Addressing unmet needs in understanding asthma mechanisms

From the European Asthma Research and Innovation Partnership (EARIP) Work Package (WP)2 collaborators

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ABSTRACT Asthma is a heterogeneous, complex disease with clinical phenotypes that incorporate persistent symptoms and acute exacerbations. It affects many millions of Europeans throughout their education and working lives and puts a heavy cost on European productivity. There is a wide spectrum of disease severity and control. Therapeutic advances have been slow despite greater understanding of basic mechanisms and the lack of satisfactory preventative and disease modifying management for asthma constitutes a significant unmet clinical need. Preventing, treating and ultimately curing asthma requires co-ordinated research and innovation across Europe. The European Asthma Research and Innovation Partnership (EARIP) is an FP7-funded programme which has taken a co-ordinated and integrated approach to analysing the future of asthma research and development. This report aims to identify the mechanistic areas in which investment is required to bring about significant improvements in asthma outcomes.

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Executive summary

This manuscript describes a state-of-the-art view of the mechanisms involved in asthma onset, asthma progression and asthma exacerbations and is an outcome of the European Asthma Research and Innovation Partnership (EARIP: www.earip.eu). Readers are also referred to companion EARIP reports including a report describing the vision and call to action resulting from the EARIP project, to explore pathways for optimising asthma research in Europe [1], a report on national and regional asthma programmes in Europe [2] and an editorial on building the case for more investment in asthma research in Europe [3].

The EARIP project was established in 2013 to harmonise efforts to reduce mortality and morbidity from asthma by agreeing the most important research priorities in Europe. The partnership has produced an evidence- and consensus-based list of the research priorities needed to reduce asthma deaths and hospitalisations [4]. This coordinated agenda for asthma research and innovation will be used to address this major health and societal challenge and will be the foundation on which future European Union, national and international research funding programmes can transform asthma outcomes throughout Europe.

International guidelines describe asthma as a chronic airway disease characterised by airway hyper-reactivity (AHR) caused by a range of processes including airway inflammation, bronchoconstriction of airway smooth muscle (ASM), airway infections, allergic responses, exposure to pollutants, exercise, stress, and nervous provocation [5]. Asthma is one of the most common chronic long-term health conditions in Europe. It is estimated that the number of people aged 45 years and under with asthma in Europe is over 30 million [6]. The prevalence of asthma varies substantially across the European Union. A key feature of asthma is reversible decreases in lung function, measured either as changes in peak expiratory flow, or a decrease in forced expiratory volume in 1 s (FEV_1). Symptoms include dyspnoea, cough, wheeze and chest tightness. Asthma is a heterogeneous, complex disease with multiple clinical phenotypes that incorporate persistent symptoms and acute exacerbations with a wide spectrum of both disease severity and control [7, 8]. The complex interplay between genetic and environmental influences that contribute to asthma pathogenesis makes management a significant ongoing challenge. The lack of preventative, curative and disease modifying management for asthma constitutes a significant unmet clinical need.

Asthma is most frequently associated with onset in early life, and is increasing in incidence in the developed world, but can also start in adulthood (that is, late-onset asthma) [9]. Current pharmacological management approaches are centred on the use of symptom relievers in the form of bronchodilators, which may be short acting or long acting and anti-inflammatory therapies, predominantly glucocorticosteroids [10], their combinations [11, 12], leukotriene receptor antagonists (LTRAs) [13–15] and the non-selective anti-inflammatory and adenosine receptor antagonist, theophylline. Innovative novel monoclonal antibody therapies in phase II/III clinical trials or in recent clinical use include antagonists of Th2 cytokines [16–20] and anti-IgE therapies [21–24]. Sub-lingual and other immunotherapy approaches are also exciting, potentially disease modifying therapies [25], that require further study and further development. These have had varying levels of success at controlling asthma symptoms, improving lung function, and in particular reducing asthma exacerbation rates; however no therapy yet adequately modifies disease, is sufficiently accessible to asthma patients or could be considered a cure. Despite these advances, translating scientific progress to new treatment avenues has been slow for asthma, considering the innovations and development of therapies that have had substantial impact on disease in other fields. Significant disconnects are observed between objective disease severity parameters and patients' experience of their disease [26, 27], and outcomes are worse in those with psychosocial disadvantage [28, 29], mediated by mechanisms that remain unclear. Behavioural problems, such as non-adherence, are common even in patients with severe disease [30] and associated with worse outcomes [31].

As medical research enters the "omics" era, a new challenge facing asthma research is how best to harness big datasets and apply them to models relevant to human disease, in order to benefit asthma treatment. This challenge is further complicated by a recent focus on asthma endotypes or phenotypes and personalised medicine applying novel mechanism-based therapies to those in which such mechanisms are active. Taking all the above into account, it is timely to ask the question "where next in asthma research?" This report reviews current scientific knowledge (state of the art) on mechanisms of asthma onset, asthma progression from mild to severe disease and asthma exacerbation and identifies current unmet needs in asthma research to indicate where future research efforts should be focused.

The important mechanisms involved in asthma onset, asthma progression and asthma exacerbation and the strength of the supportive evidence for each mechanism are summarised in table 1. The important methodologies or technologies that are required to beneficially impact on asthma research in future years are summarised in table 2. Key unmet needs in understanding asthma mechanisms that we have identified are listed in table 3 and mechanisms of asthma onset, asthma progression from mild to severe disease and asthma exacerbation are pictorially represented in figures 1, 2 and 3 respectively. These are the major areas

TABLE 1 Mechanisms involved in asthma inception, progression and exacerbation

Phase	Mechanism and strength of supportive evidence
Onset	Genetics[#,##,¶¶]
	Allergen exposure and sensitisation, Th2 pathways[§§§]
	Viruses[++,§§§,##,¶¶]
	Bacteria[#,¶¶]
	Pollution[+,¶¶]
	Diet[§§,¶¶]
	Hormones[¶¶]
	Stress and psychological factors[¶¶]
Progression	Allergen exposure and sensitisation, Th2 pathways[+++,§§§,###,¶¶¶]
	Bacteria[¶¶]
	Pollution[+,¶¶]
	Airway remodelling[+++,§§§,###,¶]
	Resolution of inflammation[+,§§,###,¶]
	Immune responsiveness *versus* tolerance[+,§§§,###,¶]
	Adverse effects of therapies[+,¶]
	Diet[§§,##,¶¶]
	Hormones[¶¶]
	Stress and psychological factors[¶¶,#]
Exacerbation	Genetics[#,¶]
	Allergen exposure and sensitisation, Th2 pathways[++,§§§,##,¶¶¶]
	Viruses[+++,§§§,###,¶¶¶]
	Bacteria[¶]
	Pollution[+,¶¶]
	Stress and psychological factors[¶]

[#]: *ex vivo* patient sample; [¶]: longitudinal cohort or cross-sectional clinical study; [+]: *in vitro* model; [§]: animal model. Three symbols: very strong evidence; two: validated or repeatable evidence; one: weak evidence.

in which future investment is needed to drive forward asthma research to hasten progress in preventing, controlling and curing asthma, and in preventing and treating asthma attacks and their associated morbidity and mortality.

In lay terms, these scientific goals can be better expressed as three broad aims that should translate into immediately understandable goals to boost:

1) Prevention (related to onset)
2) Cure or control (related to progression)
3) Attack avoidance/treatment (related to exacerbations)

Objectives

To identify unmet needs in understanding mechanisms of asthma, to help discover and develop new therapeutic targets and better define the role of existing targets. To produce a state of the art review article summarising existing knowledge and make recommendations on what is needed to move forward in asthma research.

Methods

The task was initiated during EARIP Work Package (WP)2 meetings at the European Academy of Allergy and Clinical Immunology (EAACI) conference on June 8, 2014; followed by a teleconference on June 18, 2014 and another meeting at the European Respiratory Society congress on September 9, 2014; where plans to convene a writing team were identified. In London, on February 11, 2015 and at the EAACI conference June 8, 2015 the writing team was assembled: M.R. Edwards, S. Saglani, C. Skevaki, J. Schwarze and J. Smith. This was followed up *via* teleconferences during the period June 2015 to December 2015. Additional writing partners were identified and invited to contribute on specific subjects considered important and without the specific expertise in the writing team. A complete advanced draft was produced and edited by S. Johnston in April, 2016. This draft was circulated widely among stakeholders for comments/revisions and discussed at a face to face meeting at the American Thoracic Society congress in May 2016. Detailed comments from this meeting were provided to S. Johnston, who then further edited

TABLE 2 Methodologies or technologies required to beneficially impact on asthma research

Method	Description
NGS eQTL epigenetic, and array platforms	RNA-seq, array techniques that capture all expressed genes in a sample. Can identify new mechanisms and suggest biomarkers. eQTL can relate SNPs to expression of genes in patient samples proving some translation of genetic effects.
GWAS and exome sequencing	GWAS identifies SNPs associated with diseases such as asthma. An association of an SNP does not necessarily inform on a mechanism. Exome sequence refers to sequencing the exons and other specified parts of that gene, such as adjacent promoter sequences.
Metabolomics	Analysis of biomarkers either by mass spectrometry or other protein analysis, *e.g.* bromotyrosine in urine.
Lipidomics	Analysis of pro-inflammatory and pro-resolving lipid networks by liquid chromatography-tandem mass spectrometry in sputum, BAL or plasma.
Volatile organic compounds	Detects organic compounds identified by mass spectrometry in non-invasive samples such as exhaled breath. Can detect viruses and bacteria in theory.
Nasosorption and Bronchosorption	Detects proteins in very small volumes of biological fluid. Has been applied to the nose and lung in detecting rare cytokines.
Mathematical modelling	Can predict the efficacy of a new therapy, or how a new model may behave. This may replace executing the actual experimental work.
Machine learning	Can interrogate big datasets in a free and non-hypothesis-driven manner. Can be useful in the formulation of new hypotheses.
Better pre-clinical models	Needed to study mechanisms of interactions between virus infections, allergen exposure, defective innate immunity and airway microbes in initiation, progression and exacerbation of asthma.
Human challenge models (allergen, virus, pollution)	Can permit early proof of concept and proof of mechanism in development of novel therapeutic approaches and enable identification of novel mechanisms of disease to lead to new therapeutic strategies.
Anti-viral therapies and vaccines	Needed for use in early life in studies on asthma prevention and for prevention and therapy of asthma exacerbations.
Pre-clinical and clinical models of disease	Needed to better understand mechanisms of neuronal dysfunction, airway remodelling, *etc*. in progression and severity of asthma. Pre-clinical models should include development of models in and reagents for guinea pigs, rats, rabbits and dogs as well as mice.
Population-based birth cohort studies	Needed to integrate genetic and epigenetic host factors with studies of virus infections, allergens and other environmental exposures and gut and airway microbes, with studies of host innate and acquired immune reactivity *versus* tolerance with systems biologic analytic methodologies, to understand mechanisms of asthma onset.
Clinic/community based studies of paediatric and adult asthmatics	Needed to investigate disease mechanisms and to validate mechanistic findings from pre-clinical models in the human setting.
Clinical trials	Needed to investigate efficacy and mechanisms of injection and sublingual immunotherapy, and of novel asthma therapies.

NGS: next generation sequencing; eQTL: expression quantitative trait loci; GWAS: genome wide association studies; SNP: single nucleotide polymorphism; BAL: bronchoalveolar lavage.

the manuscript to a near final draft. This near final draft manuscript was circulated among all authors and members of EARIP WP2 working group for final comments and revisions and the final version was prepared by S. Johnston, taking into account all submitted final comments and revisions.

Asthma onset

Introduction

Asthma onset frequently occurs early in life. Lung function is reduced by school age [32] and this reduction is permanent and tracks to adulthood [33, 34]. The key pathological abnormalities of asthma, including eosinophilic inflammation and airway remodelling, also frequently develop early and may be established by school age [35, 36]. Therefore, to achieve disease modification, early intervention is essential. The ideal intervention would allow primary prevention. Disappointingly, even secondary prevention is currently not on the horizon.

In order to achieve primary prevention it is necessary to accurately predict which child will develop asthma. Although one third of preschool children wheeze, only a third will go on to develop asthma [37]. Unfortunately, accurate prediction of asthma during the preschool years remains a significant challenge. Many clinical prediction scores, incorporating known risk factors, such as atopy and family history, have

TABLE 3 Key unmet needs in understanding asthma mechanisms and the nature of the unmet need

Key unmet need	Where needed	Nature of the unmet need[#]
Mechanisms of onset of both allergic and non-allergic asthma	Onset	Birth cohort studies, pre-clinical models, novel targets, new therapies, clinical studies
Role of early life viruses and bacteria as causative agents of asthma	Onset	Pre-clinical models, novel targets, new therapies, clinical studies
Understanding mechanisms underlying the progression of virus-induced early wheezing to later well-defined asthma	Onset Progression	Birth cohort studies, pre-clinical models, novel targets, new therapies, clinical studies
Identifying early life risk factors to enable accurate prediction of which early wheezers will later develop asthma to permit primary prevention	Onset Progression	Birth cohort studies, pre-clinical models, novel targets, new therapies, clinical studies
Mechanism of progression to treatment refractory severe asthma	Progression	Pre-clinical models, targets, therapies, clinical studies
Role of pro-Th2 cytokines IL-25, IL-33 and TSLP and of PGD_2/CRTH2	Onset Progression Exacerbation	Pre-clinical models, targets, therapies, clinical studies
Understanding mechanisms of development of immune reactivity *versus* immune tolerance in asthma	Onset Progression Exacerbation	Pre-clinical models, novel targets, new therapies, clinical studies
Mechanisms of non-allergic asthma	Onset Progression	Pre-clinical models, targets, therapies, clinical studies
Role of air pollution, occupational and other environmental exposures, second hand smoke	Onset Progression Exacerbation	Pre-clinical models, targets, therapies, clinical studies
Protective *versus* pathogenic role of gut and lung microbiomes	Onset Progression Exacerbation	Pre-clinical models, targets, clinical studies
New anti-viral therapies and vaccines	Onset Exacerbation	Pre-clinical models, therapies, clinical studies
Understanding mechanisms of increased susceptibility to viral and bacterial infection in asthma	Progression Exacerbation	Pre-clinical models, novel targets, new therapies
Understanding asthma phenotypes expressing deficient anti-viral immunity, and the mechanisms involved	Progression Exacerbation	Pre-clinical models, novel targets, new therapies
Understanding mechanisms of action of pro-resolving lipid mediators in asthma	Progression Exacerbation	Pre-clinical models, novel targets, new therapies
Understanding neural pathways	Onset Progression Exacerbation	Pre-clinical models, novel targets, new therapies, clinical studies
Understanding role of obesity, diet, hormones and pregnancy	Onset Progression Exacerbation	Pre-clinical models, novel targets, new therapies, clinical studies
Mechanisms of airway remodelling	Progression	Pre-clinical models, novel targets, new therapies, clinical studies
Mechanisms underpinning relationship between psychological factors and asthma	Onset Progression Exacerbation	Pre-clinical models, clinical studies
Mechanisms of adverse effects of asthma therapies	Progression Exacerbation	Pre-clinical models, clinical studies
Translating genetic findings to disease mechanisms	Onset Exacerbation	Pre-clinical models, clinical studies

[#]: pre-clinical models may include *in vitro* studies in human cells as well as *in vivo* studies in animal models; clinical studies may include human challenge (virus, allergen, pollution) models as well as mechanistic and interventional clinical studies.

been proposed [38], but they are either applicable too late; or have a poor predictive value [39]. It is known that host and environmental influences interact to contribute to asthma risk.

In addition to studying disease mechanisms, following children who grow out of asthma and identifying differences between those who do and those who do not at the molecular level may provide insight into mechanisms and potentially point towards a cure of asthma.

A further significant problem is late onset asthma, which is frequently severe, often appears non-atopic (absent skin-prick test positivity or raised serum specific IgE) in pathogenesis and which may have other

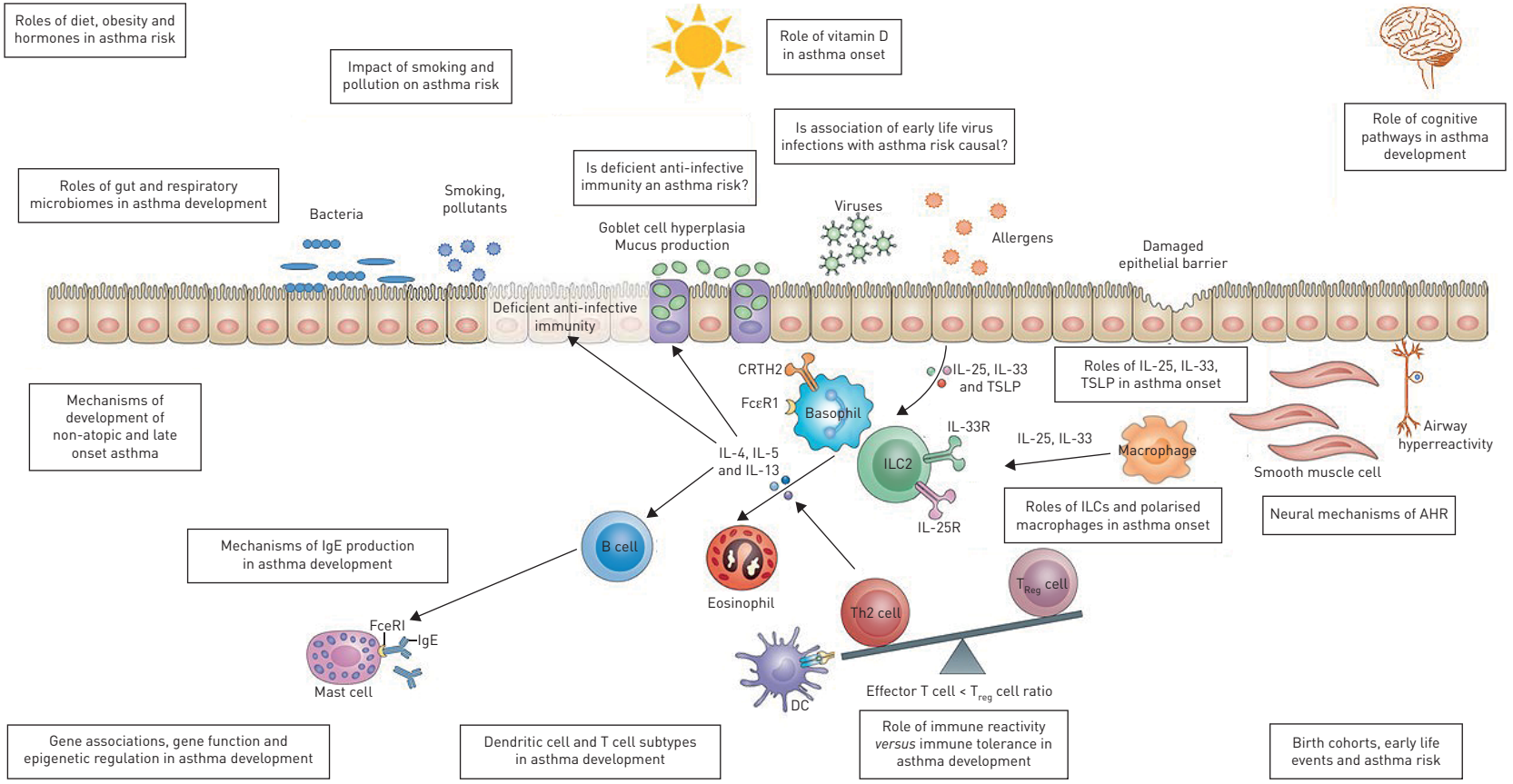

FIGURE 1 Major mechanisms of asthma onset. The major cell types, cytokines and mechanisms involved in asthma onset are depicted, with major mechanisms/research areas requiring substantial further investment highlighted in boxes.

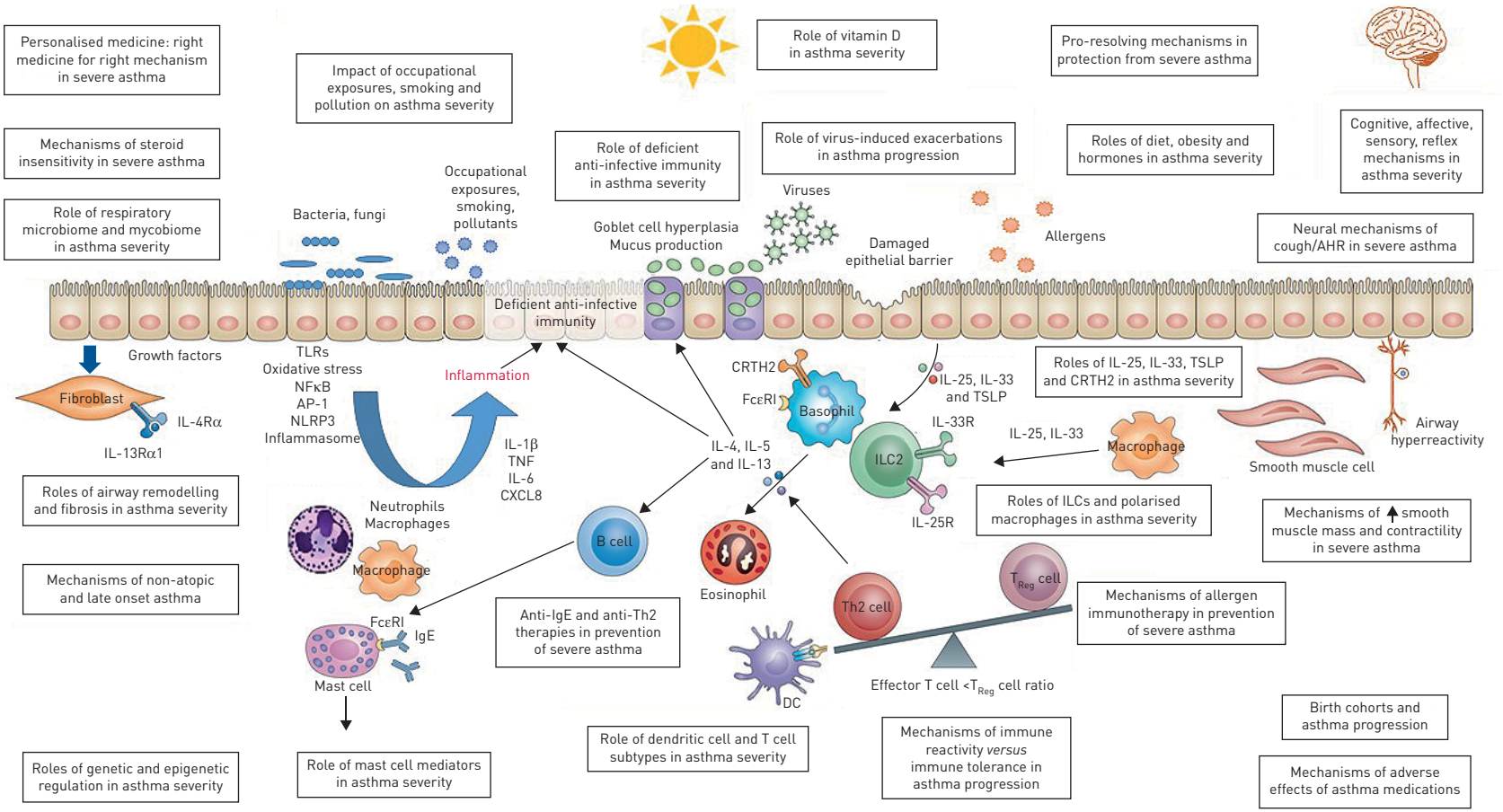

FIGURE 2 Major mechanisms of asthma progression and severity. The major cell types, cytokines and mechanisms involved in asthma progression are depicted, with major mechanisms/research areas requiring substantial further investment highlighted in boxes.

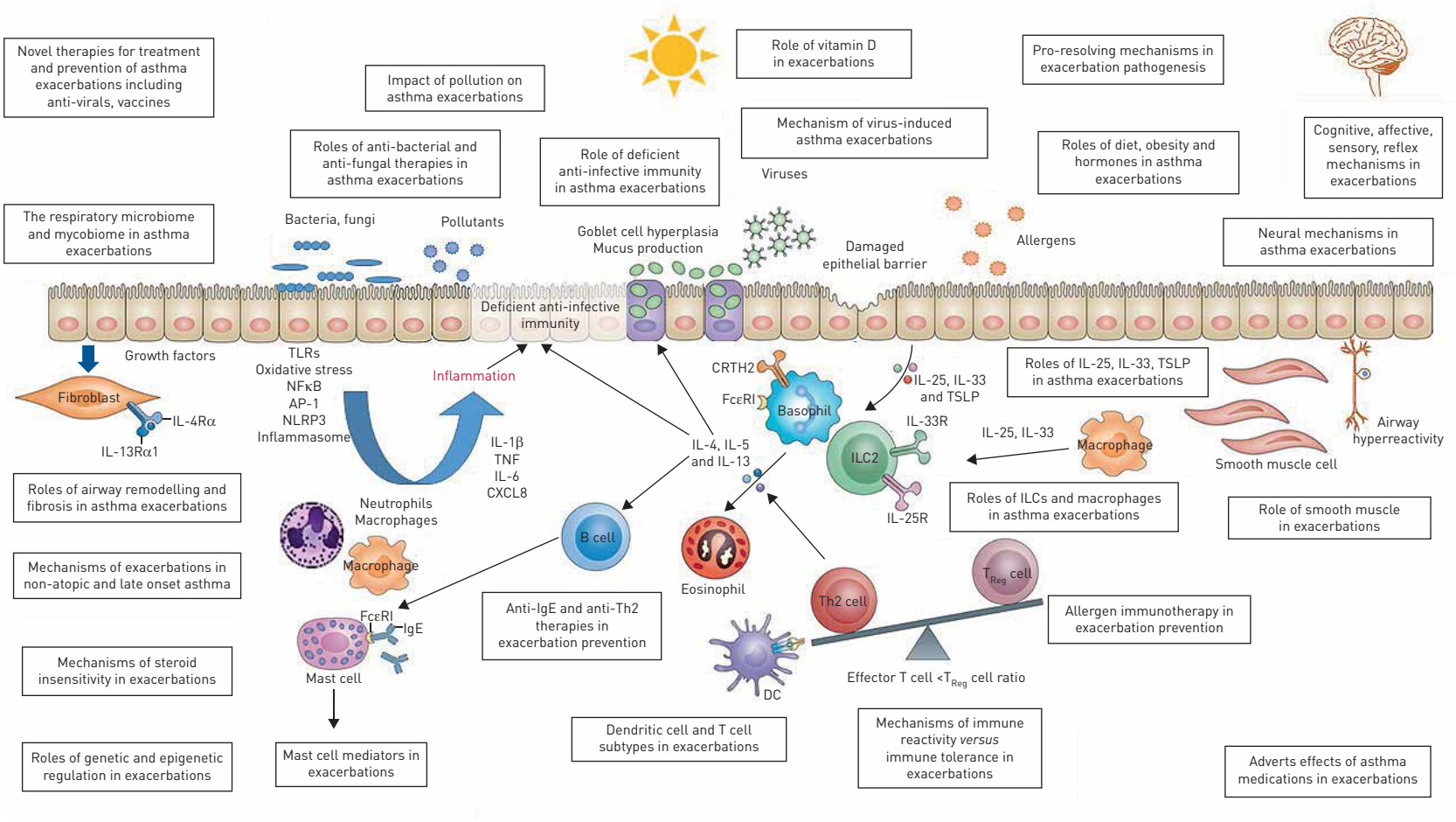

FIGURE 3 Major mechanisms of asthma exacerbations. The major cell types, cytokines and mechanisms involved in asthma exacerbations are depicted, with major mechanisms/research areas requiring substantial further investment highlighted in boxes.

risk factors, can be quite a difficult differential diagnosis (sometimes overlapping with chronic obstructive pulmonary disease (COPD)) and may represent a different disease spectrum from early onset asthma. Also asthma can arise in adulthood as a result of occupational and irritant exposures frequently resulting in non-Th2 asthma.

Asthma is more likely to be diagnosed in people with anxiety and depression [40, 41]. Research strategies therefore need to incorporate multiple risk factors and include complex interactions in order to reflect the disease and identify effective interventions [42]. See figure 1 for a summary of important mechanisms involved in asthma onset.

Asthma onset: state of the art

Viruses

Viral infections represent critical events that likely contribute to asthma onset. Early life respiratory syncytial virus (RSV), human rhinovirus and human metapneumovirus lower respiratory infections have all been strongly associated with progression to clinically defined asthma by age 6 [43–47]. However, little is known about the mechanisms underlying the progression of virus-induced early wheezing to later life well-defined asthma. Indeed, there is considerable debate over whether these virus infections are important causative agents of later asthma development, and/or are markers of risk for asthma development independent of causation. It is possible that different viruses fulfil different roles that are not obvious in the available cohort studies. Interactions between viral infections and underlying genetic susceptibility identified from Genome Wide Association Studies (GWAS) have been reported [45]. Although this has shed light on gene–environment interactions, and identified several candidate genes with reproducible and strong associations, they remain associations and mechanistic advances are needed. For example polymorphisms spanning *CDHR3* are associated with childhood asthma with severe exacerbations [48]. *CDHR3* encodes cadherin-related family member 3 (CDHR3), which is involved in epithelial polarity and cell-cell interactions as well as being a receptor for rhinovirus C, the most common respiratory virus associated with asthma exacerbations and the key variant identified in GWAS modulates levels of CDHR3 providing a putative mechanism [49]. Some mechanistic evidence has come from available small animal models. Infection of newborn mice with RSV, and the mouse equivalent pneumovirus, show striking long term changes to the airway and induction of pro-Th2 responses to allergens later in life [50, 51]. The interactions between virus infection with allergen exposure, and also the beneficial effects of environmental and airway microbes are important avenues to pursue for future research. (see table 2 for an explanation of new methodologies in asthma research).

Allergen exposure and Th2 pathways

T helper (Th)2 immunity mediated by interleukin (IL)s -4, -5, -9 and -13 after sensitisation and exposure to allergens drives allergic asthma, one of the major subtypes of asthma, inducing IgE production, eosinophilia and tissue remodelling [52]. Targeted therapies in subtypes of established Th2-mediated asthma have been successful in reducing exacerbations [53, 54]. Interventions to prevent initial sensitisation may provide a long-lasting alternative to Th2-targeted therapies. As yet, initiating processes are incompletely understood, but likely involve dendritic cells, basophils, eosinophils, innate lymphoid cells (ILCs) and macrophages and airway epithelial cells secreting the newly identified pro-Th2 cytokines IL-33, TSLP and IL-25, which thus may be potential alternative candidates for intervention.

ILCs are of particular interest as they have been recently described as tissue-resident and circulating innate immune cells [55], with three major subsets: ILC1s, ILC2s and ILC3s, which mirror Th1, Th2 and Th17 cells, respectively [56]. There is little detailed information on the role of ILCs in asthma onset and progression, though roles for both IL-33 and IL-25, which act as growth factors and activators of ILC2s, have recently been proposed in acute exacerbations [57, 58]. A particular shortcoming in our present understanding is information from experimental studies in humans.

Mouse models of asthma have provided evidence for ILC2s in development of allergic asthma [59–61] and ILC3s in a model of obesity-associated AHR [62]. Whether these data can be translated to asthma depends on whether these models truly reflect human disease. In addition, asthma is a heterogeneous disease with respect to onset (early *versus* late), response to treatments and concomitant pathology (allergic *versus* non-allergic, obesity), which likely reflects differences in underlying pathobiology. So, the contribution of ILCs may vary between the various subtypes of asthma. There is evidence that IL-33, IL-25 and TSLP, and their receptors, are involved in response to allergens and respiratory viruses [57, 58]. This work provides at least preliminary evidence for a role of ILCs in asthma, as well as other innate and adaptive immune cells like eosinophils, basophils [63], mast cells and T cells, which may also be responsive to IL-33, IL-25 and TSLP.

Air pollution

The role of air pollution in asthma development is unclear [64]. Three European birth cohort studies have reported positive relationships between traffic-related pollution and doctor-diagnosed asthma [65–67]. The Southern Californian Children's Health Study has reported that traffic-related pollutants can cause asthma in children [68, 69], as has a Dutch study in which traffic-related pollution levels at the birth address and incidence of asthma were considered during the first 8 years of life [70]. Exposure to nitrogen dioxide (NO_2) and particulate matter ($PM_{2.5}$) at birth correlated with asthma development in childhood and adolescence [71] and a 5 ppb increase in NO_2 levels during the first year of life with physician-diagnosed asthma (OR 1.17) [72]. In women, a 3.6 $\mu g \cdot m^{-3}$ increase in $PM_{2.5}$ correlated with greater odds (OR 1.20) of incident asthma, whereas a 3.6 $\mu g \cdot m^{-3}$ increase in $PM_{2.5}$ (OR 1.14) and 5.8 ppb increase in NO_2 (OR 1.08) correlated with incident wheeze [73]. Not all studies, however, show such a relationship [74], and such inconsistencies may be due to incomplete exposure assessment or insufficient study power.

A recent report from the Royal College of Physicians London summarised the state of the art and also states that exposure of young children to second-hand tobacco smoke remains one of the most important sources of indoor air pollution and is associated with asthma prevalence, although the effects of exposure to maternal smoking in pregnancy may be stronger than for childhood exposure [75]. Occupational exposures as well as second-hand smoking may also be related to late onset adult asthma.

Host characteristics that have been reported to influence the effects of air pollution on asthma development include nutritional status, atopy, and social stress [76–82]. Polymorphisms in glutathione S-transferase (GSTP1) that facilitates the elimination of reactive oxygen species have also been associated with a greater risk of asthma [83] and sensitisation to allergen in association with traffic-related NO_x during the first year of life [84]. As the environment is a potentially modifiable factor in asthma development [64], funding is urgently needed to study the effects of the environment on asthma development, to reveal new approaches to reduce future development of asthma.

Bacteria and the microbiome

During and immediately following birth neonates are exposed to microbes that progressively colonise the gut, the skin and airways. Indeed, within a very short time frame *post partum*, one moves from being 100% human, to a human super organism that lives with vast numbers of microbes [85, 86]. Although much research to date has focused on the gut, host-microbe interactions in the lung may also be powerful determinants of the presence or absence of asthma [87].

The human microbiome describes the totality of microorganisms (bacteria, viruses, fungi and parasites) associated with the human body, in both health and disease. Molecular techniques that include 16S rRNA gene sequencing, microbial whole genome sequencing and metagenomics are revolutionising our understanding of the diversity and function of the human microbiome [88, 89]. The lower airways are now known to carry a characteristic community of microorganisms [90–92] with a similar density of bacteria to the upper intestines [91].

Presence of certain bacteria may be protective or harmful. A rich microbial environment in early life is associated with protection against development of asthma and allergies [93–95] and the protection appears to be mediated *via* signals transmitted *via* innate immune signalling [95]. Molecular studies have since shown that particular genera, such as Bacteroidetes and Actinobacteria appear to be protective [91, 96]. In support of the above, experimental animal models have shown that the early life formation of the lung microbiome provides signals to the immune system that shape its maturation and influences susceptibility to asthma [97–99]. Several studies have reported that past exposure to tuberculosis or BCG vaccination may be associated with protection from development of asthma and allergies [100, 101], though others do not agree [102] and causal relationships in man are difficult to establish [103]. Preclinical studies with BCG and bacterial products derived from *Mycobacterium tuberculosis* have shown protection from eosinophil recruitment and AHR in mouse models of allergic airway inflammation [104, 105].

It is clear that the microbiome influences the trajectory towards or away from asthma [106], so how could this be harnessed to reduce the onset of asthma? Spending the first year of life in a farming environment leads to a reduced risk of asthma and allergies [107]. This protection is associated with increased microbial diversity [94], and could be related to bacterial components that are inhaled, as exposure of the lung to farm dust and endotoxin protect against house dust mite-induced asthma through A20 induction in lung epithelial cells [108] or to the constituents of raw cow's milk [109]. This latter observation may be particularly relevant from the standpoint of future interventions. In addition to a high microbial load, raw cow's milk contains a plethora of "prebiotics", that is to say "energy sources" for bacteria, which can support bacterial growth and function in the gut. Early life nutrition, and perhaps maternal nutrition, are key areas where more research is needed in order to identify strategies through which early life microbial

exposure and colonisation can be shaped. Answering which microbial signals, when and how we can provide them, holds the potential for reducing the onset of asthma.

Molecular investigations also reveal that the bronchial microbiota in asthma is rich in pathogens [91, 110–114] (typically Proteobacteria such as *Moraxella*, *Neisseria* and *Haemophilus* spp.). Birth cohort studies have shown that the presence of the same pathogens in throat swabs predicts the later development of asthma [115], and that these bacteria have been associated with asthma exacerbations [116]. A recent study applying 16S rRNA sequencing of the nasopharynx microbiome of 234 children in their first year of life showed that transient infections of *Streptococcus*, *Moraxella*, or *Haemophilus* were associated with virus-associated acute respiratory infections. Importantly, asymptomatic infection with *Streptococccus* was a predictor for asthma [117]. The role these bacteria play is still unclear, and additional studies show that they may be independent risk factors for acute wheezing episodes in young children aged <3 years, as well as viruses [118]. The interactions between viruses and bacteria to promote wheeze and asthma onset are largely unexplored.

Finally, the role of antibiotics remains widely debated. While maternal use of antibiotics has a dose-related association with the risk of asthma in offspring [119]; there is evidence for efficacy of antibiotics in the treatment of asthma and related syndromes [120–123]. The idea that the microbiome represents both harmful and protective genera, that help shape the early immune system; and that bacteria have a dual role in initiating and protecting against disease, may partly explain this controversy.

Diet and hormones

The prevalence of obesity has more than doubled since 1980, such that it is now the commonest nutritional disorder worldwide [124]. The concomitant rise in the prevalence of asthma triggered speculation that obesity may be causally related to asthma incidence. Obesity is a major risk factor for asthma development, with the strength of association greater in females than in males [125]. However, understanding of the pathophysiological mechanisms underlying this relationship remains incomplete.

Cluster analyses have identified two phenotypes of obesity-related asthma (ORA) distinguished primarily by age of onset [126]. The early onset phenotype is characterised by classic, Th2-driven inflammation, and is complicated by the subsequent development of obesity [126]. Conversely, the late onset phenotype is typically non-atopic, exhibits lower markers of Th2 inflammation, occurs more frequently in women and develops consequent to obesity [127]. Little is understood about how obesity may affect asthma onset. The increased AHR observed in late onset ORA is likely multifactorial with contributions from mechanical, inflammatory, metabolic and dietary factors in addition to co-morbidities such as gastro-oesophageal reflux disease and sleep-disordered breathing. Studies to enhance comprehension of the mechanisms underpinning ORA onset should be prioritised to facilitate the development of phenotype-specific therapies.

Evidence suggests that obesity and asthma share common developmental origins [128]. Common genetic, epigenetic, maternal and pre-natal factors may predispose to both conditions by influencing *in utero* foetal programming. Subsequent early life events and exposures in the post-natal period may subsequently modify this risk. Further research efforts are warranted to help in the prevention of the development of both disorders.

We are cognisant of the fact that we have not adequately addressed the roles of a number of other dietary factors including vitamins, oxidants/anti-oxidants, fatty acids, *etc.*, in asthma onset. For example epidemiological evidence highlights strong associations with vitamin D status [129, 130], assessed as circulating levels of 25-hydroxyvitamin D, with an increased incidence, severity and poor control of asthma [131], and these data appear strongest in children [129, 130]. A recent Cochrane systematic review concluded that vitamin D is likely to reduce the risk of severe asthma exacerbation and healthcare use [131]. Two large trials of vitamin D supplementation in pregnancy show strong trends for improvement of respiratory outcomes in the infant [132, 133], through likely effects on lung maturation, appropriate immune system development and improved handling of respiratory infections. Vitamin D and oxidants are additionally addressed in part in the following section, but further research into these factors and their roles in asthma prevention are also needed, including further clinical studies of vitamin D throughout the life course.

Further studies on the roles of fatty acids are also clearly needed, as a recent study on the effects of n-3 long-chain polyunsaturated fatty acids (fish oil) *versus* placebo (olive oil) in the third trimester of pregnancy reduced the absolute risk of persistent wheeze or asthma at three to 5 years of age in the offspring by 31%, with a greater reduction of 54% seen in offspring of mothers whose blood levels of eicosapentaenoic and docosahexaenoic acid were in the lowest third of the trial population at randomisation [134].

Immune reactivity versus *immune tolerance*

Immune tolerance is an active process involving specialised regulatory T-lymphocyte (Treg) subsets [135, 136]. The airways are continuously exposed to harmful and non-harmful environmental antigens

and in health these elicit immune responsiveness to achieve the effective handling of pathogens, but importantly also immune regulation to prevent potentially damaging immune responses to non-harmful antigens [135–137].

Allergic asthma is provoked by inhalation of otherwise innocuous aero-allergens such as house dust mite, mouse, cockroach, cat and dog proteins [138] accompanied by a lack of immune tolerance, leading to inappropriate immune responses to these harmless allergens. A major $CD4^+$ Treg population expresses the transcription factor Foxp3; young boys with a rare, X-linked, genetic mutation causing loss of $Foxp3^+$ Tregs, suffer from multisystem autoimmunity, and severe atopy including eczema, food allergy, and eosinophilic inflammation, highlighting the importance of $Foxp3^+$ Tregs in preventing aberrant immune responses to allergens in early life [139]. A second major Treg population synthesises the anti-inflammatory cytokine IL-10. Blood cells from individuals with severe allergy/asthma show reduced IL-10 synthesis in response to allergen stimulation [136, 140].

Inappropriate immune responses/breaches of immune tolerance can result from failure in generation of Tregs, or inhibition of their actions, and both can be influenced by the environment. For example, vitamin D promotes both $IL\text{-}10^+$ and $Foxp3^+$ Tregs [141], whilst air pollution is associated with impaired $Foxp3^+$ Treg function [142]. Many other environmental factors, such as smoking and oxidative stress, can stimulate production of mediators that inhibit the action of Tregs [143]. The manner in which antigen is presented by dendritic cells also profoundly affects the immune response, including whether effector or regulatory lymphocyte responses are generated, and dendritic cells are similarly affected by environmental factors [144, 145]. The inappropriate immune response to innocuous allergens in asthma causes further immune dysregulation as the Th2-biased allergic response inhibits antiviral responses [146].

Understanding critical mechanisms of development of naturally occurring immune tolerance offers the potential for early life interventions to dramatically reduce the incidence of allergies [147]. The same potential therefore exists for asthma. Funding is needed to study the effects of the environment on immune regulation, and to reveal new approaches to reduce future development of asthma.

Psycho-social and behavioural factors

Although frequently unrecognised, psychological dysfunction (particularly anxiety) is up to six times as common in people with asthma as in matched controls [28, 148] including in children, with evidence of a bi-directional relationship, in that although asthma may induce anxiety, anxiety may also precede asthma. Longitudinal cohort studies in adults have reported that the presence of anxiety and panic significantly increased the odds of subsequent asthma [40], and a birth cohort study reported that behavioural problems in the child at the age of 3 years was a significant risk factor for both a subsequent asthma diagnosis and for late-onset wheezing [148]. The mechanisms underlying this relationship are not understood, although it is known that stress can have significant but complex effects on immunological function [149]. Psychological stress can affect the release of cortisol and the expression of inflammatory mediators in a complex and time-dependent way, with increased airway inflammation associated with stress [150]. There is some evidence that psychological stress may predispose to the development and severity of atopic conditions, including asthma, through effects on the immune system [151]. Although it is clear that stress can result in measurable neuroimmunological effects that can be associated with asthma expression and morbidity, the precise significance of these biological effects and the ways in which they interact with other stress-related factors, such as health beliefs and behaviours, is not fully understood [151].

Addressing the unmet need

The study of early life asthma onset presents some unique challenges that separate it from stable asthma progression and asthma exacerbation. A great unmet need in understanding mechanisms of asthma onset is the lack of available complex mouse models that accurately reflect human asthma development in early life. Allied to this are the difficulties in obtaining valuable early life human samples from human birth cohort studies to validate findings in the models.

The discovery of age appropriate, efficacious interventions in preventing asthma onset is completely dependent on the development of age appropriate models that will allow discovery of critical mechanisms. Not only are some susceptibility genes different for childhood and adult onset asthma [152, 153], but the effects of immune maturation and lung development must also be considered [98]. Effective prevention strategies that are clinically translatable will only become apparent if data is obtained from correct animal models and validated in early life/childhood studies of asthma onset. This is highlighted by the stark differences in allergic airways disease that result depending on age at first allergen exposure in mice [98]. The significantly different responses that result from allergen exposure at different ages can also be explained by influences of the developing airway microbiome and the maturing immune system. Recently marked

differences between human adult and neonatal immune systems have been highlighted by the demonstration of a unique group of CXCL8/IL-8 producing T cells in preterm babies that were particularly at risk of infections [154]. Immune mechanisms that predict neonatal infection have also been investigated using a systems biology approach and investigating immune-metabolic networks in babies [155]. Blood volumes as small as 0.5–1 mL were sufficient to identify a 52 gene cluster; predictive of bacterial infection. However, an accurate prediction was only achieved when immune and metabolic pathways were integrated, emphasising the need to use complex models, and combine information accordingly.

Any distinction between the mechanisms of early- and adult-onset asthma may reflect not so much differences in the host response but in the timing of first exposure to an aetiological allergen. For example, certain types of occupational asthma, arising through specific IgE-associated responses to protein allergens encountered only in the workplace, appear clinically and mechanistically to be indistinguishable from atopic asthma acquired early in life. Moreover, there is good evidence that the incidence of occupational asthma can be related directly to the levels of allergen exposure in the workplace, and that the disease can be effectively controlled by reducing such exposures [156].

There is a similar dearth of complex mouse models that accurately reflect human asthma development in later life. Also allied to this are the difficulties in obtaining valuable human samples from human cohort studies in adulthood, or studies of incident late onset asthma, to validate findings in the models.

Another need is that of lower airway samples from accurately phenotyped patients (representing all the different asthma phenotypes/subtypes) and controls, from which meaningful information can be obtained from relatively small sample sizes [35, 157, 158]. We also need to determine the degree to which information from more easily accessible peripheral blood or nasal airway samples, reflects lower airway changes. Mechanistic studies that allow manipulation of contributory factors and truly reflect human disease are needed, but currently are not being undertaken. In children, this is likely because of the difficulties inherent in obtaining appropriate tissue samples that allow functional analyses to be performed. One example of circumventing these limitations is the identification of asthma susceptibility genes through large scale, multi-centre studies that have incorporated patients from different cohorts, with data analysis being performed by a single centre with the required expertise [159]. This approach of collaboration and getting access to the targeted patients and specific sampling from multiple centres and ensuring the correct expertise in processing and analysis also needs to be adopted to allow mechanistic advances from more invasive or difficult samples. However, this approach, to some extent, does not differentiate the different phenotypes or endotypes and may lead to some genes being missed. Combined GWAS plus expression quantitative trait loci analysis for different phenotypes or endotypes may provide solutions.

The use of novel techniques that allow detailed information to be obtained from small and limited samples, while challenging, is not impossible. These include single cell transcriptome profiling [160] and multi-component immunohistochemistry. Models using human cells also need to be innovative and optimally reflect *in vivo* conditions. Utilisation of co-cultures of epithelial cells or their supernatants with leukocytes [57, 161] will improve functional information obtained. The limitations of animal models are recognised [162] but if efforts are made to optimally reflect human conditions by allowing for critical factors such as age, route of allergen exposure, appropriate species and strain, findings can be translated [163].

With respiratory viral infections likely being important in asthma onset, an important unmet need in asthma onset is how best to target these viruses. Palivizumab is an anti-RSV antibody effective in reducing RSV induced bronchiolitis and it may reduce subsequent wheezing [164–166]. Recently, two new oral RSV antivirals performed well in double-blind, placebo-controlled studies of experimental RSV infection in healthy subjects, showing impressive reductions in symptom score and virus load [167, 168]. Development of new small molecule inhibitors or other anti-virals that can be used in early life or childhood is an area that must receive further support.

The role of non-pharmacological approaches to asthma prevention, including environmental manipulations and psycho-social approaches, remains uncertain.

Progression of stable asthma

Introduction

Mechanisms that promote disease activity and progression in established, but stable asthma overlap with, but may also be distinct from, those involved in asthma inception, and may thus require different scientific insights and treatments. This next section deals with stable asthma and asthma progression including the dynamics of the microbiome, and whether modifications may serve as future useful therapeutic targets, the process of airway remodelling and its impact on lung function over time, the role of airway nerves, of adverse effects of asthma treatments and of disease modifying therapies such as anti-Th2 cytokine biologics, the identification and mechanisms of other non-Th2 asthma endo-phenotypes, and immunoregulatory

processes such as resolution of airway inflammation and the development of immunological tolerance. See figure 2 for a summary of important mechanisms involved in asthma progression and severity.

Progression of stable asthma: state of the art

Bacteria, fungi and the micro/mycobiome

Detection of pathogenic *Proteobacteria*, particularly *Haemophilus* spp., are more frequent in bronchi of asthmatics than controls [91]. Only 3/11 adult asthmatic subjects had severe asthma, though all were on inhaled corticosteroids (ICS). Similar data have been reported in other studies and the bacterial burden correlated with AHR [110], longer asthma disease duration, lower FEV_1 and higher sputum neutrophils [112]. In another study in sputum in severe and non-severe asthma *Bacteroidetes* and *Fusobacteria* were reduced in non-severe and severe asthmatic groups compared to healthy controls, *Proteobacteria* were more common in non-severe asthmatics compared to controls (OR 2.26) and *Firmicutes* were increased in severe asthmatics compared to controls (OR 2.15). Among *Firmicutes,* streptococcal operational taxonomic units (OTUs) were associated with recent onset asthma, rhinosinusitis and sputum eosinophilia [169].

Potentially pathogenic bacteria have also been identified by culture in sputum of 15% of subjects with stable asthma and this was associated with greater airway inflammation [170], while in stable severe asthma, sputum was positive for bacteria in 52% of patients [171]. IgE positivity to *Staphylococcus aureus* enterotoxin was significantly greater in patients with severe asthma (59.6%) than in healthy controls (13%) and enterotoxin IgE-positive subjects had increased risk of severe asthma (OR 11.09, 95% CI 4.1–29.6), greater oral steroid use and hospitalisations and lower FEV_1 [172]. Further case-control studies identified patients with severe asthma with non-tuberculous mycobacteria infections, and infected subjects were older, had lower FEV_1, and had used ICS for longer than controls [173, 174]. Additionally roles for the atypical bacteria *Mycoplasma* and *Chlamydophila pneumoniae* in asthma pathogenesis have long been postulated, as reviewed in [175, 176], however data to date are inconclusive and further work is needed to better understand the possible contribution of these organisms to asthma pathogenesis.

There is clear evidence of increased susceptibility to bacterial infections in asthma. Increased risk (approximately three-fold) of invasive pneumococcal disease in non-severe asthma has been confirmed in two separate studies [177, 178], while that risk increases to 12-fold in severe asthma [177]. Studies of mechanisms of increased susceptibility to bacterial infection in asthma report that toll-like receptor (TLR) 5 and TLR7 expression is decreased in the lung in severe asthma, thus severe asthmatics may suffer from insufficient TLR signalling during bacterial infections leading to impaired defence mechanisms [179–181]. Effective antibacterial immunity requires type II IFN-γ and Th1 immune responses and mouse model data report that host defence against bacteria is also mediated by type I interferons [182, 183]. There is extensive data that type I, II & III interferon induction by viral and bacterial stimuli is deficient in asthma and that deficiency relates to underlying asthma severity [157, 180, 184–187]. IL-12 and IL-18 induction by lipopolysaccharide in macrophages are also deficient in asthma [186, 188, 189] and these are important for induction of IFN-γ and Th1 immune responses. Reduced phagocytosis of bacteria is reported in patients with severe asthma, thus persistence of bacteria in the lower airways in asthma may result from this defect [190]. The mechanisms for increased susceptibility to bacterial infections in asthma are only partially understood, and further research in this field is clearly needed. The role ICS play in this increased susceptibility also needs further investigation.

The microbiota in established asthma is different to that in health [91], and recent preclinical evidence argues that the airway microbiota found in diseased lungs can influence chronicity and progression of airway inflammation [191], while treatment with certain microbes or their products can reduce asthma severity [108]. Microbial manipulation has been spectacularly successful in treating bowel inflammation [192, 193], and if asthma is driven by airway dysbiosis then manipulation of the airway microbiota may also ameliorate disease progression.

A pragmatic and potentially highly effective means of controlling asthma progression could be *via* the diet. A diet high in fermentable fibre fed to adult mice was effective at reducing the severity of allergic airway inflammation *via* fermentation of dietary fibre by gut microbiota, which directed the immune system away from allergic responses [194]. The logical next steps are intervention studies in humans, where individuals would eat a diet high in fermentable fibres, or to receive supplements of certain downstream metabolites generated following microbial fermentation of fibre (*e.g.* short chain fatty acids). Alternative approaches whereby individuals are exposed to bacteria (probiotics) and/or their substrates (prebiotics) with the goal of directing an individual's immune system away from asthma, hold promise.

Fungal sensitisation and long term fungal infection/colonisation are associated with increased asthma severity and complications such as bronchiectasis and chronic pulmonary aspergillosis. Estimates suggest that many millions of people have severe asthma with fungal sensitisations and allergic bronchopulmonary

aspergillosis and a substantial proportion of adult asthmatics attending secondary care have fungal sensitisation. Little is known about which fungi and fungal allergens are relevant to asthma pathogenesis, and there is little data on the most effective management strategies. Further studies are needed on fungal exposure, sensitisation and infection/colonisation and the role of host defence against fungi in asthma, as well as studies on the role of the mycobiome and of effective interventions [195].

Virus infections

Virus infections precipitate the great majority of asthma exacerbations and exacerbation frequency in asthma is strongly correlated with rates of decline in post-bronchodilator lung function (FEV_1) [196–198] and loss of bronchodilator reversibility [197]. It therefore seems likely that virus induced exacerbations likely lead to structural alterations in the lung, and permanently worsened airflow and poor health status, and although the mechanisms are unclear, the effects are likely long lasting and may be irreversible [199]. It is not known whether impaired host defence against virus (and bacterial) infections is associated with progression from mild to severe asthma.

Air pollution

There is substantial evidence linking traffic-related air pollution, exposure to second hand smoke and occupational exposures to progression of both allergic and non-allergic asthma to severe disease and perhaps to COPD. Diesel exhaust heightened lower airway eosinophilic inflammation in allergic subjects [200] and timing and duration of traffic-related air pollution exposure were found to be effect modifiers as early-life traffic related exposure correlated with persistent wheezing (OR 2.31) in children [201]. The first 8 years of life represent a susceptible period, as exposure to traffic-derived particulate matter in that period has been associated with impaired lung function growth [202]. Furthermore, in a study of asthmatic patients walking on a London polluted street for 2 h, the reduction in FEV_1 and the degree of neutrophilic lung inflammation observed after the walk was associated most consistently with exposures to ultrafine particles and elemental carbon, and the reduction in FEV_1 was greater in the moderate compared to the mild asthmatics [203]. Fine particulate matter was associated with increased respiratory resistance in children [204] and lifetime exposure to PM_{10} and NO_2 was associated with retarded lung volume growth in elementary school age children [205]. However, since no specific pollutant or combination conferred more detrimental effects, it is important to use multi-pollutant models to analyse these associations [206]. Many developing countries still employ open fires for cooking, which correlated with greater risks (OR 2.12) of asthma symptoms in children [207]. The detrimental effects of pollution are readily demonstrated in certain subgroups. Young males are 3-times more likely to have asthma symptoms associated with exposure to truck traffic-related air pollution than those without [208], whereas being overweight (OR 4.36) or obese (OR 3.06) increases susceptibility to exercise-related asthma symptoms associated with exposure to $PM_{2.5}$ [209].

Mechanistic experimental exposure studies point to interactions between diesel exposure and allergen, in increasing airway inflammation [200] and suggested a possible mechanism contributing to epithelial wall damage following allergen exposure [210].

Epidemiological evidence supports a role for air pollutants in contributing to the spread of respiratory viral infections [211]. This may be relevant to the mechanisms by which increased ambient ozone, nitrogen dioxide, $PM_{2.5}$ and sulphur dioxide levels are associated with increased admission for asthma exacerbations particularly in children [212].

Airway nerves and neural pathways

The role of airway innervation in asthma and the potential contribution of neuronal dysfunction to asthma pathophysiology are rarely studied. Human airways are richly innervated by parasympathetic efferent nerve fibres, as well as various sensory (afferent) nerve fibres [213], whereas contribution from the sympathetic nervous system is scant [214, 215]. Afferent airway nerve activation mediates the noxious sensations patients associate with asthma, *e.g.* chest tightness, air hunger, airway irritation, congestion and breathlessness [216] but also initiates reflexes producing cough, bronchospasm, AHR and mucus hypersecretion. Neuronal dysfunction therefore has the potential to play a major role in the pathogenesis of asthma symptoms.

Dysfunction of afferent and efferent nerves plays a major role in the pathogenesis of airway obstruction and AHR in pre-clinical asthma models [217–221] and in a major asthma symptom, cough [222]. Cough in asthma is *the* archetypal airway reflex and is not only a common [223] and troublesome symptom [224], but is also associated with disease severity [225] and poor prognosis [226]. Of the symptoms experienced by asthma patients cough is also the most readily objectively quantified and thus can provide insights into neuronal dysfunction. Recent work suggests that different airway diseases including asthma exhibit differing cough responses to a range of inhaled tussive agents, suggesting distinct neuro-phenotypes can be identified [222]. Moreover, compared with healthy controls, different phenotypes of mild/moderate

stable asthma exhibit heightened cough responses to the inhaled irritant capsaicin, which directly activates vagal afferent fibres. These responses were most exaggerated in females with non-atopic asthma, suggesting neuronal dysfunction may be particularly relevant in non-Th2 disease [227]. Cough responses were unrelated to treatment with inhaled corticosteroids, exhaled nitric oxide, airflow obstruction or AHR, also supporting the hypothesis that neuronal hyper-responsiveness is a key feature contributing to treatment-resistant and non-allergic phenotypes.

Defensive respiratory reflexes, such as cough and bronchospasm, are regulated by vagal afferent nerves [228–231]. Ion channels on the termini of airway sensory fibres located under the airway epithelium make them capable of directly responding to a diverse range of agents, many of which correspond to triggers of asthma symptoms identified by patients, including changes in temperature, humidity, pollution and irritant chemicals such as cigarette smoke, cleaning products, perfumes, *etc.* [228, 232], as well perhaps to changes in airway calibre during bronchospasm [233], Neuronal dysfunction in animal models of asthma can also be induced by eosinophils [234], viruses [235] and mediators such as neurotrophins [236, 237] and can mediate induction of AHR by β_2-agonists [238].

Gastroesophageal reflux disease and a history of rhinitis or sinusitis are clearly identified risk factors for development of severe asthma [239], however the mechanisms involved are poorly understood, and the possible involvement of neural pathways requires further study.

The importance of nerves in asthma is highlighted by the effectiveness of muscarinic antagonists in the treatment of asthma [240, 241], blocking reflex bronchospasm mediated by acetylcholine release from parasympathetic efferent airway fibres. Animal models of asthma have demonstrated that in addition to anti-muscarinics, the late response to inhaled allergen can be prevented by blockade of afferent sensory nerves [219], implicating airway nerve function in allergic as well as irritant induced asthma symptoms. Although translation in clinical allergen challenge studies is still required. Of note AHR, airway obstruction and cough often persist despite treatment with highly effective anti-inflammatory drugs, perhaps as a consequence of persisting neuronal dysfunction [220]. Therefore, there is a need for further research on the role of nerves in the pathogenesis of asthma and on new therapeutic approaches targeting neural dysfunction in this disease.

A substantial proportion of new or relapsing asthma in adulthood can be attributed to exposures encountered in the workplace [242]. The paradigm of occupational asthma induced by classic allergic responses to workplace proteins such as flour or detergent enzymes is evidence that age itself is no protection against the development of IgE-associated respiratory disease. A substantial proportion of occupational asthma, however, arises in response to exposure to chemical agents that are of too low a molecular mass to act alone as antigens. In some cases these agents appear to conjugate with human proteins to form a hapten-protein allergenic complex; in many others this appears not to be the case and other mechanisms, such as neuronal dysfunction acquired through repeated exposure to respiratory irritants require exploration. The public health implications may be substantial since irritant exposures encountered at work – by, for example cleaners who consistently report high rates of asthma-like symptoms to cleaning products [243], or swimming pool attendants who regularly encounter chlorine [244] – can have very significant "down-stream" effects in consumers.

Research on the role of nerves in asthma has been hampered by the different innervation of murine and human airways [245], by limited access to neuronal tissues (nerve fibres or ganglia) from patients with asthma, and by methodological challenges in analysis of these tissues. Therefore, more funding is needed for neuronal studies of human tissues, such as analysis of nerves in human whole-mount biopsies [215], to provide urgently needed data on the precise nature of airway innervation in asthma [162]. In addition, experimental non-murine, *e.g.* guinea pig, asthma models are needed to provide more relevant functional information on airway nerves in asthma [246–248]. Models and reagents in other species such as rats, rabbits and dogs would also aid both safety and efficacy studies. Finally, clinical studies of emerging therapies specifically targeting neuronal dysfunction in asthma [249, 250] are needed to identify new therapeutic opportunities [222].

Airway remodelling

Airway remodelling represents one of the most challenging problems in asthma and is linked to disease progression. Airway remodelling includes epithelial cell shedding, goblet cell hypertrophy, basement membrane (BM) thickening and ASM cell hyperplasia, leading to progressive decline in lung function [251, 252]. Another prominent feature of lower airway remodelling is increased vascularisation that correlates with airflow limitation and AHR [251, 252]. Growing evidence suggests that inherent changes in lung-resident cells are the principal drivers of fibrotic processes. Support for this concept comes from studies demonstrating that airway remodelling is observed in preschool children without signs of inflammation, and is comparable to that documented in adults [251, 252].

Other mechanisms involved in promoting airway remodelling processes include notch2 [253], IL-13 [254], the gamma-aminobutyric acid system [255], transcription factors regulating goblet cell differentiation [256] and many others. Further studies investigating mechanisms involved in promoting airway remodelling processes are required to lead to better therapies to prevent its development. Once developed, novel therapeutic approaches such as bronchial thermoplasty can ameliorate severity [257], however mechanisms involved are poorly understood, and better understanding of these mechanisms could lead to less invasive approaches to ameliorate severity.

Mainstay anti-inflammatory therapies, such as ICS, do not significantly affect airway remodelling processes, emphasising the notion that uncontrolled structural changes may be a cause as well as a consequence of progressing asthmatic disease and future funding should be channeled to studies aiming to dissect the mechanisms involved [258, 259]. Large-scale proteomic and genomic profiling of lung-resident cells may also guide the identification of novel mechanisms for airway remodelling. In addition, mathematical modelling approaches aimed at elucidating the effects of ASM contraction and the causes of altered airway mechanics in asthma may greatly facilitate the understanding of airway remodelling pathogenesis [258, 259].

Immune cells infiltrating the airways of asthmatics are potent sources of pro-fibrotic factors, including transforming growth factor (TGF)-β, and therefore a role for immune cells in promoting structural changes is likely. In fact, eosinophils are a rich source of TGF-β and eosinophil depleted mice are protected from peri-bronchial collagen deposition and increased ASM mass in response to chronic allergen exposure [258]. Recent studies have also uncovered key roles for other TGF-β superfamily members, including activin-A, and bone morphogenic proteins and the epithelial cell-derived cytokines, IL-33, TSLP and IL-25, in airway remodelling in asthma [260–262]. Hence, the development of biologics targeting the effects of these cytokines on the control of airway remodelling represents a fruitful avenue for future research that may be translated to better treatment options for asthmatics [261, 262].

A major hindrance to our understanding of the mechanisms underlying airway remodelling is the lack of animal models that can effectively recapitulate structural changes [251, 252]. It is, thus, essential that future funding should be targeted on the development of better animal models and novel *in vitro* assays that incorporate multiple cell types and mimic the intrinsic mechanical forces occurring in the airways. Finally, studies investigating the effects of environmental changes, such as diet and microbiota composition, on airway remodelling are warranted and future funding should be channeled in this area.

Immune reactivity versus *immune tolerance*

Because development of tolerance to allergens depends on the context (including dose, timing and route of exposure as well as presence of co-exposures such as lipopolysaccharide) of exposure to environmental antigens, this is amenable to therapeutic manipulation. The best example of this is allergen immunotherapy, which involves administering allergen to patients to induce long-term, allergen-specific, immune tolerance. This can be achieved by injection immunotherapy, which has been shown to induce benefits lasting long after treatment has stopped [263], but which requires repeated attendance at allergy clinics for 3 years. This can also be achieved by sublingual immunotherapy [25], which is newer and therefore has unknown duration of long-term benefit, but which has the advantage of being administered by the patient at home.

Allergen immunotherapy increases Treg frequency and particularly of those Tregs that synthesise IL-10 [264]. Efforts are ongoing to standardise immunotherapy protocols, improve safety and efficacy, reduce costs and duration of treatment, and to better understand immune mechanisms involved to further improve its utility in treating asthma [264]. Further current challenges in immunotherapy include understanding how to maintain immune tolerance and memory regulatory cell populations over extended periods of time.

If factors that underlie loss of such immune tolerance in asthma can be identified by research, then future therapies and environmental policies can be formulated to tackle the underlying immune dysregulation in asthma. Research is needed to study mechanisms underlying loss of immune tolerance and dysregulation in different asthma endotypes with the aim of designing better targeted treatments that restore normal immune responses, reducing asthmatic inflammation without the side-effects of suppressing healthy immune responses as seen with high dose corticosteroids. As the environment is a potentially modifiable factor in immune dysregulation, funding is also needed now to urgently study the effects of the environment on immune regulation, to reveal new approaches to treat asthma.

Resolution of airway inflammation

Chronic inflammation underlies the pathogenesis of asthma, the intensity of symptoms and progression of disease. Yet, efforts to tackle chronic inflammation in asthma have mostly explored the activation of anti-inflammatory or immunoregulatory responses on the assumption that the dampening of pro-inflammatory responses would suffice for inflammation to fade away.

Recently, it has become apparent that resolution of inflammation is by itself an active and highly orchestrated process, of similar complexity to the onset and progression of inflammation, responsible for the catabolism of the inflammatory response, the egress of immune cells and the restoration of tissue homeostasis [265]. Among the multiple pathways involved, the production of specialised pro-resolving lipid mediators (SPMs) such as resolvins, protectins and maresins seems to be particularly important [266]. These are generated through complex biosynthetic pathways from ω-3 and ω-6 polyunsaturated fatty acids and act in a stereospecific manner through G protein-coupled receptors to reverse vasodilation and suppress leukocyte infiltration, de-activate inflammatory cells, promote apoptotic cell and tissue debris clearance and repair damaged tissue [266].

In asthma, emerging evidence suggests that SPMs play a prominent role in the resolution of airway inflammation [267]. SPM networks are altered in exhaled breath condensate, bronchoalveolar lavage, sputum or blood of patients with asthma [268–272], while administration of synthetic SPMs such as lipoxin A4, protectin D1, resolvin D1 (RvD1) or resolvin E1 (RvE1) promotes resolution of airway inflammation in experimental mouse models [270, 271, 273–277]. The pro-resolving actions of SPMs in this context may additionally involve activation of macrophage efferocytic function and suppression of Th2 cytokines, IgE and ILC2 function, although their spectrum of activities remains largely uncharacterised [267]. This raises the possibility of a key role of SPMs in the termination of airway inflammation with significant implications for the therapeutic potential of these molecules in halting asthma progression. This largely unexplored area is therefore in need of urgent funding.

Adverse effects of asthma therapies

β_2-agonists are almost universally used therapies in asthma as single agents, and as combination therapy with ICS. Inhaled short- (SABA) and long-acting (LABA) β_2-agonists mediate their protective effects by inducing cyclic adenosine monophosphate (cAMP) in smooth muscle cells (SMCs) leading to smooth muscle relaxation and thereby, bronchodilatation. Although β_2-agonists have undoubted beneficial effects, safety concerns have been repeatedly raised regarding the use of SABAs and LABAs in asthma.

Regular use of SABAs four times daily in stable asthma results in worse asthma control than use only when needed to relieve wheezing [278, 279] and overuse of SABAs (without ICS) in asthma exacerbations has been repeatedly associated with increased risk of hospitalisation or mortality [280, 281]. LABA use without ICS has also been linked to increased asthma mortality [282–284] and the US Food and Drug Administration has cautioned medical providers about risks associated with LABA use without ICS [285]. The fact that LABA use, when combined in the same inhaler with an ICS, is safe was confirmed in two recent large studies in both adults [286] and children [287]. Concerns about use of β_2-agonists in the absence of ICS persist as excessive use of SABAs in the absence of ICS was recently identified in 40% of deaths and use of LABAs without ICS in five deaths [281]. The mechanisms behind these safety concerns and the mechanisms through which ICS therapy is protective are poorly understood.

Fenoterol is a SABA linked with an epidemic of asthma mortality in the 1980s [288] and reductions in hospitalisations due to asthma exacerbations following withdrawal of fenoterol suggested this was due to a beneficial effect of withdrawal on asthma severity [289].

β_2-agonists relax smooth muscle by raising levels of cAMP in SMCs. However, the most numerous cells in the airway and the cells most accessible to inhaled β_2-agonists are not SMCs, but bronchial epithelial cells (BECs) and airway macrophages. It therefore seems likely that respiratory adverse effects of β_2-agonists might be mediated *via* effects on these cells.

The pro-inflammatory cytokine IL-6 is induced in BECs by β_2-agonists alone, and importantly in relation to overuse of β_2-agonists in asthma exacerbations, IL-6 induction by rhinovirus infection was further augmented by β_2-agonists. Promoter studies revealed that LABA augmentation of rhinovirus-induced IL-6 occurred *via* a cAMP response element (CRE) in the IL-6 promoter [290], indicating that an adverse effect of β_2-agonists is mediated *via* cAMP elevation in BECs, just as are their beneficial effects in SMCs.

Two independent clinical trials have confirmed induction by β_2-agonists of the asthma-related mediators brain-derived neurotrophic factor (BDNF) [291] and matrix metalloprotease (MMP)-9 [292] in humans and both mediators are induced *via* cAMP/CREs [293, 294]. Many other pro-inflammatory mediators with potential adverse effects in asthma have CREs in their promoters and are therefore likely to be induced by β_2-agonists including IL-17, COX-2, amphiregulin, MMP-2, MUC5AC, MUC5B and MUC8 [295]. However, these have not to date been studied with β_2-agonists. A genome-wide study suggests the number of human genes potentially inducible by β_2-agonists *via* CREs in their promoters might extend into the hundreds [296]. Thus β_2-agonists have the potential to induce many genes implicated in asthma pathogenesis.

Induction of and augmentation of rhinovirus-induced IL-6 by LABA were both abolished by ICS [290]. ICS also blocked LABA induction of BDNF both *in vitro* and *in vivo* [291]. IL-17, MMP-2, MUC5AC and MUC8 are all suppressed by steroids [295]. These data suggest that use of β_2-agonists/ICS in combination inhalers would result in the ICS component blocking direct genomic adverse effects of β_2-agonists while maintaining the beneficial bronchodilator effects [295]. This interpretation supported by the demonstration that taking both together, combined in a single inhaler, is clearly safe [286, 287]. Mechanistic studies investigating adverse effects of β_2-agonists and protective effects of ICS in BECs and airway macrophages *in vitro*, and *in vivo* in people with asthma, to identify genes induced by β_2-agonists and suppressed by ICS, are urgently needed.

Use of LTRA therapy has been associated with an increased incidence of Churg–Strauss syndrome (CSS) [297]. How LTRA therapy may interact with the pathogenesis of CSS is unknown. Potential mechanisms for an association between LTRAs and the CSS have been postulated including potential for allergic/hypersensitivity drug reactions and leukotriene imbalance resulting from leukotriene receptor blockade. Further studies monitoring incidence of CSS in asthma patients receiving LTRAs are needed, including studies on the possible role of steroid/ICS withdrawal. Mechanistic studies investigating how LTRA therapy may interact with the pathogenesis of CSS are also needed.

Inhaled corticosteroids are widely used in COPD and their use is associated with an increased risk of pneumonia, but the mechanisms of this effect remain unclear [298]. There is clear evidence of increased susceptibility to bacterial infections in asthma [177, 178], but this increased risk has not been directly linked to ICS use. The mechanisms for increased susceptibility to bacterial infections in asthma and the role ICS plays in this increased susceptibility are poorly understood. ICS also suppress antiviral immunity in the absence of asthma [299], and are associated with impaired innate interferon responses in asthma [300], but ICS also reduce exacerbation frequency [301], the majority of which are induced by viruses. Therefore, ICS presumably have mixed effects in asthma, and the mechanisms involved clearly need better understanding in order to guide future drug development.

Human genetics research will be critical to the development of genetic profiles for personalised medicine in asthma. Genetic profiles that predict individual disease susceptibility and risk for progression, may predict which pharmacologic therapies will result in a maximal therapeutic benefit, and may also predict whether a therapy will result in an adverse response in a given individual. Pharmacogenetic studies of the glucocorticoid, leukotriene, and β_2-adrenergic receptor pathways have identified genetic loci associated with therapeutic responsiveness [302]. Future studies, are needed to identify genetic profiles permitting personalised approaches to maximise therapeutic benefit for an individual, while minimising the risk for adverse events.

Diet and hormones

Obesity has been associated with both over and under diagnosis of asthma [303], and with worse asthma control, impaired response to ICS therapy, increased exacerbation frequency, increased healthcare utilisation and diminished asthma-specific quality of life relative to normal-weight asthma [304]. Patients with ORA are more likely to have obesity-related comorbidities such as obstructive sleep apnoea and gastroesophageal disease that can influence responsiveness to asthma therapies [305]. Obesity, although reportedly not associated with airflow obstruction [306], adversely affects pulmonary mechanics by reducing lung tidal volume, functional residual capacity and AHR [307], however, the mechanisms involved require clarification. Additional studies addressing the effect of obesity on airway remodelling may also help identify novel therapeutic targets.

Obesity is associated with chronic, low-grade, systemic inflammation and adipose tissue produces numerous pleiotropic adipokines in addition to serving as an energy storage depot [308]. The two most widely studied adipokines are leptin (pro-inflammatory) and adiponectin (anti-inflammatory) and both have been implicated in the pathogenesis of ORA [308]. However, our understanding of the roles of adipokines within obese airways warrants further research. Adipokines are promising therapeutic targets as they are differentially expressed in obesity and several have been shown to exhibit immunomodulatory effects [308].

Although obesity is a state generated by positive calorie imbalance, dietary constituents, such as ω-3 and ω-6 polyunsaturated fatty acids and saturated fats, may also affect ORA pathogenesis [309]. Furthermore, insulin resistance and oxidative stress may also play an important role [310]. In addition to enhancing AHR obesity is thought to potentiate airway inflammation and reactivity to environmental stimuli such as ozone and particulate matter [127], with associated negative consequences on asthma control. Results from recent mouse studies have emerged to suggest potential mechanisms [311]; however, further work is required.

The most rational strategy to improve the health status of individuals with ORA is weight reduction and several studies have demonstrated improvements in pulmonary function, asthma control, health status, AHR and systemic and airway inflammation following weight loss interventions [312, 313]. However,

whilst weight optimisation confers many health benefits and is recommended in the treatment of ORA, randomised controlled trial (RCT) data evaluating the efficacy of weight loss interventions (both surgical and non-surgical) in ORA are limited [314].

Evidence addressing the optimal pharmacological strategy for the treatment of ORA is lacking. Practical ORA management is complicated by the vicious cycle generated by obesity-induced poor asthma control and corticosteroid-associated weight gain. Greater understanding of the mechanisms underpinning obesity-associated corticosteroid resistance (in addition to promotion of weight loss strategies) is key to breaking this cycle and improving clinical outcomes. Thus further work in this area should be prioritised.

Asthma is more common in males from birth until puberty [315, 316] but becomes more prevalent [317, 318] and more severe [319, 320] in women after puberty. Women are more likely to develop difficult-to-treat or steroid refractory asthma [316] and women >25 years of age account for >62% of hospitalisations and 64% of asthma deaths [316, 321]. In addition to puberty, menstruation [322], pregnancy [323, 324], menopause [325, 326] and oral contraceptive use [327] have been associated with asthma outcomes in women [316]. Peri-menstrual cyclic changes in lung function have been reported in women [328] and asthma symptoms and peak expiratory flow can deteriorate during high levels of oestrogens [329]. These data suggest a role for sex hormones, most likely a pathogenic role for oestrogen, and most immune cells involved in asthma express the oestrogen receptors (ER) ERα, ERβ. The hypothesis that sex hormones play a role in the pathogenesis of asthma is further supported by the higher prevalence of asthma in women with early menarche [330] which is associated with higher oestrogen concentrations [331]. Oestrogen levels have also been associated with the incidence of asthma [332] and women with a history of asthma who use oral contraceptives have reduced risk of current wheeze [333]. But there are also developmental differences: female fetal lung development is more rapid than that of males [334] and male lungs are smaller with fewer respiratory bronchioles at birth [335] while at puberty boys have ∼25% higher lung volumes than girls of identical height [336] and lung function development ends earlier in girls than in boys [337]. Understanding gender differences in asthma which might in part be regulated by sex hormone levels appears important to optimise individualised treatment. Furthermore, a better understanding of gender differences in asthma pathogenesis and progression might lead to new treatment modalities.

Among the chronic diseases of adolescence, asthma has the highest prevalence and healthcare usage [338]. During puberty children experience a shift in cognitive abilities from more concrete to more abstract thinking [339, 340] and children with asthma feel more lonely and depressed compared to healthy peers [341]. Absenteeism or poor school performance can disrupt peer relationships and endanger development of independence [340]. While self-care generally increases in children it may decrease again in adolescence [340, 342]. Girls are more likely to incorporate asthma into their social and personal identities compared to boys who try to avoid this [343]. Age and low emotional quality of life correlate with body mass index and level of asthma symptoms [340]. Thus, in addition to hormonal changes discussed above which influence asthma severity, developmental changes in cognition and behaviour during puberty can severely affect asthma outcomes. Most management interventions do not account for the challenges faced by psychosocial and physiological needs during puberty [340] and age specific programmes are needed [340]. Such programmes could have vast influences on the future course of individual patients' asthma and could be instrumental in improving long term outcomes.

Around 10% of women of childbearing age suffer from asthma [324]. Asthma prolongs time to pregnancy and has negative effects on fertility that increase with age and asthma severity [344]. Pregnancy is associated with a large increase in circulating oestrogen levels that drop to baseline levels after delivery. The course of asthma during pregnancy is variable, with approximately one third reporting unchanged asthma control, one third an improvement and one third worsening asthma [324]. Analysis of pulmonary function *versus* symptoms suggests that some reported deteriorations in symptoms might not be reflected in changes in airway function [324, 345]. A small increase in the risk of congenital malformations in the offspring of patients with asthma has been reported with a higher incidence in more severe asthma [324]. Preterm labour, low birth weight, small for gestational age and preeclampsia have been associated with pregnancies in women with asthma [324]. Understanding mechanisms that lead to deteriorations of asthma control during pregnancy and programmes to monitor asthma control during pregnancy have a potential to avoid adverse outcomes during pregnancy.

Psycho-social and behavioural factors

Despite advances in pharmacotherapy that are theoretically capable of achieving high levels of asthma control for most patients, asthma outcomes have remained suboptimal and many patients remain symptomatic despite intensive pharmacological therapy. Psychological conditions such as anxiety and depression are common in people with asthma and associated with poor control [28]. Consistent evidence from cross-sectional surveys using a variety of methodologies suggests that symptomatic asthma control

and asthma-related health status are impaired when anxiety or depression are also present [29, 346–348]. This relationship is independent of confounding factors such as age, gender, socio-economic status, objective asthma severity and prescribed treatment level, with one study [348] estimating that the presence of psychiatric co-morbidity accounted for 29% of the variance in Asthma Control Questionnaire score. Anxiety and depression are associated with poor asthma outcomes across a range of different outcome measures. These include impaired asthma-related quality of life [349, 350], higher asthma-related health resource utilisation [351], increased asthma-related health costs [351] and increased use of rescue medication [352]. Patient reported outcomes correlate poorly with objective physiological measures of asthma control, while measures of psychological state are strongly predictive of most outcomes. The mechanisms underlying these associations are not well understood, and there is a paucity of interventional studies to show whether the recognition and treatment of co-morbid psychological dysfunction is effective in improving asthma control, with variable evidence supporting other behavioural interventions.

Breathing control exercises now have ever-increasing evidence to support their use as an adjuvant treatment for those uncontrolled on standard pharmacotherapy and are advocated in guidelines [353, 354], although the mechanism of action is incompletely understood. Behavioural interventions to support adherence [355] and internet-based behaviour change and self-management support interventions for asthma show promise [356] but require further research to clarify which patients may benefit and in the optimal format of delivery. An accurate multidisciplinary assessment of adherence and psychological state are considered key parts of the multidimensional phenotyping of asthma required in a difficult asthma clinic before embarking on expensive treatment with new biological therapies. Other non-pharmacological interventions (including relaxation, mindfulness, biofeedback and cognitive-behavioural based) have some supportive evidence but an inadequate evidence-base and mechanisms of effectiveness are generally poorly understood [357]. In view of how commonly the co-morbidity between psychological problems and asthma occurs, it is perhaps surprising that the evidence base for treatment is so meagre.

To effectively complement pharmacological asthma management, there is a need to understand the neurocognitive, affective and behavioural mechanisms that impact asthma outcomes, particularly those relevant to anxiety and depression. Breathlessness is the fundamental symptom of asthma, with brain processing pathways possessing a strong affective component that can cause anxiety and enhance symptom perception [358] as well as negatively impacting cognition and behavioural coping mechanisms [359]. Neurocognitive studies have reported activation in the ventrolateral periaqueductal gray (vlPAG) associated with respiratory threat [360] and prefrontal activity may reflect stress-related inflammation [361]. Investigation of neural pathways in asthma shows differential responses to a cognitive task in subgroups with different levels of inflammatory response to an allergen challenge, suggesting the possibility of neurophenotypes for asthma, with the potential of targeted interventions [362]. Differential neural reactivity is related to disease severity in brain areas related to emotion-processing, indicating that neurophenotypes may exist within asthma populations. Such neurocognitive evidence supports cognitive-behavioural models [363] suggesting that some people with asthma may have dysfunctional cognitions (*e.g.* symptom perception) that interact with physiological mechanisms leading to the increased asthma onset risk noted in longitudinal studies. Cognitive-affective models have emphasised the importance of symptom perception in reduced asthma-specific quality of life [363]. Anxiety is the strongest predictor of the unpleasantness of breathlessness during bronchoconstriction in people with asthma [364], and anxiety has a stronger relationship with asthma-related health status than lung function [365].

Understanding of the mechanisms underpinning these relationships is needed to efficiently design and target appropriate interventions.

Addressing the unmet need

New therapies and emerging biologicals now target Th2 asthma phenotypes, with success, showing that disease modifying agents have efficacy, even though asthma is clearly established. Limited information exists regarding other asthma endo-phenotypes particularly non-Th2 asthma and treatment-resistant phenotypes. Therefore, novel approaches incorporating pre-clinical and clinical models are required in order to develop new therapeutic approaches for these neglected varieties of asthma.

Many of the agents known to precipitate asthma symptoms are also capable of activating airway nerves leading to the hypothesis that neuronal hyperresponsiveness is a key feature of some asthma endotypes, contributing to treatment-resistant phenotypes, particularly in non-Th2 asthma. The role of airway innervation has received very little attention to date and has the potential to identify novel therapeutic targets. Major investment in consortia aimed at understanding and treating severe asthma has focused on inflammatory profiling in the various sub-phenotypes identified with little investment in the characterisation of neuronal function and associated targets.

Anti-IgE and IL-5-targeted therapies are currently finding their place in the treatment of asthma. A word of caution may be required as long-term effects are unknown as yet. And further, both eosinophils (depleted by anti-IL-5 and anti-IL-5 receptor) and basophils (depleted by anti-IL-5 receptor) may exercise protective properties and thus their depletion may cause unwanted effects. A major challenge for these interventions will be to decide optimal timing of their use, particularly as sensitisation and the start of asthma progression frequently occurs early in life. While much effort is put in obtaining antibodies that are highly effective, it should also be considered that dampening responses might be effective and at the same time may preserve useful functions of the targeted mediator. Thus, while anti-Th2 cytokine therapy will continue to impact on stable asthma and perhaps on asthma progression, there is still a lot of basic information missing on the best way to harness these therapies.

The successes with Th2-targeted therapy indicate that this approach is viable in the treatment of asthma in which these pathways are active. Intervention with the inhibition of Th2 responses is still in its infancy and development would be greatly helped by relevant animal models. Efforts like U-BIOPRED and SARP, to obtain detailed molecular insights into various endotypes and phenotypes of severe asthma, should be pursued in parallel with the development of animal models to substantiate the functional relevance of specific molecular pathways for specific endotypes.

Airway epithelial cells and macrophages are an important source of IL-25, IL-33 and TSLP, which act on Th2 cells, ILC2s and dendritic cells to enhance Th2 responses and we need to clarify whether epithelial cells and macrophages from individuals with asthma produce greater amounts of these Th2-promoting cytokines compared to those without disease. We also need to develop inhibitors of IL-25, IL-33 and TSLP [366] to permit comparisons of their relative abilities to inhibit Th2 cell, ILC2 and dendritic cell-mediated enhancement of Th2 responses. These studies will need to be carried out in pre-clinical models to permit greater understanding of their biology, as well as in RCTs with detailed phenotyping, to permit better understanding of which sub-phenotypes of asthma might respond to each therapy. Blocking these pathways holds great promise, as this should block Th2 responses more effectively than targeting individual Th2 cytokines, and they should also be more broadly applicable across different asthma phenotypes. Recent studies have also revealed that ILCs show plasticity. ILC1s can trans-differentiate into ILC3s, and vice versa [367] and ILC2s into ILC1s and *vice versa* [368]. Therefore trans-differentiation is an option for restoring the balance between ILC subtypes and with that adjust effector responses.

There is a need for more intensive investment in mechanisms of resolution of inflammation. Key areas that will need to be investigated include the more global lipidomic analysis of ω-3 and ω-6 polyunsaturated fatty acid-derived lipid mediators, the better understanding of their regulation and biological functions at the "system level", the effect of age, diet, environmental influences and infections in their generation, and their deregulation during disease. The identification of asthma subtypes that would most likely benefit from SPMs, and the development of appropriate therapeutic agents with desirable pharmaceutical properties will ultimately determine whether such rational new treatments aiming at pushing inflammation "forward" towards its resolution can bring about significant improvements in asthma outcomes.

Studies analysing the effects of the disease itself, as well as treatments such as ICS, on host immunity against viral and bacterial infections in asthma are urgently needed. These should include *ex vivo* studies in human primary cells as well as *in vivo* studies in models of asthma, investigating the effects of disease/ICS on host defence against virus and bacterial infections in BECs, macrophages and dendritic cells. Human experimental rhinovirus infection should be used to determine whether virus infection alters the microbiome and impairs host immunity to bacteria in patients with asthma. This model would then offer a model to study the effects of disease/ICS on the interactions between virus infection, host immunity, and the microbiome in small numbers of patients over a short period. By comparison of the effects of virus infection between asthmatic/healthy patients and patients using ICS and ICS-naive patients, experimental rhinovirus infection has the potential to identify mechanisms of increased susceptibility to viral/bacterial infections related to disease itself and to ICS use. Studies are also needed to investigate relationships between viral and bacterial infections and progression from mild to severe asthma, as well as studies on host immunity against these infections and asthma progression.

In relation to air pollution, the observation that exposure to traffic-derived PM during the first 8 years of life can impair lung function growth [202] must make us focus on the mechanisms by which traffic related air pollution, as well as perhaps work related exposures and second hand smoking may increase the severity of asthma. Epidemiological studies need to be pursued to find out whether there are any threshold safe levels of exposure or not in this respect. In addition, we need to find out what determines susceptibility to adverse consequences of exposure, the cellular and molecular mechanisms involved, and to devise and test preventive measures and treatments (for those particularly susceptible), as the problem

of pollution will take time to be controlled at source. We need GWAS and epigenetic studies in exposomics, and extension of these studies into asthma in particular, to better understand how asthma underlies increased susceptibility to stressors like cigarette smoking, occupational exposures and traffic related (*e.g.* diesel exposure) and other sources of pollution.

We need to refine the understanding of mechanisms underlying the worsening of asthma progression with particular focus on oxidative stress pathways, Th2 and non-Th2 pathways (including the role of sensory nerves). We need to determine whether there is a particular endotype of asthma that results from high susceptibility to pollutant effects and we need to establish treatments (and life style measures, *e.g.* dietary) to combat the effects of pollutants on asthmatic diathesis in the most susceptible individuals.

Studies are additionally needed to determine whether direct induction by β_2-agonists *via* cAMP, as well as potentiation by β_2-agonists of virus-induction, of pro-inflammatory mediators in airway epithelial cells and macrophages, may explain the adverse effects of β_2-agonists in stable asthma and in asthma exacerbations. These studies should be carried out *in vitro* using human primary cells from people with asthma and should use methods such as whole genome transcriptomics or RNA-seq to identify all genes which are up-regulated by β_2-agonists. Further studies should test the hypothesis that these adverse effects will be resolved by the addition of ICS. Similar studies should be carried out *in vivo*, in people with asthma with both LABAs and SABAs delivered alone at therapeutic doses and should sample airway lining fluid using the novel method of bronchosorption to measure soluble mediators, as well as airway BECs and macrophages for gene expression analyses. Again these studies should include β_2-agonists alone, and β_2-agonists in combination with ICS. Such studies are urgently needed to better inform safest use of these ubiquitous therapies.

A major hindrance in our understating of the mechanisms underlying airway remodelling is the lack of animal models that can effectively recapitulate structural changes. This is due to the disparities in lung morphology between species and to the fact that most of the experimental protocols utilised are tested as preventive, rather than therapeutic, approaches [251, 252, 258]. It is, thus, essential that future funding should be targeted to the development of better animal models, to mapping of different models to different asthma phenotypes and development of novel *in vitro* assays that incorporate multiple cell types and mimic the intrinsic mechanic forces occurring in the airways. In addition, more attention should be directed to the contribution of the remodelling of the small airways in asthma, the mechanisms of which remain incompletely defined.

For the microbiome, future studies are required to establish the range of commensals and *Proteobacteria* spp., fungi and other pathogens in the lower airway microbiome of normal and asthmatic subjects, to establish the degree of individuality, stability and anatomic variability of airway microbial communities, to relate the microbiota to severity and duration of asthma, current therapy, and measures of inflammation and prospectively study the effects of antibiotics on the asthmatic airway microbiome. Further work in pre-clinical models as well as in man is needed to understand the role of probiotics, prebiotics and the gut microbiome in regulating allergic airway inflammation and asthma. Finally, studies investigating the effects of environmental changes, such as diet and microbiota composition, on asthma progression are warranted and future funding should be channeled in this area. Pragmatic approaches, such as diet changes, have the potential to rapidly be tested and implemented, and consequently represent an opportunity for influencing asthma progression in the medium term. To achieve this, directed funding ensuring high quality and rigorous clinical intervention studies is needed.

Better understanding of the mechanisms underpinning the relationship between psychological and neurological factors and asthma outcomes is needed to help clinical care and to understand the complexity of asthma. The effectiveness of psychological and non-pharmacological interventions and the way such interventions fit in with pharmacological strategies need to be established, and the mechanisms of effectiveness need to be understood to allow efficient targeted delivery of appropriate interventions to the right patient. Patients consistently stress the need for a holistic approach and for better integration of non-pharmacological strategies, and a personalised medicine approach demands that a holistic strategy encompassing these factors be established.

Asthma exacerbations

Introduction

Asthma exacerbations are universally accepted as the major cause of asthma morbidity, they contribute hugely to the healthcare costs associated with asthma and they are responsible for unacceptable asthma mortality [369]. The pharmaceutical industry, academia and governing bodies all recognise the important impact of asthma exacerbations and the need for new asthma treatments that specifically address the burden of asthma exacerbations [5].

Respiratory viral infections precipitate the vast majority of asthma exacerbations, however a number of other factors also contribute to exacerbation risk including allergen exposure [301, 370], air pollution [371], bacterial infection/reactivation [118, 372], eosinophils [373, 374], total IgE [375], asthma severity [376], genetic background [49, 159], dampness [377], temperature variation [378], vitamin D deficiency [379, 380] medication adherence [381], absence of health insurance [376] and risk perception [382]. Indeed, identification of other precipitants of asthma exacerbation is key to reduce asthma morbidity and healthcare utilisation. Additional studies of risk scores for asthma exacerbation may help guide management of asthma patients [383, 384] while predictors of hospitalisation and/or relapse in patients already experiencing an exacerbation are also being examined [385]. See figure 3 for a summary of important mechanisms involved in asthma exacerbations.

Asthma exacerbations: state of the art

Viruses

In adults and children, respiratory viruses cause approximately 80% of asthma exacerbations. The most common precipitants are rhinoviruses, but all respiratory viruses can do so. The roles of respiratory viruses, which may work both additively/synergistically with other stimuli including allergens and pollutants [301, 370, 371], and bacteria and atypical bacteria which are also important triggers of asthma exacerbation have been extensively covered in other recent reviews [386–388].

Interferons are anti-viral cytokines that induce >300 individual genes which aim to limit virus replication and dissemination [389]. Many studies have reported deficient type I (β) and type III (λ) interferon production in BECs cultured from both children and adults with asthma, in response to rhinovirus infection [157, 180, 184, 185, 187, 390, 391]. Studies have been extended to include IFN-α, other viruses/viral mimics and other cell types including airway macrophages [185, 392], peripheral blood mononuclear cells [300, 393], and blood-derived dendritic cells [394, 395]. The degree to which this deficiency relates to asthma severity and/or control [300], or is found only in certain asthma phenotypes, but not others [396, 397], requires further study.

Impaired interferon responses in asthma are thought to be caused at least in part, by increased expression of the negative regulator suppressor of cytokine signalling (SOCS)-1 [398]. Various cytokines including IL-4, IL-13 [146] and TGF-β [399, 400] can suppress virus-induced interferons *in vitro*, however whether this is solely dependent upon SOCS1 remains to be established. Additionally TLR7 (a viral RNA sensor and potent interferon-inducer) expression [179, 180] and function [401] are reported to be deficient in asthma. Further studies on mechanisms of interferon deficiency in asthma are required.

Augmenting deficient interferon production in asthmatic patients could be a novel approach for therapeutic intervention in the treatment of virus induced asthma exacerbations. Recently, inhaled recombinant IFN-β therapy was studied in naturally occurring colds in mild-moderate asthmatics and it reduced moderate/severe exacerbations, symptoms and improved lung function in a sub-group of moderate asthmatics [402]. Whether or not IFN-β was acting as an anti-viral cytokine, and/or was also effective due to antagonistic properties on Th2 cytokine pathways [403, 404] is currently unclear. Azithromycin has been shown to augment interferon responses during rhinovirus infection and reduce viral load *in vitro* [405]. This property of azithromycin however has not been confirmed in clinical trials.

Neuronal dysfunction in asthma can be amplified by respiratory viral infections [406], but this is an area that requires further investigation to determine the degree to which this is important in man.

Bacteria, fungi and the microbiome

Bacteria are also associated with asthma exacerbations, specifically the atypical bacteria (*Mycoplasma* and *Chlamydophila pneumoniae*) with serological positivity rates as high as 40–60% in some studies [175, 372, 407–409], indicating that viral and atypical bacterial infections may act together to increase the risk of asthma exacerbations.

While asthmatics have increased susceptibility to respiratory bacterial infections [177, 178, 410], increased carriage of pathogenic respiratory bacteria identified by culture [411] and molecular techniques [91], in depth microbiome studies pre and post asthma exacerbation in longitudinal studies are lacking. Studies in mouse models have highlighted potential immune deviation properties of atypical bacteria that may help promote Th2 immunity [412], and people with asthma have impaired IFN/Th1 responses to bacterial polysaccharides [185, 186, 188, 189]. In addition, viral infection impairs anti-bacterial innate immune responses [413] and increases bacterial adherence to BECs [414]. There is therefore good evidence that respiratory bacterial infections are more common and more severe in asthma, and that viral infection can increase susceptibility to bacterial infection.

Acute wheezing episodes in children aged <3 years were associated with both bacterial and virus infection [118], however guidelines recommend that antibiotics should not be administered routinely in asthma exacerbations [415]. Adults with asthma exacerbations treated with telithromycin showed statistically significantly greater reductions in asthma symptoms, improvement in lung function and faster recovery compared to placebo [409]. However, acute liver toxicity limits telithromycin to severe life threatening infections. A recent study reported azithromycin treatment resulted in no statistically or clinically significant benefit in acute asthma exacerbations in adults, however, for each patient randomised, more than 10 were excluded because they had already received antibiotics, thus widespread antibiotic usage prevented firm conclusions being drawn from this study [416]. Further studies of antibiotics in adults and children, in setting of low antibiotic use, are needed to determine which asthma patients may benefit from antibiotic therapy during asthma attacks [417].

Azithromycin treatment reduced the duration of acute episodes of asthma-like symptoms in 1–3 year old children [418]. Furthermore, in 1–6 year old children with history of recurrent severe lower respiratory tract infections (LRTIs), azithromycin early during an apparent respiratory tract infection reduced the likelihood of severe LRTI [419]. In adults low-dose azithromycin prophylaxis for 6 months in subjects with exacerbation-prone severe asthma significantly reduced the rate of severe exacerbations and LRTIs requiring treatment with antibiotics in a predefined subgroup analysis of subjects with non-eosinophilic severe asthma [420]. Further studies of antibiotics in both adults and children, in carefully phenotyped patient groups, are needed to determine in which asthma patient groups antibiotic therapy may prevent asthma attacks.

Emerging evidence indicates that the microbiota can influence the host's ability to fight respiratory infections [421], which could have profound implications for controlling exacerbations of asthma. Further studies of the effects of virus infections and of antibiotic therapy [422] on the airway microbiota and on risk and severity of asthma exacerbations are needed.

The role of fungi in precipitating asthma exacerbations is likely under-appreciated. Exacerbations of allergic bronchopulmonary aspergillosis may be fungal in origin, yet clinical trials are few, and with disappointing results [423]. Epidemics of severe exacerbations in the summer months are also thought likely related to alternaria sensitisation and exposure [424], while epidemics of exacerbations related to thunderstorms may be related to pollen and/or fungal exposures in sensitised individuals [425]. Further research into the role of fungi in the aetiology of asthma exacerbations is needed.

Allergen exposure and Th2 pathways

An important mechanistic process not completely understood is how virus infections exacerbate existing Th2 immunity. Risk of hospital admissions for asthma exacerbations increases dramatically when allergen sensitisation and exposure occur together with a respiratory viral infection [301, 370] and mechanistic studies in human and mouse models of rhinovirus infection show induction of the pro-Th2 cytokines IL-25 and IL-33 by rhinovirus, thus potentiating Th2 immunity *via* increased IL-4, IL-5, IL-13 and eosinophilia within the airways [57, 58, 186]. How important human respiratory viruses impact on the newly identified ILC2s is very understudied, but two studies suggest virus induced IL-25 and IL-33 potentiated Th2 immunity *via* actions on these cells [57, 58].

While corticosteroid based therapies impact only moderately on the severity of asthma exacerbations, anti-Th2 biologics and anti-IgE therapies have shown a surprising efficacy [16, 18, 20, 23]. Whether or not this added value is simply *via* suppressing allergen-induced Th2 pathways, or is affecting other aspects of virus-allergen interactions (such as restoring deficient anti-viral immunity [24]) with a net beneficial effect on the outcome of virus-induced asthma exacerbations requires further exploration.

Pollution

A short-term increase in pollution exposure levels has been associated with asthma exacerbations: a 10 $\mu g \cdot m^{-3}$ increase in $PM_{2.5}$ correlated with emergency room visits and hospitalisation [426], while long-term exposure to NO_2 increased asthma hospitalisation in the elderly [427]. Exposure to low dose air pollution acting directly on airway cells may cause chronic production of inflammatory mediators in asthmatics. These mediators may cause increased infiltration of airways tissue by immune cells and changes to the airways parenchyma leading to chronic remodelling [428]. This could be responsible for a lowered threshold for other stimuli to cause asthma exacerbations. Acute exposure to high levels of air pollution alone may directly provoke asthma exacerbations in asthmatic individuals [429] either by action on innate and structural cells, and/or actions on the adaptive immune system [430].

There is emerging evidence providing a link between the increasing amounts of air pollutants/allergens in the environment and the worsening of symptoms in asthma [431]. For example, air pollution has been

linked to increases in hospital visits for respiratory disease [432]. Synergy has also been suggested between air pollutants and allergens in the risk and exacerbation of asthma (especially in children), suggesting an advantage of avoiding co-exposure [371, 431].

Many epidemiological studies have identified ambient pollutant gases and airborne particles as risk factors for asthma exacerbations. However, the pollutants responsible remain unclear and causal relationships have often not been established. Among the most consistent observations are the direct effects of particle components on the generation of reactive oxygen species and induction of oxidative stress and inflammatory responses [433].

Diet and hormones

Individuals with ORA are at increased risk of asthma exacerbations [434]. To the best of our knowledge, no studies have assessed the effect of obesity on the innate or adaptive immune response to respiratory viruses in asthmatics. Given that ~80% of asthma exacerbations are virus-induced [435], *ex vivo* and human challenge studies in this area may facilitate the development of novel preventative or therapeutic strategies.

Up to 20% of pregnant women with asthma experience asthma exacerbations during pregnancy and up to 6% require hospitalisations [324]. During pregnancy the severity of asthma can change due to extrinsic as well as unknown factors. Uncontrolled asthma has a higher likelihood to deteriorate during pregnancy and previous exacerbations predict emergency room visits during pregnancy [436], but its course is unpredictable and might even vary between pregnancies [324]. Exacerbations have been reported to be more frequent during the early third trimester. Pregnancy has been reported to be associated with impaired anti-viral immunity in the presence [437] as well as absence [438] of asthma. Understanding the mechanisms that lead to deteriorations of asthma control during pregnancy has potential to improve adverse outcome associated with poor asthma control during pregnancy.

Resolution of airway inflammation

A surprising recent observation has been that protectin D1 is induced by viral infection [439, 440] and viral RNA-sensing TLRs [277], and protectin D1 exhibits potent antiviral activity against influenza [439] while several resolvins and protectins can also enhance antibacterial defences [441]. This raises the possibility of a dual role of SPMs in the regulation of inflammation and antiviral immunity in the respiratory tract with significant implications for the therapeutic potential of these molecules in the treatment or prevention of asthma exacerbations.

Psycho-social and behavioural factors

Stress, anxiety and depression are associated with an increased risk of exacerbation, less successful emergency treatment, and increased asthma hospitalisation rates [442, 443], and psychological co-morbidity may be associated with increased asthma mortality risks [444]. The mechanisms underlying this increased risk are uncertain. Non-adherence with regular medication is common even in those with severe disease and exacerbation risk, and is associated with an increased risk of severe asthma attacks [31]. Non-adherence occurs even in the period of deteriorating symptoms prior to an asthma attack [445]. Risk stratification and targeted strategies to reduce exacerbation risk may be possible [446, 447].

Addressing the unmet need

While it is widely acknowledged that respiratory tract infections, particularly viral, account for the vast majority of asthma exacerbations (approximately 80%), a number of other factors are also likely to play a role. Identification of these other factors is vital if we are to reduce asthma morbidity and healthcare resource utilisation. Our incomplete understanding of asthma exacerbation pathophysiology and mechanisms is preventing the development of effective treatments and preventive strategies. Many research programmes are seeing cross-European collaboration to integrate data, tools and techniques in order to explore related mechanisms and identify factors that play a decisive role in asthma. Similar cross-European collaboration is now needed to better understand aetiology and mechanism of asthma exacerbations.

At present there are few available therapies that target respiratory infections either non-specifically or specifically by virus type. A growing body of evidence suggests that augmenting deficient interferon production in asthmatic patients could be a novel target for therapeutic intervention in the context of virus-induced asthma exacerbations. In addition, despite there being much interest and activity in developing viral inhibitors, no anti-virals have specifically been trialled in models of asthma exacerbations, despite the fact that such models exist and appear suitable [57]. Again, the paucity of available anti-virals and a timely and effective model in which to test their efficacy must be considered an important unmet need. While interferon therapy is attractive in that it may target many viruses non-specifically, this

argument can be extended to support the use of anti-viral therapies for asthma exacerbations. The recent developments in anti-viral therapy for important respiratory viruses have been covered elsewhere [448] and will not be systematically reviewed here. At the time of writing no anti-virals have specifically been trialled in models of asthma exacerbations, despite the multiple lines of evidence supporting the role of viruses as the most obvious asthma exacerbation trigger. Such studies are urgently needed.

Historically there has been much interest and activity in developing viral inhibitors. Small molecules and antibodies to viruses themselves or their receptors have been developed, or are currently undergoing clinical trials for rhinovirus [449, 450], RSV [451, 452] and influenza [453, 454]. However, there has been slow progress in applying these to asthma. One question that is yet to be satisfactorily answered is in which model should they be tested? While the study of natural infections requires hundreds of subjects and is therefore expensive and time consuming, a useful alternative could be experimental infection models, which are performed with as few as 30 subjects. These have been successfully developed for rhinovirus [57, 186], and as rhinovirus is the most important viral trigger, there is an urgent need for these treatments to be tested in experimental rhinovirus challenge models to enable careful study of both efficacy and mechanisms. Another important consideration is which type and strain of virus should be used, and whether the experimental strains available behave in a manner comparable to wild type strains. This case is of particular importance for rhinovirus. The discovery of rhinovirus group C (RV-C) [455, 456], and its rise as probably the most clinically important group of rhinoviruses responsible for the majority of asthma exacerbation admissions [457–459] makes it a clear target for anti-viral therapy. There are several roadblocks concerning RV-C, however; this is a difficult virus group to culture with a unique receptor [49]. Few laboratories have this expertise and there is as yet no RV-C stock available for experimental human challenge. Taken together, the case for the implementation of anti-viral therapy in asthma exacerbations, while clearly supported by the available scientific evidence, is hampered by several practical issues that requires further funding, careful planning and co-ordination between academia and industry to overcome.

Viral vaccines also represent an important unmet need, with either vaccines for some respiratory viruses completely unavailable or when available, of unclear benefit. New vaccine candidates against RSV including live attenuated vaccines are currently being or have recently been tested in clinical trials [460–462]. Immunisation of mice with a rhinovirus capsid protein (VP0) containing highly conserved regions has been shown to induce cross-reactive cellular and humoral immune responses and, therefore, such capsid domains may prove useful for the development of a subunit rhinovirus vaccine [463]. In the same model, rhinovirus VP1 vaccination has also been tested and provided some promising results in terms of cross-serotype antibody responses for future vaccine development [464]. Further work to develop an effective and broadly cross-serotype protective vaccine against rhinovirus infections is urgently needed.

Regular vaccination against seasonal influenza is recommended for asthmatic children and adults [465, 466]. A 2013 Cochrane review on the potential protective effect of the inactivated flu vaccine concluded that there was no significant reduction in the number, duration or severity of influenza-related asthma exacerbations in asthmatic children or adults [467]. However, vaccinated children did display better symptom scores during influenza-positive weeks [467]. Accumulating evidence now supports the concept of alternative influenza virus vaccine development strategies that will stimulate an immune response towards the conserved stalk rather than head domains of the virus haemagglutinin [468, 469]. Such a universal vaccine would allow cross-immunisation against several influenza virus subtypes; however protective efficacy remains to be confirmed.

Our understanding of how asthma exacerbation frequency and severity relates to underlying asthma endotypes and phenotypes is also lacking. An important network running for over a decade is the National Institutes of Health-sponsored Severe Asthma Research Program (SARP), which is dedicated to the study of children and adults with severe asthma and aims at better understanding asthma endotypes with a focus on molecular and cellular processes. Subjects participating in the programme undergo detailed characterisation including clinical, physiological, radiological and genomic assessment and investigators seek to explore related mechanisms and identify factors that play a decisive role in disease history [470]. In Europe, the BIOAIR and the U-BIOPRED severe asthma networks aim at improving our understanding of mechanisms and provide insight into endotypes or phenotypes such as those of frequent exacerbators [471], asthma exacerbation indicators [472], biomarkers [473] and pathophysiology of disease [474]. These phenotypes need to be cross validated in different cohorts before they can be used to define patients. Asthma phenotyping has not been extensively studied among various neglected patient groups including smokers, drug users and the elderly. Identification of clusters may help recognise patients particularly prone to developing asthma exacerbations. Recent work towards this direction has defined phenotypic groups among elderly patients [475–477] and has attempted to profile asthma exacerbations in drug users [478]. Finally, there is great potential for novel diagnostic technologies (in particular

non-invasive techniques and biomarkers) to be developed to support prediction [479], assessment and management of asthma exacerbations [480, 481] and future studies in the field would help personalise intervention strategies.

The existing evidence points to the fact that asthma exacerbations are likely a result of synergistic and/or additive interactions between several factors. Perhaps most evidence is available for virus and Th2 interactions, and how these precipitate asthma exacerbations in allergic asthma. No study has yet linked impaired interferon responses with a known asthma phenotype, however most studies observed this phenomenon in atopic asthma [157, 185, 187, 390, 392, 394]. Sputum eosinophils in the past have been related to asthma exacerbation risk [482] and impaired IFN-λs in cultured cells negatively correlated with sputum eosinophilia [185] and both serum IgE and IL-4 staining levels in biopsies [187]. Mechanistically, much evidence definitively shows that type I and III interferons and Th2 cytokines can exhibit potent negative regulation on the expression or actions of each other. IFN-α for example, can suppress Th2 cell polarisation in pure T cell and mixed leukocyte culture systems [404, 483]. IFN-λ (IL-28) when given to the airways can suppress the generation of allergic airways inflammation in the ovalbumin sensitisation and challenge model in mice, which is Th2 driven [403]. Conversely, crosslinking FcεR1 on dendritic cells prior to influenza virus or rhinovirus challenge down regulates IFN-α [394, 395]; thus the overall evidence strongly underscores many potential mechanisms of counter-regulation of Th2 cytokines and type I and III interferons. Interestingly, SOCS1, linking with impaired interferon is a Th2 inducible negative regulator and in the airway epithelium, correlates with serum IgE [398]. There is still a lot to learn about how asthma exacerbations are precipitated, including how the underlying pathophysiology of asthma can be subtly affected by another insult or stimulus. This therefore represents an unmet need. The interactions between pathogen sensing, environmental microbiota and genetics are likely also important in asthma exacerbations and future research should explore the role of non-Th2 pathways and other processes including inflammasome activation in airway remodelling and how these processes affect steroid resistance.

Further studies investigating the effectiveness of macrolide (including derivatives with and without antibiotic properties) and other antibiotics in treatment and prevention of acute exacerbations of asthma in adults and children in settings of low antibiotic usage, and studies including stratification on blood/sputum cell counts, are required. Further studies investigating the effects of viral infection on secondary disturbances of the microbiome in adult and children with asthma exacerbations are clearly needed, as well as studies investigating the mechanisms of impaired host defence against bacterial infections in asthma.

Asthma exacerbations constitute major outstanding challenges to treatment. Given the generation of danger signals during an exacerbation, the role of alarmins and of ILCs in exacerbations needs to be clarified. Understanding the role of these new ILC populations could eventually lead to new therapeutic targets. Similar work is needed on the role of neuronal mechanisms and respiratory reflexes.

The role of pollution in precipitating asthma exacerbations is also incompletely understood. Polymorphisms in glutathione S-transferases (GSTM1 and GSTP1) that facilitate the elimination of reactive oxygen species have been associated with breathing difficulties and respiratory symptoms in asthmatic children following increases in ambient O_3 concentrations [484] and an altered response to combined exposures to ragweed pollen and diesel exhaust particles [485]. How the innate immune response of asthmatics to infections is altered by air pollution should also be studied. Further research examining the influence of gene polymorphisms which could predict asthmatic patients that will benefit from antioxidant supplementation would be of interest.

Patients at higher risk of exacerbations may be identified through viable clinical strategies, with exacerbation prevention strategies targeted to improve exacerbation outcomes. Psychological and behavioural factors, for example substance abuse [486], may increase exacerbation risk, and may be amenable to intervention, although the mechanisms underlying this increased risk are not fully understood.

Summary and conclusions

Asthma research now has loftier aims than in previous times, in that we now aim to prevent disease occurring, to cure it when it does occur, and if these are not possible, to treat and control asthma to prevent progression to severe disease, as well as to prevent and treat acute attacks of asthma. There is also a focus on studying mechanisms in different asthma phenotypes, including atopic and non-atopic asthma, so that new therapies can be best targeted towards the most appropriate phenotype.

This analysis of available literature on asthma mechanisms has delineated the most important unmet needs in asthma research and proposes how these might be addressed. A stratification into asthma onset, progression of stable asthma, and asthma exacerbations allows identification of where in the continuity of asthma research future efforts need to be directed, indicating who would benefit and to what extent such efforts would impact on people with asthma. Our review of the literature and discussions with colleagues

have clearly identified many important unmet needs. The most important mechanisms involved in asthma onset, asthma progression and asthma exacerbation and the strength of the supportive evidence for each mechanism are summarised in table 1. The most important methodologies or technologies that are required to beneficially impact on asthma research in future years are summarised in table 2. Key unmet needs in understanding asthma mechanisms that we have identified are listed in table 3 and are pictorially represented in figures 1–3. These are the major areas in which future investment is needed to drive forward asthma research to hasten progress in preventing asthma developing, in controlling and curing established asthma, and in preventing and treating asthma attacks and their associated morbidity and mortality.

First, we have shown that research into asthma onset suffers from several shortcomings in research effort. Importantly, this is an area that if addressed, could have the biggest impact, in both the short and long term, by preventing asthma occurring in the first place. We need to better understand the onset of both allergic and non-allergic asthma, including the role of air pollution. The development of new models, new sampling methods, and the structuring of research in productive, collaborative initiatives will deliver new information in the longer term and with time, new therapeutic targets for asthma inception. Funding this area of asthma research to amend these shortfalls should thus be considered a long term proposition. However, there are also more immediate goals to be achieved. The study of new vaccines and anti-virals, or application of existing ones to determine whether viruses are functionally associated with asthma onset could lead to exciting opportunities for asthma prevention. Even reducing respiratory viral infections by 20% in young infants could have vast financial effects on the healthcare burden associated with asthma. Prevention of acute respiratory viral infections could prevent wheeze and perhaps allergic sensitisation and asthma onset. Funding this research would deliver a short term gain, as anti-virals are already in phase II/III (for RSV) and would have to be applied to the right population at highest risk and at the optimal age window in clinical trials. For rhinovirus the prospects of pathway to impact are further away, as anti-viral programmes are still in development. However vaccine candidates while still experimental, do hold promise and need to be prioritised.

Targeting mechanisms of progression of stable asthma to severe disease requires more basic research to hasten development of a more comprehensive array of disease modifying therapies that better address the needs of severe therapy resistant asthma. The studies should include the different endotypes and phenotypes of asthma and specific mechanisms should include neural pathways, airway remodelling and the understudied adverse effects of asthma therapies. The longer term prospects of such programmes would be to achieve better asthma control with better targeted novel treatments, to reduce progression to severe asthma and to cure established asthma. Other areas that need more research include the role of co-morbidities, better clinically defined groups and models, and also aspects of the microbiome and bacteria-virus interactions, together with their interactions with pollutants. These last two subjects would benefit specifically from basic research including new *in vitro* and *in vivo* models. The value in funding these areas should be considered a longer term proposition, with the initial aim of developing models and techniques, and applying them to gain new disease insights, which would eventually lead to new targets or agents for therapy.

Asthma exacerbations cause major morbidity, mortality and healthcare costs. Much more research funding needs to be channelled into anti-viral therapy and vaccine development programmes to benefit asthma exacerbations, which have a clear viral aetiology. Funding also needs to be directed at development of better pre-clinical models of virus infections, and of interactions between virus infections, allergen exposure and airway microbes to study mechanisms of exacerbation. A major priority is the development of human challenge models in asthma with viruses, allergens and pollutants to enable identification of novel mechanisms of disease to lead to new therapeutic strategies. Development of human challenge models will also permit early proof of concept and proof of mechanism studies in development programmes of novel therapeutic approaches for prevention/treatment of exacerbations. Funding also needs to prioritise better understanding of the mechanisms of increased susceptibility to viral and bacterial infection in asthma and better identification of the endotypes and phenotypes of asthma to which these specific mechanisms apply. More research is also needed to better understand of the interactions between Th2 pathways and anti-viral immunity in exacerbation pathogenesis.

In summary, this paper describes a compelling picture of unmet need in asthma. Researchers, funders and the pharmaceutical industry need to work together to address these important priorities to prevent, treat and eventually cure asthma.

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