Programming of the lung by early-life infection

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Many important human diseases, such as asthma, have their developmental origins in early life. Respiratory infections in particular may alter the course of asthma and may either protect against or promote the development of this disease. It is likely that the nature of the effects depends on the type and age of infection and is determined by the impact of infection on the immune and respiratory systems. Immunity in early life is plastic and can be moulded by antigen encounter, which may enhance or reinforce the asthmatic phenotype of early life, or induce protective responses. Chlamydial respiratory infections have specific effects and may increase asthma severity in early life by promoting systemic interleukin 13 responses and causing permanent changes in lung structure. Respiratory viral infections, such as those of respiratory syncytial virus and rhinovirus, promote pro-asthmatic responses in early life that contribute to the induction of asthma. By contrast, probiotics or infection or exposure to certain bacteria, such as *Streptococcus pneumoniae*, may have protective effects in asthma by increasing the numbers and activity of regulatory T cells. Here, we review the impact of infections on the developmental origins of asthma. Understanding these effects may lead to new therapeutic approaches for asthma that either target deleterious infections or utilize beneficial ones.

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Role of infections in the developmental origins of asthma and other diseases

Infections in early life have long-term consequences on respiratory and general health. This is because immunity in early life is plastic and can be altered and moulded by factors and events such as exposure to infections and allergens, as well as different diets and obesity.^{1–8} Infections in early life alter immunity by affecting the phenotype of immune responses, especially of T helper cells, macrophages and other immune and structural cells.^{9–11} Infections may also reduce lung function, which becomes irreversible as the lungs mature during the first 2 years of life. Thus, infections may play a major role in the developmental origins of health and disease. They may either protect against or predispose to the development of many other conditions, for example, asthma.^{3,4,10–17}

The nature of immune responses to infectious agents and allergens is the result of coordinated responses of immune cells that regulate each other through complex signalling networks. Innate and adaptive immune cell activity and cytokine release may be critically important in driving pathogenic responses to infections and to allergens. In asthma, T helper type 2 (Th2) cells and the cytokines they release, interleukin (IL)-4, IL-5 and IL-13, mediate pathogenesis (reviewed in¹⁸). IL-4 is necessary for Th2 cell differentiation, promotes immunoglobulin E production, allergic inflammation and the development of

mucus hypersecretion and airway hyper-responsiveness (AHR). IL-5 is required for the development and influx of eosinophils and may contribute to AHR. IL-13 is a critical mediator of Th2 responses and hallmark features of allergic airway disease (e.g. inflammation, mucus hypersecretion and AHR). Antigen-presenting cells (APCs), plasmacytoid and myeloid dendritic cells (DCs) and alveolar macrophages, are innate immune cells that are critical mediators of adaptive responses to infection and are important in the mechanisms that lead to alterations in T-cell programming. APCs recognize pathogens and present antigen as part of the major histocompatibility complexes to T cells, which drives the development of effector T cells with particular functions (e.g. protective or destructive).^{19–21} Infections may alter the phenotype of APCs by altering surface molecule expression, inducing Th2 and inflammatory responses that promote allergic airway diseases, such as asthma.

Numerous pathogens that infect the lung are associated with the development of asthma. Early-life infections with viruses (respiratory syncytial virus, rhinovirus, influenza) and bacteria (*Chlamydia* and *Mycoplasma*) have deleterious effects and may drive the development of wheezing, asthma and stress and potentially have systemic effects.^{3,4,22–24} Others, such as probiotics, and as we have shown *Streptococcus pneumoniae*, may be beneficial and suppress pro-asthmatic responses.^{12,25–29} There has also been substantial recent interest in the role of the entire lung microbiome in the development of asthma.^{30–32} There is an increase in the numbers and species (i.e. the bioburden) of the lung microbiome in asthma, and reduced lung function is related to bioburden and diversity. Nevertheless, the mechanisms that underpin the effects of

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infections remain poorly understood. If we can elucidate the mechanisms, we may be able to develop new treatments for asthma that either target deleterious pathogens or utilize beneficial ones.

The association of chlamydial respiratory tract infections in early life and asthma

Chlamydial respiratory tract infections (RTIs) are an example of how infections in early life may promote the developmental origins of disease and asthma. RTIs with *Chlamydia pneumoniae* are very common and frequently cause asymptomatic lung infection and acute respiratory disease in children and adults. At any particular time, 5% of children and adults have asymptomatic chlamydial respiratory infection.^{33,34} Importantly, 80% of adults have anti-chlamydial antibodies, indicating that most individuals have been infected at some stage of their lives.³⁵ Substantial epidemiological, clinical and experimental evidence associates infection with *C. pneumoniae* with the development and exacerbation of asthma, particularly severe asthma in children and adults.^{3,13,36–40} Recent studies specifically implicate early-life chlamydial infection in the development of reduced lung function and asthma later in life.^{10,11,13,37–40} Early *C. pneumoniae* infection in children is strongly associated with acute wheezing, disease progression and asthma.^{13,38,39,41–45} Indeed, 50% and 70% of children with severe asthma have culturable and detectable *C. pneumoniae* and/or *Chlamydia trachomatis* in their lungs, respectively. The role of *Chlamydia* in asthma is almost certainly underappreciated because of the following: the need to assess lower respiratory tract tissues, difficulties in detection, lack of standardized methods, assessment not being routine in clinical laboratories, infections often being asymptomatic and the effects of infection persisting after the clearance of the bacteria.^{3,46,47} Given that many individuals are infected in the respiratory tract with *Chlamydiae*, it is likely that 'two hits' are required for asthma development and both environmental and genetic factors are likely to contribute.⁴⁸ Such factors include the status of maturation of the immune system and allergic sensitization. Indeed, some individuals may be genetically predisposed to aberrant immune responses (i.e. Th2 responses) to environmental factors (e.g. chlamydial RTIs) that may inhibit the clearance of infectious agents and promote the development of allergy and asthma.³

The mechanisms of the association between early-life chlamydial RTI, reduced lung function and enhanced asthma in later life remain little known and there are currently no effective treatments for infection-associated asthma. It is likely that infection induces permanent immune and structural alterations that play crucial pathogenic roles,⁴⁹ and we have investigated these possibilities (see subsequent sections). In addition, we, and others, have also shown that *Chlamydia* can infect and activate airway epithelial cells and induce the release of IL-8, IL-13 and granulocyte macrophage-colony-stimulating factor that could enhance airway

inflammation and asthma severity.^{18,50–52} *Chlamydiae* can also infect endothelial cells and induce the release of pro-fibrotic factors such as basic fibroblast growth factor and cytokines such as IL-8 and monocyte chemotactic protein-1, as well as soluble factors that may contribute to inflammation and remodelling in asthma.^{53–55} These bacteria also have the capacity to infect airway smooth muscle cells,⁵⁶ which may alter muscle tone and induce AHR. *Chlamydiae* may also affect the innervation of the airways and can infect cells of the nervous system such as microglia and astrocytes and promote the release of pro-inflammatory cytokines such as IL-6, which may induce Th2 cell development and expansion, as well as AHR.⁵⁷ Detailed studies that determine how infections promote the development and exacerbation of asthma are required to facilitate the design of effective interventions.⁵⁸

Chlamydial RTI in early life causes permanent alterations in immunity and lung structure and function, and increases the severity of asthma

Our group, and others, have investigated the mechanisms of how chlamydial RTIs are associated with the induction and increased severity of allergic airway disease in later life using mouse models.^{10,11,59} We developed a model of neonatal chlamydial RTI and combined it with the classic model of ovalbumin-induced allergic airway disease. We used *Chlamydia muridarum*, which is a natural mouse respiratory pathogen and is the most appropriate chlamydial species to investigate host-pathogen relationships and the impact of infection on allergic airway disease in mice.^{10,11} We infected neonatal mice on the 1st day of life with 400 inclusion-forming units of *C. muridarum*. The bacteria replicated in the lungs and infection peaked after 10 days, declined after 15 days and was cleared by 21 days.¹⁰ Infection was mirrored by histopathological evidence of inflammation in the lung. Infection was allowed to resolve and allergic airway disease was induced on day 45. We then assessed the impact of infection on allergic airway disease.^{10,11} Controls were: (1) infection without allergic airway disease, (2) allergic airway disease without infection and (3) no infection or allergic airway disease.

The induction of allergic airway disease resulted in the development of hallmark features of asthma with increased numbers of mucus-secreting cells around the airways, IL-13 expression in the lungs and increased AHR. Neonatal chlamydial RTI increased the severity of allergic airway disease in later life by further increasing mucus-secreting cell numbers, IL-13 expression and AHR. The mechanisms involved were then investigated. The development of allergic airway disease was also characterized by eosinophilic inflammation of airway tissue, increased Th2 cytokine release (IL-5 and IL-13) from ovalbumin-restimulated T cells in the lung-draining mediastinal lymph nodes (MLN) and the influx of activated mDCs into the lungs. Surprisingly, all of these pro-inflammatory responses that are normally associated with enhancing allergic airway disease were suppressed by the prior neonatal chlamydial RTI, with

infection instead inducing a mixed Th2/Th1 response. The effects of infection on systemic DC-induced T-cell responses were then assessed. DCs were isolated from spleens of adult (9-week-old) mice that were infected or sham-exposed as neonates. DCs were pulsed with ovalbumin and cultured with ovalbumin-peptide T-cell receptor-specific transgenic (ovalbumin TCR Tg) T cells. Neonatal infection resulted in the increased release of IL-13 in DC and T-cell co-cultures. IL-13 can induce all the hallmark features of asthma in mice, and this effect of infection may account for some/all of the increases in severity of allergic airway disease. The effect of infection on lung structure was also assessed. Neonatal infection had striking and permanent effects on lung structure. Infection resulted in alveolar wall destruction, which substantially increased alveolar diameter in adulthood, which was reminiscent of emphysema in the parenchyma. These destructive effects were permanent as they were present in adult mice 9 weeks after neonatal infection. Infection had little observable effect on airway structure (thickness of airways-associated basement membrane, epithelial cell or smooth muscle layers), apart from mucus-secreting cell hyperplasia. These effects on parenchymal structure have recently been confirmed by others⁵⁹ and may contribute to the induction of AHR and reduced lung function by neonatal infection. It is not known how these specific changes in lung structure enhance AHR, which was detected by increases in transpulmonary resistance and decreases in dynamic compliance (i.e. the inverse of elastic recoil). It is likely that reductions in alveolar attachments to the airway wall decreased airway support and elastic recoil, leading to alterations in resistance and compliance. Furthermore, the level of resistance is mostly driven by the larger airways, although the smaller airways contribute, whereas the smaller airways are primarily responsible for compliance. The consequences of infection that resulted in parenchymal destruction may have led to a stiffening of the smaller airways, which could enhance their contribution to resistance and reduce compliance. It is also possible that infection of the larger airways and smooth muscle may have altered their reactivity without changing their structure, although this possibility remains untested.

We also examined the effect of chlamydial infection during the infant and adult stages.¹¹ Infant mice were infected at 3 weeks of age just after weaning, which equates to a 2-year-old human infant, whereas adult mice were infected at 6 weeks of age. Allergic airway disease was induced 6 weeks after infection (i.e. when mice were 9 or 12 weeks old). Infant infection also increased the severity of allergic airway disease by increasing mucus-secreting cell numbers, IL-13 expression and reducing lung function. However, these effects occurred through very different mechanisms compared with neonatal infection. Infant infection increased Th2-mediated allergic inflammation (increased IL-5 and IL-13 release from MLN T cells stimulated with ovalbumin, and eosinophil influx into the airways) and systemic DC-induced IL-13 release, but had no effects on lung structure. By contrast, adult infection had no effect on lung structure and/or any of the features of allergic airway disease that were altered by neonatal and/or infant infections.

Thus, early-life infection increases the severity of allergic airway disease and potentially asthma, but occurs through different mechanisms in different age groups.

Chlamydial RTI, IL-13 and asthma

The role of IL-13 in chlamydial infections was further investigated.^{19,51} *Chlamydia* is known to grow in innate immune cells such as neutrophils and macrophages,⁶⁰ and these observations were extended to show that bone marrow DCs could also be infected.¹⁹ Infection induced the production of IL-13 by DCs and increased the proliferation of ovalbumin TCR Tg T cells and promoted a Th2 phenotype. Adoptive transfer of infected and ovalbumin-pulsed DCs along with ovalbumin TCR Tg T cells into naïve adult mice increased IL-13 release into bronchoalveolar lavage fluid and AHR compared with similarly treated but uninfected DCs.¹⁹ These studies suggest that chlamydial RTI may subvert DC function to promote Th2 responses and AHR in asthma. These Th2 responses may be ineffective in removing the bacteria, which requires interferon (IFN)- γ and Th1 responses for clearance.^{61,62}

We have also used adult IL-13-deficient ($-/-$) mice to demonstrate that infection and disease are markedly reduced in the absence of IL-13.⁵¹ Compared with their wild-type counterparts, IL-13 $-/-$ mice did not lose weight and had reduced infection and airway inflammation. Depletion of CD4 $+$ T cells did not affect the clearance of *Chlamydia* in IL-13 $-/-$ mice, suggesting a lack of CD4 $+$ T-cell involvement. We then showed that a lack of IL-13 increased the uptake of *Chlamydia* by macrophages and decreased the infection of lung epithelial cells (i.e. LA4 murine lower respiratory epithelial cells).⁵¹

Thus, IL-13 promotes chlamydial RTI, and as IL-13 is increased in the airways of asthmatics this may increase their susceptibility to infection and worsen the severity of asthma. We have also shown that an ongoing, rather than cleared, chlamydial RTI in adults increases the severity of asthma by inducing a phenotype resembling neutrophilic asthma that is dependent on infection-induced neutrophils.⁶³ We have made similar observations with *Haemophilus influenzae* that may be driven by infection-induced IL-17/Th17 responses.^{64,65}

Other infections and the developmental origins of asthma

Infections with respiratory syncytial virus and rhinovirus in early life have also been widely associated with the induction and exacerbation of asthma, which we have extensively reviewed recently,⁴ and it is not necessary to repeat the details here. This probably occurs through increases in airway inflammation and inappropriate Th2 responses that are induced by these infections, and which are reinforced upon re-infection. A recent study has shown that infection of

neonatal mice with pneumonia virus of mice, the murine equivalent of respiratory syncytial virus, also increased the severity of subsequent allergic airway disease.⁶⁶ This also occurred through the augmentation of Th2 responses. Again, infection was associated with the development of mucus hypersecretion and AHR, and the effects were dependent on signalling through the IL-4 receptor α .⁶⁶

Many studies have investigated the use of including probiotics in early life to inhibit the development of asthma and other allergic diseases. Probiotics can activate common mucosal surfaces, and effects in the gastrointestinal tract can alter responses in the airways.⁶⁷ They have immune-modulating capacity and can alter DC responses, induce the development of Tregs and attenuate allergic responses. Administration of probiotics in children may have benefits in eczema but trials investigating their capacity to suppress asthma have had variable results and are, as yet, not recommended for management of allergic diseases in children.^{67,68}

We have performed a series of studies that suggest that the Gram-positive bacteria *S. pneumoniae* may also have beneficial effects in asthma.^{12,26–29} Although this bacterium is a common respiratory pathogen, it is surprising that it has not been widely associated with asthma. The studies that have been performed have shown that infection suppresses peripheral eosinophilia⁶⁹ and that *S. pneumoniae* vaccination reduces asthma exacerbations.^{70,71} Furthermore, asthmatic children do not necessarily seroconvert upon infection and have low *S. pneumoniae* antibody levels.^{72,73} Thus, infection or immunization with *S. pneumoniae* may boost immune responses and moderate allergic responses, thereby reducing the severity of asthma.¹² We have shown that *S. pneumoniae* infection, the killed bacteria or its human vaccines, suppress the asthma phenotype in our models of allergic airway diseases. This occurs through the induction of increased numbers of regulatory T cells that have enhanced suppressive capacity and reduce the proliferation of Th2 cells and their release of cytokines. It is known that *S. pneumoniae* induces the production of regulatory T cells to a greater extent than other Gram-positive bacteria.⁷⁴ Others have shown similar suppressive effects with daily gavage of mice with *Lactobacillus*,¹⁵ *Helicobacter* infection¹⁶ and a range of other bacteria.¹²

Summary

Infectious challenge in early life can promote the developmental origins of both health and disease, and our hypothesis is that the outcome depends on the nature and age of infection and perhaps also the timing of infection relative to allergen exposure. Respiratory viruses and some bacteria, particularly *Chlamydia* and *Mycoplasma*, are associated with the induction or increased severity of asthma in early life. In contrast, exposure to probiotics, *S. pneumoniae* or other bacteria may be protective. Whether the effects are beneficial or deleterious are likely to result from the phenotype of immune responses to infection, the nature of the interaction

of the infectious agent with the immune response and the impact of infection on lung structure and function. Elucidating the relationships between infections, immunity and lung structure in early life is likely to identify new potential avenues for the development of therapeutic approaches in order to prevent the emergence of disease in early life.⁷⁵

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