Development of Allergic Immunity in Early Life

Clare M Lloyd[1] & Sejal Saglani[1,2]

[1]Inflammation, Repair & Development Section, National Heart and Lung Institute, Faculty of Medicine, Imperial College, London; [2] Dept of Paediatric Respiratory Medicine, Royal Brompton Hospital, Royal Brompton Harefield NHS Foundation Trust, London, UK

Contact:

Inflammation, Repair & Development Section

National Heart and Lung Institute

Sir Alexander Fleming Building

Faculty of Medicine

Imperial College

South Kensington

London SW7 2AZ. UK

+442075943102

c.lloyd@imperial.ac.uk

Running Title: Allergic immunity in Early Life

Abstract

The growth and maturity of the peripheral immune system and subsequent development of pulmonary immunity in early life is dictated by host, environmental and microbial factors. Dysregulation during the critical window of immune development in the postnatal years results in disease which impacts on life-long lung health. Asthma is a common disease in childhood and is often preceded by wheezing illnesses during the preschool years. However, the mechanisms underlying development of wheeze and how and why only some children progress to asthma is unknown. Human studies to date have generally focused on peripheral immune development, with little assessment of local tissue pathology in young children. Moreover, mechanisms underlying the interactions between inflammation and tissue repair at mucosal surfaces in early life remain unknown. Disappointingly, mechanistic studies in mice have predominantly used adult models. This review will consider the aspects of the neonatal immune system which might contribute to the development of early life wheezing disorders and asthma, and discuss the external environmental factors which may influence this process.

Key words: Asthma, inflammation, wheezing, immune development

Introduction

Asthma is a childhood onset disease, with 1.1 million children currently affected in the UK. Children diagnosed with asthma by age 7 years already have a deficit in lung function and increased bronchial responsiveness as neonates (1). Furthermore, longitudinal birth cohort studies have shown wheezing and bronchial hyper-responsiveness in early childhood are predictors of newly diagnosed asthma in early adulthood (2), and a prior diagnosis of asthma is associated with an increased risk for COPD among never smokers(3). The fundamental pathophysiological features of allergic asthma in both adults and children include airway hyperresponsiveness, resulting in airway obstruction that is usually reversible, chronic inflammation that is predominantly eosinophilic and structural changes of the airway wall, termed remodelling. We have shown eosinophilic inflammation and structural airway remodelling (4) are already established during the preschool years and become maximal and equivalent to the changes seen in adulthood by school age (5). Birth cohort and cross-sectional studies of preschool children with wheezing have uncovered strong associations between lung function and immune responses in early life. Furthermore, children who apparently "outgrow" early wheezing illnesses remain at increased risk for relapse or recurrence during midlife(6).

Although approximately one-third of all infants and preschool children have wheezing disorders, we do not know why some but not all pre-school children wheeze; and why some wheezy children develop asthma, whereas others spontaneously remit. No current medication, including inhaled corticosteroids (ICS), can prevent the development of asthma. Therefore, a focus on the understanding of the early life pulmonary immune environment is essential not only to prevent the development of asthma, but also in the long term to contribute to the prevention of COPD (7). This review will consider the impact of various intrinsic and extrinsic factors which may impact the development of wheezing and progression to asthma (Fig 1).

Severe asthma in children

Although the majority of children with asthma achieve good control with only low-moderate doses of maintenance inhaled steroid therapy, approximately 5% remain poorly controlled despite maximal prescribed therapy. This small proportion with persistent poor control are a significant clinical challenge as they utilise up to 50% of all healthcare resources for asthma (8). All children with on-going asthma symptoms despite maximal conventional treatments are described as having Problematic Severe Asthma (9). The sub-group in whom persistent symptoms result from a failure of basic asthma management have “Difficult Asthma” (DA) (10), whilst those who remain symptomatic despite these factors having been addressed have “Severe Therapy Resistant Asthma” (STRA) (11). A common definition and guidelines for management of severe asthma have recently been proposed for all patients aged 6 years and over (10). Although a significant advantage of common guidelines is the ability to trial novel treatments using similar criteria, some key differences underlying the evolution and pathophysiology of severe asthma between children and adults must be considered, and an automatic extrapolation of findings from adult clinical trials to children may not always be appropriate.

Pathophysiology of paediatric asthma

Asthma is characterised by eosinophilic inflammation, airway hyperresponsiveness (AHR) and structural airway remodelling, including increased thickness of the subepithelial reticular basement membrane, increased airway smooth muscle mass and angiogenesis (12). All of these changes are established in children by school-age, and are present regardless of disease severity (13),(14) (5). Asthma has traditionally been thought of as a disease of type 2 immunity due to increased levels of IL-13, IL-4 and IL-5. As such therapy has been directed towards mitigating the effects of these cytokines (15, 16). However, asthma is notoriously heterogeneous, with numerous clinical and pathological phenotypes (17) and

there is evidence that not all patients fit this restrictive, Th2 biased categorisation of disease pathology (18). In particular, patients with severe disease may not all have a Th2 phenotype.

A feature of severe asthma in young children is the early presence of airway remodelling – including thickened basement membrane (4). It has been proposed that a normal inflammatory response to harmful stimuli is followed by a phase of repair and regulation, whereas during chronic disease repeated cycles of inflammation result in exaggerated repair culminating in tissue remodelling. However, increasing evidence now confirms that at mucosal surfaces, the barrier epithelial cells and underlying stromal cells are active immunologically and cooperation with immune effector cells results in development of inflammation and repair in parallel, or in disease exaggerated inflammation and abnormal remodelling (19). Using both our neonatal model of inhaled allergen exposure and paediatric bronchoscopic airway samples we have shown early and parallel onset of inflammatory and structural changes is apparent in airway mucosal diseases such as preschool wheezing and asthma (20) (4, 21) and cystic fibrosis (22). Specifically, we were among the first to show an absence of tissue eosinophilic inflammation or remodeling in infants with recurrent respiratory symptoms and wheeze at a year of age (21), but increased inflammation and remodeling by 3 years (4) which increased further with age and was established to a similar degree to adults by school-age (5, 23). Early life events that result in altered tissue repair are likely to be sustained across the life-course and result in down-regulation of cell defence mechanisms and impact the ageing trajectory. Although associations between inflammation and remodelling are apparent during early life mucosal diseases, nothing is known about the interactions between the maturing immune system and remodelling pathways, and whether repair pathways are more easily triggered in early life and thus lead to exaggerated repair.

Th2 mediators in paediatric severe asthma

Children with STRA have a persistent airway luminal and tissue eosinophilia and airway remodelling despite high dose maintenance steroid therapy. Another key feature of

childhood severe asthma is the presence of severe atopy with multiple aero-allergen sensitisation. This has been found repeatedly in cohorts as a feature of severe disease (24) (23, 25). However, in our study, in which evidence of type 2 inflammation was sought in induced sputum supernatant and bronchoalveolar lavage fluid (two different platforms) and immunohistochemistry of bronchial biopsies, evidence for the presence of IL-4, IL-5 and IL-13 was scarce (23). Furthermore, in a separate unrelated cohort, the paediatric Severe Asthma Research Programme (SARP), found neither Type 1 nor Type 2 inflammation dominated in children with severe asthma (26). The airway pathology is therefore present despite a relative absence of elevated levels of Th2 cytokines. Using an age appropriate neonatal mouse model of inhaled allergen challenge, we have shown that the Th2 cytokines, IL-5 and IL-13 in particular, are steroid sensitive (27). An explanation for the paucity of Th2 mediators in paediatric STRA may be that since significant care is taken to ensure patients are compliant with maintenance steroid therapy, levels of Th2 mediators may be dampened.

Innate mediators and severe asthma with fungal sensitisation

The fact that children with STRA remain symptomatic, even while on inhaled steroids, and in 50% of cases while on maintenance oral steroids (23) indicates that other molecular pathways underlie disease pathology. Although IL-4, IL-5 and IL-13 levels were difficult to detect, we did find an increased frequency of patients with elevated IL-33^{+} cells in the submucosa of the airway wall (27). IL-33 is an innate cytokine derived from the airway epithelium that has been associated with the initiation and potentiation of the pathobiology of allergic asthma (28, 29). We have shown using endobronchial biopsies from children with STRA that submucosal IL-33 expression was associated with increased thickness of the reticular basement membrane, a specific feature of airway remodelling (27). Moreover, *in vitro* culture of primary fibroblasts from the STRA patients' biopsies showed increased secretion of collagen following IL-33 stimulation compared to controls, even in the presence of the steroid budesonide. Intranasal administration of rIL-33 to non-allergic mice resulted in increased collagen production in the lung, as well as the expected increase in AHR. Using

our neonatal murine model of inhaled house dust mite exposure, we showed levels of IL-33 remained elevated in allergen challenged mice, despite steroid therapy, and this was associated with persistent AHR and remodelling. Interestingly, levels of IL-13 were significantly reduced. Thus it was apparent that IL-33 was relatively steroid resistant and was associated with airway remodelling, both of which are features of severe disease in children with STRA (27). Conversely, remodelling was absent in HDM-exposed $ST2^{-/-}$ mice that lack a functional receptor for IL-33. The fact that IL-33, but not IL-13, was maintained following steroid treatment of neonatal HDM-exposed mice, as well as in biopsies from children with STRA suggests that IL-33 is resistant to the actions of steroids. This is important because IL-33 is a cytokine with wide ranging effects on a variety of cells and is thought to bridge innate and adaptive immune pathways (30). Numerous experimental models have shown that IL-33 induces the development of type 2 innate lymphoid cells (ILC2s) (31) (32). These cells belong to a recently discovered population that displays none of the usual lineage markers for leukocytes but produce effector cytokines. Like effector T cells, ILCs have now been classified according to the cytokines that they secrete and the transcription factors that they express (33). ILC2 cells are defined by the production of type2 cytokines IL-13, IL-5 and IL-4 as well as expression of Gata3. We have shown that children with STRA have significantly elevated levels of ILC2 cells (defined as $Lineage^{-}CD45^{+}CRTH2^{+}$) in their blood, broncho-alveolar lavage and in induced sputum (34). Although present in the blood, numbers were very small, and there were significantly higher proportions of ILC2 in the airways, demonstrating the propensity of these cells to remain at local tissue sites.

Although as a group, children with STRA had increased tissue expression of IL-33, the heterogeneity of the disease was reflected in the spread of IL-33 levels within the group, thus highlighting the need to identify sub-phenotypes of patients in whom any mediator, such as IL-33, is the predominant driver of disease. Having shown using our adult murine model that exposure to the fungal allergen *Alternaria alternata* resulted in IL-33 driven exacerbation of allergic airways disease (35), we investigated the role of this mediator in the very specific clinical sub-phenotype of severe asthma with fungal sensitisation (SAFS) (36). We have

shown that children with SAFS had evidence of worse allergy than STRA without SAFS, despite being prescribed more steroid treatment (37). Using our neonatal mouse model we compared disease severity following inhaled HDM exposure to *Alternaria* exposure, and also showed higher serum IgE levels and greater eosinophilia in animals sensitised to *Alternaria*, which was maintained despite administration of steroids. Although levels of Th2 cytokines were not different between HDM and *Alterrnaria* exposure, IL-33 was significantly higher in the *Alternaria* exposed neonatal mice. Moreover, on dividing patients into those with and without fungal sensitisation we showed IL-33 was increased in BAL, and immunohistochemistry of bronchial biopsies in children with SAFS (37). Interestingly, these patients also had higher levels of MMP-9 which is known to be important in regulation of extracellular matrix molecules such as collagens within tissues. To date, trials of anti-fungal agents have been disappointing in patients with SAFS (38), but these data suggest this sub-phenotype may be the most amenable to therapeutics that block the action of IL-33.

Neutrophils in childhood severe asthma

Unlike adults, in whom neutrophilic asthma is a recognised steroid resistant phenotype of severe disease (39), we have shown that children with STRA do not have increased neutrophils or mast cells in their airways or in bronchial biopsies (23, 40). However, we have recently demonstrated that intra-epithelial neutrophils are present in some children with STRA (40). Although children with STRA had increased intra-epithelial neutrophils compared to controls, what was most interesting was that the epithelial neutrophil expression was high in a sub-group of STRA patients and low in others. Challengingly, the neutrophil-high children had better asthma outcomes (higher FEV_1 percent predicted, better symptom control) while being prescribed lower maintenance inhaled steroid doses. However, despite the presence of intra-epithelial neutrophils, levels of tissue and luminal IL-17A were not increased in STRA, while, epithelial expression of the IL-17R was increased and stimulation of primary bronchial epithelial cells with IL-17A led to steroid resistant IL-8 secretion (40) .

Interestingly, children who grow up in cattle farms and are protected from the development of asthma also have increased peripheral neutrophils (41). These data highlight two critical issues pertaining to discovery of novel therapies for paediatric STRA. The first is the inability to extrapolate findings from adult studies to children and the need for age appropriate mechanistic studies, and secondly that targeting inflammatory mediators or cells without confirmation of findings from experimental models in human tissue is unlikely to be successful as has been demonstrated by initial trial of blocking IL-17 in adult patients (42).

Clinical features and risk factors specific for paediatric disease

Preschool wheeze and its progression to asthma

An important clinical phenotype that is very distinct and unique to children is that of wheezing in preschool children. Cohort studies have shown that there are several clinical phenotypes of wheezing in preschool children with different outcomes by school-age(43). Preschool wheezers who develop asthma have a permanent reduction in lung function by school-age (44), persisting into adulthood (45) and increasing susceptibility to chronic obstructive pulmonary disease (COPD) (7). The factors that initiate asthma are very different from those that perpetuate the disease. No current medication, including inhaled corticosteroids (ICS), can prevent progression from preschool wheeze to asthma, but ICS are able to control the symptoms of established asthma. Thus investigating the molecular mechanisms underlying the inception of preschool wheeze and its progression to asthma is essential to identify therapeutic targets that will prevent early and sustained lung function abnormalities. Early viral infections (46), bacterial colonization (47) and allergen sensitization (48) are important in causing wheeze and the subsequent development of asthma. These early life exposures, coupled with genetically determined susceptibility, have a major impact on the natural history of the disease. Cumulatively, the data highlight the critical nature of this early period in which the maturing immune system meets new environmental exposures, and subsequent immune/inflammatory responses in the lung are initiated. This underscores

the need for animal models of asthma to include young, as well as adult animals, particularly when considering the induction of disease. Two clinical phenotypes of preschool wheeze have been described (49). In episodic (viral) wheeze, children only wheeze with respiratory infections (usually viral), and do not have symptoms at other times. In multiple trigger wheeze, children wheeze both with infections, and are symptomatic between infections. Importantly, the natural history and clinical management of each phenotype is different (49, 50). Multiple trigger wheezers are more likely to be atopic, respond to regular inhaled steroids, develop an early and permanent reduction in lung function and go on to develop asthma in school age. Conversely, episodic wheezers are less atopic, only respond to intermittent therapy at the time of symptoms, and are less likely to develop asthma. However, the distinctive molecular mechanisms that result in development of each of these wheezing subtypes and the factors that dictate progression to allergic asthma remain unclear.

Pathology of preschool wheeze

Preschool multiple trigger wheezers have a distinct airway inflammatory profile to episodic wheezers (51). Multiple trigger wheezers have evidence of eosinophilic airway inflammation (52). Children with this phenotype tend to have a good response to treatments for allergic asthma, in particular they have reduced symptoms and exacerbations if treated with maintenance low dose inhaled steroids (53). Although the inflammatory profile is eosinophilic, numbers of mast cells are similar in wheezers and non-wheezers. Little is known about the cytokines driving preschool multiple trigger wheeze, although increased expression of IL-4 in the submucosa has been reported (51). Since leukotriene-receptor antagonists have variable benefit, there are few other therapeutic targets currently available for those with severe preschool wheeze that persists despite high-dose inhaled steroids. It is known that in addition to eosinophilia, features of airway remodelling, specifically increased thickness of the reticular basement membrane, are already present in children with persistent, severe wheeze (51, 52). However, neither of these features are predictive of

asthma development by school-age. The only pathological abnormality that predicts future asthma is airway smooth muscle(54), but at present, no biomarkers that represent smooth muscle function in preschoolers are known, so this feature cannot be used to identify future asthmatics; and in any event, we lack interventions to prevent the evolution of preschool wheeze to asthma.

Early sensitisation (55), in particular to inhalant and perennial allergens with high levels of specific IgE (56), is an important risk factor determining progression to asthma in school age and early, multiple sensitisation predicts a severe disease trajectory (57). In addition, genetic susceptibility is an important factor that contributes to childhood severe asthma (58). Several genes identified from GWAS have specifically been associated with childhood asthma including IL-33, which has also been associated with severe disease in both children (58) and adults (59). In addition, when the sub-group of children with severe exacerbations are considered, IL-33 was again identified as a susceptibility locus.

It is clear that there are significant early changes in lung pathology that occur in children with these wheezing phenotypes, but what events precipitate these changes and crucially what factors mediate progression and propagation of disease through the life course are not well understood. Given that it is recognised that asthma and probably preschool wheeze, reflect the pathological consequences of a disordered immune system, it seems important to consider the immense changes that occur to the immune system during this early life period, and how these may impact the lungs, which also continue to develop postnatally.

Development of immunity in early life

Newborn babies are particularly vulnerable to infection in early life because the immune system is not yet developed, but is shaped and educated during this immediate postnatal period. Although transfer of maternal antibodies across the placenta provides some

protection against pathogens, the potential antigenic burden is very high compared to the relatively sterile semi-allogeneic environment in utero. It was perceived that babies were born in a state of immune tolerance in order to protect them from developing overwhelming immune responses upon exposure to the wealth of pathogens encountered on leaving the protected in utero environment. This antigenic burden can be intrinsic as well as extrinsic - as birth triggers exposure to previously unseen, benign self-antigens to which the neonate must develop tolerance in order to prevent later autoimmune reactions. Similarly, immune responses must be restrained upon exposure to environmental antigens, such as allergens. Robust responses to these antigens would lead to hyper-inflammation that would be dangerous and destructive to organs such as the lungs that are still developing postnatally. Thus, immune reactions against most antigens are kept under tight control, but mature adaptive immune responses can be mobilised when the baby is faced with life threatening, dangerous pathogens (60). It is apparent that early life represents a window of both vulnerability and opportunity that impacts both immune development and tissue homeostasis, therefore it is important to understand how immunity develops in the normal lung in order to try and appreciate how allergic immunity develops.

Innate immune mechanisms are compromised during the first months of life. There is evidence that expression of TLRs that recognise key molecular signatures on the surfaces of pathogens are either absent or expressed at very low levels in the new-born. In fact, it has been documented that TLR2 and 4 are largely absent from the murine foetal lung, but expression is rapidly upregulated after birth (61). Human studies are much harder to perform, but although data in human neonatal lung are rare, nasal mucosal explants taken from young children showed enhanced allergen-induced T cell reactivity and proliferation, increased production of Th1 cytokines, IL-10 production and TLR4 expression in response to TLR stimulation (62). Investigators have also taken advantage of cord blood as well as some studies using peripheral blood from babies. A comprehensive longitudinal profiling study of innate immunity over the first 2 years of life revealed qualitative and quantitative

differences in TLR responses in neonates compared to those in adults. Comparison of responses to TLR stimulation in adult and neonatal peripheral blood determined that monocytes and dendritic cells from neonates have a reduced capacity to secrete IL-12p70, IFN-α and IFN-c, but a greater facility to secrete IL-10 than adult cells(63). These findings suggest that rather than neonates being in a state of immune suppression responses to TLR stimulation are enhanced at birth, but differences are age specific and progression is not linear. Studies with human cord blood have shown that perinatal TLR responses are increased – particularly in newborns who subsequently develop allergic disease. Neonates from allergic mothers, or those that subsequently developed allergies showed increased secretion of TNFα following TLR3, 4, 5 stimulation and enhanced IL-6 after TLR, 4, 5, ligands. Crucially, however, T cell derived IFNγ responses to mitogen or allergen were suppressed compared to non-allergic subjects (64). Subsequent longitudinal analysis of TLR responses in allergic versus non allergic children during the first 5 years of life revealed significant differences over time. From birth non-allergic children exhibit increased production of IL-10, TNFα and IL6 in response to TLR stimulation over the first five years of life (65). In comparison, allergic children show enhanced cytokine production at birth but this decreased over time so that by age 5, this hyperresponsiveness to stimuli had declined and cytokine responses were reduced in comparison to similar aged non-allergic children. A comprehensive study of immune development in urban preschool children determined that early life environmental exposures are vital in the stimulation of cytokine responses (66). Importantly, although cytokine production increased with age, these responses at birth were poorly predictive for later responses at aged 1 or 3. Interestingly, exposure to common environmental allergens, such as cockroach, mouse or dust mite was associated with enhanced cytokines responses at age 3 years, including production of IFN-α and IL-10. However, reduced LPS-induced IL-10 responses as birth were related with recurrent wheeze, while RSV-induced IL-8, allergen-induced IL4 were both associated with atopy. Clearly the cytokine balance during early life is critical in the development of allergic

responses and is influenced by a variety of external factors. In future, it will be important to determine which cells secrete particular cytokines and establish how this fluctuates over time as well as determine whether the cellular source of the cytokine during development influences pathology. For example, development of allergic disease in the first year of life in high risk children has been associated with reduced responses of T regulatory cells to microbial stimuli (67). Collectively, studies show that TLR patterning occurs during the perinatal period and that the mother's allergic status influences this. Although these differences in TLR responses may protect the neonate from over-exuberant inflammation to microbial infection, they may contribute to the development of inappropriate reactions to environmental antigens such as allergens.

Neutrophils are a vital component of the innate immune system and are the most prevalent leukocyte in human blood. However, both neutrophil numbers and functionality are reduced in neonates compared with adults (63). Neonatal neutrophils have reduced expression of TLR4, defective MyD88 and p28 signalling (68), and lower levels of CD11b/CD18 which reduces their capacity for transmigration and chemotaxis (69). In addition, neonatal neutrophils have reduced phagocytic capacity and impaired intracellular killing (70). Collectively, these deficiencies in numbers, mobilization and function increase the susceptibility of neonates to sepsis.

Pulmonary macrophages in early life

The lungs are colonised with macrophages in the early postnatal period. Macrophages are the most predominant leukocyte within the lung and there are at least two distinct populations resident at homeostasis: Airway macrophages (AM) that are resident within the airspaces and interstitial macrophages (IM) that are situated within the lung parenchyma. Each are characterized by their unique location, properties and functions, and populations may be identified on the basis of their expression patterns of the integrins CD11b and CD11c (71). AMs have high surface expression of CD11c but lack CD11b expression, distinguishing them from macrophages present in other tissue compartments. Conversely,

IMs express high levels of CD11b but only low levels of CD11c. AMs have a critical influence in maintaining immunological homeostasis and are able to sense the local environment to maintain suppression or initiate the appropriate host defence response. In contrast, IMs are thought to exert a regulatory function within the lung tissue. Whilst AM employ non-specific lines of defence (such as high phagocytic ability, the secretion of antimicrobials, nitric oxide (NO), tumour necrosis factor (TNF)-α and interferon (IFN)-γ), it has been suggested that interstitial macrophages have a greater capacity to release specific cytokines associated with the adaptive immune response, such as interleukin (IL)-10 (72).

Although once thought to be derived from mononuclear phagocyte precursors recruited from the bone marrow it is now accepted that airway macrophages derive from yolk sac macrophages and are established prenatally. Experiments with mice show that monocytes do not make a significant contribution to tissue macrophage populations under homeostatic conditions and furthermore, following depletion of lung macrophages, repopulation of the lung occurred by proliferation in situ, rather than replacement from the bone marrow (73-75). Given the recognition that tissue residency has a specific impact on macrophage phenotype, experiments examining cord blood monocytes are unlikely to be reflective of airway and lung macrophage phenotypes. However, these samples are particularly difficult to obtain and there is scant information regarding phenotypes of interstitial macrophages in newborns or how they impact on development of allergic immunity. Some studies have used bronchial lavage samples from babies requiring mechanical ventilation during chronic infections, and suggested that phenotypic maturation of monocyte populations was associated with gestational age. Preterm infants had increased proportions of non-classical $CD14^{+}CD16^{+}$ monocytes at birth, and immature macrophage phenotypes were associated with progression from respiratory distress syndrome to chronic lung disease of prematurity (76). Preterm infants with resolving lung injury had greater proportions of mature macrophages, than those with progressive lung disease. Since these types of studies rely on lavage of ventilated, intubated babies, there is no information regarding the phenoptype of

macrophages during normal human perinatal development or on the impact of phenotype on development of asthma in subsequent childhood.

Perinatal Dendritic cells in the lung

Neonatal murine lungs show fewer conventional dendritic cells, coupled with a lower ratio of $CD103^+$ to $CD11b^+$ DC (77). However, neonatal lungs matured and were able to prime adult naïve CD4T cells as effectively as adult DC. Interestingly, both adult and neonatal BCG-primed DC induced Th2 cytokine responses from neonatal lymph node T cells, indicating that Th2 skewing is an intrinsic feature of neonatal T cells (77, 78). Similarly, the ratio of plasmacytoid (pDC) to myeloid dendritic cells (mDC) is reversed in human cord blood dendritic subsets compared to those in adult peripheral blood (79). Both mDC and pDC were found to exhibit a more immature phenotype compared to those from adult blood, with lower expression of key markers such as MHC class II and ICAM1 as well as the costimulation molecules CD80 and CD86. In vitro stimulation assays using TLR ligands such as LPS, poly I:C or CpG showed that neonatal (cord blood) mDC failed to mature to the extent of adult cDC (80, 81). The failure of neonatal DC to initiate efficient Th1 responses may rest with a specific defect in mDC whereby IL-12(p35) is transcriptionally repressed at the chromatin level (82). Human neonatal APC also show impaired production of type I interferons in response to TLR agonists. Allergic children show higher numbers of $TLR2^+$DC at birth, and these enhanced levels are maintained throughout the first year of life. This is important because these cells are known to produce enhanced levels of inflammatory cytokines such as IL-6 (65). Collectively, these defects in innate immune signalling may contribute to the propensity of neonatal T cells to generate immune reactions involving type 2 cytokines rather than type 1 and with persistent allergen exposure may contribute to the development of allergic disease and asthma in children.

T helper cells in early life

Early life exposure history shapes development of the immune system - however it is not known whether pathology progresses because of an imbalance in regulatory cells vs. effector cells, and if so how microbial exposures affect this imbalance. This is important since accumulating evidence suggests that these early life microbial exposures are linked to subsequent development of wheeze and asthma (83). Although the ontogeny of CD4 subsets has been examined in peripheral blood during the first year of life there is less information regarding the phenotypes of key lymphoid cells within the pulmonary mucosa of neonates.

The relatively limited exposure to antigen in utero dictates that newborns are more reliant on innate immune pathways for protection against infections. However, neonatal mice have been shown to be able to mount mature adaptive immune responses in vivo (60, 84). Foetally derived CD4+ T cells with an effector memory phenotype have been shown to be present in cord blood (85). These cells develop during foetal life within the uterine environment which is thought to be relatively sterile, but occur even in the absence of pathology or infectious history. Importantly, they exhibit a variety of different effector inflammatory functions associated with CD4 T helper cells at birth. Moreover, foetal cells are thought to be an important source of type-2 cytokines in early life in both mice and humans (60, 86).

Nasal associated lymphoid tissue (NALT) is established before birth, while bronchus associated lymphoid tissue (BALT) develops postnatally (87). This immature immune system is shaped following postnatal exposure to bacteria, viruses and allergens, influencing its development and may result in skewing towards health or disease. The majority of studies to date have used peripheral blood cells to examine early life immune cell phenotypes. These studies have determined that a T helper 2 (Th2) cell preference is required for a healthy pregnancy (88), but also that this skew is maintained during the neonatal period, reducing gradually during the first 2 years of life (89). In contrast, atopic children retain these foetally derived allergen-specific responses (89). This deviation from the physiological in utero Th2

skewing, with exaggerated Th2 responses in either pregnancy or the first 3 months of life has been associated with an increased risk of subsequent childhood asthma or wheeze (90).

Interestingly, a skewed Th2 cytokine response was initiated from either adult or neonatal BCG-primed DC using neonatal lymph node T cells, indicating that Th2 skewing is an intrinsic feature of neonatal T cells (77). In fact, it seems that this skewing of neonatal T cell immunity towards type 2 reactions limits inflammatory damage while permitting colonisation with neonatal commensal microbes in the intestine. One of the major effector functions of human neonatal T cells has been shown to be production of the chemokine CXCL8, which can activate antimicrobial neutrophils and γδT cells. Importantly these T cells were found to be rare in adults – highlighting the distinct nature of the neonatal immune system and provides a potential protective mechanism against bacterial infection in newborns (91). Similarly, γδT cells from newborns show enhanced pleiotropic functional responses when compared to αβT cells, and importantly lack the characteristic deficit in IFNγ production. Therefore, in the absence of a mature αβT cell compartment, γδT cells are poised at birth to contribute to both immuno-protection and immuno-regulation (92).

Although the mechanisms that facilitate persistence of the neonatal type2 environment are unclear, transcriptional profiling of circulating Th2 cells in mice and humans has revealed sets of genes that are likely to confer pathological features to Th2 cells that may be either unique to specific allergic diseases such as asthma or rhinitis, while others maybe common to a range of atopic disorders (93, 94). Interestingly, Th2 cells from asthmatic patients specifically displayed increased expression of genes which were predicted to support enhanced Th2 survival, proliferation/activation and cytokine production. Furthermore, the IL-33/ST2 axis seems to selectively licence memory Th2 cells to promote allergic type inflammation via production of IL-5 and thus stimulate eosinophilic inflammation (95, 96). These pathogenic effector Th2 cells seem to be a specific subpopulation of Th2 cells with enhanced pro-inflammatory function and have been described in patients with eosinophilic

gastrointestinal disease as well as atopic dermatitis (97). The latter patient population were children, but the point during immune development when this subpopulation develops has yet to be determined. T cell phenotypes are widely acknowledged to be plastic with receptor expression and cytokine production influenced by a wide range of intrinsic and extrinsic factors – including allergen exposure as well as age, so it is possible that the balance of subsets changes over the lifespan according to particular exposures. In the future, it will be important to examine the temporal shifts in Th subsets of the lifespan in order to identify pathways for novel treatment.

Regulation of inflammation at mucosal sites is controlled by specialized populations of tissue resident regulatory T cells that express RORgt or GATA3 - key transcription factors normally associated with effector Th populations (98) (99). Described in murine models of gut inflammation, these regulatory populations are held in balance by the local microbiota. In addition, expression of the IL-33 receptor is thought to define a population of T regulatory cells in the gut that is important for accumulation and maintenance of T regulatory cells in an inflammatory environment (100). As yet, it is unclear whether they are also present at human mucosal sites – including the lung. Normal pregnancy is associated with skewing towards a type 2 immune environment which is thought to afford protection to the developing foetus, and maybe due to an altered ratio of effector to regulatory T cells (101). Although this inequity normally corrects during the first year of life, there is evidence that prolongation of the type 2 bias increases the risk of allergy and atopic diseases. Functional $FoxP3^{+}$ cells with enhanced expression of PD-1 are generated following in vitro activation of naïve cord blood derived $CD45RO^{+}CD25^{+}CD^{+}$ T cells with APC, compared to cells derived from adult peripheral blood (102). Longitudinal analysis of infant peripheral blood has determined that perinatal Th cells have an enhanced propensity to develop into FoxP3+ T regulatory cells in the first 12 months of life but that Th17 cells can already be induced at 3 months (103). Additionally, the proportion of both resting naıve T regulatory cells and activated Tregs isolated from peripheral blood increase markedly from birth to 6 months of age (104). In

contrast little is known regarding the phenotypes of cells within the airways of infants. However, it is important to remember that neonatal thymectomy in mice results in development of autoimmune disease, highlighting the importance of a competent regulatory population even early in life. $CD4^+CD25^+$ T cells are important for the development of neonatal tolerance to transplantation antigens.

Innate lymphoid cells in early life

Innate lymphoid cells (ILCs) are considered to play an indispensable role in immune homeostasis as well as the initiation, regulation and resolution of inflammation (105). ILCs are thought to exert particular influence at mucosal surfaces including the airways and gastrointestinal (GI) tract, with ILC2s promoting repair following influenza infection in the lung, and ILC3s mediating tissue repair following intestinal inflammation (106). They are also key players mediating interactions between innate and adaptive type 2 immune pathways (107). Despite the proposed importance of these effector cells in immune regulation and disease, and the intimate link with T helper cells, nothing is known about their role in immune maturation and early life development. Recent evidence indicates that the ILC2 population undergoes rapid expansion in the neonatal lung during the first few weeks of life (108) (109),(110).

Factors that may result in accentuated or prolonged Th2 skewing include maternal allergy (111) but environmental exposures are also critical as has become apparent from the farming exposure studies (112). Growing up in close proximity to cows seems to provide protection and incidence of allergic diseases are greatly reduced in children who have grown up in farming communities (83). Similarly, diet has a profound influence on the systemic microbiome which impacts the developing immune system and thus the trajectory towards disease or health (113). Although it is apparent that the composition of inhaled exposures has a direct impact on the airway immune profile, mechanistic studies in experimental laboratory models in the context of a developing immune system are scarce, while studies in children have predominantly used peripheral blood rather than cells directly isolated from the

lung. Recent data in mice suggests that enhanced neonatal skewing of Th2 immunity after exposure to allergens results from hyperactivity of the IL-33-pathway (108). IL-33 production during the postnatal phase of lung development is postulated to drive Th2 type immunity, thus lowering the threshold for innate immune response to allergen. Similarly, it has been shown that the process of birth is enough to trigger IL-33 production from the lungs of alveolar epithelial cells of newborn mice which drives the expansion of IL-33R expressing innate lymphoid cells (ILC), concomitant with IL-3 driven polarization of airway macrophages into an M2 phenotype (109). It is thought that this homeostatic type 2 pathway delays antibacterial effector responses, as well as prolonging the in utero derived type 2 environment. However, it is not known whether human neonates experience a similar wave of hyperactivity in IL-33 responses. Thus, it is not known how the balance between effector T cells or innate cell subsets differ in the lung following exposure to viruses, bacteria or allergens in early life.

We have recently shown that ILC2s are present in the airways of children with severe therapy resistant asthma (34). Proportions of ILC2 were determined by flow cytometry in BAL samples from children with STRA and compared to samples collected from children undergoing investigations for recurrent lower respiratory tract infections. Whereas ILC2 were extremely rare or absent in this latter group, they were present in BAL from asthmatic children. Interestingly, there were relatively higher proportions of ILC2 in the airway samples (either BAL or sputum) compared to peripheral blood.

These are descriptive studies, but mechanistic studies in mouse models have focused solely on the use of adult models with environmental exposures in the context of a mature immune system. However, since it is known that the neonatal immune system is physiologically skewed towards type 2 immunity, and an exaggeration of this skewing results in later disease, it is possible that in early life ILCs are less important in disease initiation, or that they may be protective. Experiments using adult mouse models have suggested that ILCs are vital for the development of an effective adaptive T cell response during classical type 2 responses initiated by allergens or parasitic worm infections (30) (114). However, given that

the neonatal immune landscape is different from that of the adult it is likely that there will be distinct differences in responses to allergens and cytokines in neonatal mice compared to adult mice. Indeed, accumulation of pulmonary ILCs at homeostasis occurs postnatally and relatively small numbers are present at birth. Conversely, ILC2 are thought to be critical in limiting inflammation and promoting tissue repair in order to maintain homeostasis (115). Although ILC2 are able to secrete a number of factors that are associated with wound repair, such as amphiregulin (116), mechanistic studies have shown that they are involved in development of pulmonary fibrosis following bleomycin injury and in patients with liver or dermal fibrosis, rather than in the specific repair of tissue following inflammation (117-119). It will be important in the future to delineate how ILC2 are able to contribute to the wound healing process without causing tissue remodelling. It is possible that those ILC2 associated with driving allergic pathology may represent a different subset or that their function may be context specific. This is a particularly important point in early life, when the lung is still developing, so the phenotypic characteristics of ILC2 during pulmonary immune development and their functional role in repair or resolution of inflammation needs to be understood.

External influences on the developing immune system

Although the mechanisms underlying development of early life wheeze are not well understood it is clear that a wide variety of external, environmental influences affect the developing neonatal immune system and therefore the development of sensitivity to allergens as well as to pathogens. It is clear that the postnatal period represents a critical window for development of aberrant immune responses, but the maternal environment has a significant impact. One of the major influences on the development of neonatal immunity and subsequent maintenance of immune homeostasis is our relationship with the microbes that live upon and within us. All of our mucosal surfaces, the skin, gut and lungs are colonized by dynamic communities of microbes that are integral for our well being. These local microbiota are essential for energy harvest from food sources, for metabolism and most importantly

when considering neonatal development, the training and education of local and systemic immunity (120). It is clear that robust immune health is associated with a flourishing and diverse microbiota, but each of these microbial ecosystems is dynamic and is exquisitely sensitive to the environment provoked by changes in diet, intake of drugs and hormonal influences.

A lack of diversity and resilience in the microbiota has been associated with the rise in autoimmune and allergic diseases documented in the developed world. For the neonate the first few years of life are associated with the acquisition of a competent microbiota, since the uterine environment is relatively sterile. This period represents a critical window for immune development, since the composition of the microbiome is affected by the birth process itself, whether the baby is breastfed, weaning as well as a whole host of external environmental influences such as living in a home with siblings and or pets, spending time in day care and living in an urban or rural environment (83). Each of these factors will impact on the diversity of the airway microbiota and therefore the education and training of the immune system. We have shown that age at first allergen exposure is critical in determining the degree of allergic immune responses generated. Neonatal mice exposed to inhaled house dust mite from day 3 of life had significantly more eosinophilia, AHR and type 2 immune responses compared to adult mice (121). Moreoever, if allergen challenge was commenced at day 14, then responses were negligible and the mice appeared protected. The formation of the lung microbiota is a key parameter in this process. During the first 2 weeks after birth, the bacterial load in the lungs increased, and there was a shift in the airway bacterial phyla from a predominance of Gammaproteobacteria and Firmicutes towards Bacteroidetes. The changes in the microbiota were associated with the decreased aeroallergen responsiveness seen at day 14 and the emergence of a Helios(-) Treg cell subset that required interaction with programmed death ligand 1 (PD-L1) for development. This demonstrated that the airway microbiota induces T regulatory cells early in life, and if its development dysregulated during a critical period of development postnatally, can lead to sustained susceptibility to allergic airway inflammation in adulthood (121).

Maternal influences

Although once considered a sterile environment the advent of more sensitive methods of detection has shown that the womb contains commensal microorganisms which may affect development of the foetal immune system, but this concept is not universally accepted (122, 123). Given that the foetus is nourished by the mothers blood supply it is understandable that key aspects of the mother's wellbeing will affect the foetus. These exposures will affect the growth and development of the foetus, but may also influence subsequent development of disease.

Maternal diet exerts a significant influence on the developing foetus. A recent study determined that the nature of maternal diet influenced epigenetic imprinting of immune cells and lung stromal cells in utero. Enhanced intake of dietary fibre by the mother was associated with increased levels of acetate which primed FoxP3 T regulatory cell mediated protection against development of asthma in mice, but critically a similar axis was observed in humans (124). Similarly, a recent study showed that supplementation during the last trimester of pregnancy with fish oil-derived fatty acids was sufficient to reduce the risk of persistent wheeze or asthma and lower respiratory tract infections by one third (125). Deficiency in certain vitamins, particularly vitamins D and A is associated with increased asthma risk. Vitamin D has wide ranging effects on immune cells, particularly development and functional capacity of T regulatory cells (126). Specifically, lower levels of serum vitamin D have been associated with increased airway remodeling and worsened asthma control in children with severe asthma (127). We have shown using a neonatal model of inhaled allergen exposure, that offspring from mother mice that were fed a vitamin D deficient diet had exaggerated airway eosinophilia and Th2 cells with reduced T regulatory cells. In addition, if pups were supplemented with a vitamin D sufficient diet at weaning this resulted in a significant reduction in serum IgE levels, reduced pulmonary eosinophilia and airway remodelling (128). Interestingly, a recent clinical trial determined that supplementation with vitamin D during pregnancy elicited a modest reduction in incidence of recurrent wheeze and

asthma in the offspring (129). Although mouse models have determined that maternal vitamin A (retinoic acid) affects development of lymphoid tissue inducer cells – thus affecting the size of secondary lymphoid organs and setting the threshold for immune responses in the offspring, supplementation studies in humans have not shown efficacy in prevention of development of asthma (130). Only a weak, inverse association was observed for maternal intake of vitamin A and E with childhood allergic rhinitis (131). Conversely, maternal obesity has profound effects on immune responses in the baby, giving rise to systemic inflammation as well as asthma. Maternal prenatal BMI is associated with increased incidence of wheezing and asthma (132).

There are multiple longitudinal studies that support the hypothesis that maternal, or paternal, smoking has a negative impact on pulmonary health in babies and greatly enhances the odds of developing recurrent wheeze or asthma (133-135). Cigarette smoke exposure has been shown to increase expression of specific microRNAs in the blood concomitant with lower numbers of T regulatory cells in maternal and cord blood, which was associated with an increased risk of atopic disease (136). Infants from smoking mothers also display attenuated innate TLR-mediate immune responses compared to infants from nonsmoking mothers (137). Similarly, a number of different studies have shown that traffic related air pollution not only affects lung growth, but is associated with an increased prevalence of asthma as well as wheezing (138).

Maternal stress is a significant risk factor for wheezing in early life as well as development of asthma (132, 139). This may be due to transfer of glucocorticoid hormone across the placenta, which has wide ranging effects on the immune system (140). Although it has been suggested that maternal stress impacts development of adaptive immune responses in neonates, with reduced humoral immunity and reduced capacity for Th1 responses, recent studies suggests that the effects may only be transient (141, 142).

Postnatal Influences

Immune homeostasis at mucosal surfaces is achieved by maintaining a balance between promoting tolerance to environmental particles and mounting rapid immune responses to pathogens including viruses and bacteria. Increasing evidence has shown that postnatal microbial colonization with commensal bacteria is vital in shaping the developing immune system, particularly at mucosal surfaces (120). Neonatal colonization by microbes begins soon after birth, is influenced by gestational age, the mother's microbiota, route of delivery at birth and use of antibiotics. Indeed prolonged use of antibiotics also has profound effects on the development of the neonatal immune system (143).

As the neonate transitions from the relative low microbe environment of the womb into the external world, rapid colonisation of mucosal surfaces by microbes takes place. These microbes and their metabolic products are arguably the strongest stimulators of early immune development and play a vital role in the maturation and education of the developing neonatal immune system. The mode of delivery of the infant has a profound effect on the microbial load experiences by the baby in the first few days and weeks of life. Caesarean and vaginal births result in different species of microbes that colonise the infant from the beginning, reflecting the normal flora of the skin and the vagina respectively (144). These microbes initially colonise the gastrointestinal tract and this intestinal microbiome undergoes dynamic changes during the immediate postnatal period, and this is associated with functional development of the immune system. As well as the differences in microbial exposure, the very nature of labour including high levels of circulating hormones, the pressure on the baby of uterine contractions and the stress of passage through the birth canal itself impacts multiple aspects of the neonatal immune system. Collectively, these factors elicit the release of a number of pro-inflammatory cytokines, including IL-6, IL-1, IFNγ and TNFα which are all measurable in both the maternal circulation and cord blood (145). Epidemiologic evidence suggests that children born via caesarian delivery have an increased risk for a range of metabolic and immune diseases, including atopic diseases, compared to than those born via vaginal delivery. A recent clinical study showed that

exposure of infants delivered by C-section exposed to maternal vaginal fluids soon after birth restored the skin, gut and oral microbial communities to a level comparable to that shown in babies after a vaginal birth (146). Although the authors did not assess the subsequent long-term consequences on health or development of immunity it is proof of concept that the microbiota can be manipulated postnatally in babies delivered by C-section. This concept is not universally accepted however, and an alternative study has suggested that the microbiota of each organ reflects that of its mother – regardless of the route of delivery. Longitudinal sampling of infants revealed that the microbiota structure and function expands and diversifies considerably from birth through the first few months. Thereafter it resembles the microbiota from the corresponding maternal body site – irrespective of the mode of delivery or other prenatal factors (147).

Breast-feeding is an important factor in neonatal immune development, apart from the obvious nutritional benefits. Human milk contains a variety of different proteins that aid digestion, facilitate maturation of the neonatal gut while also stimulating neonatal immunity and providing early antimicrobial protection during this period of immune development (133),(140). As well as providing leukocytes and complement proteins from the mother, breast milk contains regulatory cytokines such as IL-10 and TGFβ, which promote tolerance to antigens, including those in food, in early life (148, 149). Other immune-modulatory molecules include immunoglobulins, lysozyme – which can degrade gram-positive bacterial cells walls and kappa-casein that blocks pathogen binding to the gut wall, as well as dietary nucleotides and lipids. Multiple epidemiologic studies have shown that in developed countries a history of breast feeding is associated with reduced risk of severe lower respiratory infections, obesity as well as other important childhood diseases, but the effect on asthma or wheezing is debated (149). This is possibly because of a lack of clarity surrounding the diagnosis of both asthma and wheeze in many studies, but it seems that breast feeding for at least 4 months, compared to formula feeding may delay development of atopic dermatitis, cows milk allergy and wheezing in early childhood (150).

Influence of early life infections

The developing immune system is shaped by the antigens that are encountered in the immediate postnatal period, resulting in colonisation and establishment of the pulmonary microbiota and subsequent expression of pattern recognition receptors (PRRs) on resident cells such as epithelial cells and airway macrophages.

Numerous epidemiologic studies have shown that growing up in a farming environment is protective against development of asthma and childhood wheeze (83). The strongest protective effect has been seen in farming communities which retain traditional farming practices with families living in close contact with animals (41, 112). Amish and Hutterite farming communities share a genetic heritage and many other cultural aspects of life – apart from the close contact that the Amish have with their farm animals. However, whereas, incidence of allergies, wheeze and asthma are incredibly low in the Amish communities, incidence in Hutterite children is similar to that in urban communities. Importantly, the strongest protection was afforded to those children that are exposed to farm dust in utero (151). Recent analysis showed that although the incidence of asthma is 4 fold lower in the Amish population compared to the Hutterites, the level of dust in their homes was much higher (41). There were significant differences in the proportions and phenoytpes of innate immune cells between the two groups - with lower eosinophils and higher neutrophils in the Amish, and the converse in the Hutterites. Cell surface markers such as CXCR4 and CD11b were raised on neutrophils from the Hutterite children and their monocytes had raised levels of HLA-DR. These data indicate that the farm dust that the Amish were exposed to from very early in life has a significant influence on the developing immune system impacting on subsequent allergic diseases.

Respiratory infections are common in childhood and the combination of infections encountered during this developmental period has an important influence on the immune status of the lung. Respiratory infections with viruses or bacteria are the most common

triggers of exacerbations in asthmatic children but there is also evidence that they may be precipitating factors in the development of wheeze as well as asthma. Rhinovirus is the most commonly attributed driver of viral induced exacerbations. However, infants who develop wheeze concomitant with HRV infection are at a significantly enhanced risk for subsequent asthma. It has been suggested that host genotype is a significant factor and it is notable that variants within the 17q21 locus are associated specifically with asthma in children who experiences HRV wheezing illness (152), and expression of *ORMDL3* and *GSDM* – two genes found at this locus - were identified in GWAS studies as asthma risk genes (153). Cadherin-related family member 3 (CDHR3) was recently identified as an asthma risk gene in children with severe asthma and exacerbations (58). This is of particular interest because expression of CDHR3 on epithelial cells facilitates both binding and replication of rhinovirus-C in those cells that would normally be unable to support infection (154). Moreover, a coding SNP associated with enhanced cell-surface expression of CDHR3 protein is related to increased risk of wheezing illnesses and hospitalizations for childhood asthma.

Although the most common infection in childhood is rhinovirus, RSV is also very common in babies, and can lead to significant asthma risk. It has been shown that babies that experience very early infection with RSV (at less than 3 months of age) have higher Th2 responses with increased levels of IL-4 (155). However, although viruses have been described as causative agents in the development of wheeze and asthma, a prospective study in children indicates that the reverse is true. Prospective, repeated characterization of a birth cohort demonstrated that allergic sensitization during early life predisposes children to more severe viral respiratory illnesses and wheezing but that wheezing respiratory illnesses do not increases the risk for subsequent development of allergic sensitization (156).

Serial viral infections are a significant risk for wheezing and asthma but in addition certain bacterial infections can also enhance risk. A prospective study collected hypopharyngeal aspirates in babies at one month and incidence of wheeze monitored for the next 5 years (Bisgaard 2007 17928596 (157). Those neonates colonised with *S.pneumoniae*, *H influenzae* or *M.catarrhalis*, either alone or in combination, were found to be at increased risk

for recurrent wheeze and asthma by 5 years of age. Thus the nature, pattern and severity of infections experienced during childhood influences development of wheezing illness and asthma in some children, particularly if they have a susceptible genetic background.

Summary and Future directions.

Early life immunity is critical in determining lifelong health. Myriad influences on development of the pulmonary immune system and the combination of factors encountered by the neonate during this window determines heath or disease. All children encounter respiratory infections from birth, yet only a proportion develop asthma. The combination of genetic together with intrinsic and extrinsic factors dictate whether the immune response is appropriately tailored to clear infection versus an exaggerated response which changes the course of pulmonary immunity, resulting in disease (Fig 2). The challenge in determining the molecular mechanisms underlying this process is to accurately phenotype patients and use age-appropriate and tissue specific clinical samples in studies. This represents a significant challenge, but with the advent of novel technologies, which allow analysis of gene and protein expression at the micro level, this should be achievable. Similarly, it is imperative that we use appropriate preclinical models that will enable accurate translation of findings to children. Moving forward in our goal of more effective treatment of childhood allergic disease will require a shift in mind-set with respect to clinical trial design and more effective collaboration between industrial partners, clinicians and academic researchers.

References

1. Bisgaard H, Jensen SM, Bonnelykke K. Interaction between asthma and lung function growth in early life. Am J Respir Crit Care Med.2012;185:1183-1189.
2. Tagiyeva N, Devereux G, Fielding S, Turner S, Douglas G. Outcomes of Childhood Asthma and Wheezy Bronchitis. A 50-Year Cohort Study. Am J Respir Crit Care Med.2016;193:23-30.
3. Svanes C, Sunyer J, Plana E et al. Early life origins of chronic obstructive pulmonary disease. Thorax.2010;65:14-20.
4. Saglani S, Payne DN, Zhu J, et al. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med.2007;176:858-864.
5. Payne DN, Rogers AY, Adelroth E, et al. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med.2003;167:78-82.
6. Stern DA, Morgan WJ, Halonen M, Wright AL, Martinez FD. Wheezing and bronchial hyper-responsiveness in early childhood as predictors of newly diagnosed asthma in early adulthood: a longitudinal birth-cohort study. Lancet.2008;372:1058-1064.
7. Tai A, Tran H, Roberts M, Clarke N, Wilson J, Robertson CF. The association between childhood asthma and adult chronic obstructive pulmonary disease. Thorax.2014;69:805-810.
8. Lane S, Molina J, Plusa T. An international observational prospective study to determine the cost of asthma exacerbations (COAX). Respir Med.2006;100:434-450.
9. Hedlin G, Bush A, Lodrup Carlsen K, et al. Problematic severe asthma in children, not one problem but many: a GA2LEN initiative. Eur Respir J.2010;36:196-201.
10. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J.2014;43:343-373.
11. Bush A, Saglani S. Management of severe asthma in children. Lancet.2010;376:814-825.
12. Saglani S, Lloyd CM. Novel concepts in airway inflammation and remodelling in asthma. Eur Respir J.2015;46:1796-1804.
13. Barbato A, Turato G, Baraldo S, et al. Epithelial damage and angiogenesis in the airways of children with asthma. Am J Respir Crit Care Med.2006;174:975-981.
14. Turato G, Baraldo S, Zuin R, Saetta M. The laws of attraction: chemokines, neutrophils and eosinophils in severe exacerbations of asthma. Thorax.2007;62:465-466.
15. Pavord ID, Korn, S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet.2012;380:651-659.
16. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med.2011;365:1088-1098.

17. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med.2012;18:716-725.
18. Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. Nat Rev Immunol.2015;15:57-65.
19. Lambrecht BN, Hammad H. The airway epithelium in asthma. Nat Med.2012;18:684-692.
20. Saglani S, Mathie SA, Gregory LG, Bell MJ, Bush A, Lloyd CM. Pathophysiological features of asthma develop in parallel in house dust mite-exposed neonatal mice. Am J Respir Cell Mol Biol.2009;41:281-289.
21. Saglani S, et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med.2005;171:722-727.
22. Regamey N, Jeffery PK, Alton EW, Bush A, Davies JC. Airway remodelling and its relationship to inflammation in cystic fibrosis. Thorax.2011;66:624-629.
23. Bossley CJ, Fleming L, Gupta A, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. The Journal of allergy and clinical immunology.2012;129:974-982 e913.
24. Sharples J, Gupta A, Fleming L, et al. Long-term effectiveness of a staged assessment for paediatric problematic severe asthma. Eur Respir J.2012;40:264-267.
25. Fitzpatrick AM. Severe Asthma in Children: Lessons Learned and Future Directions. J Allergy Clin Immunol Pract.2016;4:11-19; quiz 20-11.
26. Fitzpatrick AM, Teague WG. Severe Asthma in Children: Insights from the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Pediatr Allergy Immunol Pulmonol.2010;23:131-138.
27. Saglani S, Lui S. Ullmann N, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. The Journal of allergy and clinical immunology.2013;132:676-685 e613.
28. Lloyd CM, Saglani S. Epithelial cytokines and pulmonary allergic inflammation. Curr Opin Immunol.2015;34:52-58.
29. Mitchell PD, O'Byrne PM. Epithelial Derived Cytokines in Asthma. Chest.2016.
30. Halim TY, Steer CA, Matha L, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. Immunity.2014;40:425-435.
31. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. IL-33-responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. Journal of immunology (Baltimore, Md : 1950).2012;188:1503-1513.
32. Kim HY, Chang YJ, Subramanian S, et al. Innate lymphoid cells responding to IL-33 mediate airway hyperreactivity independently of adaptive immunity.2012.
33. Spits H, Artis D, Colonna, M, et al. Innate lymphoid cells--a proposal for uniform nomenclature. Nat Rev Immunol.2013;13:145-149.
34. Nagakumar P, Denney L, Fleming L, Bush A, Lloyd CM, Saglani S. Type 2 innate lymphoid cells in induced sputum from children with severe asthma. The Journal of allergy and clinical immunology.2016;137:624-626 e626.
35. Snelgrove RJ, et al. Alternaria-derived serine protease activity drives IL-33-mediated asthma exacerbations. The Journal of allergy and clinical immunology.2014;134:583-592 e586.
36. Denning DW, Pasley C, Hartl D, et al. Fungal allergy in asthma-state of the art and research needs. Clin Transl Allergy.2014;4:14.
37. Castanhinha S, Sherburn R, Walker S, et al. Pediatric severe asthma with fungal sensitization is mediated by steroid-resistant IL-33. The Journal of allergy and clinical immunology.2015;136:312-322 e317.
38. Agbetile J, Bourne M, Fairs A, et al. Effectiveness of voriconazole in the treatment of Aspergillus fumigatus-associated asthma (EVITA3 study). The Journal of allergy and clinical immunology.2014;134:33-39.

39. Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. The Journal of allergy and clinical immunology.2015;135:299-310; quiz 311.
40. Andersson CK Adams A, Nagakumar P, et al. Intraepithelial neutrophils in pediatric severe asthma are associated with better lung function. The Journal of allergy and clinical immunology.2016.
41. Stein MM, Hrusch CL, Gozdz J, et al. Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children. N Engl J Med.2016;375:411-421.
42. Busse WW, Holgate S, Kerwin E, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. Am J Respir Crit Care Med.2013;188:1294-1302.
43. Savenije OE, Granell R, Caudri D, et al. Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA. The Journal of allergy and clinical immunology.2011;127:1505-1512 e1514.
44. Morgan WJ, Stern DA, Sherill DL, et al. Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence. Am J Respir Crit Care Med.2005;172:1253-1258.
45. Sears MR, Greene JM, Wilan AR, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med.2003;349:1414-1422.
46. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. Am J Respir Crit Care Med.2008;178:667-672.
47. Bisgaard H, Hermansen MN, Bonnelykke K, et al. Association of bacteria and viruses with wheezy episodes in young children: prospective birth cohort study. BMJ.2010;341:c4978.
48. Illi S, et al. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. Lancet.2006;368:763-770.
49. Brand PL, Baraldi E, Bisgaard H, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J.2008;32:1096-1110.
50. Brand PL, Caudri D, Eber E, et al. Classification and pharmacological treatment of preschool wheezing: changes since 2008. Eur Respir J.2014;43:1172-1177.
51. Turato G, Barbato A, Barraldo S, et al. Nonatopic children with multitrigger wheezing have airway pathology comparable to atopic asthma. Am J Respir Crit Care Med.2008;178:476-482.
52. Saglani S, Payne DN, Zhu J, et al. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med.2007;176:858-864.
53. Castro-Rodriguez JA, Rodrigo GJ. Efficacy of inhaled corticosteroids in infants and preschoolers with recurrent wheezing and asthma: a systematic review with meta-analysis. Pediatrics.2009;123:e519-525.
54. O'Reilly R, Ullmann L, Irving S, et al. Increased airway smooth muscle in preschool wheezers who have asthma at school age. JAllergy ClinImmunol.2012.
55. Lazic N, Roberts G, Custovic A, et al. Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts. Allergy.2013;68:764-770.
56. Simpson A, Lazic N, Belgrave DC, et al. Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years. The Journal of allergy and clinical immunology.2015;136:1224-1231.
57. Belgrave DC, Buchan I, Bishop C, Lowe L, Simpson A, Custovic A. Trajectories of lung function during childhood. Am J Respir Crit Care Med.2014;189:1101-1109.
58. Bonnelykke K, Sleiman P, Nielsen K, et al. A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations. Nat Genet.2014;46:51-55.
59. Moffatt MF, Gut IG, Demanais, F, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med.2010;363:1211-1221.

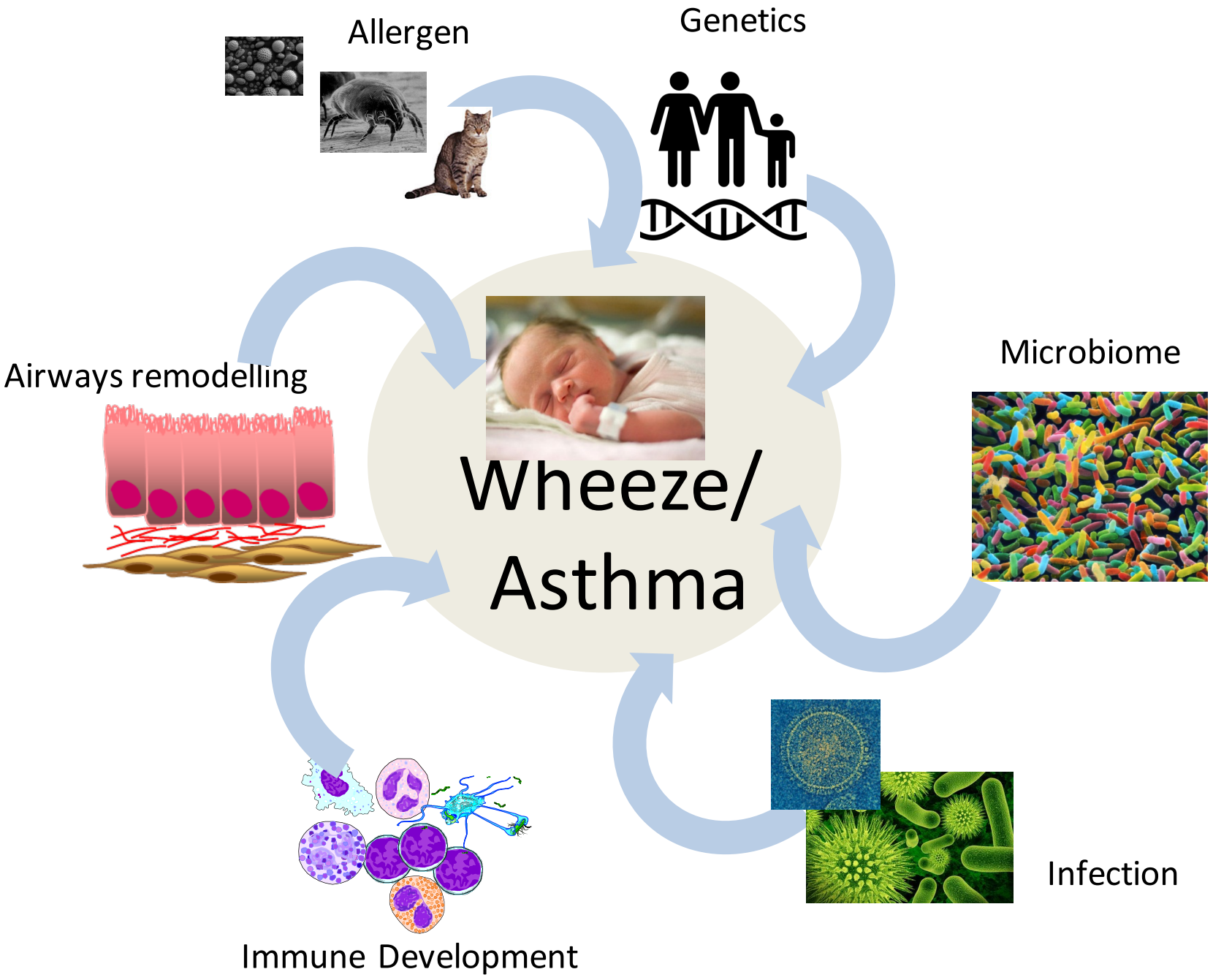
60. Adkins B, Leclerc C, Marshall-Clarke S. Neonatal adaptive immunity comes of age. Nat Rev Immunol.2004;4:553-564.
61. Harju K, Ojaniemi M, Rounioja S, et al. Expression of toll-like receptor 4 and endotoxin responsiveness in mice during perinatal period. Pediatr Res.2005;57:644-648.
62. Tulic MK, Fiset PO, Manoukian JJ, et al. Role of toll-like receptor 4 in protection by bacterial lipopolysaccharide in the nasal mucosa of atopic children but not adults. Lancet.2004;363:1689-1697.
63. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. Nat Rev Immunol.2007;7:379-390.
64. Prescott SL, Noakes P, Chow BW, et al. Presymptomatic differences in Toll-like receptor function in infants who have allergy. The Journal of allergy and clinical immunology.2008;122:391-399, 399 e391-395.
65. Tulic MK, Hodder M, Forsberg A, et al. Differences in innate immune function between allergic and nonallergic children: new insights into immune ontogeny. The Journal of allergy and clinical immunology.2011;127:470-478 e471.
66. Gern JE, Caltroni A, Jaffee KF, et al. Patterns of immune development in urban preschoolers with recurrent wheeze and/or atopy. The Journal of allergy and clinical immunology.2017.
67. Ismail IH, Boyle RJ, Mah LJ, Licciardi PV, Tang ML. Reduced neonatal regulatory T cell response to microbial stimuli associates with subsequent eczema in high-risk infants. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology.2014;25:674-684.
68. Al-Hertani W, Yan SR, Byers DM, Bortolussi R. Human newborn polymorphonuclear neutrophils exhibit decreased levels of MyD88 and attenuated p38 phosphorylation in response to lipopolysaccharide. Clin Invest Med.2007;30:E44-53.
69. Kim SK, Keeney SE, Alpard SK, Schmalstieg FC. Comparison of L-selectin and CD11b on neutrophils of adults and neonates during the first month of life. Pediatr Res.2003;53:132-136.
70. Miller ME. Phagocyte function in the neonate: selected aspects. Pediatrics.1979;64:709-712.
71. Hussell T, Bell TJ. Alveolar macrophages: plasticity in a tissue-specific context. Nat Rev Immunol.2014;14:81-93.
72. Bedoret D, Wallemacq H, Marichal T, et al. Lung interstitial macrophages alter dendritic cell functions to prevent airway allergy in mice. J Clin Invest.2009;119:3723-3738.
73. Yona S, Kim KW, Wolf Y, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity.2013;38:79-91.
74. Hashimoto D, Chow A, Noizat C, et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity.2013;38:792-804.
75. Guilliams M, De Kleer I, Henri S, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J Exp Med.2013;210:1977-1992.
76. Prince LR, Maxwell NC, Gill SK, et al. Macrophage phenotype is associated with disease severity in preterm infants with chronic lung disease. PLoS One.2014;9:e103059.
77. Roux X, Remot A, Petil-Camurdan A, et al. Neonatal lung immune responses show a shift of cytokines and transcription factors toward Th2 and a deficit in conventional and plasmacytoid dendritic cells. Eur J Immunol.2011;41:2852-2861.
78. Willems F, Vollstedt S, Suter M. Phenotype and function of neonatal DC. Eur J Immunol.2009;39:26-35.
79. Hunt DW, Huppertz HI, Jiang HJ, Petty RE. Studies of human cord blood dendritic cells: evidence for functional immaturity. Blood.1994;84:4333-4343.
80. De Wit D, et al. Impaired responses to toll-like receptor 4 and toll-like receptor 3 ligands in human cord blood. J Autoimmun.2003;21:277-281.

81. De Wit D, Tonon S, Olislagers V, et al. Blood plasmacytoid dendritic cell responses to CpG oligodeoxynucleotides are impaired in human newborns. Blood.2004;103:1030-1032.
82. Goriely S, Van Lint C, Dadkhah R, et al. A defect in nucleosome remodeling prevents IL-12(p35) gene transcription in neonatal dendritic cells. J Exp Med.2004;199:1011-1016.
83. von Mutius E. The microbial environment and its influence on asthma prevention in early life. The Journal of allergy and clinical immunology.2016;137:680-689.
84. Forsthuber T, Yip HC, Lehmann PV. Induction of TH1 and TH2 immunity in neonatal mice. Science.1996;271:1728-1730.
85. Zhang X, Mozeleski B, Lemoine S, et al. CD4 T cells with effector memory phenotype and function develop in the sterile environment of the fetus. Sci Transl Med.2014;6:238ra272.
86. Prescott SL, Macaubas C, Holt BJ, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. Journal of immunology (Baltimore, Md : 1950).1998;160:4730-4737.
87. Brugman S, Perdijk O, van Neerven RJ, Savelkoul HF. Mucosal Immune Development in Early Life: Setting the Stage. Arch Immunol Ther Exp (Warsz).2015;63:251-268.
88. Marzi M, Vigano A, Trabattoni D, et al. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. Clin Exp Immunol.1996;106:127-133.
89. Prescott SL, Macaubas C, Smallacombe T, et al. Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. Clin Exp Allergy.1998;28 Suppl 5:39-44; discussion 50-31.
90. Rothers J, Halonan M, Stern DA, et al. Adaptive cytokine production in early life differentially predicts total IgE levels and asthma through age 5 years. The Journal of allergy and clinical immunology.2011;128:397-402 e392.
91. Gibbons D, Fleming P, Virasami A, et al. Interleukin-8 (CXCL8) production is a signatory T cell effector function of human newborn infants. Nat Med.2014;20:1206-1210.
92. Gibbons DL, Haque SF, Siberzahn T, et al. Neonates harbour highly active gammadelta T cells with selective impairments in preterm infants. Eur J Immunol.2009;39:1794-1806.
93. Seumois G, Chavez L, Gerasimova A, et al. Epigenomic analysis of primary human T cells reveals enhancers associated with T(H)2 memory cell differentiation and asthma susceptibility. Nature immunology.2014;15:777-788.
94. Seumois G, Zapardiel-Gonzalo J, White B, et al. Transcriptional Profiling of Th2 Cells Identifies Pathogenic Features Associated with Asthma. Journal of immunology (Baltimore, Md : 1950).2016;197:655-664.
95. Endo Y, Hirahahra K, Inuma T, et al. The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. Immunity.2015;42:294-308.
96. Endo Y, Hirahara K, Yagi R, Tumes DJ, Nakayama T. Pathogenic memory type Th2 cells in allergic inflammation. Trends in immunology.2014;35:69-78.
97. Mitson-Salazar A, Yin Y, Wansley DL, et al. Hematopoietic prostaglandin D synthase defines a proeosinophilic pathogenic effector human T(H)2 cell subpopulation with enhanced function. The Journal of allergy and clinical immunology.2016;137:907-918.e909.
98. Ohnmacht C, Park JH, Cording S, et al. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through RORgammat(+) T cells. Science.2015;349:989-993.
99. Sefik E, Geva-Zatorsky N, Oh S, et al. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. Science.2015;349:993-997.
100. Schiering C, Krausgruber T, Chomka A, et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. Nature.2014;513:564-568.

101. Thome JJ, Bickham KL, Ohmura Y, et al. Early-life compartmentalization of human T cell differentiation and regulatory function in mucosal and lymphoid tissues. Nat Med.2016;22:72-77.
102. de Roock S, Hoeks SB, Meurs S, et al. Critical role for programmed death 1 signaling and protein kinase B in augmented regulatory T-cell induction in cord blood. The Journal of allergy and clinical immunology.2011;128:1369-1371.
103. Dijkstra KK, Hoeks SB, Prakken BJ, de Roock S. TH17 differentiation capacity develops within the first 3 months of life. The Journal of allergy and clinical immunology.2014;133:891-894 e895.
104. Collier FM, Tang ML, Martino D, et al. The ontogeny of naive and regulatory CD4(+) T-cell subsets during the first postnatal year: a cohort study. Clin Transl Immunology.2015;4:e34.
105. Sonnenberg GF, Artis D. Innate lymphoid cells in the initiation, regulation and resolution of inflammation. Nat Med.2015;21:698-708.
106. Sonnenberg GF, Artis D. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. Immunity.2012;37:601-610.
107. Guo L, Huang Y, Chen X, Hu-Li J, Urban JF, Jr., Paul WE. Innate immunological function of TH2 cells in vivo. Nature immunology.2015;16:1051-1059.
108. de Kleer IM, Kool M, de Bruijn MJ, et al. Perinatal Activation of the Interleukin-33 Pathway Promotes Type 2 Immunity in the Developing Lung. Immunity.2016;45:1285-1298.
109. Saluzzo S, Gorki AD, Rana BM, et al. First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment. Cell Rep.2017;18:1893-1905.
110. Steer CA, Martinez-Gonzalez I, Ghaedi M, Allinger P, Matha L, Takei F. Group 2 innate lymphoid cell activation in the neonatal lung drives type 2 immunity and allergen sensitization. LID - S0091-6749(17)30237-3 [pii] LID - 10.1016/j.jaci.2016.12.984 [doi]. Journal of Allergy and Clinical Immunology.2017.
111. Fu Y, Lou H, Wang C, et al. T cell subsets in cord blood are influenced by maternal allergy and associated with atopic dermatitis. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology.2013;24:178-186.
112. Ege MJ, Mayer M, Normand AC, et al. Exposure to environmental microorganisms and childhood asthma. N Engl J Med.2011;364:701-709.
113. Marsland BJ, Salami O. Microbiome influences on allergy in mice and humans. Curr Opin Immunol.2015;36:94-100.
114. Oliphant CJ, Hwang YY, Walker JA, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. Immunity.2014;41:283-295.
115. Tait Wojno ED, Artis D. Emerging concepts and future challenges in innate lymphoid cell biology. J Exp Med.2016;213:2229-2248.
116. Monticelli LA, Sonnenberg GF, Abt MC, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. Nature immunology.2011;12:1045-1054.
117. Hams E, Armstrong ME, Barlow JL, et al. IL-25 and type 2 innate lymphoid cells induce pulmonary fibrosis. Proc Natl Acad Sci U S A.2014;111:367-372.
118. McHedlidze T, Waldner M, Zopf S, et al. Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. Immunity.2013;39:357-371.
119. Wohlfahrt T, Usherenko S, Englbrecht M, et al. Type 2 innate lymphoid cell counts are increased in patients with systemic sclerosis and correlate with the extent of fibrosis. Ann Rheum Dis.2016;75:623-626.
120. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell.2014;157:121-141.
121. Gollwitzer ES, Saglani S, Trompette A, et al. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. Nat Med.2014;20:642-647.
122. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med.2014;6:237ra265.

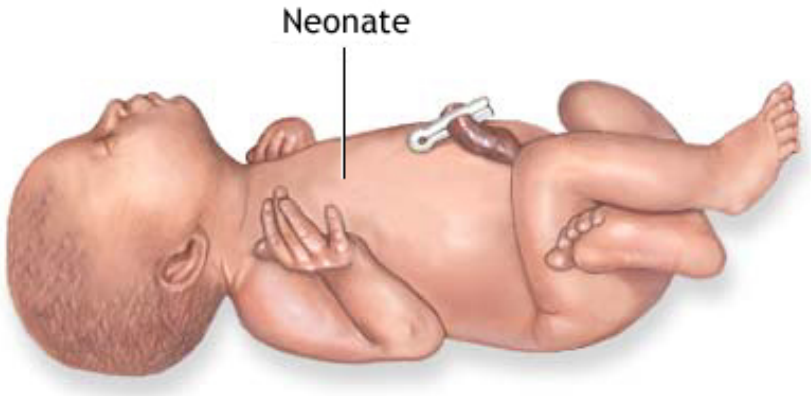
123. Kliman HJ. Comment on "the placenta harbors a unique microbiome". Sci Transl Med.2014;6:254le254.
124. Thorburn AN, McKenzie CI, Shen S, et al. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. Nat Commun.2015;6:7320.
125. Bisgaard H, Stokholm J, Chawes BL, et al. Fish Oil-Derived Fatty Acids in Pregnancy and Wheeze and Asthma in Offspring. N Engl J Med.2016;375:2530-2539.
126. Pfeffer PE, Mann EH, Hornsby E, et al. Vitamin D influences asthmatic pathology through its action on diverse immunological pathways. Ann Am Thorac Soc.2014;11 Suppl 5:S314-321.
127. Gupta A, Sjoukes A, Richards D, et al. Relationship between serum vitamin D, disease severity, and airway remodeling in children with asthma. Am J Respir Crit Care Med.2011;184:1342-1349.
128. Vasiliou JE, Lui S, Walker SA, et al. Vitamin D deficiency induces Th2 skewing and eosinophilia in neonatal allergic airways disease. Allergy.2014;69:1380-1389.
129. Litonjua AA, Carey VJ, Laranjo N, et al. Effect of Prenatal Supplementation With Vitamin D on Asthma or Recurrent Wheezing in Offspring by Age 3 Years: The VDAART Randomized Clinical Trial. JAMA.2016;315:362-370.
130. van de Pavert SA, Ferriera M, Domingues RG, et al. Maternal retinoids control type 3 innate lymphoid cells and set the offspring immunity. Nature.2014;508:123-127.
131. Maslova E, Hansen S, Strom M, Halldorsson TI, Olsen SF. Maternal intake of vitamins A, E and K in pregnancy and child allergic disease: a longitudinal study from the Danish National Birth Cohort. Br J Nutr.2014;111:1096-1108.
132. Rosas-Salazar C, Hartert TV. Prenatal exposures and the development of childhood wheezing illnesses. Curr Opin Allergy Clin Immunol.2017;17:110-115.
133. MacGillivray DM, Kollmann TR. The role of environmental factors in modulating immune responses in early life. Front Immunol.2014;5:434.
134. den Dekker HT,Sonnenshein-van der Vort AM, Jongste JC, et al. Tobacco Smoke Exposure, Airway Resistance, and Asthma in School-age Children: The Generation R Study. Chest.2015;148:607-617.
135. Vardavas CI, Hohmann C, Patelrou E, et al. The independent role of prenatal and postnatal exposure to active and passive smoking on the development of early wheeze in children. Eur Respir J.2016;48:115-124.
136. Herberth G, Bauer M, Gasch M, et al. Maternal and cord blood miR-223 expression associates with prenatal tobacco smoke exposure and low regulatory T-cell numbers. The Journal of allergy and clinical immunology.2014;133:543-550.
137. Noakes PS, Hale J, Thomas R, Lane C, Devadason SG, Prescott SL. Maternal smoking is associated with impaired neonatal toll-like-receptor-mediated immune responses. Eur Respir J.2006;28:721-729.
138. Brandt EB, Myers JM, Ryan PH, Hershey GK. Air pollution and allergic diseases. Curr Opin Pediatr.2015;27:724-735.
139. van de Loo KF, van Gelder MM, Roukema J, Roeleveld N, Merkus PJ, Verhaak CM. Prenatal maternal psychological stress and childhood asthma and wheezing: a meta-analysis. Eur Respir J.2016;47:133-146.
140. Gollwitzer ES, Marsland BJ. Impact of Early-Life Exposures on Immune Maturation and Susceptibility to Disease. Trends in immunology.2015;36:684-696.
141. O'Connor TG, Winter MA, Hunn J, et al. Prenatal maternal anxiety predicts reduced adaptive immunity in infants. Brain Behav Immun.2013;32:21-28.
142. Ramratnam SK, Visness CM, Jaffee KF, et al. Relationships Among Maternal Stress and Depression, Type 2 Responses, and Recurrent Wheezing at Age 3 Years in Low Income Urban Families. Am J Respir Crit Care Med.2016.
143. Deshmukh HS, Liu Y, Menkiti OR, et al. The microbiota regulates neutrophil homeostasis and host resistance to Escherichia coli K1 sepsis in neonatal mice. Nat Med.2014;20:524-530.

144. Dominguez-Bello MG, Costell EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A.2010;107:11971-11975.
145. Malamitsi-Puchner A, Protonotariou E, Boutsikou T, Makrakis E, Sarandakou A, Creatsas G. The influence of the mode of delivery on circulating cytokine concentrations in the perinatal period. Early Hum Dev.2005;81:387-392.
146. Dominguez-Bello MG, De Jesus-Laboy KM, Shan N, et al. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nat Med.2016;22:250-253.
147. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nature Medicine.2017;23:324-326.
148. Field CJ. The immunological components of human milk and their effect on immune development in infants. J Nutr.2005;135:1-4.
149. Verhasselt V. Neonatal tolerance under breastfeeding influence. Curr Opin Immunol.2010;22:623-630.
150. Greer FR, Sicherer SH, Burks AW, American Academy of Pediatrics Committee on N, American Academy of Pediatrics Section on A, Immunology. Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics.2008;121:183-191.
151. von Mutius E. Trajectories of childhood wheeze. The Journal of allergy and clinical immunology.2011;127:1513-1514.
152. Caliskan M, Bochkov YA, Kreiner-Molleret al. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. N Engl J Med.2013;368:1398-1407.
153. Moffatt MF, Kabesch M, Liang L, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. Nature.2007;448:470-473.
154. Bochkov YA, Watter K, Ashraff S, et al. Cadherin-related family member 3, a childhood asthma susceptibility gene product, mediates rhinovirus C binding and replication. Proc Natl Acad Sci U S A.2015;112:5485-5490.
155. Kristjansson S, Bjarnarson SP, Wennergren G, et al. Respiratory syncytial virus and other respiratory viruses during the first 3 months of life promote a local TH2-like response. The Journal of allergy and clinical immunology.2005;116:805-811.
156. Jackson DJ, Evans MD, Gangon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. Am J Respir Crit Care Med.2012;185:281-285.
157. Bisgaard H, Hermansen MN, Bruchvald F,et al. Childhood asthma after bacterial colonization of the airway in neonates. N Engl J Med.2007;357:1487-1495.


Allergen
Genetics
Airways remodelling
Wheeze/
Asthma
Microbiome
Immune Development
Infection

Protective Signals

Diverse Microbiome
Exposure to Farm dust
Pets
Siblings
Diet rich in PUFA, fibre



Harmful Signals

Serial viral infections
Pollution
Smoke exposure
Poor diet
Allergen exposure

Gene x environment interactions

WHEEZE

?

ASTHMA