Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease

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Clinical asthma is very widely assumed to be the net result of excessive inflammation driven by aberrant T-helper-2 (Th2) immunity that leads to inflamed, remodelled airways and then functional derangement that, in turn, causes symptoms. This notion of disease is actually poorly supported by data, and there are substantial discrepancies and very poor correlation between inflammation, damage, functional impairment, and degree of symptoms. Furthermore, this problem is compounded by the poor understanding of the heterogeneity of clinical disease. Failure to recognise and discover the underlying mechanisms of these major variants or endotypes of asthma is, arguably, the major intellectual limitation to progress at present. Fortunately, both clinical research and animal models are very well suited to dissecting the cellular and molecular basis of disease endotypes. This approach is already suggesting entirely novel pathways to disease—eg, alternative macrophage specification, steroid refractory innate immunity, the interleukin-17–regulatory T-cell axis, epidermal growth factor receptor co-amplification, and Th2-mimicking but non-T-cell, interleukins 18 and 33 dependent processes that can offer unexpected therapeutic opportunities for specific patient endotypes.

Introduction
In recent years, the morbidity and mortality of asthma have decreased, probably as a result of improved management. Some evidence suggests that the relentless rise in disease incidence and prevalence is now also reaching a plateau.1 However, although contemporary treatment approaches are indisputably effective, many patients have substantial residual disease and some, with very severe asthma, respond suboptimally even to high-dose oral steroids (figure 1).2-4 Furthermore, asthma—the most common serious chronic lung disease afflicting around 150 million people worldwide—remains both unpreventable and incurable.3 Despite decades of intensive research, little progress in identification of new treatments has been made since the introduction of inhaled β2 adrenoceptor selective agonists (1969) and inhaled glucocorticosteroids (1974).

This Review aims to advance the argument that the way in which we think about the pathogenesis of asthma is flawed (or incomplete), which in turn is preventing the discovery of better treatments, prevention, and cures. Clear evidence now suggests that asthma is a heterogeneous and genetically complex disease (>100 genes have already been implicated) that cannot be explained by one mechanism alone. To order this heterogeneity and the volume and complexity of clinical and basic research data, the new notion of disease endotypes (panel 1), identifying definable subpopulations of asthma with discrete pathogenic pathways, is introduced and a conceptual framework to model endotypes is presented.

This Review is structured into four sections: weaknesses of the current T-helper-2 (Th2)-inflammation

Search strategy and selection criteria
hypothesis; asthma heterogeneity in terms of ontogeny, clinical phenotypes, and molecular patterns; asthma endotypes; and novel mechanisms, with particular emphasis on alternative macrophase specification programmes and the role of innate immunity as determinants of more severe and steroid-refractory asthma endotypes. By understanding asthma endotypes, and their molecular determinants, effective therapies, and possibly cures, can be developed that are highly effective in targeted patient subgroups.

Limitations of the Th2-inflammation hypothesis

Since the mid-1990s, asthma research has been propelled forward by innovations stemming from the Th2-inflammation hypothesis, providing a molecular framework for understanding the well known associations of atopy or IgE and eosinophilic lung inflammation with asthma. A helper T-cell population induced by interleukin 4 is able to produce a panel of cytokines—such as interleukin 4 or 13 (causing B-cell IgE production, mucus secretion, and fibrosis), interleukin 5 (causing eosinophilic inflammation and damage), and interleukin 9 (promoting mast cell growth)—which induce traits associated with classic asthma (figure 2). The Th2-inflammation hypothesis6-9 coincided with the rise of genetically modified mouse technology and molecular profiling methods. Thus, lung Th2 immunity is now understood in fine molecular detail: from the nature of antigen, through the co-stimulation topology of antigen-presenting dendritic cells, to the language of transcription factors and chromatin reshaping that controls gene programmes governing the emergence and persistence of Th2-biased lymphocytes and their trafficking patterns in vivo.10-12

Th2 immunity is undoubtedly important in some asthma endotypes. But even from its inception, concerns have arisen about whether the Th2-inflammation hypothesis would lead to improved treatments.6-9 These concerns have now been heightened. The main reasons for questioning the Th2-inflammation hypothesis is that it cannot explain why airway hyper-responsiveness and tissue remodelling are not clearly linked to inflammation; why existing T-cell immunosuppressives and new Th2-targeted treatments, which often worked well in Th2 disease models, have no or marginal effectiveness in the clinic; why many patients have recurrent exacerbations; why substantial residual disease remains when anti-inflammatory therapy is optimised; why asthma shares some genetic risk factors with chronic obstructive pulmonary disease (COPD); and why some patients have severe asthma. Moreover, the Th2-inflammation model cannot account for the substantial clinical and molecular heterogeneity that has now been unequivocally documented in human asthma. Therefore, this intellectual framework needs to be revised. Indeed, that the entry criteria for patients into almost all clinical trials for asthma does not reflect the pattern of actual asthma in the community is remarkable.13

Several problems exist. If T cells were fundamentally important, T-cell inhibitors should be very effective treatments; however, T-cell-directed therapies have uniformly failed in clinical trials.6-9 Th2 immunity is fundamental to atopy, but although atopy is a risk factor for asthma in populations, it has poor sensitivity and specificity as a predictor of disease. Eosinophilic inflammation is the Th2 driven trait that most consistently tracks with disease activity, exacerbation susceptibility, treatment responses, and as a useful biomarker to guide treatment.14-17 However, airway inflammation is much the same between non-asthmatic atopics, allergic rhinitics, and atopic asthmatics.9 An almost identical pattern of response occurs after allergen challenge in people allergic to house-dust mites with and without asthma.10-12 The Th2-inflammation model predicts that eosinophilic inflammation should drive airway hyper-responsiveness, but no clear relation exists.18,19 Furthermore, population studies show atopy and airway hyper-responsiveness are not concordant.14 Neutrophils, mast cell infiltration of
airway smooth muscle, intensity of inflammation, and inflammation of airway smooth muscle, might discriminate between inflammation in atopy versus that in asthma, but the sensitivity and specificity of these putative co-determinants has not been formally proven.\textsuperscript{25-26} Lung Th2 cytokines are found as often in atopy as in asthma, and interferon γ (a Th1 cytokine) is actually upregulated in human asthma together with interleukins 4 and 5 in sputum but not in blood.\textsuperscript{27}

T-cell immunosuppressive drugs (eg, cyclosporine and methotrexate) have measurable but very weak effects in asthma, and immunosuppressive therapy after allograft transplantation does not prevent asthma or allergy in children and adolescents.\textsuperscript{28} Furthermore, glucocorticosteroids (and β₂ agonists), which are highly effective in atopic asthma with eosinophilic inflammation, paradoxically consolidate and intensify Th2 immunity and increase IgE.\textsuperscript{29} At the molecular level, steroids preferentially suppress interleukin 12 and T-bet (negative regulators of Th2 immunity) and spare STAT6 (which induces Th2 genes).\textsuperscript{30,31} Steroids also dampen expression of T-bet, the transcription factor controlling Th1 (but not Th2) immunity.\textsuperscript{32} Steroid sensitivity wanes in severe asthma; however, genetic manipulation to enhance steroid effectiveness does not suppress experimental asthma and instead favours Th2 immunity.\textsuperscript{33} In children, steroids worsen Th2 immunity and increase IgE, which has been related to the genetic association of the low affinity IgE receptor (FCER2) with risk of severe exacerbations.\textsuperscript{34} Considered together with the weak effects of therapies of anti-interleukins 4 and 13 (eg, pitrakinra) and anti-interleukin 5 (eg, mepolizumab) that are in trials,\textsuperscript{35,36} it is reasonable to suggest that steroids exert their beneficial effects in asthma at loci other than Th2 immunity. Steroids suppress the end effects of Th2 immunity but consolidate the underlying aberration.

Despite these caveats, Th2 immunity is clinically important, specifically for childhood asthma with atopy and mild allergic adult asthma, and Th2 directed therapy will be effective in some asthma endotypes.\textsuperscript{37} However, present treatments are effective in mild atopic asthma (figure 1), and Th2-directed therapies are unlikely to ameliorate the residual disease burden of more severe disease.

**Heterogeneity of clinical asthma and treatment responses**

Asthma continues to elude specific definition and can therefore currently only be characterised in functional terms (panel 2). Although evidence has suggested the pronounced heterogeneity of asthma for decades, little interest has focused on understanding its basis. Most asthma trials and research protocols have used inclusion criteria—typically predicted forced expiratory volume in 1 second (FEV\textsubscript{1}), degree of reversibility, inflammation, eosinophilia, and often IgE, because they can be measured objectively and accord well with disease notions. However, because asthma is heterogeneous, these criteria have resulted in patients being selected to trial new asthma drugs who are not representative of asthma in general practice.\textsuperscript{38} Advances in asthma epidemiology including longitudinal outcomes studies, population genetics, and molecular profiling methods; application of statistical methods, such as clustering and principal component techniques, to show distinct asthma patterns and subsets; and compelling new clinical research, have all contributed to a reawakening of interest in understanding disease heterogeneity.

Paediatricians have defined three major patterns of wheezing in infants that have also been assessed for their effect on subsequent persistent asthma in adulthood: transient infant wheeze, non-atopic wheezing in toddlers, and IgE-mediated wheeze or asthma. A fourth category has also been introduced: late-onset childhood asthma.\textsuperscript{39} Evidence now suggests that transdermal sensitisation associated with polymorphism in filagrin, a molecule important in maintaining cutaneous integrity, rather than aeroallergen links asthma risk with atopy and atopic dermatitis.\textsuperscript{40-41}
Wheeze in early life in the absence of atopic sensitisation almost invariably resolves to normal lung function by 13 years of age, whereas atopic sensitisation, particularly before 3 years of age, is associated with a much higher chance of reduced lung function and worsened airway hyper-responsiveness, which occur despite the airway biopsy pathology of non-atopic asthma being almost indistinguishable from atopic asthma in children. This finding suggests that very early intervention with steroids would be beneficial, but this is not the case, perhaps because steroids actually worsen Th2 immune deviation. Long-term follow-up studies also suggest that up to half of asthma in adolescence or early adult life represents a relapse of previously quiescent disease. Asthma affects more boys than girls in childhood, but more women in later life. At the cellular and molecular level, children who are predisposed to life-long asthma might have substantial changes early in disease, including oxidative stress and acetylation. IRAK-M (a negative regulator of innate immunity), epidermal growth factor (EGF), interleukin 6, and prostaglandin E2 are known to be involved in the pathogenesis of asthma.

The heterogeneity of asthmatic inflammation is well documented; Golash first suggested sputum eosinophilia as a hallmark of disease in the 1890s. Woolcock and Peat noted that airway hyper-responsiveness was unimodally distributed in the general population and only partially overlapped with atopy, precluding atopy as a cause of all airway hyper-responsiveness. Wardlaw and colleagues have reported the absence of a clear relation between intensity of inflammation (eosinophils) and severity of asthma. They also noted that only airway smooth muscle infiltration by mast cells differentiated the airway pathology of eosinophilic bronchitis from asthma and that this trait, not airway remodelling, is associated with airway hyper-responsiveness.

More recently, Simpson and colleagues have identified distinct inflammatory endotypes in sputum samples from patients with clinical asthma, forming the basis of a simple classification schema: (1) eosinophilic; (2) neutrophilic; (3) mixed (ie, both neutrophils and eosinophils found); and (4) paucigranulocytic (few or no granulocytes in the sputum).

Since these investigators noted evidence of upregulated toll-like receptors, they have specifically linked neutrophilic asthma to innate immunity. Haldar and Pavord have independently replicated the identification of neutrophil variant asthma (which has been suggested for many years). These heterogeneous inflammatory patterns are entirely consistent with the identification of a distinct cytokine profile in patients with asthma. Similarly, application of factor analysis to molecular genetics studies tends to segregate, rather than cluster, atopic and asthmatic disease traits.

The biology of very severe asthma is almost certainly distinct from milder forms of disease, and severe asthma is heterogeneous. Severity should be viewed as a separately regulated biology rather than as one end of the spectrum of disease or the result of an inexorably progressive process. Results from long-term epidemiological studies in asthma have shown that the severity grade of asthma tends to be established early in life, and disease seldom progresses to a more severe grade.

Two major subdivisions of severe asthma have been proposed on the basis of discordant inflammatory patterns. Furthermore, Pavord and colleagues have reported severe asthma in the absence of eosinophilic inflammation and, by inference, Th2 immunity. Brasier and co-workers applied mathematical pattern-recognition methods to compare cytokine patterns in bronchoavleolar lavage from 43 patients with mild or moderate asthma with 43 patients with severe disease, and noted four distinct profiles to predict methacholine responsiveness. However, few molecular mechanisms exist that unequivocally distinguish severe asthma. Possibilities include CREB (cyclic AMP response element binding protein 1), which regulates gene expression in response to increases in cyclic AMP, RIP-2 (receptor-interacting serine-threonine kinase 2), an intermediate in toll-like receptor signalling; reduced generation of pro-healing lipoxin A4; or anti-inflammatory interleukin 10.
Combination therapy (bronchodilator plus inhaled glucocorticosteroid) achieves good outcomes for many, but not all, patients with mild to moderately severe asthma and can reduce the rate of severe exacerbations by up to a third (figure I). Other therapies have also proven to be of measurable, but lesser and variable benefit—eg, cysteinyl-leukotriene receptor antagonists and anti-IgE monoclonal antibodies.\(^1\) Other therapeutic options, such as selective phosphodiesterase isozyme inhibitors, selective chemokine and cytokine antagonists, vaccines, and T-cell-directed or general immunosuppressives, are ineffective or of marginal benefit.\(^6\)

Primary prevention has not proven possible so far, since a meta-analysis shows that avoidance of house-dust mites is effective.\(^7\) The \(\beta\) adrenoceptor is polymorphic and its Arg16 variant might adversely affect response to regular short-acting drugs but not long-acting \(\beta\) agonists.\(^6\)

About 15% of patients with asthma respond well to leucotriene antagonists and, although urinary concentrations of leucotriene do not predict responses, polymorphism in 5 lipoxgenase (ALOX) and the receptor (CYSLTR2) can establish drug sensitivity.\(^8\) Steroid responses are very heterogeneous in childhood and adult asthma.\(^6\)

**An asthma endotype model**

In view of the manifest problems with the Th2-inflammation model and the pronounced heterogeneity of asthma, how is the disease to be modelled and how will new treatments be found? Gibson’s four inflammatory patterns provide one simple and useful model.\(^6\)

A more extensive rational approach is to assess components of disease that could be considered in the definition of endotypes. The asthma endotype model shown in figure 3 maps inter-relations between clinical determinants that are known to be important in the manifestation and expression of asthma across its diverse patterns and severities. The table lists candidate genes linked to endotype determinants. The model is non-linear, but does not make a priori assumption about the weight of a specific factor—ie, inflammation—to expression of disease. Some of the evidence base for the model is summarised below.

**Airway smooth muscle**

Airway narrowing, which is largely caused by contraction of airway smooth muscle, is the mechanism that can be most clearly linked to symptoms, as can be inferred from the additional benefit of combining long-acting bronchodilators with steroids and the remarkably effective benefits of killing muscle by bronchial thermoplasty in severe disease.\(^7,7\) More smooth muscle exists in airways of asthmatic patients, but alone it might not be a cause;\(^5\) modelling studies suggest that increased bulk might even protect against excessive closure. Airway smooth muscle in asthmatic patients also secretes inflammatory cytokines including stem-cell factor (or kit-ligand), a mast cell growth and activation protein that is now also implicated in dendritic cell activation.\(^7\) Increased mast cell number and degranulation have been suggested as pathological indicators of changed function.\(^2,15\)

Specific molecular changes affecting contractility—such as changes in myosin light chain kinase isoforms or cross talk between signalling \(G\) proteins—remain controversial,

<table>
<thead>
<tr>
<th>Genetic</th>
<th>Biology</th>
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<tr>
<td>Lung function: basal FEV(_1), airway hyper-responsiveness, airway smooth muscle</td>
<td>EDN1, ADAM33, B2ADR, CREB, CCR5, COL2A1, CTA, CYSLTR1, CYSLTR2, EP2, FEVR2, GSTM1, HNMT, KCN51, LELP1, MMP, MUC7, MLCK, NOS3, PDGFRB, PLA2, PLAU, PTGDR, PTGER3, PTGER4, TXB2, VDR</td>
</tr>
<tr>
<td>Immunity</td>
<td>FP8, IL17, TGF(<em>B), IL6, KORs, ROR(</em>\alpha), RONF, chemokines, CD14, CD40, CD86, DPP10, FGL2, HLA-A, ICOS, IGSH, IL12B, IL4, IL6, IL9, IL10, IL13, IL18, IL22, IL33, IRAK1, ITK, IL1, MMP, MRPS1, MUC1, NOD, NPY1R, PLA2, PPPARG, PTGDR, ROR1, RUNX1, SFTP7, Socs5, SPP1, STAT6, TBX21, TIM1, VDR, VEGFR</td>
</tr>
<tr>
<td>Inflammation and remodelling</td>
<td>EDN1, ADAM33, IL17A, IL17F, NOS2, SOD2, CREB, VDR, CAT, chemokines, COL2A1, CTA, DPP10, ECP1, EP2, FYN, GSTM1, IGSH, IL2, IL5, IL9, IL13, IL17, IL18, IL33, PLA2, PLAU, Socs, STAT6, TNFA, UTG, VEGFR</td>
</tr>
<tr>
<td>Resolution and repair</td>
<td>VDR, LEP, IL10, FAS, NR3C1, RAGE, TIMP, Lipoxin A(_4) (15LOX, 5LOX), presqualene phosphates</td>
</tr>
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<td>Exacerbations, smoke, and environmental imitants</td>
<td>VDR, ADH1, CAT, CYP24A1, GSNOR, HLA-G, IL12B, IL2, IL6, IL12, IL17, IL23, IL33, IRAK1, MMP, MRPS1, NOD2, SFTP7, UTG, NOS2F2</td>
</tr>
<tr>
<td>BMI and nutrition</td>
<td>ADRB2, FABP3, NR3C1, VDR, FABP4, NR3C1</td>
</tr>
<tr>
<td>Perception</td>
<td>KCN51, GAD65</td>
</tr>
<tr>
<td>Genes of unknown function from linkage</td>
<td>DCN1, GCLM, ORMDL3, SCGB3A2</td>
</tr>
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Candidate genes selected from linkage and microarray studies (genetic) or from the known biology of established disease processes (biology) are shown as putative determinants of asthma endotype trait elements (see also figure 3). Definitions of these genes can be found via the NCBI website OMIM database (http://www.ncbi.nlm.nih.gov/sites/entrez).
and microarray profiling and genetic linkage studies have not suggested strong candidates for changed function. However, increased rate of shortening has been shown, and although the specific mechanism of this effect is disputed, faster or excessive shorting will probably be the major functional defect causing airway narrowing and symptoms. Clinical asthma is unlikely to occur in almost all cases without the contribution of airway smooth muscle. All known bronchodilators are functional antagonists, or direct pharmacological antagonists of contractile mediators, and they are not fundamentally able to prevent contraction to intense stimuli.

Airway hyper-responsiveness and dynamic inhomogeneity
Alexander and Paddock first described airway hyper-responsiveness to systemic pilocarpine in 1921. People with asthma often show pronounced airway hyper-responsiveness with loss of plateau that is orders of magnitude greater than in healthy controls. Airway smooth muscle responds differently to inflammation in patients with asthma, with substantial variation in degree and site of bronchoconstriction during the late-phase rise in inflammation that follows antigen challenge. This heterogeneity of constriction has been linked directly to the loss of bronchoprotective response to deep inspiration, which might indicate a defect in responses of airway smooth muscles to cyclic stretch, leading to risk of catastrophic bronchoconstriction. Dynamic hyperinflation caused by patchy constriction of large airways is also thought to contribute greatly to airway hyper-responsiveness. This dynamic inhomogeneity has been directly recorded by tomography (with hyperpolarized 3He-MRI or CT).

Steroids are able to reverse this defect only in the mildest disease. Airway hyper-responsiveness can be worsened by inflammation, but the main component is inherited separately. Palmer and colleagues noted that serum IgE concentrations, blood eosinophil counts, and airways responsiveness to inhaled agonist were inherited separately in human beings. Inflammation actually correlates poorly with airway hyper-responsiveness. Perhaps the greatest misunderstanding in basic (mouse) and applied asthma research is that intrinsic (inherited) and antigen/inflammation-induced airway hyper-responsiveness arise from different mechanisms. This notion is particularly ironic in asthma models in mice because they clearly show that basal airway reactivity is a heritable trait, whereas the small and transient labile component indicates changes in access of agonists to airways smooth muscle that is secondary to inflammation.

FEV<sub>1</sub> decline and fixed obstruction risk
Long-term outcome studies show that basal FEV<sub>1</sub> is set early in life and few patients with asthma have excessive rates of decrease. However, some patients—particularly those with adult onset asthma, smokers, and those with persistent uncontrolled eosinophil inflammation, airway hyper-responsiveness, or with inherited polymorphisms in ADAM33—can have rapid decline and progress to fixed obstruction. Some evidence suggests that inhaled steroid use can reduce an excessive decline, but this notion is controversial. Linkage studies have shown that inheritance of FEV<sub>1</sub> has no known genetic determinants in common with asthma severity or symptom score. Bisgaard and colleagues have noted that the rate of lung function decline and airway hyper-responsiveness in children is associated with lower lung function, delayed use of steroids for symptomatic disease, smoking, and positive allergic skin-prick test, which accords with Grol and co-workers’ earlier work. In childhood, lower lung function and lower increase in FEV<sub>1</sub>, predicts worse airway hyper-responsiveness in adulthood, but the molecular basis for this finding remains unknown.

Immunity, inflammation and remodelling, resolution, and repair
The interplay of immunity, inflammation, and remodelling has been a central theme in asthma research for decades. Eosinophilic inflammation is the trait that is best linked to symptoms and treatment responses, but alone it is not enough to cause asthma, which is absent in atopic, eosinophilic bronchitis, and Crohn’s disease (for which airway inflammation also occur). Similarly, atopic rhinitis with allergen exposure produces pathological changes similar to asthma in airways and cytokine release without asthma symptoms, and anti-interleukin-5 antibodies seem to have some benefit but only in a patient subset with a high eosinophil load. Lung eosinophilia is a useful biomarker to titrate steroid responsiveness, and decreasing eosinophils reduce the risk of exacerbations. Inflammation is heterogeneous in asthma. Steroids have little effect in neutrophilic asthma, and basal eosinophilia predicts steroid effect. Airway pathological changes are established early in life as basement membrane pseudo-thickening and angiogenesis are evident in children with asthma (and in atopic children without asthma). Similar early changes probably occur in lung nerves that undergo remarkable plastic changes in asthma, which could partly relate to increased cough. Good evidence also suggests that resolution processes are defective in asthma. Resolvin E1 (18R-trihydroeicosa-5,8,11,14-tetraenonic acid) suppresses experimental asthma in vivo, and pro-resolving lipoxins are diminished as asthma severity worsens. Production of interleukin 10, which exerts an inflammation-suppression effect under some conditions, is also dampened.

Exacerbations
Acute asthma exacerbations—usually but not uniformly caused by rhinovirus infection—are a major cause of morbidity in asthma. In severe asthma, five risk factors have been identified for recurrent exacerbations: severe nasal sinus disease, gastro-oesophageal reflux, recurrent...
respiratory infections, psychological affective disorders, and obstructive sleep apnoea. Rhinovirus infection affects lower airways even in healthy people, causing narrowing and inflammation. Eosinophilia is a known risk factor for asthma exacerbations. Patients with asthma do not have more frequent infections but rather more intense reactions. This finding has been linked to interplay of the EGF receptor (EGFR) with matrix metalloproteases and external regulated kinase (ERK) signalling, and primary or acquired interferon α and λ deficiency. Conventional plasmacytoid-dendritic cells govern lung viral immunity. The dendritic cell growth factor FLT3 induces conventional plasmacytoid and plasmacytoid-dendritic cells, and suppresses respiratory syncytial virus infection; however, little is known about variations across endotypes or the contribution of inhibitory pathways such as CD200.

Body-mass index, nutrition, and obesity

Thomas Platts-Mills is credited with proposing that watching television was the cause of the asthma epidemic in the 1980s. Analysis of 20016 children (aged 6–7 years) showed that high bodyweight, salty diet, and time spent watching television were independent risk factors for asthma. This finding is supported by a prospective multiple logistic regression analysis of 932 children in Boston. Body-mass index affects treatment outcome. Vitamin D deficiency, due to less sun exposure and indoor inactivity, has been proposed as an asthma cause. Vitamin D receptor expression in the lung is needed for inflammation and expression of experimental asthma, suggesting that vitamin D might have a stronger effect on host defense than inflammation does. Importantly, both the β, adrenocortico-steroid and glucocorticosteroid receptor, which are closely associated with asthma, are obesity candidate genes. Furthermore, asthma risk almost certainly begins in utero. Prenatal stress, smoke, and exposure to air pollutants all increase asthma risk.

Asthma–COPD overlap

Many asthmatics smoke, which worsens their disease and impairs steroid responses. Lapperre and colleagues used factor analysis statistics in 117 patients with COPD by measuring lung function, DLCO (the single-breath diffusing lung capacity for carbon monoxide), PC_{a}MeCh (the concentration of inhaled methacholine causing a 20% fall in FEV1), total IgE, exhaled nitric oxide, and differential cell counts in induced sputum. They noted that airflow inflammation and inflammation of the airways, and systemic features commonly associated with asthma (eg, IgE and eosinophils) were separate and predominantly independent contributors to COPD. These data are entirely consistent with genome-wide scans of COPD susceptibility and severity, and underscore the certainty that shared co-determinates of asthma and COPD exist in some patients. Interleukin-13 promoter polymorphism is associated with adverse effects of smoke on lung function, smoking, airway hyper-responsiveness, and eosinophils interact positively for respiratory symptoms. As in asthma, lung eosinophilia predicts smokers who will benefit from steroids; smoking greatly impedes the activity of steroids. The newly discovered intermediate pendrin, like EGF, induces mucin in both asthma and COPD. Furthermore, the interplay of interleukin β and tumour growth factor β—recently identified as a cause of small airways disease in COPD—could be a cause of fixed airflow restriction in asthma. Additionally, vitamin D biology, immune defense mediated by serpins and collectins, dysregulation of oxidative stress and apoptosis with decreased clearance of senescent cells, and secondary necrosis and impaired repair capacity are probably co-determinants of asthma and COPD.

Novel disease mechanisms

Asthma has a heritable component that is estimated to be between 36% and 94%. More than 100 plausible candidate genes have been suggested, but each has a
Interleukin-18 polymorphisms have been replicated in several genetic linkage studies. This cytokine is known to induce IgE by causing NKT cells to upregulate the co-stimulation molecule CD40 and interleukin 4. Interleukin 18, which potently induces interferon γ, has been implicated in processes relevant to neutrophilic and mixed inflammatory patterns because exogenous interleukin 18 triggers bystander memory cells—the types of cells that would reflect past viral infections—to release not only interferon γ, causing neutrophilia, but also interleukin 13, inducing airway remodelling. This mechanism might relate to asthma endotypes in which recurrent infection drives accelerated lung function decline or which show extensive neutrophilia. However, the role of NKT cells in asthma, where they have been implicated in processes relevant to neutrophilic and mixed inflammatory patterns because exogenous interleukin 18 triggers bystander memory cells—the types of cells that would reflect past viral infections—to release not only interferon γ, causing neutrophilia, but also interleukin 13, inducing airway remodelling. This mechanism might relate to asthma endotypes in which recurrent infection drives accelerated lung function decline or which show extensive neutrophilia. However, the role of NKT cells in asthma, where they have been linked to allergic (via interleukin 4) and neutrophilic (via interleukin 17) endotypes, is controversial.

Interleukin 33 is an interleukin-1-like cytokine that was identified initially as the ligand to an orphan receptor called T1/ST2, which is preferentially expressed on Th2 cells. However, interleukin 33 induces airway hyper-responsiveness and goblet hyperplasia and...
eosinophilic inflammation dependent on ST2 binding and transduction via the innate immunity transducer, MyD88, concurrently with induction of interleukines 4, 5, and 13. These effects occur in Rag−/− mice that do not have a functional adaptive immune system. These data suggest that Th2 mimicking pathological changes can be induced entirely in the absence of an adaptive immune system defining a second adaptive immunity independent endotype.46

Rhinovirus infection is the main cause of asthma exacerbations. Epidemiological studies have shown that patients with asthma do not have more frequent infections but rather more severe inflammation. Such patients also overexpress the EGFR. Liu and colleagues97 have identified a new mechanism governing the intensity of inflammatory responses to rhinovirus. Rhinovirus binds to ICAM-1, inducing upregulation of EGF and triggering an inflammatory response in the infected cell. In the presence of raised matrix metalloproteinase, excessive EGF is cleaved from the cell surface and binds to unregulated EGFR, sending a signal via ERKs that synergises with the direct response to rhinovirus, greatly enhancing inflammation.97 This mechanism is an example of how an asthma candidate gene (matrix metalloproteinase) with weak attributable risk can exert a stronger effect in an altered disease context. Because EGFR also mediates mucus induction, these results suggest the use of EGFR-tyrosine kinase inhibitors (eg, gefitinib or erlotinib) or antihuman epidermal growth factor receptor 2 family antibodies or blockers in some asthma endotypes. Whether EGFR affects the macrophage CD200 pathway, which limits viral inflammation, is not known.103

The role of macrophages in asthma has probably been greatly underestimated, and this cell lineage is increasingly researched as the role of innate immunity emerges. Macrophages do not always undergo classic activation, but might adopt alternative phenotypes96,142 associated with hallmark features such as induction of AMCases (chitinases), which are linked to asthma severity.141 Alternatively, macrophages are the main candidates for asthma endotypes that do not need T cells.

Much of the evidence that alternatively specified macrophages can achieve these effects comes from genetically manipulated mice. SHIP-1 is a negative regulator (ie, turns off) inflammatory cytokine and surface receptor signalling. SHIP-1 deficiency causes spontaneous asthma in mice with a Th2-like pattern,144 including AMCases induction.144 Similarly, mice with activated Hck—a Src family kinase—develop an aggressive T-cell–independent eosinophilic lung inflammation associated with progressive airway fibrosis.145,146 Lyn is related to Hck. A profound and multi-trait severe asthma syndrome develops in Lyn-deficient mice who display hyper-IgE, enhanced bronchoconstriction, mast cell, and eosinophil degranulation; very persistent inflammation associated with deficient apoptosis; and Th2-like cytokines together with enhanced interferon γ.149

Biochemically, alternative macrophage activation has been linked to tumour growth factor β and interleukin 13.146 Because these responses can occur in the complete absence of T cells, interleukin 13 probably exerts its effect via the type II interleukin-4 receptor.149 These findings might explain why interleukin 13 is a candidate gene in both asthma and COPD.150

Summary and implications

This Review has developed the argument that the Th2-inflammation hypothesis, although useful, is not adequate to understand the substantial heterogeneity of asthma. The first iteration of an open-frame asthma endotype model has been presented and discussed in the context of entirely novel disease pathways, many of which are independent of adaptive immunity. The role of innate immunity, which is intrinsically insensitive to steroids and can be driven by tissue damage, has been emphasised. There are important implications. Definition of asthma endotypes opens the possibility of much more precise disease classification and definition of biomarkers that meet formal diagnostic or prognostic criteria. From analysis of several asthma candidates across many endotype components, effective future therapies will be used as an adjunct to existing medicines or will be combinations of activities, since few known candidates affect enough crucial endotype components to be effective in their own right. Some asthma endotypes will be reclassified as orphan diseases. Because the inclusion criteria and endpoints for clinical trials directed at some novel endotypes are likely to be unvalidated, we will probably increasingly use adaptive clinical trial methods to identify responding patient endotypes. In adaptive clinical trials, responsive patients (and non-responding patients) are identified during the course of the trial itself (allowing the trial design to be adapted while running) rather than during retrospective statistical analysis after the trial has closed. Specific definition of asthma endotypes should also spur and redirect basic research to discover the mechanisms and highly innovative ideas that are required to improve asthma therapy and ultimately prevent or cure the disorder or disorders.

Conflict of interest statement

I declare that I have no specific conflict of interest with the material presented in this Review. Currently, or within the past 3 years, I have received consultancy, travel, and speaker fees from AstraZeneca Pharmaceuticals in relation to β-agonist steroid combination products. I have received consultancy fees from Roche Pharmaceuticals in relation to review of preclinical investigational concepts and compounds. My laboratory has several full-time staff who undertake fee-for-service testing of compounds in preclinical animal models of asthma and COPD, for which the surplus arising is used to support basic research. The University of Melbourne has licensed patented intellectual property arising partly from research in my laboratory on GM-CSF to MorphoSys AG, Germany, for the treatment of chronic inflammatory disorders including lung disease.
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