

## Pulmonary Immunity during Respiratory Infections in Early Life and the Development of Severe Asthma

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### Abstract

Asthma affects 10% of the population in Westernized countries, being most common in children. It is a heterogeneous condition characterized by chronic allergic airway inflammation, mucus hypersecretion, and airway hyperresponsiveness (AHR) to normally innocuous antigens. Combination therapies with inhaled corticosteroids and bronchodilators effectively manage mild to moderate asthma, but there are no cures, and patients with severe asthma do not respond to these treatments. The inception of asthma is linked to respiratory viral (respiratory syncytial virus, rhinovirus) and bacterial (*Chlamydia*, *Mycoplasma*) infections. The examination of mouse models of early-life infections and allergic airway disease (AAD) provides valuable insights into the mechanisms of disease inception that may lead to the development of more effective therapeutics. For example, early-life, but not adult, *Chlamydia* respiratory infections in mice permanently modify immunity and

lung physiology. This increases the severity of AAD by promoting IL-13 expression, mucus hypersecretion, and AHR. We have identified novel roles for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and IL-13 in promoting infection-induced pathology in early life and subsequent chronic lung disease. Genetic deletion of TRAIL or IL-13 variously protected against neonatal infection-induced inflammation, mucus hypersecretion, altered lung structure, AHR, and impaired lung function. Therapeutic neutralization of these factors prevented infection-induced severe AAD. Other novel mechanisms and avenues for intervention are also being explored. Such studies indicate the immunological mechanisms that may underpin the association between early-life respiratory infections and the development of more severe asthma and may facilitate the development of tailored preventions and treatments.

**Keywords:** early life; *Chlamydia*; IL-13; tumor necrosis factor-related apoptosis-inducing ligand; asthma

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### Asthma

Asthma is a chronic allergic inflammatory condition of the airways that affects more than 300 million people worldwide. The inflammation, at least in patients with allergic asthma, is driven through the actions of activated mast cells, eosinophils, and Th2 lymphocytes. These activated inflammatory cells and the mediators they release promote inflammatory responses that damage the airways and result in mucus-secreting cell (MSC) hyperplasia and metaplasia and structural changes in lung

tissue. These events are accompanied by exaggerated sensitivity of the airways to nonspecific stimuli, known as airway hyperresponsiveness (AHR). These pathological processes result in a narrowing of the airways leading to widespread airflow obstruction and breathing difficulties associated with asthma. Combination therapies with inhaled corticosteroids and bronchodilators effectively manage mild to moderate asthma, but there are no cures, and patients with severe asthma often do not respond to these treatments.

### Early-Life Infections and Asthma Inception

Asthma prevalence has increased in recent decades, which cannot be accounted for by changes in genetic predisposition alone. This has prompted the development of hypotheses that implicate environmental factors, and the “hygiene hypothesis” has received most attention. This suggests that the risk of developing asthma is altered by exposure to infections in early life (1). Improved sanitation, increased use of antibiotics and vaccines, and smaller families may reduce exposure to infection

and increase the prevalence of allergic diseases (2–5).

Both the nature and age of infection are crucial in protecting against or predisposing to the development of asthma (6–11). Increasing evidence supports a role for early-life infections in molding the phenotype of immune responses to antigens later in life (6, 12). The immune system of early life is polarized to respond in a Th2-biased fashion (13–16). It is believed that the Th2-biased immune system in early life must be exposed to Th1- or regulatory T cell (Treg)-inducing infections to develop a normal balanced Th1/Th2/Treg phenotype. The absence of these infections may promote the persistence of a Th2 phenotype and predispose to the development of asthma (Figure 1). Neonatal mice vaccinated with the Th1-inducing bacillus Calmette-Guérin were protected from eosinophilic lung inflammation and AHR in allergic airways disease (AAD) (17). Moreover, *Helicobacter pylori* induced Tregs that protected mice from developing AHR, mucus hypersecretion, and the infiltration of eosinophils and Th2 cells into bronchoalveolar lavage fluid (BALF) (18, 19). We have shown that exposure to *Streptococcus pneumoniae* bacteria, components, or vaccines induce Tregs that suppress AAD, which could also be effective in early life (20–24). In contrast, several respiratory viral infections, including respiratory syncytial virus (RSV) and rhinovirus (RV), predispose to the

development of asthma, and this has been extensively reviewed (6, 12).

Respiratory infections in early life may also induce irreparable damage to lung structure resulting in reduced lung function in later life. The lungs are unique in that they continue to undergo significant maturation during the first 2 years of life, with only ~10% of the adult pulmonary alveolar compartment present at birth (25). Therefore, airway damage caused by early life infection may be irreversible, leading to fixed alterations in lung structure and function.

Investigations of the mechanisms that underpin the differential effects of both the nature of infection and the age at which infection occurs may identify potential avenues for the development of therapeutics that protect against the development of asthma. The intracellular bacterium *Chlamydia* has been shown in recent studies, including several from our laboratory, to influence the inception of AAD, and these models have provided insights into the mechanisms of disease development that may inform the development of more effective therapeutics for asthma.

## ***Chlamydia* Respiratory Infections and Asthma**

### **Prevalence of Early-Life *Chlamydia* Respiratory Infections**

*Chlamydia* infections are a primary cause of pneumonia in early life. *Chlamydia*

*trachomatis* is one of the most common causes of neonatal pneumonia, whereas *Chlamydia pneumoniae* accounts for a significant proportion of community-acquired pneumonia in children and young adolescents (6, 26–29). Transmission of *C. trachomatis* from mother to child occurs during passage through the infected cervix, with an estimated 100,000 neonates exposed annually in the United States and pneumonia developing in up to 20% of newborns born to infected women (30, 31). Children who develop pneumonia as a result of *C. trachomatis* infection in early life are more likely to have abnormal lung function and chronic cough in later life compared with noninfected children (32). *C. pneumoniae* is responsible for ~10% of community-acquired pneumonias (33, 34). Moreover, several studies have shown that *C. pneumoniae*-specific antibodies occur in more than 75% of healthy young adults, suggesting a high prevalence of asymptomatic infection within the population (35, 36).

### **Clinical Association between Early-Life *Chlamydia* Respiratory Infection and Asthma**

Recent clinical studies have demonstrated an association between *C. pneumoniae* infection and asthma in children. Teig and colleagues showed that ~24% of children with lung disease, but no healthy control subjects, were polymerase chain

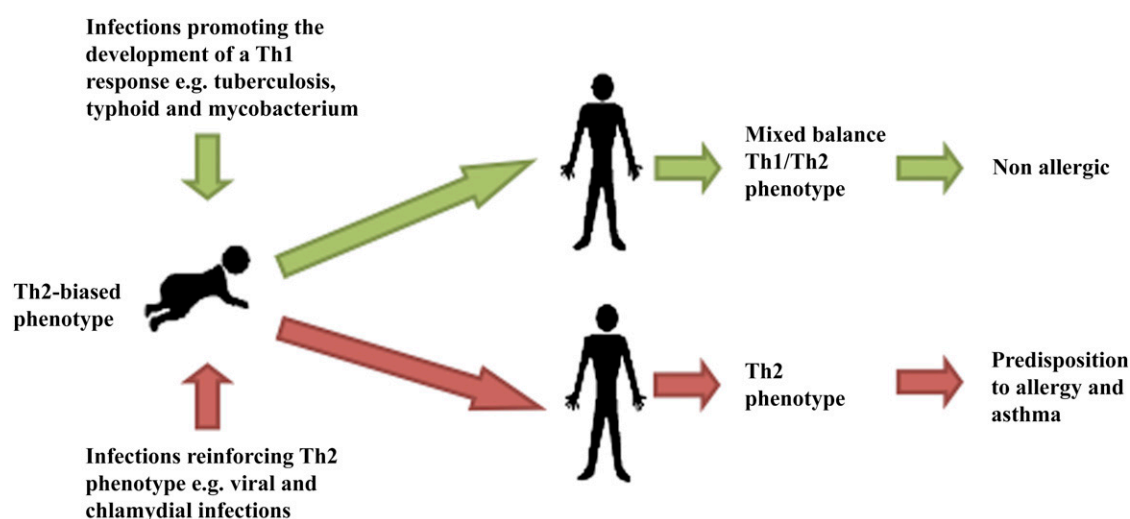


**Figure 1.** Role of early-life infections in predisposition to asthma. Exposure to some microbial infections that induce Th1 or regulatory T cell (Treg) responses may shift the Th2-biased immune system of early life to a more balanced Th1/Th2/Treg phenotype. By contrast, certain infections, particularly respiratory viral infections, may reinforce the Th2 immune phenotype of early life and predispose to the development of asthma in later life.

reaction-positive for *C. pneumoniae*, indicating that the infection was present in a substantial proportion of children with stable asthma or chronic bronchitis (37). Webley and colleagues showed that 67% of children with asthma were polymerase chain reaction positive, and 33% were culture positive for *C. pneumoniae* (38). Interestingly, they also observed that *C. pneumoniae*-positive BALF cultures correlated with elevated serum levels of *Chlamydia*-specific IgE, which was detected at higher levels and more frequently in the BALF of pediatric patients who did not respond to corticosteroid treatment (35, 36). This indicates that *C. pneumoniae* infection may be associated with steroid-resistant asthma. Another study demonstrated that infants with diagnosed wheeze are more likely to develop asthma after *C. pneumoniae* infection than noninfected wheezing infants (39). Collectively, these studies indicate that *Chlamydia* respiratory infections may significantly influence the development and severity of asthma in children.

#### Experimental Evidence for the Association between Early-Life *Chlamydia* Respiratory Infection and Asthma

Our group has investigated the mechanisms of how early-life *Chlamydia* respiratory infections are associated with the induction and increased severity of AAD using mouse models (40–42). We developed models of neonatal and infant *Chlamydia* respiratory infection and combined them with a model of ovalbumin (Ova)-induced AAD. We used *Chlamydia muridarum*, which is a natural mouse respiratory pathogen and is the most appropriate *Chlamydia* species to investigate host–pathogen relationships and the impact of infection on AAD in mice (40, 41). Mice were infected as neonates, infants, or adults, and the infection and associated inflammation were allowed to resolve. The impact of resolved infections on subsequent AAD was assessed. Neonatal and infant, but not adult, *Chlamydia* infection increased the severity of AAD by increasing MSC numbers, IL-13 expression, and AHR (40). Neonatal, but not infant or adult, infection also had striking and long-lasting effects on lung structure, inducing emphysema-like increases in alveolar diameter in adulthood (41).

#### Immune Mechanisms of Early-Life Infection-induced Asthma

##### Tumor Necrosis Factor-related Apoptosis-Inducing Ligand and IL-13 Promote *Chlamydia* Respiratory Infection

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces inflammation and apoptosis that could be involved in increasing alveolar diameter. IL-13 is a Th2 cytokine that drives many of the features of asthma (mucus hypersecretion and AHR). We demonstrated that both TRAIL and IL-13 increase the effects of *Chlamydia* respiratory infection in early life (43, 44). TRAIL-deficient (*Tnfsf10*<sup>−/−</sup>) mice had less severe weight loss, pulmonary inflammation, histopathology, and mucus hypersecretion compared with wild-type (WT) mice after neonatal *C. muridarum* infection (44). However, the absence of TRAIL had minimal effects on *Chlamydia* load. Therefore, the major impact of TRAIL is on immune responses to *Chlamydia* and infection-induced pathology rather than on the infection itself. This shows a previously unrecognized role for TRAIL in immune responses to early-life *Chlamydia* respiratory infection.

In contrast, TRAIL is protective against pneumococcal and influenza lung infections (45–47). Steinwede and colleagues demonstrated that *Tnfsf10*<sup>−/−</sup> mice experienced more severe pneumococcal infection with impaired bacterial clearance and increased mortality, which was associated with reduced apoptosis of alveolar macrophages (45). TRAIL deficiency or treatment with anti-TRAIL antibody increased morbidity associated with influenza and delayed viral clearance (46, 47).

Thus, TRAIL-mediated responses may be important in the clearance of certain infections but detrimental in others. Thus, in certain infections (e.g., pneumococcal infection, influenza), therapeutics that enhance TRAIL-mediated apoptosis (e.g., recombinant [r] TRAIL, TRAIL receptor agonists) may be beneficial, whereas in others (e.g., *Chlamydia*), inhibition of TRAIL-mediated apoptosis (e.g., anti-TRAIL) would be the goal.

We have also shown that *Chlamydia* respiratory infection in early life decreases

the levels of the IL-13 decoy receptor (IL-13Rα2). This removes an important regulatory mechanism that controls IL-13 signaling and enables constitutive IL-13 to promote more severe infection, infection-induced pulmonary inflammation, histopathology, and mucus hypersecretion.

Previous studies from our laboratory have shown that IL-13 mRNA expression increased rapidly in the lungs of adult BALB/c mice after *C. muridarum* infection and promoted susceptibility to infection (48). Adult *Il13*<sup>−/−</sup> mice had significantly reduced disease severity, *Chlamydia* load, and inflammation compared with WT mice, and administration of rIL-13 into the lungs reversed these effects. IL-13<sup>−/−</sup> macrophages had enhanced uptake of *C. muridarum* *in vitro* and *in vivo*. Moreover, the depletion of IL-13 during infection of lung epithelial cells *in vitro* decreased the percentage of infected cells and reduced bacterial growth. Infection altered the phenotype of dendritic cells (DCs) *in vivo* to increase Th2 cytokine secretion, including IL-13, and infection of murine bone marrow-derived DCs *in vitro* also resulted in increased secretion of IL-13 (35, 43). Coculture of infected DCs with Ova-specific T-cell receptor transgenic CD4<sup>+</sup> T cells promoted a Th2 phenotype, and adoptive transfer of these cells into naive BALB/c mice increased the levels of IL-13 in the airways (49). Relevant to early-life infections, systemic DCs isolated from the spleens of adult mice infected as neonates or infants, but not as adults, increased IL-13 release from Ova T-cell receptor transgenic CD4<sup>+</sup> T cells compared with control DCs (41).

Collectively, these data suggest that *Chlamydia* infection alters the phenotype of DCs to deviate the induced immune response from a protective Th1 to a nonprotective Th2 response that is permissive for chronic infection and consequently increases susceptibility to Th2-driven asthma.

##### Early-Life *Chlamydia* Respiratory Infection Promotes Persistent AHR via TRAIL and IL-13

We showed that neonatal and infant, but not adult, *Chlamydia* respiratory infection led to persistent AHR even in the absence of allergen sensitization and challenge (40, 41). We extended these studies to show that it is both TRAIL and IL-13 responses that are induced during early life *Chlamydia*

respiratory infection that promote persistent AHR (42, 43) (Figure 2). Both *Tnfsf10*<sup>-/-</sup> and anti-TRAIL-treated WT mice were protected against infection-induced AHR. However, administration of rTRAIL or agonistic death receptor (DR) 5 antibody to naive neonatal mice did not induce persistent AHR, identifying that increased TRAIL responses in combination with infection is required. TRAIL has been previously shown to promote AHR in three different models of AAD (49, 50). Interestingly, genetic deletion or siRNA silencing of TRAIL abolished Ova- and house dust mite-induced AHR, whereas delivery of rTRAIL into the airways of naive mice induced AHR. These TRAIL-mediated effects were IL-13 dependent, as administration of rTRAIL to the lungs of *Il13*<sup>-/-</sup> mice did not induce AHR, whereas delivery of rIL-13 into the airways of *Tnfsf10*<sup>-/-</sup> mice did. These data suggest that IL-13 is required for TRAIL-induced AHR. This is consistent with our data that show that early-life infection-induced persistent AHR is both TRAIL and IL-13 dependent.

We also showed that that neonatal infection-induced alveolar enlargement is TRAIL dependent, as it did not occur in *Tnfsf10*<sup>-/-</sup> mice (43). Infection-induced TRAIL-mediated alveolar enlargement may contribute to reduced lung function. Increased alveolar diameter results in an overall loss of alveolar attachments to airway walls and, therefore, loss of elastic support. Decreased alveolar attachments are associated with deformity of the peripheral airways and decreased airflow (51).

We also show that *Chlamydia* respiratory infection in infancy results in persistent AHR in the absence of AAD, which was dependent on IL-13 and signal transducer and activator of transcription (STAT)-6 (42). Furthermore, increased AHR in adulthood was restored by administration of rIL-13 during early-life infection in *Il13*<sup>-/-</sup> mice. However, similar to our observations with rTRAIL, the administration of rIL-13 in the absence of infection did not induce AHR. However, it is not infection-induced production of IL-13, but rather a decrease in the decoy

receptor IL-13Rα2, that promotes exaggerated IL-13 signaling and AHR (43).

Respiratory viral infections also induce AHR in an IL-13- and/or STAT-6-dependent manner (52–56). Treatment of RSV-infected mice with anti-IL-13 inhibited AHR, and AHR did not develop in *Stat6*<sup>-/-</sup> mice (55). Importantly, similar to our observations with *Chlamydia*, RSV infection of 7-day-old mice results in persistent AHR in adulthood (52).

Collectively, these studies demonstrate that IL-13 responses induced during respiratory bacterial and viral infections in early life induce persistent AHR. Thus, IL-13 inhibitors, which are already in clinical trials as asthma treatments, could be a potential therapeutic strategy for suppressing infection-induced AHR in early-life.

### IL-13 Responses during Early-Life Infection Increase the Severity of Subsequent AAD

We previously showed that early-life *Chlamydia* respiratory infection increased the severity of subsequent AAD by increasing the number of MSCs, IL-13 production, and AHR (40, 41). We recently described more detailed mechanisms, highlighting the importance of constitutive IL-13 responses during early-life infection as a key contributor to the increased severity of AAD (43). Infection of WT mice in early life did not increase the production of IL-13 or the expression of its primary signaling receptor, IL-13Rα1, but reduced the levels of its decoy receptor, IL-13Rα2. This indicates that infection suppresses decoy receptor expression promoting exaggerated IL-13 responses. Neutralization of IL-13 by administration of anti-IL-13 monoclonal antibody during early life infection prevented the development of subsequent infection-induced severe AAD by reducing the number of MSCs and AHR (43). Lukacs and colleagues also showed that the preferential production of IL-13 during a primary RSV infection plays a critical role in the exacerbation of cockroach allergen-induced disease in later life (57). Similar to what we observed with early-life *Chlamydia* infection, the depletion of IL-13 during RSV infection inhibited the development of severe allergen-induced AHR in later life. Another study by You and colleagues showed that neonatal exposure to RSV resulted in persistent AHR, pulmonary

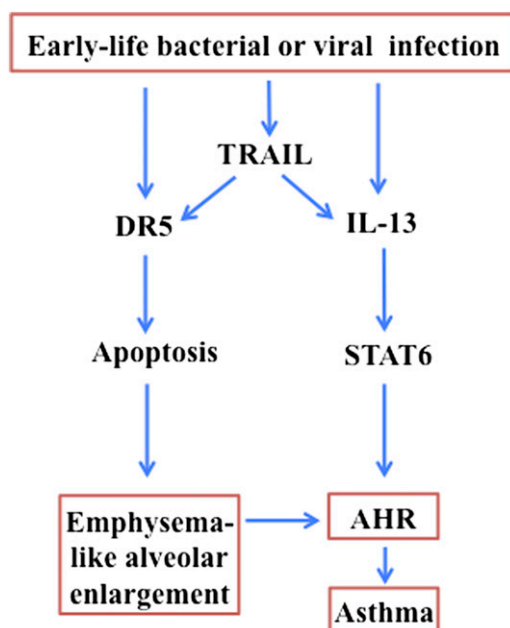


**Figure 2.** Early-life respiratory infections increase tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and IL-13 responses, which promote the development of more severe asthma. Bacterial (e.g., *Chlamydia*) and viral (e.g., respiratory syncytial virus, rhinovirus) infections increase TRAIL, which can induce apoptosis of alveolar epithelial cells downstream of its death receptor. This leads to emphysema-like alveolar enlargement and a loss of alveolar attachments to the airways. These infections also induce IL-13 responses and signal transducer and activator of transcription 6 (STAT6) signaling that drives airway hyperresponsiveness (AHR). Importantly, TRAIL may be upstream of IL-13 and require IL-13 to induce AHR. DR5 = death receptor 5.

inflammation, and subepithelial fibrosis and more severe subsequent Ova-induced AAD (52). Similar effects were also observed after neonatal RV infection, which resulted in increased severity of subsequent AAD by inducing pulmonary inflammation, MSC metaplasia, and AHR (54). These effects were again IL-13 dependent.

Collectively, these studies suggest that a prior respiratory infection can have substantial effects on airway responses and function and can promote more severe AAD and asthma. Respiratory infection in early life with *Chlamydia* or common respiratory viruses such as RSV and RV may induce TRAIL or IL-13-dependent changes in immunity, structure, and function contributing to the development of more severe asthma. This identifies TRAIL and IL-13 as

novel therapeutic targets for early-life infection-induced asthma, and anti-IL-13 may be developed for this in the short term.

## Future Directions

Studies of such models of infection-induced asthma in early life enable the investigation of other novel mechanisms of pathogenesis. microRNAs and epigenetic changes could be important, and there is a resurgence of interest in innate immune pathways (e.g., toll-like receptors) that could be important (58). Studies of other models of early-life respiratory infection (e.g., RSV, RV, *Haemophilus influenzae*, influenza, etc.) will identify the generalizability of mechanisms of pathogenesis. It is also

important to translate experimental findings into human studies.

## Conclusions

Early-life *Chlamydia* and viral respiratory infections have long-lasting consequences for lung health and may be a significant risk factor for the development of chronic lung diseases such as asthma and emphysema. Investigations of the mechanisms involved identify novel therapeutic targets such as TRAIL and IL-13. The development of humanized monoclonal antibodies such as anti-IL-13 hold great promise and may be efficacious in early-life respiratory infections and subsequent asthma. ■

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).

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