional risk of development of RSV-specific Th2-polarized immunological memory, which may render the effects of subsequent infections even more severe by blunting capacity for Th1-mediated defences, and by further enhancing the Th2 (in particular eosinophilic) component of the host response to the virus. The likelihood of developing initial Th2-polarized immunity to RSV infection during infancy will be further heightened in AFH^+ children at high genetic risk of atopy, in whom this normal developmental deficit in Th1 function is exaggerated. The worst case scenario, in which an AFH^+ infant develops sensitization to inhalant allergens and additionally experiences repeated RSV infections commencing in early infancy (wheezing lower respiratory illnesses; wLRI in Fig. 1), is illustrated as the synergism resulting from the confluence of these two pathways, as predicted by the sliding scale of Odds Ratios for asthma in infected and/or atopic children in the inset in Fig. 1.

While this scheme provides a biologically plausible model for integration of many of the key findings from the mouse RSV model with the relevant human asthma and allergy literature, a number of specific issues remain to be resolved. First, the role of maternal antibody requires clarification. The epidemiological literature indicates that risk for RSV bronchiolitis peak between 2 and 6 mo of age, and it is plausible that mild (often subclinical) infections may be occurring very early in infancy, which are prevented from spreading beyond the upper respiratory tract by maternal antibody, but which nevertheless covertly "prime" the Th2-polarized immune system aided by the adjuvant effect of the maternal antibodies. Second, the sequelae of perinatal RSV priming revealed in the mouse (1) need to be balanced against findings discussed above which point to the relatively short-lived nature of RSV-specific Th-memory in both children and adults. However, it remains possible that in some subjects, the Th2-polarized RSV-specific Th-memory which develops during early infections may persist into later life, in some cases into adulthood. In this context, it is also important to consider the potential contributions of other viruses. For example, bronchiolitis resulting from parainfluenza type 3 infection, like RSV bronchiolitis, occurs primarily in the first 6 mo of life, but with a lower frequency (3). How-

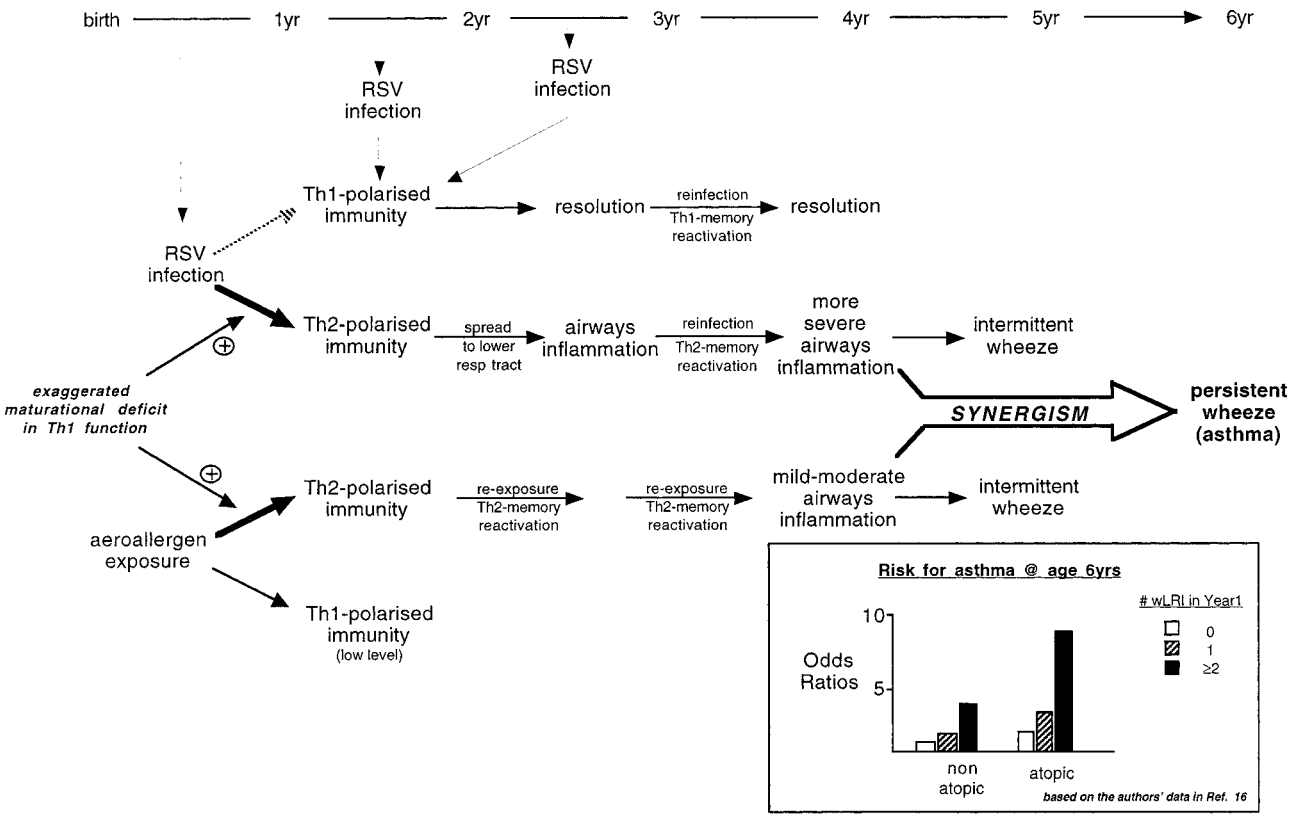

Figure 1. Age-related changes in adaptive immune responses to RSV infection as potential aetiologic factors in acute bronchiolitis and later asthma. In this scheme, the Th1/Th2 "bias" in the adaptive immune response to initial infection with RSV is determined by age, in particular, the contribution of the Th1 component is inversely related to postnatal age at infection onset. The degree of the Th2 bias during infancy is exaggerated in a subset of subjects, increasing their risk for severe RSV infection which spreads to the lower airways and triggers wheeze, and also increasing their risk for development of respiratory allergy. Epidemiological data from large birth cohort studies (exemplified by the data in the inset from reference 16) indicate that maximal risk for early development of persistent asthma involves a history of concomitant wheezing lower respiratory tract infections(wLRI) during infancy together with atopic sensitization to aeroallergens, suggesting that damage to developing airways via these two pathways acting in synergy, drives the disease process.

ever, parainfluenza types 1–3 are a frequent cause of other respiratory illnesses which do not require hospitalisation, and may covertly contribute to airway damage via the model in Fig. 1, albeit at a lower intensity than RSV. Recent findings implicating metapneumovirus as a cause of severe bronchiolitis in young children (27) raise the possible involvement of a potentially growing range of other viruses in this process. Finally, as noted above, airway biopsy samples from adults with nonatopic (intrinsic) asthma, in which virus infection is viewed as a major trigger, also display evidence of activated Th2 cells and eosinophils (18), suggesting local expression of Th1-polarized memory against the triggering virus.

Many of these issues can only be resolved by more detailed and targeted prospective studies in humans, but as illustrated in the publication from Culley et al. (1), the mouse model has unique potential to yield specific information to help sharpen the focus of the human studies. Importantly, their findings also highlight the necessity for studying RSV-mediated disease and its sequelae in the specific developmental context in which the infection primarily manifests in its human host.

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