

Opinion

Opening the Window of Immune Opportunity:
Treating Childhood AsthmaClare M. Lloyd ^{1,*,@} and Sejal Saglani^{1,*}

Asthma is an increasingly common childhood disease and although most patients can control their symptoms with medication, a proportion experience life-threatening symptoms. The advent of novel biologic therapies represents a giant leap forward for asthma treatment, but efficacy is rarely tested in children. Recent mechanistic work in mice suggests that early life is a key period for immune development and, therefore, allergen sensitization. Although children with severe asthma experience significant comorbidities and are at increased risk for serious diseases such as chronic obstructive pulmonary disease as adults, no specific investigation into tailored treatment for young children with severe asthma exists. Here, we propose how new information regarding early life immunity could be used to inform modified treatments for children.

Early Life Pulmonary Immunity Is a Barometer for Lifelong Lung Health

Asthma (see [Glossary](#)) is the most common chronic disease affecting children, representing significant morbidity and a high financial burden, accounting for 1–2% of the healthcare budget in developed countries [1]. Population birth cohort studies have shown that children with recurrent wheeze and severe **exacerbations**, as well as early allergic sensitization have a persistently low lung function trajectory that continues into adulthood and increases the risk of developing **chronic obstructive pulmonary disease** [2]. Thus, the potential lifelong impact of poor childhood asthma control on lung health is increasingly recognized [3–5], and the need for treatments that will improve lung function during childhood is apparent. Although the rapid discovery and licensing of novel **biologics** is a big step forward for asthma care, especially for those with severe disease, it is essential that efficacy and mechanistic data for novel biologics derived from studies in adults are not applied to children by extrapolation [6,7]. Currently, licensed biologics predominantly target **type 2 immune responses** including anti-interleukin (IL)-5 antibody (mepolizumab and reslizumab), anti-IL-5 receptor antibody (benralizumab), anti-IL-4 receptor α (dupilumab), anti-thymic stromal lymphopoietin (TSLP) antibody (tezepelumab), and anti-IgE antibody (omalizumab) (Table 1 and Figure 1). However, there are significant pitfalls associated with assuming the drivers of childhood and adult disease are the same. For example, it is clear that although school-age severe asthma is predominantly characterized by multiple and severe aeroallergen sensitization, with associated airway eosinophilia, airway type 2 mediators, including IL-5 and IL-13, are difficult to detect, especially when adherence to maintenance steroid therapy is ensured [8,9]. In addition, the noninvasive biomarkers used to determine response to T helper (Th)-2-mediated biologics in adults, such as blood eosinophils, may not be applicable to children because type 2 inflammation varies with age [10], and, there is little relationship between airway and peripheral eosinophilia in children with severe asthma [11,12].

Mechanisms must be investigated in children taking into consideration the impact of a maturing immune system and the developing lungs. The increasing array of targeted treatments being licensed for asthma presents an opportune time to increase our understanding of the basic mechanisms of

Highlights

Mechanistic studies using early life murine models are increasingly confirming key distinctions in type 2 immune responses between children and adults.

Cohort studies have confirmed the impact of early life lung damage on adult lung health.

Therapeutic interventions specifically targeting immune dysregulation in early-onset disease remain an unmet need.

The recent licensing of biologics for asthma, largely targets type 2 immunity, but mechanisms of action, or biomarkers of response have not been tested in early-life animal models, or in children.

Increasing recognition of the need for age-appropriate models is enabling a rapid trajectory towards achieving disease modification for chronic obstructive lung diseases.

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Table 1. Summary of Status for Novel Biologics for Treatment of Severe Asthma.

Target	Drug	Licensed use	Use in children	Refs
IL-5	Mepolizumab (GlaxoSmithKline)	Adults and children ≥ 12 years with severe eosinophilic asthma – licenced 2015	Approved August 2018 for children aged ≥ 6 years	[63] [64]
IL-5	Reslizumab (Teva Pharmaceuticals)	Adults with severe eosinophilic asthma – licenced 2016	No licence	[65] [66]
IL-5 Receptor	Benralizumab (MedImmune/AstraZeneca)	Adults with severe eosinophilic asthma Licenced – 2017	Approved by FDA for children ≥ 12 years	[67] [68] [69]
IL-13 (IL-4R α binding epitope)	Lebrikizumab (Genentech/Roche)	Not licenced	Not licenced	[70] [71]
IL-13R $\alpha 1$ IL-13R $\alpha 2$	GSK679586 (GlaxoSmithKline)	Not licenced	Not licenced	[72]
IL-13R $\alpha 1$ IL-13R $\alpha 2$	Tralokinumab (MedImmune/AstraZeneca)	Not licenced	Not licenced	[73] [74]
IL-4R α	Dupilumab (Regeneron Pharmaceuticals/Sanofi)	Adults with moderate to severe asthma – approved by FDA 2018 and EMA ^a 2019	Children ≥ 12 years with severe asthma	[75] [76]
TSLP	Tezepelumab (MedImmune/AstraZeneca)	Currently undergoing Phase III trials in adults with severe asthma		[77]
CRTH2 antagonist	Fevipirant (Novartis)	Undergoing Phase III trials in severe, uncontrolled adult asthma		[78]

^aAbbreviation: EMA; European Medicines Agency.

this disease in children, which might allow novel targeted therapeutic approaches to mitigate pathology and enable disease modification. We describe increasing evidence from early-life murine studies highlighting distinctions between mechanisms initiating allergic airways disease in neonatal, compared with adult models. We also review human findings, and propose that childhood studies are critical to achieve disease modification, or a ‘cure’ for asthma.

Understanding the Impact of Age and Disease Heterogeneity on Immune Responses to Achieve Precision Medicine

A number of key murine studies have recently focused on immune mechanisms underlying allergic airways disease in early life. They have shown that *in utero* exposures, such as undernutrition, can influence CD4⁺ T cell proliferation and activation, with a skewing towards susceptibility to experimental asthma [13]. Unsupervised cluster analyses from various databases have been applied in human birth cohort studies, relating clinical phenotypes to immune profiles; these have determined a high-risk atopic cluster with elevated house dust mite (HDM)-specific IgE and IgG4 concentrations, and multiple allergen sensitization that goes on to develop persistent wheeze and asthma [14]. Thus, risk factors for early Th2 skewing and the mechanisms underlying this are being evaluated. Of relevance, direct comparisons between adult and neonatal models have shown distinct pathways that are important for the inception of allergic airways disease, whereby CD4⁺IL-13⁺ T cells are critical to initiating neonatal allergic airways disease, while type 2 innate lymphoid cells are important for adult onset disease [15]. The use of inhaled allergen in experimental models for both sensitization and acute exposure is also important as this is the likely route of sensitization seen in humans, and which can affect the type of antigen-presenting cells that first encounter allergen. Thus, the perennial allergen HDM has improved modeling of the disease to reflect pathology seen in children. In addition, advancing techniques, including RNA sequencing (RNAseq) and single cell analysis that allow assessments of immune

Glossary

Airway hyper-reactivity (AHR):

defining physiological characteristic of asthma; hyper-reactivity of the airways, triggered following exposure to a particular stimulus usually tolerated by healthy individuals, but causes bronchoconstriction in asthmatic individuals.

Airway remodeling: collectively refers to the structural changes in both large and small airways during asthma. These changes may include subepithelial fibrosis, enhanced smooth muscle mass, enlargement of mucus glands, and neovascularization.

Asthma: overarching term to describe an airways disease characterized by shortness of breath, wheezing, or whistling noises from the chest and the presence of reduced, obstructed lung function, which is reversible. There are numerous different clinical and pathological phenotypes (manifestations) of asthma. Most commonly, asthma is associated with allergic sensitization to aeroallergens, such as HDM and by eosinophilic airway inflammation.

Atopy: genetic tendency to develop allergic diseases such as allergic rhinitis, asthma, and atopic dermatitis (eczema). It is associated with heightened immune responses to common allergens, especially inhaled and food allergens. There may or may not be symptoms of allergic disease, but there is evidence of sensitization.

Biologic: a drug made of recombinant therapeutic proteins targeting a specific inflammatory cytokine (ligand) or its receptor.

Butyrate: salt of the short-chain fatty acid butyric acid.

Chronic obstructive pulmonary disease: airway disease characterized by neutrophilic inflammation, associated with breathlessness, cough, and sputum production; commonly associated with a history of tobacco smoke exposure and characterized by a fixed reduction in lung function, not reversing to normal, even with treatment.

Exacerbation: acute or sudden loss of asthma control, with a dramatic increase in symptoms and a precipitant fall in lung function, often precipitated by viral infection or high allergen exposure (i.e., acute asthma attack).

Hygiene hypothesis: originally, deemed an increased incidence of atopic diseases in westernized countries

phenotyping in small airway or blood samples are also increasingly being used to understand more about immune development in early life.

Modeling Childhood Asthma

Modeling early life asthma is not straightforward because although the predominant clinical manifestation is of wheeze, this condition is heterogeneous. Specifically, although wheezing disorders are common in preschool children, affecting approximately one-half of all children under 5 years of age, only about one-third of those go on to develop asthma by school age [16]. Moreover, the majority of wheezing attacks in preschool children is driven by infection [17], not allergens, but early sensitization is a significant risk factor for low lung function and progression to school-age asthma [18]. Given this clinical heterogeneity, unbiased analyses and machine-learning techniques are increasingly being applied in samples from children with wheezing disorders as an approach to define different clinical and mechanistic phenotypes, which can subsequently be explored using specific neonatal murine models, or *in vitro* using primary pediatric cells [19]. For instance, this approach was used to identify immune responses induced by rhinovirus infection *in vitro*, as well as their relationship to clinical wheeze phenotypes from children in a birth cohort [18]. Although two clinical clusters were considered to be at high-risk of developing asthma and allergic sensitization, when related to immune responses to rhinovirus, only one cluster [interferon (IFN) low, inflammation high, Th2 low and regulatory moderate] was associated with early-onset asthma and sensitization, and with the highest risk of asthma exacerbations [18]. Detailed lung function and clinical phenotyping have also been applied with immune phenotyping to identify three distinct trajectories of progress to asthma and allergy; specifically, a high-risk atopic cluster with persistent wheeze for increased propensity to allergic diseases throughout childhood was identified [14]. The association between early-life, severe **atopy**, persistent wheezing, and progression to atopic **asthma** by school age is a reproducible and robust finding from independent birth cohorts in Australia, UK, and USA. Multiple allergen sensitization and severe atopy are also associated with early loss in lung function which persists throughout childhood [2].

Molecular Profiling of Childhood Asthma

Transcriptomic profiling has recently been applied to understand the molecular mechanisms underlying exacerbations of asthma in children since it is apparent that not all upper respiratory viral infections lead to clinical exacerbation [20]. In one study, longitudinal blood and nasal lavage samples were collected prospectively from a cohort of 208 school-age children aged 6–17 years, who were exacerbation prone. Samples were collected both during exacerbations and stable disease for RNAseq, inflammatory cell differential counts, and viral PCR detection [20]. Patterns of gene expression induced during clinical exacerbation of asthma were compared with those with development of a cold, but without clinical asthma exacerbation. Common molecular pathways were found to underlie both viral and nonviral clinical exacerbations in nasal lavage cells. Specifically, genes encoding epithelial-associated **SMAD3 signaling** were upregulated, and lymphocyte response pathways were downregulated early in exacerbation; this was followed by upregulation of pathways that included epidermal growth factor receptor (EGFR) signaling, extracellular matrix protein production, mucus hypersecretion, and eosinophil activation [20]. The exacerbation pathways identified, specifically SMAD3, EGFR, and fibroblast growth factor (FGFR) signaling, are of particular interest as they include mediators associated with the development of **airway remodeling** [20]. This suggests that each clinical exacerbation increases the development of airway remodeling, and that a fall in lung function might occur in parallel. We propose that having identified pathways and molecules of interest, the next steps would be to undertake hypothesis-driven mechanistic studies in age-appropriate experimental animal models and confirm findings in airway samples from children to identify novel putative therapeutic

due to living in an overly clean environment, so that children not exposed to infectious agents, symbiotic microorganisms or parasites, might develop an overactive immune system responding inappropriately to innocuous antigens such as pollens, dust, or animal dander (i.e., allergy).

Memory-like phenotype: T cells that express markers of immune memory, such as CD45RO.

Microbial dysbiosis: microbial imbalance or maladaptation of bacteria inside the host.

nBreg: a type of B cells found in neonates, that express high levels of CD5 and exhibit regulatory properties.

Ovalbumin murine model of allergic airway inflammation: intraperitoneal injection with the allergen ovalbumin and alum as an adjuvant is used to generate allergic sensitization; subsequent inhaled challenge with ovalbumin is used to mimic allergic airway inflammation as seen in asthma.

Precision medicine: identifying the optimal treatment for an individual based on the clinical manifestation of their disease (phenotype), the mechanisms driving their disease (endotype) and genetic and environmental factors.

Preschool wheeze: associated with breathlessness and cough occurring in children aged 1–5 years, commonly presenting as acute attacks, or can be associated with persistent symptoms.

SMAD3: protein of the cellular network system TGFβ signaling cascade.

Training of immune responses: 'trained immunity' is a specialised form of innate immune memory that is separate from the classical adaptive immune memory.

Type 2 immune responses: induced following exposure, for example, to allergens, and are characterized by the production of IL-4, IL-5, and IL-13. They play a critical role in the pathophysiology of allergic diseases such as asthma and allergic rhinitis. Type 2 responses are also critical against helminths invading cutaneous or mucosal sites.

Type 2 innate lymphoid cells (ILC2s): cells of lymphoid origin, derived from common lymphoid progenitors; defined by the absence of antigen specific B or T cell receptor due to a lack of recombination activating gene (RAG). ILC2s express the GATA3 transcription factor, are commonly involved in type 2 immune responses (defined above), and secrete type 2 immune mediators, such as IL-4, IL-5, and IL-13.

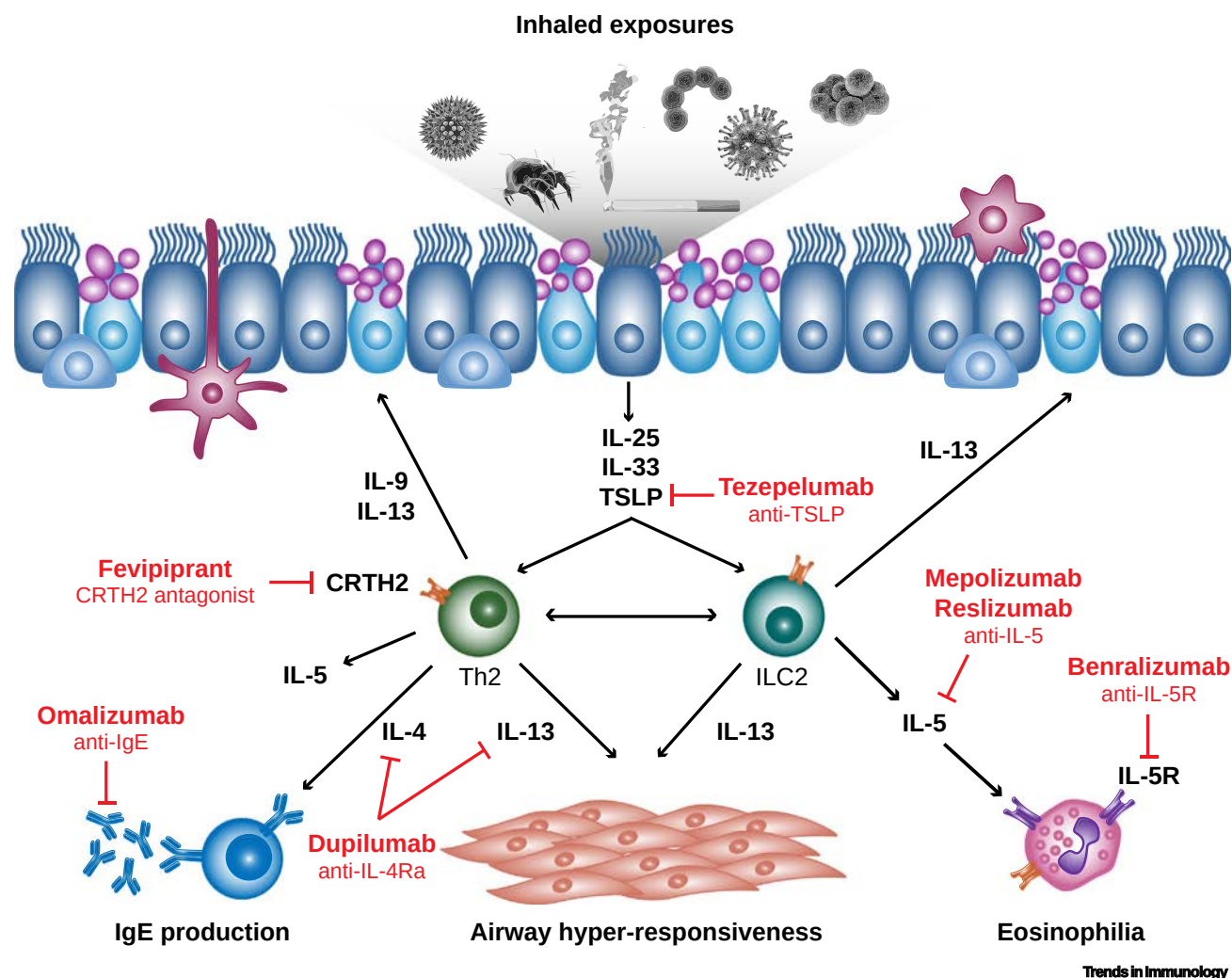


Figure 1. Novel Biologic Agents Targeting Key Type 2 Immune Pathways. Type 2 immune inflammation manifests as eosinophilic inflammation, airway hyper-responsiveness, IgE production, and goblet cell hyperplasia. These pathological features develop as a consequence of enhanced production of key cytokines during inflammation: interleukin (IL)-4, IL-5, and IL-13, secreted by a range of immune and stromal cells in the lung, but primarily T helper 2 (Th2) cells and innate lymphoid type 2 cells (ILC2s). Novel biologics (depicted in red) have been designed to target these cytokines. Abbreviations: IL-5R, IL-5 receptor; TSLP, thymic stromal lymphopoietin.

interventions. An approach incorporating: (i) deep molecular phenotyping of samples from children; (ii) mechanistic studies in age-appropriate animal models; and (iii) bioinformatics and machine-learning techniques, is needed to facilitate the application of **precision medicine** and individualized therapies.

Immune Development in Early Life: Using Systems Biology

Although it is accepted that the human immune system matures postnatally, the molecular mechanisms underlying this maturation are not well understood. The neonatal immune system shows a skew to type 2 immunity [21], in part to mitigate potential reactivity to maternal alloantigens during pregnancy, but also to prevent immune overload from exposure to extrinsic and intrinsic antigens following birth. Immune responses can occur in the fetus as early as the second trimester, orchestrated by fetal dendritic cell (DC) populations that are related to their adult counterparts

in terms of phenotype. These human fetal DCs, isolated from fetal spleen, thymus, skin, and lung, are able to respond to Toll-like receptor (TLR) ligation and migrate to lymph nodes [22]. However, they differ in their response to alloantigens, promoting a strong regulatory T cell response and inhibiting potentially harmful T cell tumor necrosis factor (TNF) α production. Similarly, human neonatal effector CD4⁺ T cells with a **memory-like phenotype** develop in the relatively sterile uterine environment, in the absence of any perceptible antigenic stimulus and are able to function as effector CD4⁺ T cells after birth [23].

The advent of systems immunology has facilitated our understanding of the immune landscape of the human neonate. A landmark study profiled blood immune cells from newborn babies over the first 3 months of life [24]. This longitudinal sample set from 100 healthy newborns, enabled investigators to examine how the immune system reacts to the environmental changes that occur within the postnatal period. These serial blood samples were analyzed by mass cytometry, proteomics, and RNASeq to gain an understanding of postnatal immune landscapes. They found a stereotypic pattern of immune development that was influenced by microbial exposure, but disturbed by **microbial dysbiosis**. Moreover, there were dramatic differences between the immune profiles of term neonates compared with those that were born prematurely, although these differences rapidly diminished postnatally. Preterm infants had lower proportions of neutrophils and exhibited a strong proinflammatory signature, characterized by high expression of chemokines CXCL11 and CXCL8, compared with term infants [24]. This initial 3-month postnatal period was found to be critical for the development of B cells, DCs, and natural killer (NK) cells, since each of these immune cell populations reached adult-like phenotypes during this time period. However, their findings in neonatal blood were not reflective of findings in the cord blood, which is surprising since cord blood is regularly used as a surrogate for neonatal cells.

Systems biology has also been used to characterize the molecular ontogeny of the immune system over the first week of life [25]. Transcriptomic, proteomic, and metabolomic analyses coupled with single cell immune phenotyping were performed in blood samples taken from newborns within the first week of life. These data have determined that the first week of human life is highly dynamic but follows a robust course that is common to newborns from different areas of the world [25]. Specifically, although most immune cell populations remain stable over the first week of life, the numbers of basophils, plasmacytoid DCs, NK cells, and neutrophils decrease. Dramatic changes in cytokines and chemokines are detected, with concentrations of CXCL10, IL-17, CCL22, and IFN increasing, while IL-10, CCL5, granulocyte colony-stimulating factor (GCSF), and IL-6 decreasing over the first week of life. Furthermore, integration of these different platforms reveals that IFN signaling, complement cascades, and neutrophil function have emerged as key drivers of the developmental trajectory that occurs during the first week of life [25]. Collectively, these studies show that the neonatal immune system undergoes considerable change in the immediate postnatal period in order for the newborn baby to cope with the environmental challenges outside the womb.

Immunity in Early Life Is Distinct from That in Adults

Since neonatal immune development is a process that is heavily influenced by intrinsic and extrinsic factors, it is perhaps not surprising that many facets of immunity in babies differs from that in adults. For example, longitudinal analysis of blood from preterm babies has revealed the presence of a population of CXCL8-producing CD4⁺ T cells that can activate antimicrobial neutrophils and $\gamma\delta$ T cells *ex vivo* [26]. In contrast, these cells are rarely present in peripheral blood samples from adults. Similarly, a population of IL-10-producing B cells are abundant in the blood of preterm and healthy term babies, but their numbers decline quickly with age, and are largely absent at 1 year of age [27]. These **neonatal regulatory B cells (nBreg cells)** could

be infected with respiratory syncytial virus (RSV) *in vitro*, leading to secretion of IL-10 and subsequent dampening of Th1 cytokine responses [27]. Moreover, these nBreg cells are found in the respiratory tract of RSV-infected infants and their frequency correlates with increased viral load and decreased peripheral blood memory Th1 cell numbers relative to those in infants without RSV infection [27]. These distinctions in the composition of the immune system in the early life period versus child or adulthood likely reflect checkpoints in place to ensure a degree of protection from pathogens, while preventing potential collateral tissue damage from overwhelming inflammation that might occur following sudden exposure at birth to myriad microbes in the external environment.

Age-Dependent Immune Development Can Affect Progression of Allergic Immunity

Although it is recognized that asthma is a disease of childhood, and that the developing immune system has facets that are unique to the neonatal period, the majority of mechanistic studies in allergic asthma models are performed using adult mice. Critically, these adult models fail to consider early-life immune development. Data from our group underscores the importance of this early-life period in development of all aspects of allergic inflammation. Specifically, we have determined that exposure of neonatal mice to inhaled HDM from day 3 of life results in robust lung eosinophilia, elevated IgE, and type 2 cytokine production (e.g., IL-13 and IL-5) from CD4⁺ T cells relative to controls [28]. This is also associated with **airway hyper-reactivity (AHR)** to methacholine challenge, as demonstrated from increased airway resistance [28]. In addition, the magnitude and degree of classical parameters of asthma: eosinophilia, mucus hypersecretion, and AHR differ depending on the age of neonatal mice at the first exposure to allergen [29]. Indeed, initial allergen exposure at days 3 and 7 of life results in the most marked airway eosinophilia and AHR, while first exposure at day 14 of life results in protection from both eosinophilia and AHR [29]. Moreover, the cellular source of IL-13 appears to be critical and differs depending on the age of first allergen exposure. In neonatal mice, IL-13⁺CD4⁺ T cells are vital for development of allergen-driven AHR following HDM exposure from day 3 of life onwards. Mice that are genetically engineered to be deficient in IL-13 derived from CD4⁺ T cells do not develop AHR relative to controls, and unlike adults, neonatal **innate lymphoid type 2 cells (ILC2s)** cannot compensate for a lack of CD4⁺ T cells in severe combined immunodeficiency (SCID) mice [15]. These data demonstrate age-specific differences in the development of allergen driven AHR. Age-dependent differences in an epithelium-expressed cascade of inflammation and remodeling factors have also been described whereby neonatal mice exposed to HDM exhibited increased 5-lipoxygenase concentrations, whereas adult mice expressed higher amounts of group 10 secretory phospholipase A₂, Wnt5a, and transglutaminase 2 [30].

Recent studies have begun to examine the mechanisms underlying allergen-driven inflammation using neonatal mouse models. Epithelium-derived IL-33 has been shown to be important in the accumulation of ILC2s within the lungs, as demonstrated using mice genetically modified with a fluorescent label on IL-33 molecules [31]. Lung inflation induced by the first intake of breath postnatally is the trigger for release of IL-33 that drives a type 2 environment in the neonatal lungs. This IL-13-rich milieu is sufficient to maintain airway macrophages (AMs) in a type 2 state during early development, but also into adulthood [31]. Similarly, perinatal activation of the IL-33 pathway has been shown to facilitate the spontaneous accumulation of ILC2s, eosinophils, basophils, and mast cells in developing lungs [32]. Exposure to HDM during this period further increases IL-33 expression, enhancing type 2 cytokine production in ILC2s, while simultaneously suppressing IL-12p35 in neonatal DCs, thereby fostering Th2 cell skewing [32]. Furthermore, other studies have also shown spontaneous production of IL-33 in the neonatal lungs and have proposed that the subsequent accumulation of ILC2s is responsible for allergic sensitization in early life

[33]. The dependence on IL-33 during this period is not universal however, since neonatal IL-33-deficient mice can develop significant AHR relative to wild-type mice [15]. The differences in findings between these studies may reflect the different genetic strain of mouse used, or the fact that previous studies did not measure AHR [31–33]. Thus, to fully appreciate the function of IL-33 in the inception and persistence of childhood-onset allergic asthma, future studies are warranted to better elucidate the mechanistic roles of IL-33, Th2 cells, ILC2s, and their interactions with stromal cells in neonatal murine models.

AMs are critical regulators of immune responses, particularly at barrier surfaces such as the lung. Evidence from mice indicates that AMs derive from fetal liver monocytes and seed the airways postnatally [34]. Their phenotype is shaped by the local environment, including their interactions with other local tissue-resident cells such as basophils [35]. In addition, the AM phenotype is likely heavily influenced by the local microbiota, concomitant with the breadth and diversity of respiratory pathogens the neonate encounters in the first months of life. Although mice genetically deficient in AM-derived transforming growth factor (TGF) β or its receptor, have shown that in young mice TGF β signaling is critical in the development and maintenance of these resident AMs, there is little information regarding their phenotype in human babies [36]. Pulmonary DCs are vital indicators to set the threshold for initiation of responses to inhaled allergen. The presence of lipopolysaccharide (LPS) in HDM suppresses allergic responses in adult but not in neonatal mice [37]. Neonatal CD11b⁺ DCs fail to upregulate the transcription factor T-bet in response to LPS, and therefore, do not secrete necessary IL-12 to suppress generation of Th2 responses, thus rendering neonatal mice more susceptible than adults to HDM driven type 2 pathology. Similarly, plasmacytoid DCs in neonatal airways protect against viral bronchiolitis and subsequent asthma, combined with pathways involving microbial metabolites that can drive protective T regulatory (Treg) responses [38]. Collectively, these data indicate that all aspects of the immune system in the lung are shaped during development and that age-specific distinction affect the nature and magnitude of immune responses to inhaled stimuli.

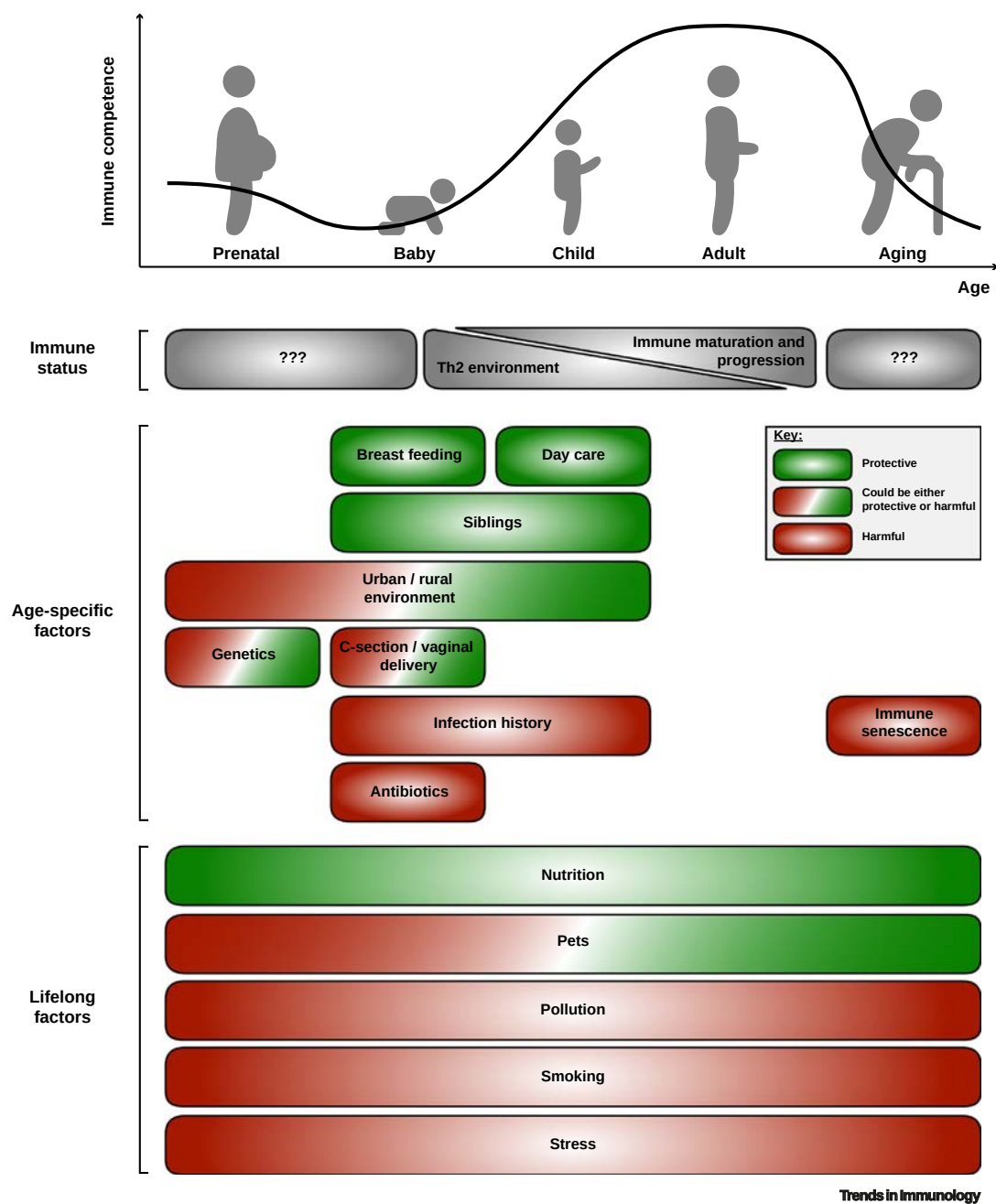
Although it is difficult to exactly model distinctions between early- and late-onset disease in humans to validate the findings from experimental models, it should be noted that many patients present with their first symptoms of allergy or asthma in adult life. Indeed, relative to children, adult-onset asthma represents a distinct phenotype, characterized by increased obesity, greater airflow limitation, and less allergen sensitization; thus, it has been proposed that mechanisms besides type 2 inflammatory pathways might inform the severe asthma phenotype observed with advancing age [39]. Moreover, both shared and distinct genetic risk factors have also been identified for childhood- and adult-onset asthma, suggesting some common, but also some different susceptibility profiles at different ages in humans [40]. Thus, it is vital to recognize that childhood asthma is distinct from the adult disease when considering the underlying mechanisms as well as potential therapies.

The External Environment Influences Immune Development

Both intrinsic and external environmental factors influence the course of postnatal immune maturation, and the nature of these influences are thought to affect lifelong immune health and facilitate the development of diseases such as asthma (Figure 2, Key Figure). In particular, it is accepted that the composition of the microbiota resident at mucosal surfaces such as the gut, skin, and lung exerts a significant influence on the developing immune system. The maternal microbiota can influence neonatal murine immune development specifically on numbers of ILC3s and mononuclear cells in the intestine [41]. As the neonate emerges from the womb with its low antigen load and into the antigen-rich external environment, contact with particular pathogen species affects the course of pulmonary immune development. In humans, postnatal

Key Figure

Immunity Is Influenced by a Variety of Factors across the Life Span



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immune education has been shown to be dependent on a range of factors that include gestational age at birth, birth order and number of siblings, living with pets, attendance at day care, as well as pollution and parental smoking [42]. All of these factors have been shown to impact respiratory health in particular. Thus, the perinatal period is thought to represent a 'window of opportunity' in which the immune system is set on a particular trajectory [43]. In humans, these first 100 days of life represent a vital transition period, and microbiota dysbiosis during this period has been associated with enhanced allergic diseases such as asthma [44].

The '**hygiene hypothesis**' proposes that exposure to microbes is necessary to mitigate the progression of inflammatory pathology, including allergy and autoimmunity [45]. The composition of the microbiota is affected by both intrinsic and extrinsic factors and neonatal respiratory infection can disturb the stability of the airway microbiome. Indeed, children who develop a higher than average number of infections during their first year of life display a disordered nasopharyngeal microbiota relative to children presenting with only 0–2 upper respiratory infections per year [46]. This instability is compounded by other factors that include mode of birth delivery, breastfeeding, as well as antibiotic use [47]. Thus, the timing of exposures to commensal microbes during very early life has long-term consequences on immune development. Colonization of neonatal, but not adult, germ-free mice with a conventional gut microbiota protects neonatal animals from accumulation of mucosal invariant NK T cells as well as from related inflammatory bowel disease [48]. Lack of exposure to commensals in early life, for example, in germ-free mice, or disruption of the microbiota by antibiotics, can increase susceptibility to allergens and promote allergic inflammation [47,48]. Hence, recognition of the importance of the microbiota in facilitating early-life immune development and subsequent immune homeostasis has led to investigating possible mechanisms by which dysbiosis may occur.

For instance, breastfeeding has been shown (in epidemiological studies) to be protective against wheeze in babies born to asthmatic mothers relative to formula feeding [49]. Moreover, a recent study in neonatal mice describes a weaning reaction that prevents susceptibility to immune pathology into adulthood [50]. Specifically, examination of the composition of gut microbiota has determined that expansion of the microbiota at the time of weaning could elicit an immune reaction involving the generation of ROR γ t⁺ Treg cells. These microbiota-induced Treg cells are necessary to prevent susceptibility to diseases later in life, including cancer and allergy [50]. Furthermore, it is well documented that early-life viral infection is associated with an increased risk of developing asthma in childhood, and this perhaps reflects aberrant pulmonary immune development as a result of a disordered microbiota. In fact, an analysis of children with **preschool wheeze** determined that microbiota-based clustering of wheezers correlates with local pulmonary inflammation in the bronchoalveolar lavage of severe wheezers. Specifically, the *Moraxella* taxa is over-represented in wheezers who also harbor elevated numbers of airway neutrophils, and this is associated with reduced bacterial diversity in bronchoalveolar lavage, reflective of microbiota dysbiosis [51].

Early-Life Opportunities for Disease Prevention

An important goal for childhood asthma management is not just alleviation of symptoms to achieve control, but to identify treatments that will achieve disease modification by: (i) preventing

Figure 2. Human immunity is influenced by a wide range of factors that may be universal across ages, or specific to a particular time of life. The influences may be protective from disease, or may be harmful, and the time of exposure may lead to either protection or harm. Babies and children are most vulnerable to these factors since immune development takes a steep developmental trajectory during early life. Although recent investigations have made key discoveries during this early-life period, we know relatively little about immunity in the fetus and during old age.

progression childhood to adult asthma; and (ii) result in a sustained improvement in lung function. Epidemiological studies have repeatedly and clearly identified environmental exposures that prevent early-life asthma development. The most striking data come from farmyard studies, initially from Europe, and more recently from farming communities in North America [52], which have convincingly shown that growing up on a farm with traditional, but not industrialized farming, protects from the development of early-onset asthma, even in children with similar genetic backgrounds [53]. Moreover, studies have been undertaken in Amish and Hutterite families, both of which have very similar genetic ancestry and agricultural lifestyles in America but have distinct farming practices. The Amish undertake traditional cattle farming involving direct exposure of the farm workers to cattle in stables, whereas the Hutterites use industrialized practices with minimum direct contact of workers with animals and time in stables. These studies reported that the prevalence of asthma was four times lower in children from Amish families compared with Hutterites, and Amish children were exposed to significantly higher endotoxin concentrations in house dust relative to Hutterites [53]. Moreover, there was an effect of this altered environment on circulating innate immune cells whereby Amish children had increased proportions of neutrophils, reduced eosinophils, and monocytes in blood with a suppressive phenotype characterized by lower expression of HLA-DR relative to Hutterite children. Inhaled exposure of house dust from Amish homes in an adult **ovalbumin murine model of allergic airway inflammation** showed protection from AHR, reduced eosinophilia, and greater neutrophilia, while house dust from Hutterite homes had no impact on ovalbumin-induced allergic airways disease [53]. This supports evidence from murine studies in adult models suggesting that the microbial and endotoxin components of inhaled products from cattle farmyards impact the mucosal epithelial immune system to skew away from allergic asthma and type 2 immunity by induction of A20 [54]. In order to determine a mechanism for protection, in a neonatal model of inhaled allergen exposure with HDM, mouse pups were exposed to a microbial extract from cattle farm dust starting at day 3 of life [15]. Specifically, lyophilized, inactivated *Acinetobacter lwoffii* was administered intranasally concomitant with HDM to neonatal mice for 3 weeks [15]. *A. lwoffii* exposure resulted in complete protection from the development of allergic airways disease, with ablation of AHR, eosinophilia, and concentrations of pulmonary IL-5 and IL-13, relative to mice exposed to HDM alone. Protection resulted from a significant reduction in pulmonary CD4⁺IL-13⁺ cells, even in the presence of elevated amounts of the innate mediator IL-33 and downstream IL-13⁺ ILC2s, relative to pups exposed to HDM without *A. lwoffii* [15]. Of note, the protection was conferred despite the use of lyophilized bacteria, suggesting that specific bacterial metabolic products may be key to providing protection, although this remains to be further tested. Short chain fatty acids, such as **butyrate**, released from the gut into the circulation following metabolism of dietary fiber by gut microbes, have been shown to protect from HDM-induced allergic airways disease, even in the offspring of mice that were fed a high fiber diet, relative to mice fed a normal diet [55]. Therefore, we posit that future experiments that investigate the role of inhaled metabolites on development of allergic airways disease may provide a potential novel avenue for disease prevention [56]. In the meantime, the role of gut microbiota in conferring disease protection from early-life wheezing attacks is currently being tested in a Phase II randomized, efficacy, interventional, clinical trial (recruiting) of mixed oral bacterial lysates OM-85 BV in children aged 6 months to 6 years, with a high risk of developing asthma (oral bacterial extract for the prevention of wheezing lower respiratory tract illness ORBEX trial - identifier NCT02148796)[†]. The primary outcome measured is the time to occurrence of the first wheezing episode in the third observation year while not receiving the study drug.

Although there is robust evidence that inhaled exposures in a traditional farming environment can confer protection from asthma, there are also data from studies of migration that indicate that protection is only maintained into later life when children move after 5 years of age [57]. Indeed,

Clinician's Corner

Asthma is the most common chronic lung disease affecting children and is associated with a lifelong impact on lung health [1].

Severe asthma in children is characterized by severe polysensitization to aeroallergens and a steroid refractory airway eosinophilia, but with few detectable type 2 immune mediators [8,60]. In contrast to adults [61], blood eosinophils do not reflect airway eosinophils in children with severe asthma [62].

Novel biologics targeting type 2 immune pathways have been licensed for adults and children in the last 5 years, but efficacy or markers of response have not been tested in children. Licensing has been granted based on extrapolation of adult mechanistic data.

Experimental studies have recently revealed important distinctions between the mechanisms initiating early-life compared with adult asthma, highlighting the influence of the maturing immune system on disease inception.

There is a unique opportunity in early life to intervene and alter the natural history of asthma to achieve disease modification, but this will only be possible if mechanistic studies are undertaken in appropriate experimental models and if specific studies of the efficacy and mechanisms of action of newly licensed biologics are appropriately undertaken in children.

migration in the first 5 years of life seems to result in children acquiring the risk of developing asthma similar to that of the indigenous population [58]. Recent studies have thus begun to define a critical period during early development in which disruption of optimal host–commensal interactions can lead to persistent and often irreversible defects in the development and **training of immune responses** [43]. There is therefore an important role of early-life education of the immune system during this window of opportunity, identified between 1 and 3 years of age in the context of developing asthma and poor lung function, which may harbor a potentially critical impact. We argue that this window underscores the need to investigate mechanisms and efficacy of novel drugs in the most appropriate experimental animal models and samples from children [6]. Since the role of early allergic sensitization appears central to the development of school-age asthma from preschool wheeze, this window to educate the immune system is currently being tested *in vivo* in a currently recruiting, Phase II clinical trial. The Preventing Asthma in High Risk Kids (PARK) study is a randomized, double-blind, placebo-controlled trial designed to test whether 2 years' treatment with omalizumab (anti-IgE) in preschool children aged 2–3 years at high risk for asthma will prevent the progression to school age asthma (NCT02570984)ⁱⁱ. Children will be followed after 2 years of intervention, off treatment, and assessed at school age to determine the drug effect on asthma prevalence.

Taken together, the studies described emphasize the importance of early-life immune education by environmental exposures that may either increase susceptibility to allergies and/or asthma, or may protect. We now need to identify specific molecular mechanisms that confer protection to allow primary prevention of disease.

Concluding Remarks

Recognition of the importance of the very early postnatal period on lifelong health has led to recent landmark studies which have investigated early-life immune development postnatally in infants and in mouse models, and the subsequent impact on onset of childhood asthma. These studies have led to a greater understanding of particular facets of immune responses that are specific to neonates. It is now accepted that the nature of immune responses in the lungs may be different in the young/neonates compared with adults, and this needs to be considered when planning to manipulate the immune system for therapeutic gain. We know that there is an important window of opportunity during which an intervention may allow favorable immune education to protect from the development of asthma and allergies. However, a key challenge is our current limited understanding of what constitutes a healthy immune system in early life. It is difficult to obtain pulmonary samples from healthy children, yet there is a critical need to define immune responses, and specifically tissue-specific responses, at steady state versus diseased conditions (see Clinician's Corner). Although novel techniques, such as single-cell RNAseq enable analyses of small samples, the limitations of obtaining pediatric healthy controls need to be accepted. Any efforts to define 'normal' using the best available samples, such as disease controls, or perhaps upper rather than lower airway samples [59] need to be acknowledged and validated. At least this will allow us to expand the limited information currently available.

An exciting highlight now is the real potential of altering the natural history of inflammatory airways diseases such as asthma and chronic obstructive pulmonary disease by better understanding the key aspects of disease initiation in early life, which may lead us to prevent irreversible lung damage. Indeed, many questions remain (see Outstanding Questions) and we now recognize the need to identify interactions between genetic susceptibility, environmental exposures, and their interplay with the host microbiome and the immune system. Consequently, mechanistic studies that pinpoint host–microbe–immune interactions are now critical in gaining one step closer to achieving the realistic possibility of curing asthma.

Outstanding Questions

What constitutes the normal immune and microbial pulmonary landscape in children?

When is the optimal time to intervene in asthma to prevent loss of lung function?

Which environmental exposures might be manipulated to prevent the development of asthma?

Which molecular immune pathways should be manipulated to allow disease modification, or treat/cure asthma in children?

Do currently licensed biologics function in the same way in children and in adults?

Can currently licensed biologics alter the natural course of pulmonary disease by allowing a long-term improvement in lung function and disease resolution?

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Resources

ⁱ<https://clinicaltrials.gov/ct2/show/NCT02148796>

ⁱⁱ<https://clinicaltrials.gov/ct2/show/NCT02570984>

References

- Serebrisky, D. and Wznia, A. (2019) Pediatric asthma: a global epidemic. *Ann. Glob. Health* Published online January 22, 2019. <https://doi.org/10.5334/aogh.2416>
- Belgrave, D.C.M. *et al.* (2018) Lung function trajectories from pre-school age to adulthood and their associations with early life factors: a retrospective analysis of three population-based birth cohort studies. *Lancet Respir. Med.* 6, 526–534
- McGeachie, M.J. *et al.* (2016) Patterns of growth and decline in lung function in persistent childhood asthma. *N. Engl. J. Med.* 374, 1842–1852
- Bui, D.S. *et al.* (2017) Childhood lung function predicts adult chronic obstructive pulmonary disease and asthma-chronic obstructive pulmonary disease overlap syndrome. *Am. J. Respir. Crit. Care Med.* 196, 39–46
- Bui, D.S. *et al.* (2018) Childhood predictors of lung function trajectories and future COPD risk: a prospective cohort study from the first to the sixth decade of life. *Lancet Respir. Med.* 6, 535–544
- Sagliani, S. *et al.* (2019) Biologics for paediatric severe asthma: trick or TREAT? *Lancet Respir. Med.* 7, 294–296
- Papadopoulos, N.G. *et al.* (2019) Pediatric asthma: an unmet need for more effective, focused treatments. *Pediatr. Allergy Immunol.* 30, 7–16
- Bossley, C.J. *et al.* (2012) Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. *J. Allergy Clin. Immunol.* 129, 974–982.e13
- Fitzpatrick, A.M. *et al.* (2017) Systemic corticosteroid responses in children with severe asthma: phenotypic and endotypic features. *J. Allergy Clin. Immunol. Pract.* 5, 410–419.e4
- Shah, S.P. *et al.* (2019) Exploring the utility of non-invasive type-2 inflammatory markers for prediction of severe asthma exacerbations in children and adolescents. *J. Allergy Clin. Immunol. Pract.* Published online May 14, 2019. <https://doi.org/10.1016/j.jaip.2019.04.043>
- Bossley, C.J. *et al.* (2016) Assessment of corticosteroid response in pediatric patients with severe asthma by using a multidomain approach. *J. Allergy Clin. Immunol.* 138, 413–420.e6
- Ullmann, N. *et al.* (2013) Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. *Allergy* 68, 402–406
- Chen, X. *et al.* (2019) Early-life undernutrition reprograms CD4(+) T-cell glycolysis and epigenetics to facilitate asthma. *J. Allergy Clin. Immunol.* 143, 2038–2051.e12
- Tang, H.H. *et al.* (2018) Trajectories of childhood immune development and respiratory health relevant to asthma and allergy. *Elife* 7, e35856
- Sagliani, S. *et al.* (2018) Inception of early-life allergen-induced airway hyperresponsiveness is reliant on IL-13(+)CD4(+) T cells. *Sci. Immunol.* Published online September 27, 2018. <https://doi.org/10.1126/sciimmunol.aan4128>
- Grannell, R. *et al.* (2016) Associations of wheezing phenotypes with late asthma outcomes in the Avon Longitudinal Study of Parents and Children: a population-based birth cohort. *J. Allergy Clin. Immunol.* 138, 1060–1070.e11
- Merckx, J. *et al.* (2018) Respiratory viruses and treatment failure in children with asthma exacerbation. *Pediatrics* 142, e20174105
- Custovic, A. *et al.* (2018) Cytokine responses to rhinovirus and development of asthma, allergic sensitization, and respiratory infections during childhood. *Am. J. Respir. Crit. Care Med.* 197, 1265–1274
- Sagliani, S. and Custovic, A. (2019) Childhood asthma: advances using machine learning and mechanistic studies. *Am. J. Respir. Crit. Care Med.* 199, 414–422
- Altman, M.C. *et al.* (2019) Transcriptome networks identify mechanisms of viral and nonviral asthma exacerbations in children. *Nat. Immunol.* 20, 637–651
- Gollwitzer, E.S. and Marsland, B.J. (2015) Impact of early-life exposures on immune maturation and susceptibility to disease. *Trends Immunol.* 36, 684–696
- McGovern, N. *et al.* (2017) Human fetal dendritic cells promote prenatal T-cell immune suppression through arginase-2. *Nature* 546, 662–666
- Zhang, X. *et al.* (2014) CD4 T cells with effector memory phenotype and function develop in the sterile environment of the fetus. *Sci. Transl. Med.* 6, 238ra72
- Olin, A. *et al.* (2018) Stereotypic immune system development in newborn children. *Cell* 174, 1277–1292.e14
- Lee, A.H. *et al.* (2019) Dynamic molecular changes during the first week of human life follow a robust developmental trajectory. *Nat. Commun.* 10, 1092
- Gibbons, D. *et al.* (2014) Interleukin-8 (CXCL8) production is a signatory T cell effector function of human newborn infants. *Nat. Med.* 20, 1206–1210
- Zhivaki, D. *et al.* (2017) Respiratory syncytial virus infects regulatory B cells in human neonates via chemokine receptor CX3CR1 and promotes lung disease severity. *Immunity* 46, 301–314
- Sagliani, S. *et al.* (2009) Pathophysiological features of asthma develop in parallel in house dust mite-exposed neonatal mice. *Am. J. Respir. Cell Mol. Biol.* 41, 281–289
- Gollwitzer, E.S. *et al.* (2014) Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat. Med.* 20, 642–647
- Dietz, K. *et al.* (2017) Age dictates a steroid-resistant cascade of Wnt5a, transglutaminase 2, and leukotrienes in inflamed airways. *J. Allergy Clin. Immunol.* 139, 1343–1354.e6
- Saluzzo, S. *et al.* (2017) First-breath-induced type 2 pathways shape the lung immune environment. *Cell Rep.* 18, 1893–1905
- de Kleer, I.M. *et al.* (2016) Perinatal activation of the interleukin-33 pathway promotes type 2 immunity in the developing lung. *Immunity* 45, 1285–1298
- Steer, C.A. *et al.* (2017) Group 2 innate lymphoid cell activation in the neonatal lung drives type 2 immunity and allergen sensitization. *J. Allergy Clin. Immunol.* 140, 593–595.e3
- Guilliams, M. *et al.* (2013) Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J. Exp. Med.* 210, 1977–1992
- Cohen, M. *et al.* (2018) Lung single-cell signaling interaction map reveals basophil role in macrophage imprinting. *Cell* 175, 1031–1044.e18
- Yu, X. *et al.* (2017) The cytokine TGF-beta promotes the development and homeostasis of alveolar macrophages. *Immunity* 47, 903–912.e4
- Bachus, H. *et al.* (2019) Impaired tumor-necrosis-factor-alpha-driven dendritic cell activation limits lipopolysaccharide-induced protection from allergic inflammation in infants. *Immunity* 50, 225–240.e4
- Lynch, J.P. *et al.* (2018) Plasmacytoid dendritic cells protect from viral bronchiolitis and asthma through semaphorin 4a-mediated T reg expansion. *J. Exp. Med.* 215, 537–557
- Teague, W.G. *et al.* (2018) Baseline features of the Severe Asthma Research Program (SARP III) cohort: differences with age. *J. Allergy Clin. Immunol. Pract.* 6, 545–554.e4
- Pividori, M. *et al.* (2019) Shared and distinct genetic risk factors for childhood-onset and adult-onset asthma: genome-wide and transcriptome-wide studies. *Lancet Respir. Med.* 7, 509–522

41. Gomez de Agüero, M. *et al.* (2016) The maternal microbiota drives early postnatal innate immune development. *Science* 351, 1296–1302
42. Beasley, R. *et al.* (2015) Risk factors for asthma: is prevention possible? *Lancet* 386, 1075–1085
43. Gensollen, T. *et al.* (2016) How colonization by microbiota in early life shapes the immune system. *Science* 352, 539–544
44. Arrieta, M.C. *et al.* (2015) Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci. Transl. Med.* 7, 307ra152
45. Bach, J.F. (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *N. Engl. J. Med.* 347, 911–920
46. Bosch, A. *et al.* (2017) Maturation of the infant respiratory microbiota, environmental drivers, and health consequences: a prospective cohort study. *Am. J. Respir. Crit. Care Med.* 196, 1582–1590
47. Russell, S.L. *et al.* (2012) Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep.* 13, 440–447
48. Olszak, T. *et al.* (2012) Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 336, 489–493
49. Azad, M.B. *et al.* (2017) Breastfeeding, maternal asthma and wheezing in the first year of life: a longitudinal birth cohort study. *Eur. Respir. J.* Published online May 1, 2017. <https://doi.org/10.1183/13993003.02019-2016>
50. Al Nabhani, Z. *et al.* (2019) A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. *Immunity* 50, 1276–1288
51. Robinson, P.F.M. *et al.* (2019) Lower airway microbiota associates with inflammatory phenotype in severe preschool wheeze. *J. Allergy Clin. Immunol.* 143, 1607–1610.e3
52. Ober, C. *et al.* (2017) Immune development and environment: lessons from Amish and Hutterite children. *Curr. Opin. Immunol.* 48, 51–60
53. Stein, M.M. *et al.* (2016) Innate Immunity and asthma risk in Amish and Hutterite farm children. *N. Engl. J. Med.* 375, 411–421
54. Schuijs, M.J. *et al.* (2015) Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. *Science* 349, 1106–1110
55. Thorburn, A.N. *et al.* (2015) Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat. Commun.* 6, 7320
56. Maniar, K. *et al.* (2018) Inhalational supplementation of metformin butyrate: a strategy for prevention and cure of various pulmonary disorders. *Biomed. Pharmacother.* 107, 495–506
57. Cabieses, B. *et al.* (2014) A systematic review on the development of asthma and allergic diseases in relation to international immigration: the leading role of the environment confirmed. *PLoS One* 9, e105347
58. Braback, L. *et al.* (2011) Migration and asthma medication in international adoptees and immigrant families in Sweden. *Clin. Exp. Allergy* 41, 1108–1115
59. Turner, S. *et al.* (2018) Pulmonary epithelial barrier and immunological functions at birth and in early life – key determinants of the development of asthma? A description of the protocol for the Breathing Together study. *Wellcome Open Res* 3, 60
60. Teague, W.G. *et al.* (2019) Lung lavage granulocyte patterns and clinical phenotypes in children with severe, therapy-resistant asthma. *J. Allergy Clin. Immunol. Pract.* 7, 1803–1812.e10
61. Wagener, A.H. *et al.* (2015) External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 70, 115–120
62. Nagakumar, P. *et al.* (2019) Pulmonary type2 innate lymphoid cells in paediatric severe asthma: phenotype and response to steroids. *Eur. Respir. J.* Published online June 4, 2019. <https://doi.org/10.1183/13993003.01809-201>
63. Bel, E.H. *et al.* (2014) Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N. Engl. J. Med.* 371, 1189–1197
64. Haldar, P. *et al.* (2009) Mepolizumab and exacerbations of refractory eosinophilic asthma. *N. Engl. J. Med.* 360, 973–984
65. Bjerner, L. *et al.* (2016) Reslizumab for inadequately controlled asthma with elevated blood eosinophil levels: a randomized phase 3 study. *Chest* 150, 789–798
66. Castro, M. *et al.* (2015) Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir. Med.* 3, 355–366
67. Bleeker, E.R. *et al.* (2016) Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta₂-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet* 388, 2115–2127
68. FitzGerald, J.M. *et al.* (2016) Benralizumab, an anti-interleukin-5 receptor alpha monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 388, 2128–2141
69. Nair, P. *et al.* (2017) Oral glucocorticoid-sparing effect of benralizumab in severe asthma. *N. Engl. J. Med.* 376, 2448–2458
70. Corren, J. *et al.* (2011) Lebrikizumab treatment in adults with asthma. *N. Engl. J. Med.* 365, 1088–1098
71. Hanania, N.A. *et al.* (2016) Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir. Med.* 4, 781–796
72. De Boever, E.H. *et al.* (2014) Efficacy and safety of an anti-IL-13 mAb in patients with severe asthma: a randomized trial. *J. Allergy Clin. Immunol.* 133, 989–996
73. Panettieri, R.A., Jr. *et al.* (2018) Tralokinumab for severe, uncontrolled asthma (STRATOS 1 and STRATOS 2): two randomised, double-blind, placebo-controlled, phase 3 clinical trials. *Lancet Respir. Med.* 6, 511–525
74. Russell, R.J. *et al.* (2018) Effect of tralokinumab, an interleukin-13 neutralising monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-severe asthma (MESOS): a multicentre, double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Respir. Med.* 6, 499–510
75. Castro, M. *et al.* (2018) Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N. Engl. J. Med.* 378, 2486–2496
76. Rabe, K.F. *et al.* (2018) Efficacy and safety of dupilumab in glucocorticoid-dependent severe asthma. *N. Engl. J. Med.* 378, 2475–2485
77. Corren, J. *et al.* (2017) Tezepelumab in adults with uncontrolled asthma. *N. Engl. J. Med.* 377, 936–946
78. Bateman, E.D. *et al.* (2017) Fevipiprant, an oral prostaglandin DP₂ receptor (CRTh2) antagonist, in allergic asthma uncontrolled on low-dose inhaled corticosteroids. *Eur. Respir. J.* 50, 1700670