

Microbes and mucosal immune responses in asthma



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The substantial increase in the worldwide prevalence of asthma and atopy has been attributed to lifestyle changes that reduce exposure to bacteria. A recent insight is that the largely bacterial microbiome maintains a state of basal immune homeostasis, which modulates immune responses to microbial pathogens. However, some respiratory viral infections cause bronchiolitis of infancy and childhood wheeze, and can exacerbate established asthma; whereas allergens can partly mimic infectious agents. New insights into the host's innate sensing systems, combined with recently developed methods that characterise commensal and pathogenic microbial exposure, now allow a unified theory for how microbes cause mucosal inflammation in asthma. The respiratory mucosa provides a key microbial interface where epithelial and dendritic cells interact with a range of functionally distinct lymphocytes. Lymphoid cells then control a range of pathways, both innate and specific, which organise the host mucosal immune response. Fundamental to innate immune responses to microbes are the interactions between pathogen-associated molecular patterns and pattern recognition receptors, which are associated with production of type I interferons, proinflammatory cytokines, and the T-helper-2 cell pathway in predisposed people. These coordinated, dynamic immune responses underlie the differing asthma phenotypes, which we delineate in terms of Seven Ages of Asthma. An understanding of the role of microbes in the atopic march towards asthma, and in causing exacerbations of established asthma, provides the rationale for new specific treatments that can be assessed in clinical trials. On the basis of these new ideas, specific host biomarkers might then allow personalised treatment to become a reality for patients with asthma.

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Introduction

The increasing incidence of allergy, atopy, and asthma in industrialised countries means that these are lifestyle diseases and a global health-care problem.^{1,2} Indeed, the asthma and allergy epidemic is occurring in urban communities throughout the industrially developed nations, with a particular increase in children.³ Notably, this increase in asthma and allergy is also accompanied by an increase in autoimmune diseases, and for the period 1950–2000 has been related to a decrease in infectious diseases.⁴

Fortunately, most patients with asthma can be treated effectively by inhaled corticosteroids or the combination of these drugs and long-acting β -adrenoceptor agonists, as recommended by the Global Initiative on Asthma (GINA) guidelines.⁵ Although this treatment is anti-inflammatory and provides symptomatic benefit, it might be needed on a lifelong basis, and many asthmatics are poorly compliant with their recommended treatment. Although allergen immunotherapy is improving continually, it only provides benefit for some patients with allergic asthma, and new strategies for allergic inflammation are being developed.⁶

Both genes and the environment affect the host immune response to microbes. In large genome-wide association studies in asthma, investigators have identified several genetic loci that affect susceptibility to various asthma types.⁷ Some genes affect epithelial barrier function and thus modify interactions between microbes and the mucosal immune system. For example, flaggrin (*FLG*) has barrier functions in the skin and airways and is associated with allergic skin and airway disease. Other genes associated with asthma have roles in mucosal immune responses, including ORM-1 (yeast) like protein 3 (*ORMDL3*), which can affect sphingolipid metabolism during inflammation.⁸ Genes for proteases involved in

tissue remodelling, including human A disintegrin and metalloprotease-33 (*ADAM33*), have been associated with asthma susceptibility. Additionally, environmental effects cause epigenetic changes⁹ through processes such as DNA methylation, histone acetylation, and micro RNA activities, all of which change the host immune response to microbes. Atopy (elevated IgE) is now generally thought to arise from gene–environment effects, starting during prenatal and early postnatal development.^{10,11}

Hence, a compelling need exists to understand the ways in which asthma is initiated and exacerbated, and to develop new treatments on the basis of these new insights.¹² In the past 15 years, a series of studies with monoclonal antibodies directed against eosinophils and other allergic cells have shown the need to target these treatments to particular patients.¹³ Although asthma is still viewed as an inflammatory disease of the airways, we believe that a renewed focus is needed on the way that aberrant responses to microbes contribute to airway mucosal inflammation in asthma.¹⁴

Microbes and man

The respiratory tract is bombarded constantly by bacteria, viruses, dust, and aeroallergens¹⁵ (figure 1). Throughout evolution, human beings have faced the threat of epidemics, plague, and pestilence, and the more frequent inhalation of organic and inorganic dusts. To meet all these challenges, the mucosal immune system needs to remain in a state of readiness to mount diverse responses to the complex and changing threats posed by pathogens. This preparedness for pathogens relies on specific interactions between the immune system and the environment, but could also contribute to mucosal inflammation in allergy and asthma.

The innate and specific immune response against parasitic worms and other macroparasites might have been

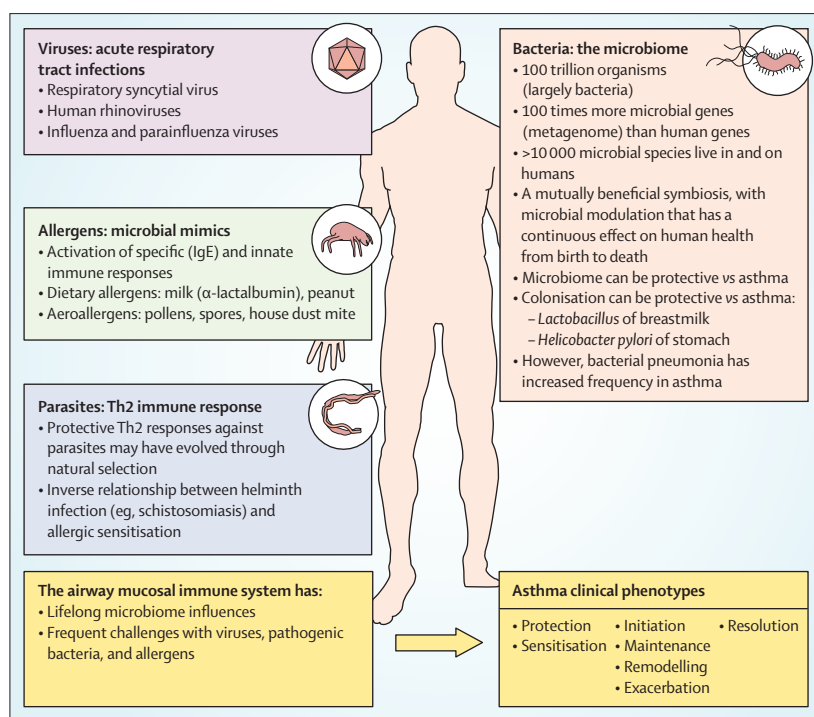


Figure 1: Microbial bombardment of the respiratory tract

The human airway is constantly affected by microbes. Bacteria contribute to the microbiome, which involves the skin and mucosal surfaces, and generally provides beneficial basal microbial stimulation. However, asthmatics are vulnerable to various bacterial pathogens that can rarely cause various types of pneumonia. Viruses generally challenge the airways more acutely than do bacteria, but asthmatics have impaired immune responses and viruses are major contributors to asthma exacerbations. Th2 immune responses are believed to have arisen during evolution as part of the immune responses to parasites, including helminths. Fungal spores act as major allergens, but many aeroallergens, such as pollens, animal danders, and house dust mites, might be acting as so-called microbial mimics, stimulating pattern recognition receptors and specific immune responses. Th2=T helper cell type 2.

important during evolution to combat infestation,¹⁶ and allergic responses could be protective against a range of environmental substances.¹⁷ The presence of worms might even protect against inappropriate allergic sensitisation.

The hygiene hypothesis proposes that low exposure to microbes in a modern environment might contribute to the increase in asthma and atopic disorders in developed countries, although the precise mechanisms involved remain unclear.¹⁸ Excessive cleanliness could lead to a decrease in the amount of microbial stimulation needed to enable normal development of the immune system. A range of gut and respiratory microbes have been found to be protective against allergy and asthma, including mycobacteria and gastric *Helicobacter pylori*. Once the disorder is established, children with asthma are vulnerable to infection with *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*, and asthmatics also have an increased risk of invasive pneumococcal disease. Experiments in mice have shown that combined *Haemophilus influenzae* infection and allergic airways disease drives a chronic infection with features of neutrophilic asthma.¹⁹ Mice have also been shown to have age-sensitive contact with commensal microbes to establish lung mucosal natural killer T cell tolerance.²⁰

Resident bacteria have been analysed in the Human Microbiome Project, which has tested thousands of specimens from healthy adults, with samples obtained from various body sites to assess the diversity of each habitat's signature microbes.²¹ The investigators used modern bacterial genomic methods that do not depend on culture, and sequenced the bacterial 16S ribosomal subunit variable region. This sequence provides a molecular barcode for each bacteria, and enables the identification of millions of bacteria within a bacterial community. Individual populations of microbes are found as the gut, respiratory, skin, and urogenital microbiota, whereas the entire set of microbes for an individual is their microbiome.²² One adult harbours an estimated 100 trillion microbes, mainly consisting of bacteria in the colon, which greatly outnumber the host cells. This number corresponds to a larger amount of microbial genes, which encode numerous antigens to act on the immune system. The gut microbiota has metabolic interactions with the host immune system, which can be studied by metabolic phenotyping.²³

Our immunological relation with this complex microflora is beginning to be defined in obstructive lung disease,²⁴ with influences from upper and lower airway flora,²⁵ and also the gut microbiome.²⁶ Reduced diversity of the gut flora might play a crucial part in the development of asthma in infancy,²⁷ and the microbiome in infants can be manipulated with supplements of *Lactobacillus casei*.²⁸ Disordered airway microbial communities are also present in adult asthma,²⁹ and can be affected by vitamin D and probiotics in the diet.³⁰ Beneficial effects of macrolides and related antibiotics in asthma could be partly due to effects on the microbiome and to anti-inflammatory actions.³¹ However, the role of microbes in initiation of asthma remains controversial and is largely based upon association; the mechanisms need to be understood.

Viruses play a major part in early-life wheezing illnesses and in causing acute exacerbations of established disease.^{32,33} Respiratory syncytial virus, human rhinoviruses, and influenza A are RNA viruses, with their own error-prone RNA polymerases that mediate rapid mutations. The respiratory viruses infect the upper respiratory tract mucosa and are prone to spread to the bronchi in patients with asthma. Various host factors affect the outcome of viral infection in the upper respiratory tract,³⁴ especially the response of the infected airway epithelial cell in contact with dendritic cells and other mucosal cells³⁵ (figure 2). Additionally, new respiratory viral infections that can potentially exacerbate asthma³⁶ are being reported.

Respiratory syncytial virus is an enveloped RNA virus of the *Paramyxoviridae* family, and is a leading cause of severe acute lower respiratory infection in early childhood, and a major contributor to global childhood mortality.^{37,38} Most infants are infected with respiratory syncytial virus in the winter of their first year of life, which usually causes a simple upper respiratory tract infection; however, a few

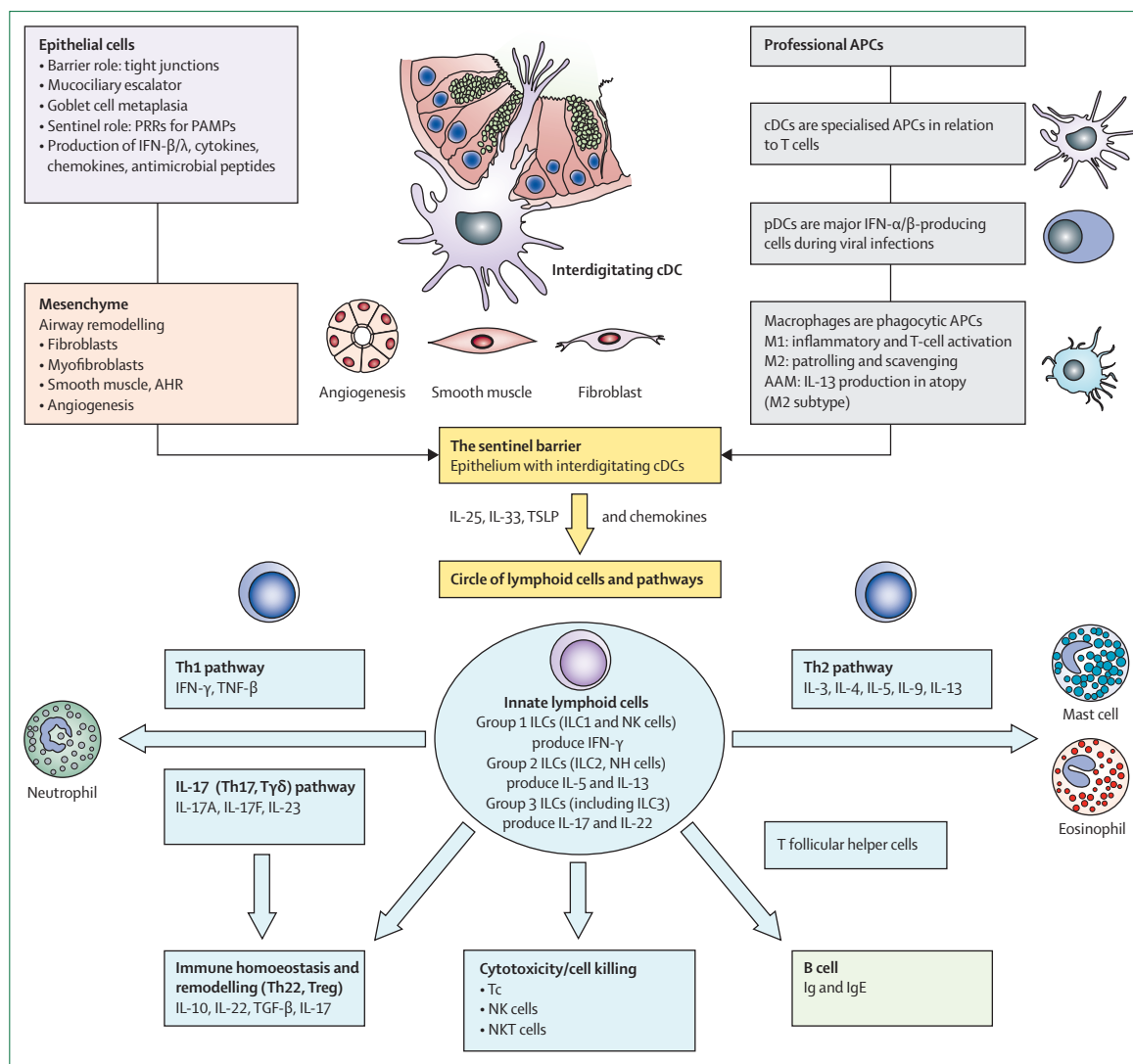


Figure 2: The mucosal immune response to microbes

The mucosal immune response initially involves the surface ciliated epithelium with interdigitating cDCs, which provides a barrier function and can monitor airway luminal events and initiate immune responses. Professional APCs also initiate immune responses. The structural mesenchymal cells of the airway are involved in two major features of asthma: airway remodelling and airway hyperreactivity. The circle of lymphoid cells describes the family of ILCs involved in acute responses, and specific T and B cell populations that act later to modulate the immune response. APC=antigen-presenting cell. PRR=pattern recognition receptor. PAMP=pathogen-associated molecular pattern. IFN=interferon. cDC=classical dendritic cell. pDC=plasmacytoid dendritic cell. M1=macrophage type 1. M2=macrophage type 2. AAM=alternatively activated macrophage. IL=interleukin. AHR=airway hyperreactivity. Th1=T helper cell type 1. Th2=T helper cell type 2. TNF=tumour necrosis factor. Th17=T helper cell type 17. ILC=innate lymphoid cell. NK=natural killer cell. NH=natural helper cell (nuocyte). Treg=T regulatory cell. TGF-β=transforming growth factor-β. Tc=T cytotoxic.

infants develop respiratory syncytial virus bronchiolitis. Infants might be vulnerable to respiratory syncytial virus bronchiolitis because their antiviral immunity is distinct from that of adults.³⁹ Children with a history of respiratory syncytial virus bronchiolitis often suffer recurrent bouts of wheeze, and almost half are diagnosed as asthmatic in the following 6 years.⁴⁰ Investigators have reported that after severe respiratory syncytial virus bronchiolitis in infancy, the prevalence of allergic asthma proceeding to adult asthma over an 18-year period increases.⁴¹ Immunity is incomplete after primary respiratory syncytial virus infection and secondary infection is common in later life.

Adult reinfection with respiratory syncytial virus generally causes symptoms of a cold, but mounting evidence suggests that the virus causes serious respiratory disease and even death in elderly patients with chronic lung disorders.⁴²

The immune response triggered by respiratory syncytial virus infection is complex and involves various components of the immune system: innate, humoral, and cellular immune responses are all thought to have key roles in both protection from infection and disease pathogenesis.⁴³ In children younger than 2 years with natural respiratory syncytial virus infection, viral load

and dynamics predict clinical severity.⁴⁴ Respiratory syncytial virus infects airway epithelial cells and triggers the release of a range of proinflammatory chemokines and cytokines. Infected epithelial cells produce thymic stromal lymphopoietin,⁴⁵ which can contribute to excessive T-helper type 2 (Th2) responses. However, severe respiratory syncytial virus bronchiolitis is characterised by airway neutrophilia, and these activated neutrophils may harbour the virus.^{46,47} The infection also involves T lymphocytes, including Th2 cells, Th17 cells, regulatory T cells, and cytotoxic T cells.

No vaccine exists for respiratory syncytial virus, partly because of the failure of a formalin-inactivated vaccine in the 1960s that led to disease exacerbation after subsequent natural viral infection.^{48,49} However, in premature and immunocompromised children, infection can now be prevented with palivizumab, a monoclonal antibody directed against the respiratory syncytial virus fusion protein.⁵⁰ Notably, antibody prophylaxis seems to reduce the frequency of subsequent recurrent childhood wheezing, suggesting that bronchiolitis is causally related to later recurrent respiratory problems.

Human rhinoviruses are the main cause of the common cold, but also cause up to 60% of asthma exacerbations.⁵¹ These viruses are RNA viruses of the *Picornaviridae* family. The genome sequences of all 99 known serotypes of human rhinovirus have been painstakingly elucidated;⁵¹ this will be useful for the development of antiviral agents and vaccines. Symptomatic human rhinovirus infections during infancy are a major risk factor for development of preschool childhood wheezing.⁵² Rhinovirus infection is second to respiratory syncytial virus in causing acute bronchiolitis of infancy, with some cases of multiple infection, but human rhinovirus is associated with more severe disease.⁵³ When human rhinoviruses cause wheezing in babies and young children, this is a major predictor of asthma at 6 years of age.^{54–56} Human rhinovirus infection is the most frequent viral cause of adult asthma exacerbations, and it is more commonly found in the lower airway in patients with asthma.⁵⁷ Compared with non-asthmatics, adults with asthma do not have more frequent rhinovirus infection, but suffer from longer-lasting lower respiratory tract symptoms.^{58,59} Experimental rhinovirus infection in asthma also causes increased lower respiratory illness and features of Th2 inflammation.⁶⁰ Influenza A is a segmented RNA virus in which eight single strands of RNA comprise the genome.⁶¹ The virus is capable of genetic reassortment if a single cell is simultaneously infected by two or more influenza A viruses. It is zoonotic and circulates in many animal hosts, including human beings, pigs, birds, horses, and dogs. The recent pandemic of influenza A H1N1 virus (originally termed “swine-origin influenza A virus [S-OIV]”) emerged in 2009 and particularly affected young adults and children.^{62–64} Although influenza A H1N1 generally causes mild illness, it can cause severe lung disease in asthmatics, people with chronic illnesses, and pregnant women.^{65–67}

Roughly one in 20 patients admitted to hospital with H1N1 virus in the UK have a variant interferon-induced transmembrane protein 3 (IFITM3) allele, which is present in only one in 400 of the general population and increases susceptibility to viral infection.⁶⁸ Endothelial cells are major sites for cytokine generation during severe influenza infection,⁶⁹ and sometimes cause a life-threatening cytokine storm. However, inhibition of the sphingosine-1-phosphate receptor could potentially decrease this virus-induced cytokine release.⁷⁰ Prediction, prevention, and management of future influenza pandemics will need coordinated international activities.⁷¹

A recent breakthrough is the recognition that allergens can drive allergic responses in patients by their capacity to stimulate the innate non-specific immune system, thus acting as so-called microbial mimics,⁷² and the protease activity of many allergens could increase their interactions with the immune system. In particular, the house dust mite allergen, *Derp2*, resembles human binding proteins for bacterial lipopolysaccharide,⁷³ which enables it to mimic lipopolysaccharide and have immunostimulatory activities on lung structural cells.⁷⁴ Finally, complex carbohydrate allergens, including β -glucans found in peanut glycoallergen *Arah1*, engage C-type lectin receptors on dendritic cells and induce allergic responses, also by activation of an innate immune response.⁷⁵

A range of environmental factors can affect the onset of asthma,² including differences between lipopolysaccharide and bacterial content in dust in rural and urban settings,^{76,77} whereas exposure to pets such as cats and dogs in the prenatal period and early infancy can be protective against allergic sensitisation.⁷⁸ Rather disappointingly, avoidance of house dust mites by the use of special bed covers was clinically ineffective as a single avoidance step.⁷⁹

Fungal (mould) sensitivity to *Alternaria alternata*, *Cladosporium herbarum*, and *Aspergillus fumigatus* is associated with allergic lower respiratory tract disease.⁸⁰ Airborne spores of *A. alternata* have been documented as serious triggers for asthma, and sensitisation predicts susceptibility to thunderstorm-associated asthma.⁸¹ Severe asthma with fungal sensitisation has recently been reported to respond to oral antifungal treatment.⁸²

Hence, complex interactions exist between the microbiome, bacterial colonisation and infection of the airways, acute respiratory virus infections, and aero-allergens and the environment. These infective and allergic factors combine to exert their dynamic effects in multi-hit interactions on the respiratory and immune systems. In the following paragraphs, we will focus on the airway mucosa, and will examine how the airways respond to microbes and mount innate and specific immune responses.

The mucosal barrier and sentinel function

The surface of the airways is lined by a ciliated epithelium, although dendritic cells have processes that interdigitate between the epithelial cells to ensure intimate contact

with the airway lumen.⁸³ Hence, the initial interactions between microbes and the host immune response involve epithelial cells and dendritic cells, although airway macrophages and intraepithelial lymphocytes and other leucocytes are also involved.

The epithelium in asthma is fragile and might be denuded, with disruption of the tight junctions between epithelial cells.⁸⁴ This disruption can result in impaired mucociliary escalator and barrier (molecular sieve) functions, but can also help the passage of allergens and other agents into airway mucosal tissue. Epithelial cells are infected by respiratory viruses, and are recognised as important for innate immune responses, since they are major sources of type I (especially interferon β) and III (interferon λ) interferons, and other cytokines and chemokines.⁸⁵ Several groups have reported that the mucosal airway interferon response to respiratory viruses is deficient in asthma,⁵⁹ although not all studies have supported this finding.^{32,85} Recent reports draw attention to deficiencies in production of interferon α , interferon β , and interferon λ in asthma.^{86–88} Deficient interferon responses to human rhinovirus can occur in both atopic children without asthma and in asthmatic children.⁸⁸ The results of a therapeutic clinical study with inhaled interferon β in asthma are awaited with interest (ISRCTN trial registration number 83107787).

Epithelial cells are recognised as contributors to the type 2 (Th2) mucosal immune response pathway.⁸⁹ Airway epithelium produces thymic stromal lymphopoietin, interleukin 25, and interleukin 33. Extensive crosstalk occurs between epithelial cells and dendritic cells, which maintains dendritic cell immune homeostasis.⁹⁰ Epithelial thymic stromal lymphopoietin has been proposed as a key regulator in allergic inflammation, whereas trefoil factor 2 is a mediator with important functions in epithelial barrier function and repair that rapidly induces interleukin 33 during allergic asthma.⁹¹ Interleukin 33 is an alarmin and a damage-associated molecular pattern that promotes both Th2 and protective antiviral CD8⁺ T-cell responses.⁹²

Dendritic cells are central in bridging innate (non-specific) and adaptive (specific) immune responses, and have also been proposed as master regulators of allergic inflammation.⁸⁹ Classical or conventional dendritic cells use their long dendrites to maintain contact with their environment, and have intermittent contact with the mixed microbial flora of the airway lumen. Conventional dendritic cells are fully equipped with receptors of the innate immune system, but are also powerful antigen-processing and antigen-presenting cells, and can present fragments of microbes in association with a range of cell surface receptors for antigen presentation. Hence, conventional dendritic cells can initiate a range of adaptive T-cell responses, including Th1, Th2, Th17, and regulatory T-cell responses (figure 2). Plasmacytoid dendritic cells are especially important in interferon α production and antiviral responses.

The circle of lymphoid cells

The initial interactions of microbes with epithelial cells, dendritic cells, and macrophages at the mucosal surface create potential to activate a wide range of different lymphoid types. We use the descriptor of a circle to express the way in which a group of different lymphoid cells can initiate several pathways, which radiate outwards and are coordinated simultaneously during a dynamic immune response (figure 2). The huge variety of chemokines and cytokines produced by the epithelium and dendritic cells, combined with the remarkable antigen-processing and antigen-presenting capacity of dendritic cells, means that a range of innate lymphoid cells and T cells can be activated.⁹³ Substantial heterogeneity and plasticity in T-cell populations, especially within Th2 cells, is now recognised.

Within the circle, a range of lymphoid cells, including T and B lymphocytes and natural killer cells, are present. When a viral infection occurs, there will be different kinetics for the involvement of various cell types, with innate lymphoid cells prominent in the early response, and the balance of cellular involvement contributes to the clinical course of the infection. Hence, a viral infection can simultaneously cause an interferon response combined with Th1, Th2, Th17, T regulatory cell, B cell, and cytotoxic responses, and airway remodelling. The airway mucosa in asthma has a complex interaction between Th2 pathways and viral immune responses,¹² whereas in health it maintains a delicate balance between fighting microbes and developing tolerance to allergens. In terms of regulation and restriction of immune responses, researchers are particularly interested in the roles of T regulatory cells and Th17 cells.

The Th2 pathway

Only about half of patients with asthma have eosinophilic manifestations,^{94,95} although the cellular and molecular details of the mucosal Th2 pathway are unravelling (figure 3). Molecular phenotyping of asthma can be done in a non-invasive way by measurement of the fraction of expiratory nitric oxide and sputum eosinophils.⁹⁴ Additionally, the Th2 pathway can be revealed in some asthmatics by gene expression analysis of bronchial cells and tissue.⁹⁶

Epithelial cells and dendritic cells are now recognised as pivotal in causing Th2-type inflammation through production of thymic stromal lymphopoietin, stem cell factor, interleukin 25 and interleukin 33, OX40 ligand, chemokine (C-C motif) ligand 17 (TARC: thymus and activation regulated chemokine), and CCL 22 (MDC: macrophage-derived chemokine). Th2 cells are orchestrators of allergic and asthmatic eosinophilic inflammation and, after activation of Th2 cells, progression then occurs down the so-called Th2 pathway. This pathway involves Th2 cells producing interleukin 4 and interleukin 13 that assist in production of IgE, with interleukin 5 acting as a terminal maturation factor for

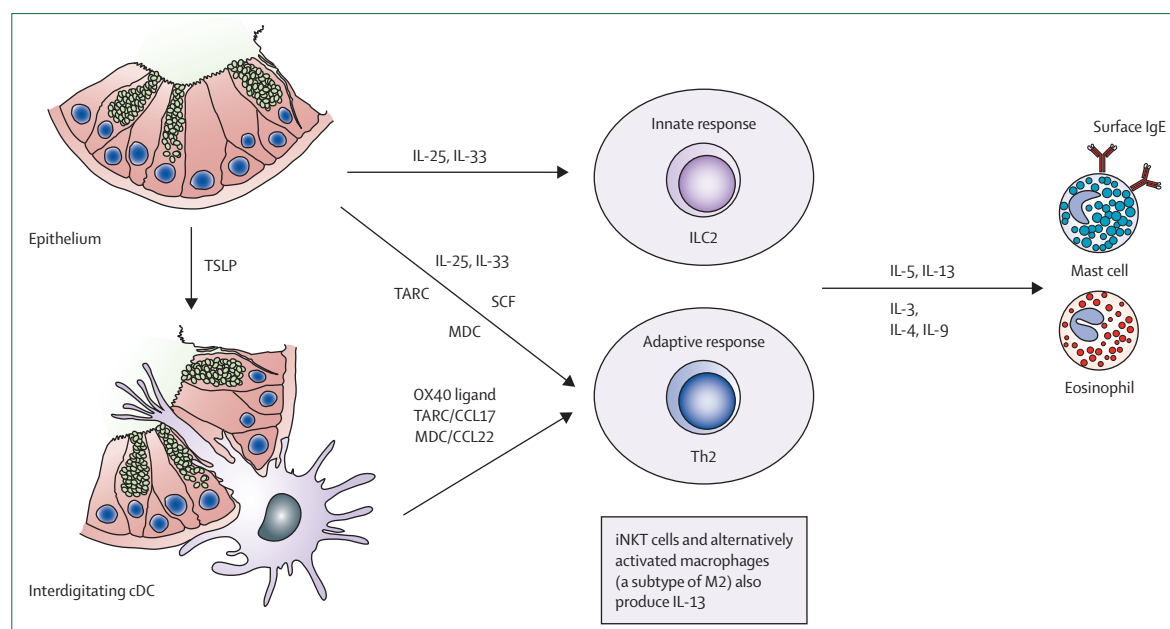


Figure 3: The Th2 pathway in allergy and asthma

The epithelium is now recognised to play a major part in initiation of the Th2 pathway through production of a family of chemokines and cytokines that act on various cells, including cDCs, which can then cause differentiation of Th2 and Th9 cells that produce IL-4, IL-5, IL-9, and IL-13. NH and other cells can also produce IL-5 and IL-13, which might be important in acute Th2 pathway responses. Mast cells and eosinophils are controlled by the cytokines and chemokines of the Th2 pathway, and are characteristic cells found in mucosal inflammation during allergy and asthma. IL=interleukin. ILC=innate lymphoid cell. TSLP=thymic stromal lymphopoietin. SCF=stem cell factor. TARC=thymus and activation regulated chemokine. MDC=macrophage-derived chemokine. Th2=T helper cell type 2. cDC=classical dendritic cell. iNKT=invariant natural killer T cell. M2=macrophage type 2.

eosinophils, and interleukin 13 being involved in mucus production, airway remodelling, and fibrosis.

Innate lymphoid cells are a family of developmentally related immune cells that are involved in inflammation and remodelling, and a uniform nomenclature has been proposed.⁹⁷ Group 2 innate lymphoid cells (ILC2) produce interleukin 4, interleukin 5, and interleukin 13, and were previously termed nuocytes or natural helper cells.⁹⁸ ILC2 can be activated by microbes acting on toll-like receptors, and do not need specific IgE-dependent triggering. Exposure of epithelial cells to a range of insults causes them to activate ILC2, and triggers interleukin 5 and interleukin 13 production, which contributes to airways hyperreactivity and could be important early in the acute allergic immune response.

Macrophage subtypes involved in active inflammatory immune responses (M1) and patrolling or surveillance (M2) have been described in mice.⁹⁹ Alternatively activated macrophages, representing a subtype of M2 macrophages, can also produce interleukin 13, and are involved in asthma exacerbations in children.⁹⁹

The effector cells of the Th2 pathway are mainly mast cells and eosinophils. Allergic or anaphylactic hypersensitivity involves allergens crosslinking IgE on the surface of the mast cell to cause degranulation.¹⁰⁰ This process is mediated by allergen-triggered cross-linking of specific IgE molecules bound to receptors for the Fc portion of IgE on the mast cell surface, which triggers

mast cell degranulation and the release of vasoactive peptides such as histamine and heparin. Mast cells and IgE are important in acute allergic reactions that can manifest as acute bronchospasm in patients with asthma.

Eosinophils are found in a substantial proportion of asthmatic patients; these patients are generally responsive to inhaled corticosteroids.¹⁰¹ Eosinophils have characteristic granules that contain major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin. Some of the highly basic eosinophil proteins can have toxic effects and cause airway hyperreactivity. Eosinophils can be long-lived tissue cells that synthesise various cytokines and take part in airway tissue remodelling. Neutrophils can also infiltrate the airways of asthmatics and are especially prominent in severe asthma in adults, and during particular asthma exacerbations.¹⁰²

Pattern recognition receptors

So far, we have discussed the complexity of genes, microbes, and the mucosal immune response. However, a unifying process is the way in which bacteria, viruses, and allergens activate the immune response through microbial pathogen-associated molecular patterns.¹⁰³ Pathogen-associated molecular patterns are contained within bacteria, viruses, and other pathogens; damage-associated molecular patterns expressed by host cells also contribute to asthma. Pathogen-associated

molecular patterns interact with host immune system pattern recognition receptors, including the toll-like receptors (figure 4). In the allergic lung, changed regulation of toll-like receptor responses can impair antibacterial immunity.¹⁰⁴

Respiratory RNA viruses (eg, respiratory syncytial virus, rhinovirus, and influenza virus) have double-stranded RNA intermediates that activate intracellular toll-like receptors, most notably toll-like receptor 3 within the endosome. RNA viruses also contain various other pathogen-associated molecular patterns that can stimulate toll-like receptors and other intracytoplasmic pattern recognition receptors. Interferon responses to viruses involve toll-like receptors, C-type lectin receptors, NOD-like receptors, and RIG-1-like receptors in the bronchial epithelium.¹⁰⁵

By contrast, lipopolysaccharide or endotoxin within the wall of Gram-negative bacteria and many allergens stimulates toll-like receptor 4. Bacterial flagellin activates toll-like receptor 5, whereas mycobacterial lipoproteins activate toll-like receptors 1, 2, and 6. Pathogen-associated molecular pattern or pattern recognition receptor pathways are important targets for new drugs to combat asthma, both in the induction and maintenance of allergy and asthma, and for exacerbations caused by excessive host response to infections.¹⁰⁶ Both viruses and bacteria can activate NOD-like receptors, and cause assembly of the inflammasome and proteolytic activation of interleukins 1 β and 18.

The Seven Ages of Asthma

Although some paediatricians hesitate to label young children as asthmatic, asthma can be diagnosed at any age and various phenotypes can be defined according to clinical and molecular approaches.¹⁰⁷ Throughout life, a continuing dynamic association is played out between immune systems, the environment, and microbes. This interplay translates to the so-called Seven Ages of Asthma (figure 5). Stages up to infancy are generally not aligned to treatments, although global guidelines are available for treatment of children up to 5 years of age, children 6–16 years of age, and adults.

Fetal development

The feto-placental unit produces a range of cytokines, and transmits various maternal immunoglobulins (including IgG), dietary factors, and cells.¹¹ However, because the maternal immune system contributes to the characteristics of the child's immune system, some effects could be from before conception. The prenatal period of growth of the airways and development of the immune system is now recognised as a crucial window when maternal exposure to environmental and nutritional factors programmes the fetus. We have already noted the protective effect of lipopolysaccharide and bacteria in dust from farms,^{76,77} and exposure to pets such as cats and dogs.⁷⁸ Low maternal dietary intake of vitamin D, vitamin E, and zinc have been associated with increased recurrent wheeze in offspring.¹⁰⁸ The fetal gut

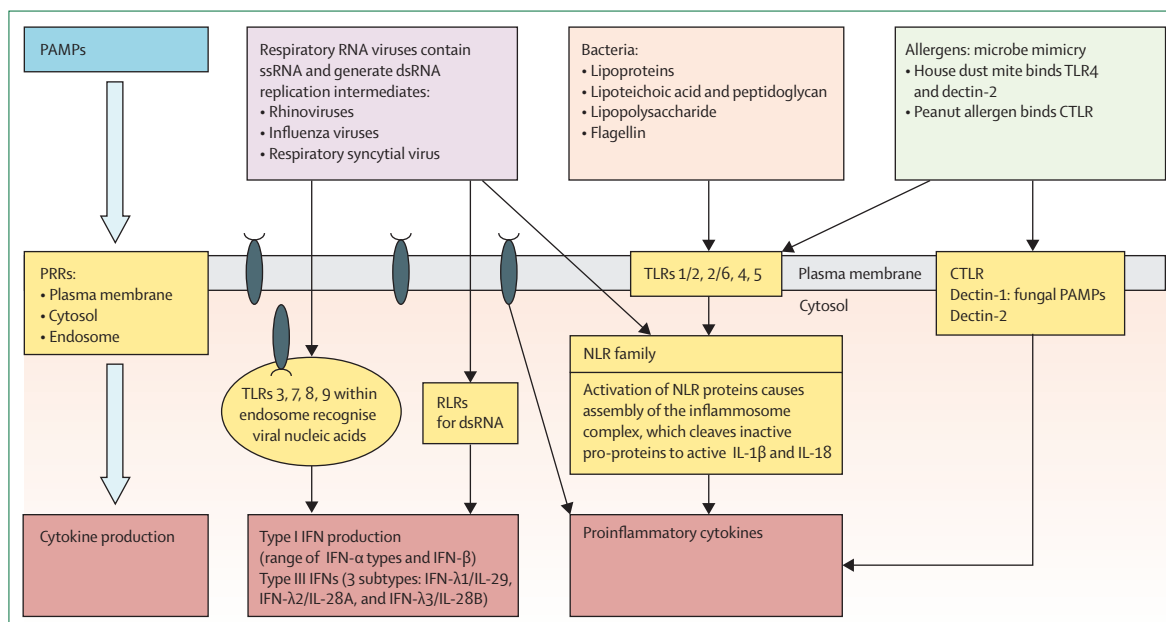


Figure 4: PAMPs and the mucosal immune response

Microbes contain PAMPs, which are important drivers of innate mucosal immune responses. The common respiratory viruses have an RNA genome and stimulate intracellular endosomal PRRs, including TLR3, TLR7, TLR9, RLRs, and NLRs. An important part of this process is initiation of type I IFN production. Bacterial PAMPs usually act on different individual PRRs, such as cell surface TLRs and NLRs, and the inflammasome is involved in release of active IL-1 β and IL-18. Allergens can mimic microbial PAMPs to stimulate PRRs. House dust mite has been especially studied in relation to TLR4 and dectin-2. PAMP=pathogen-associated molecular pattern. ssRNA=single-stranded RNA. dsRNA=double-stranded RNA. TLR=toll-like receptor. CTLR=C-type lectin receptor. PRR=pattern recognition receptor. RLR=RIG-1-like receptor. NLR=NOD-like receptor. IL=interleukin. IFN=interferon.

was previously thought to be sterile, but at birth about 100 microbial species populate the colon.²³

Birth

Umbilical cord blood from babies who go on to develop asthma already has increased concentrations of Th2 pathway chemokines. Cord blood chemokine (C-C motif) ligand 22 (macrophage-derived chemokine) concentrations are associated with elevated total IgE in babies at 1 year and in children at 6 years.^{109,110}

Furthermore, cord blood innate deficiencies in interferon γ production in response to respiratory syncytial virus predict respiratory tract illnesses in the first year of life.¹¹¹

A rural environment, maternal socioeconomic status, and the mode of delivery at birth are all factors in the development of allergy and asthma.

Infants (including neonates) up to 18 months of age

During the first year of life, the immune system is programmed in response to environmental, bacterial,

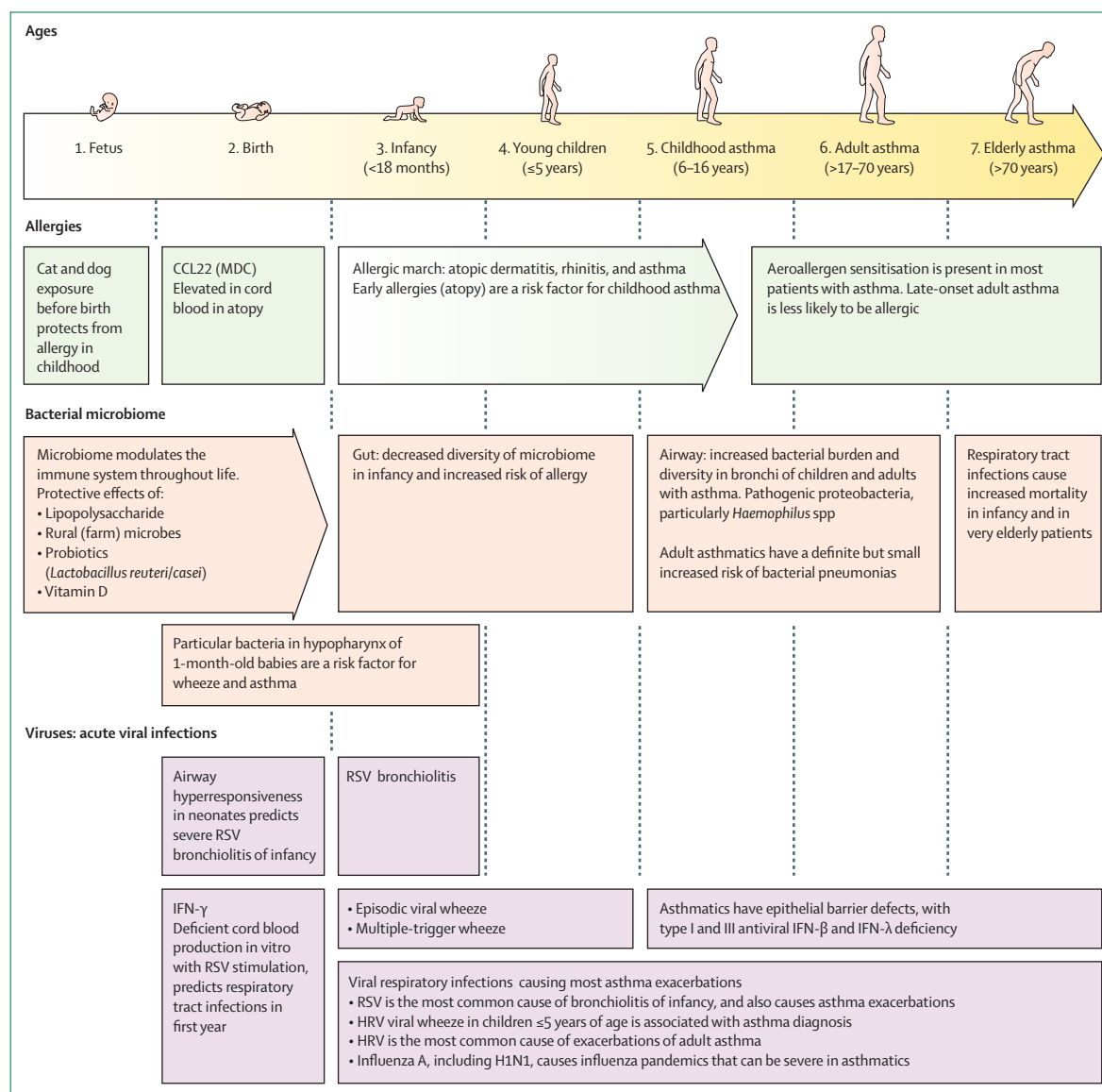


Figure 5: Microbial effects during the Seven Ages of Asthma

Seven ages of asthma have been defined to represent different clinical stages. The materno-placental unit enables the transfer of maternal antibodies and immune cells to the fetus. Thus, at birth, the umbilical cord blood shows features of the atopic and asthmatic immune status of the mother. From birth, rapid colonisation of the skin and mucosal surfaces of the infant occurs. The microbiome has important modulatory effects on the host immune response throughout life, and asthmatics are vulnerable to a range of bacterial pneumonias. Bronchiolitis of infancy caused by RSV is an important cause of childhood wheeze leading to asthma. The allergic march describes the sequence of atopic manifestations that can occur in childhood. HRV infection is the most common cause of adult asthma exacerbations, although influenza and a range of viruses can also act as acute causative agents. CCL22=chemokine (C-C motif) ligand 22. MDC=macrophage-derived chemokine. RSV=respiratory syncytial virus. IFN=interferon. HRV=human rhinovirus.

and viral effects.¹¹² Shortly after birth, the gut is colonised with bacteria, and breastmilk could also have beneficial effects through colonisation with *Lactobacillus reuteri*.¹¹³ By contrast, neonates with colonisation of the hypopharynx involving particular bacteria are at increased risk for recurrent wheeze and asthma early in life,¹¹⁴ and neonates with atopic mothers have a specific nasal mucosal lining fluid cytokine signature.¹¹⁵ Respiratory syncytial virus infection is the most common cause of severe acute bronchiolitis of infancy,¹¹⁶ which is associated with wheeze and asthma in later life.^{40,41} Bronchial hyperresponsiveness has recently been shown to precede acute severe viral bronchiolitis in infants.¹¹⁷

Children up to 5 years of age

A European Respiratory Society Task Force has proposed the term “episodic (viral) wheeze” to describe children who have intermittent wheeze but are well between these episodes. By contrast, “multiple-trigger wheeze” is present chronically in children who wheeze both during and outside discrete episodes, and is more likely to continue into asthma of childhood.¹¹⁸ In children up to 5 years of age, viral respiratory infections in the first 3 years are the most important risk factor for asthma at age 6 years.^{54,55} Allergic sensitisation at an early age is also a major risk factor for childhood asthma. The appearance of atopic manifestations can follow the so-called allergic march—the sequential appearance of atopic dermatitis, food allergy, allergic rhinitis, and asthma during childhood.¹¹⁹

Children 6–16 years of age

Reduced diversity of the intestinal microbiota is associated with allergy in this age group²⁷ and exposure to rural environmental dust microorganisms is protective against childhood asthma.⁷⁶ Children with severe asthma are often atopic,¹²⁰ but remain vulnerable to virally induced exacerbations, and one in four patients continue to have asthma symptoms into adulthood.¹⁶

Adults

The bronchial airway microbiota are associated with the amount of airway hyper-responsiveness in adults with suboptimally controlled asthma,¹²¹ and the microbiota of the bronchial tree is disturbed in asthmatic airways.²⁹ In children and adults, viruses, especially human rhinovirus,¹⁴ are the most important cause of asthma exacerbations.

Elderly patients

Despite the high prevalence of asthma in elderly people, the management of asthma in these patients is often neglected.¹²² Elderly patients frequently have a range of concomitant diseases that need poly-pharmacy, ageing itself causes loss of lung function with changes to the immune and endocrine systems, and elderly patients often have decreased mobility and changed diet. These patients are particularly vulnerable to viral infections, as

shown by influenza vaccination being recommended for this age group.

Studies with monoclonal antibodies

In the past 15 years, a series of informative clinical studies have been done with highly specific monoclonal antibodies targeting individual components of type 2 mucosal immune responses in asthma.¹³ However, the clinical actions of monoclonal antibodies against elements of type 2 immune responses in asthma are mainly with regard to exacerbations, despite the use of biomarkers to guide this treatment,¹²³ which suggests that other mechanisms are also important in the pathophysiology of asthma.

Anti-IgE is the first monoclonal antibody to be licensed for use in asthma, specifically for severe refractory allergic asthma with raised serum IgE concentrations. Anti-IgE has relatively little direct action on lung function parameters, but has been proven to be effective in reducing the number and severity of asthma exacerbations.¹²⁴ Anti-IL-5 causes profound suppression of blood eosinophil counts in asthma and significantly prevents exacerbations in refractory severe asthma with sputum eosinophilia.^{125–127} Efficacy of anti-IL-5 in severe eosinophilic asthma has been confirmed in a larger multicentre study.⁹⁵ Anti-IL-13 improved lung function and symptoms in asthma, but was more effective in selected patients with high serum periostin concentrations.¹²⁸ The key to usefulness of anti-IL-13 is likely to be through targeting therapy to specific phenotypes,¹²⁹ which could be helped by non-invasive sampling methods, such as absorption of nasal mucosal lining fluid.¹³⁰

Challenge models

Several inhaled challenges that have been done on patients have been fundamental to our understanding of the pathophysiology of allergy and asthma. An airway challenge has the particular advantage of giving a defined stimulus to an accessible tissue, to cause a controlled tissue reaction. Inhalation of specific allergens may be performed in patients with mild allergic asthma,¹³¹ which is the classical model to detect effects on the early and late asthmatic reactions.¹³² However, live viral challenges also need to be studied in patients with asthma,¹³³ and studies have been done with human rhinovirus,¹³⁴ respiratory syncytial virus,¹³⁵ and influenza A virus.¹³⁶ Challenge with pathogen-associated molecular patterns that stimulate toll-like receptors and other innate receptors is also possible, and lipopolysaccharide has been used extensively in human challenge models, including those in which it has been given by inhalation.¹³⁷

A major breakthrough in the study of human inflammatory diseases has been the introduction of techniques for analysis of gene expression (transcriptomics, mRNA), metabolites (metabolomics), and proteomics (including advances in immunoassay). Assessment of gene expression with metabolomics and proteomics in diseased tissue

is important to study networks in diseases that involve complex gene–environment interactions.^{138,139} These exciting molecular analytical methods are being applied to human asthma phenotypes in a wild-type state, but an integrated systems approach is needed.¹⁴⁰

To help and accelerate the development of new drugs for asthma, one approach is to do ex-vivo experimental challenge studies with microbes and their components on human cells and tissue, followed by clinical studies incorporating in-vivo challenges, and by sampling and assessment of related biomarkers. In asthmatic patients of defined phenotypes and genotypes, a range of challenges will be needed in conjunction with various mucosal sampling methods before application of network biology. The introduction of highly specific treatments for asthma, along with companion biomarkers, would represent personalised medicine in which highly specific appropriate biologic therapy can be guided to the individual asthmatic patient.^{6,141}

Conclusions

Throughout life, the human immune system interacts dynamically with microbes, allergens, dietary constituents, and metabolites. Asthma can be viewed as an abnormal airway mucosal inflammatory reaction to environmental and microbial stimuli that arises because of innate or acquired variations in the host response. Both good and bad bacteria, viruses, and allergens occur in relation to initiation and exacerbation of asthma for particular patients at key times in their lives. In view of the complexity of the microbial and immunological interactions, the high number of asthmatic immune phenotypes is not surprising. Indeed, clinical studies with biological agents suggest that asthma arises from diverse pathways, and that specific treatments should be targeted to every patient according to their asthma phenotype. Since microbes have a key role in asthma, future advances in understanding might depend on the development of a range of sampling methods, challenges, and biomarkers. Sequential studies on human cells in vitro and clinical studies in vivo might give additional information to that provided by preclinical animal asthma models. However, the main goal is to develop molecular biomarkers to stratify disease pathways and personalise therapy for the individual patient, leading to optimum treatment of those patients with difficult-to-treat asthma.

Contributors

TTH, SLJ, and PJO all contributed to this report. TTH was the principal author, wrote the original draft, and designed the figures. The report was developed following rounds of discussion and review by SLJ and PJO, who contributed equally.

Conflicts of interest

In the past 5 years, TTH has been Principal Investigator for respiratory clinical studies sponsored by Novartis, Merck, GlaxoSmithKline, and Daiichi Sankyo. He has been participated in setting up a spin-off company with Imperial Innovations called Mucosal Diagnostics, and has received support to attend the American Thoracic Society from Boehringer Ingelheim. In the past 5 years, SLJ has received research

grants from AstraZeneca, Centocor, GlaxoSmithKline, MedImmune, Sanofi Pasteur, and Synairgen, and he does consultancy work for these companies. He also holds share options in Synairgen. PJO declares that he has no conflicts of interest.

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