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.

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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).[2–6] Furthermore, asthma—the most common serious chronic lung disease afflicting around 150 million people worldwide—remains both unpreventable and incurable.[7] Despite decades of intensive research, little progress in identification of new treatments has been made since the introduction of inhaled β_2 adrenoceptor selective agonists (1969) and inhaled glucocorticosteroids (1974).

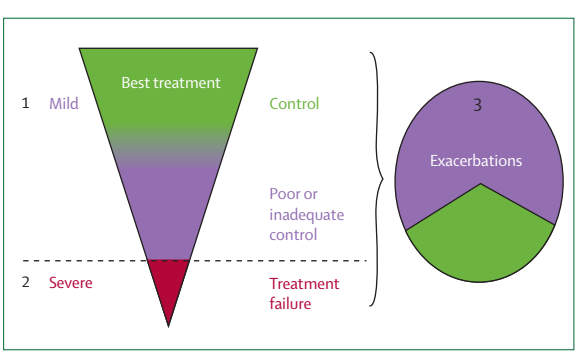

Figure 1: **Residual disease burden in optimally treated asthma**
The diagram shows that as severity increases (purple), the number of afflicted individuals decreases (represented as an inverse triangle). Exacerbations are represented as a pie chart. Severe asthma (red triangle), which is defined as failure of optimised treatment, is rare and estimated at about 5% of all disease. Notional best possible treatment (green) shows (1) residual disease in patients with moderate disease, (2) refractory severe asthma, and (3) breakthrough acute exacerbations. The purple and red regions represent the targets for future therapies. Th2-directed therapies will most probably compete with present therapies for mild asthma.

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, preventions, 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

National Center for Biotechnology Information (NCBI) public domain databases, Pubmed, and OMIM (On-line mendelian inheritance in man) were searched by combining the key term "asthma" sequentially with each of the following descriptors: "phenotype", "subtype", "factor analysis", "principal component analysis", "cluster", "clade", "classification", "pattern recognition", "severe", "severity", "refractory", "complex disease", "heterogeneity", "variant", "inflammation", "sputum", "biopsy", "eosinophil", "neutrophil", "lymphocyte", "Th2", "IL4", "IL13", "GATA3", "TBET", "macrophage", "immunity", "immunity-adaptive", "immunity-innate", "Treg", "IL17", "IL23", "biomarker", "exhaled", "condensate", "exacerbation", "virus", "COPD", "atopy", "epidemiology", "genome-wide scan", "candidate gene", "ontology", "childhood", "remission", "lung function", "FEV1 decline", "airway reactivity", "bronchial hyperreactivity", "airway smooth muscle", "inhomogeneity", " resolution", " repair", "resolving", " lipoxin", "obesity", "comorbidity", "perception", and "dyspnea". Very recent papers within the past 3 years have been preferentially selected. Human asthma candidate genes, or mouse genes with human homologues, identified in the primary search from gene profiling studies, linkage studies, or from basic and clinical biology were assigned to endotype component categories on the basis of gene ontogeny (GO) categories, OMIM, and published work proximity to relevant processes with the iHoP (Information hyperlinked over Protein), search tools, and biological plausibility.

***Panel 1:* What is an endotype?**

Endotype—a contraction of endophenotype—is a subtype of disease defined functionally and pathologically by a molecular mechanism or by treatment response. Asthma, like many chronic disorders, is a heterogeneous and genetically complex disease, meaning that many genes (>100 have been identified) are likely to contribute, variably, to its different manifestations. Asthma is likely to have several specific endotypes associated with distinct clinical features, divergent underlying molecular causes, and distinct treatment responses.

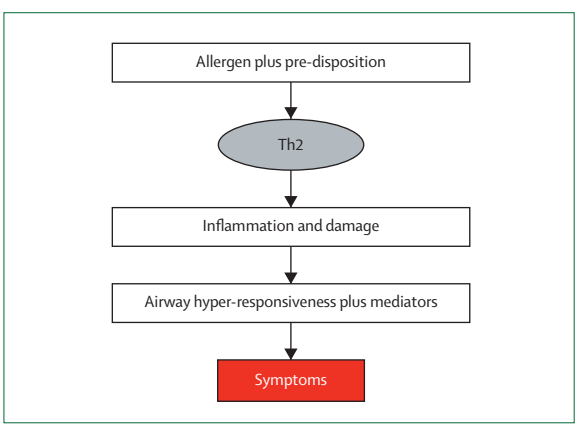

***Figure 2:* Linear representation of the Th2-inflammation hypothesis**
The diagram shows the most common view of asthma, which is that allergen exposure in sensitive individuals induces Th2 immune deviation. Th2 immunity drives eosinophilic inflammation and tissue damage, resulting in airway hyper-responsiveness and mediator release. These, in turn, cause symptoms. The hypothesis is simple but does not accord well with current evidence and cannot explain the heterogeneity of asthma.

hypothesis; asthma heterogeneity in terms of ontogeny, clinical phenotypes, and molecular patterns; asthma endotypes; and novel mechanisms, with particular emphasis on alternative macrophage 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 hypothesis[8,9] 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.[8,9] 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.[14] 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.[15–17] However, airway inflammation is much the same between non-asthmatic atopics, allergic rhinitics, and atopic asthmatics.[18] An almost identical pattern of response occurs after allergen challenge in people allergic to house-dust mites with and without asthma.[19–21] The Th2-inflammation model predicts that eosinophilic inflammation should drive airway hyper-responsiveness, but no clear relation exists;[22,23] furthermore, population studies show atopy and airway hyper-responsiveness are not concordant.[24] 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.[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.[27]

T-cell immunosuppressive drugs (eg, ciclosporine 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.[28] Furthermore, glucocorticosteroids (and β_2 agonists), which are highly effective in atopic asthma with eosinophilic inflammation, paradoxically consolidate and intensify Th2 immunity and increase IgE.[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).[30,31] Steroids also dampen expression of T-bet, the transcription factor controlling Th1 (but not Th2) immunity.[32] Steroid sensitivity wanes in severe asthma; however, genetic manipulation to enhance steroid effectiveness does not suppress experimental asthma and instead favours Th2 immunity.[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.[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,[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.[36] 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_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.[13] 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.[38] 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.[39,40]

Panel 2: Definitions of asthma and indices of severity

The first systematic attempts to define asthma were made in the 1970s but, despite decades of effort, there is still no specific definition of, or validated diagnostic algorithm for, the disease. Instead, asthma is defined functionally as an inflammatory disorder linked to hyper-responsiveness that causes symptoms:

"Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment."[37]

Similarly, no current methods exist to objectively define disease severity other than by functional criteria:

- day-time symptoms
- limitations of activity
- nocturnal symptoms and wakening
- need for rescue/reliever drugs
- lung function as percentage predicted
- exacerbations
- treatment responses (success or failure to improve or control these factors)[37]

These functional or operational criteria are, however, practical, pragmatic, and very well thought out because they are simple, work in the context of global asthma guidelines, and allow the disease to be managed even in health-care systems with constrained resources.

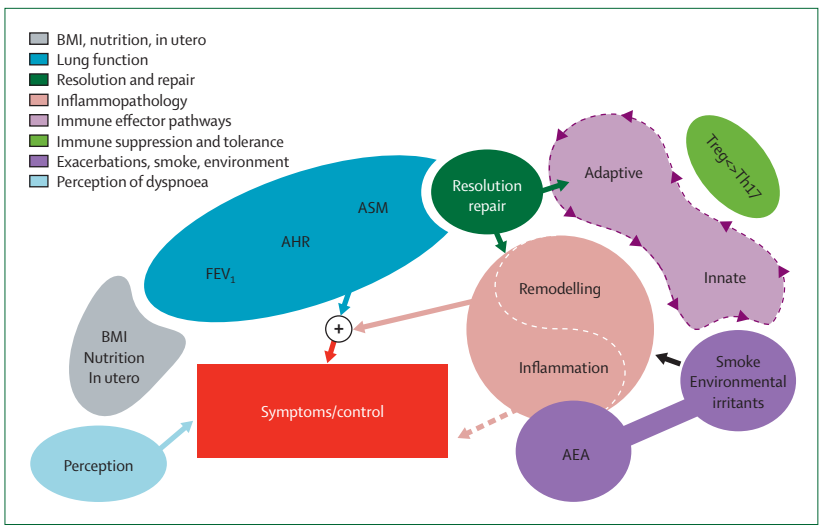

Figure 3: **Open framework asthma endotype model**
The diagram represents processes that are known to be involved in the diverse presentations of asthma as colour-coded elements. Distinct endotypes arise through the variable interplay of these components. In the model, asthma symptoms almost always arise through the interaction of altered lung function (forced expiratory volume in 1 second [FEV_1], airway hyper-responsiveness [AHR], and airway smooth muscle [ASM]) and immunopathologies (remodelling/inflammation) but these symptoms arise and interact independently. Modifying process such as body-mass index (BMI), diet, and in utero exposures; CNS sensitivity to dyspnoea (perception); acute asthma exacerbations (AEA); cigarette smoke (smoke) and environmental irritants are shown impinging on lung function on remodelling and inflammation. Both divisions of the immune system, (adaptive and innate), are shown closely interconnected (arrowed dotted line) and regulated by immune tolerance (Treg) in equilibrium with Th17 immune activation. Defective healing is represented by resolution/repair. Immunity, together with smoke and environmental contaminants, and also exacerbations, are shown interacting with inflammation and remodelling, but no assumption is made that remodelling is dependent on inflammation. Because asthma is defined by symptoms, defects in lung function are essential, and their necessary interaction with other components is indicated by +. Candidate genes linked to these processes are listed in the table.

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,[41] which occur despite the airway biopsy pathology of non-atopic asthma being almost indistinguishable from atopic asthma in children.[42] This finding suggests that very early intervention with steroids would be beneficial, but this is not the case,[43] perhaps because steroids actually worsen Th2 immune deviation.[29] 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.[44] Asthma affects more boys than girls in childhood, but more women in later life.[45] 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,[46,47] IRAK-M (a negative regulator of innate immunity),[48,49] epidermal growth factor (EGF), interleukin 6, and prostaglandin E2.[50]

The heterogeneity of asthmatic inflammation is well documented; Golash first suggested sputum eosinophilia as a hallmark of disease in the 1890s. Woolcock and Peat[24] 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[25] 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[25,26] and that this trait, not airway remodelling, is associated with airway hyper-responsiveness.[51]

More recently, Simpson and colleagues[52] 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) paucigranuloctic (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.[53] Haldar and Pavord[54] 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.[55] Similarly, application of factor analysis to molecular genetics studies tends to segregate, rather than cluster, atopic and asthmatic disease traits.[56] Segregation of inflammatory subtypes by non-invasive exhaled breath methods is not yet possible.[57]

The biology of very severe asthma is almost certainly distinct from milder forms of disease, and severe asthma is heterogeneous.[14] 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.[58] Two major subdivisions of severe asthma have been proposed on the basis of discordant inflammatory patterns.[59,60] Furthermore, Pavord and colleagues[61] have reported severe asthma in the absence of eosinophilic inflammation and, by inference, Th2 immunity. Brasier and co-workers[55] 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 responses to increases in cyclic AMP;[62] RIP-2 (receptor-interacting serine-threonine kinase 2), an intermediate in toll-like receptor signalling;[63] reduced generation of pro-healing lipoxin A4; or anti-inflammatory interleukin 10.[64,65]

Combination therapy (bronchodilator plus inhaled glucocorticsteroid) 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 1). Other therapies have also proven to be of measurable, but lesser and variable benefit—eg, cysteinyl-leukotriene receptor antagonists and anti-IgE monoclonal antibodies.[2–6] 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.[66] Primary prevention has not proven possible so far, since a meta-analysis shows that avoidance of house-dust mites is not effective.[7] The β_2 adrenoceptor is polymorphic and its Arg16 variant might adversely affect response to regular short-acting drugs but not long-acting β agonists.[67] About 15% of patients with asthma respond well to leucotriene antagonists and, although urinary concentrations of leucotriene do not predict responses, polymorphism in 5 lipoxygenase (ALOX) and the receptor (CYSLTR2) can establish drug sensitivity.[68,69] Steroid responses are very heterogeneous in childhood and adult asthma.[31,70]

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.[52] 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: it 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.[71,72] More smooth muscle exists in airways of asthmatic patients, but alone it might not be a cause;[51] 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.[73] Increased mast cell number and degranulation have been suggested as pathological indicators of changed function.[25,51]

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

	Genetic	Biology
Lung function: basal FEV_1, airway hyperresponsiveness, airway smooth muscle	*EDN1, ADAM33, B2ADR, CREB, CCR5, COL29A1, CSTA, CYSLTR1, CYSLTR2, EP2, FCER2, GSTM, HNMT, KCNS1, LELP1, MMP, MUC7, MLCK, NK2R, PDGFRA, PLA2, PLAU, PTGDR, PTGER, PTGIR, TBX21, VDR*	CREB, GSNOR, NOS, NR3C1
Immunity	*FLG, IL17F, TGFβ, IL6, RORα, RORγ, BDNF,* chemokines, *CD14, CD40, CD86, DPP10, FCER2, FLG, HLA-G, ICOS, IGHG, IL12B, IL2, IL4, IL6, IL9, IL10, IL13, IL16, IL17, IL18, IL27, IL33, IRAKM, ITK, MICB, MMP, MRP1, MUC1, NOD, PHF11, PLA2, PPARG, PTGDR, PTGER, RIP2, RUNX1, SFTPC, SOCS, SPP1, STAT6, TBX21, TIM1, VDR, VEGFR*	CREB, HCK, IL23, IL33, LYN, NFATc, NOS, NR3C1, PTEN, RIP, ROR, SHIP, SHP, TSLP
Inflammation and remodelling	*EDN1, ADAM33, IL17A, IL17F, NRF2, SOD, CREB, VDR, CAT,* chemokines, *COL29A1, CSTA, DPP10, ECP, EP2, FYN, GSTM, IGHG, IL2, IL5, IL9, IL13, IL17, IL18, IL33, PLA2, PLAU, SOCS, STAT6, TNFA, UTG, VEGFR*	AMCase, ARG, C3AR1, c-kit, C3\5, EGFR, CSF2(=GM-CSF), HCK, HMGB1, LYN, NOS, NR3C1, NRF2, PTEN, RAGE, RIP, SCF, SHIP, SHP, SOD, TIMP, TSLP.
Resolution and repair	*VDR, LEP*	IL-10, FAS, NR3C1, RAGE, TIMP, Lipoxin *A4* (15LOX, 5LOX), presqualene phosphates
Exacerbations, smoke, and environmental irritants	*VDR, AOAH, CAT, CYP24A1, GSNOR, HLA-G, IL12B, IL2, IL6, IL12, IL17, IL23, IL33, IRAKM, MMP, MRP1, NOD, SFTPC, UTG, NRF2*	CD200, EGFR, IFN, NR3C1, SOD, TIMP
BMI and nutrition	*ADRB2, FABP, NR3C1, VDR, FABP4, NR3C1*	
Perception	*KCNS1, GAD65*	
Genes of unknown function from linkage	*DCNP1, GCLM, ORMDL3, SCGB3A2*	

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 OMIN database (http://www.ncbi.nlm.nih.gov/sites/entrez).

Table: **Putative contribution of asthma candidate genes to endotype elements**

and microarray profiling and genetic linkage studies have not suggested strong candidates for changed function.[74] 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.[75] 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.[76] 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.[77,78] This heterogeneity of constriction has been linked directly to the loss of bronchoprotective response to deep inspiration,[79] which might indicate a defect in responses of airway smooth muscles to cyclic stretch, leading to risk of catastrophic bronchoconstriction.[80] Dynamic hyperinflation caused by patchy constriction of large airways is also thought to contribute greatly to airway hyper-responsiveness.[81] This dynamic inhomogeneity has been directly recorded by tomography (with hyperpolarited 3He-MRI or CT).

Steroids are able to reverse this defect only in the mildest disease.[82] Airway hyper-responsiveness can be worsened by inflammation, but the main component is inherited separately.[83] Palmer and colleagues[84] 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.[85] 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.[84,85]

FEV_1 decline and fixed obstruction risk

Long-term outcome studies show that basal FEV_1 is set early in life and few patients with asthma have excessive rates of decrease.[58,86,87] However, some patients—particularly those with adult onset asthma, smokers, and those with persistent uncontrolled eosinophilic inflammation, airway hyper-responsiveness, or with inherited polymorphisms in *ADAM33*—can have rapid decline and progress to fixed obstruction.[88] Some evidence suggests that inhaled steroid use can reduce an excessive decline, but this notion is controversial.[87] Linkage studies have shown that inheritance of FEV_1 has no known genetic determinants in common with asthma severity or symptom score.[89] Bisgaard and colleagues[90] 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,[90] which accords with Grol and co-workers' earlier work.[87] In childhood, lower lung function and lower increase in FEV_1 predicts worse airway hyper-responsiveness in adulthood,[91] 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 atopy, eosinophilic bronchitis, and Crohn's disease (for which airway inflammation also occur).[92] Similarly, atopic rhinitis with allergen exposure produces pathological changes similar to asthma in airways and cytokine release without asthma symptoms,[21] 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.[93] 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).[94] 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-trihydroeicosapentaenoic acid) suppresses experimental asthma in vivo,[95] and pro-resolving lipoxins are diminished as asthma severity worsens.[96] Production of interleukin 10, which exerts an inflammation-suppression effect under some conditions, is also dampened.[65]

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.[97] Rhinovirus infection affects lower airways even in healthy people, causing narrowing and inflammation.[98] Eosinophilia is a known risk factor for asthma exacerbations.[16] 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,[99] and primary or acquired interferon α and λ deficiency.[100,101] 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;[11] however, little is known about variations across endotypes or the contribution of inhibitory pathways such as CD200.[97,102]

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.[103] Analysis of 20 016 children (aged 6–7 years) showed that high bodyweight, salty diet, and time spent watching television were independent risk factors for asthma.[104] This finding is supported by a prospective multiple logistic regression analysis of 932 children in Boston.[105] Body-mass index affects treatment outcome.[106] Vitamin D deficiency, due to less sun exposure and indoor inactivity, has been proposed as an asthma cause.[107] 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.[108,109] Importantly, both the β_2 adrenoceptor and glucocorticosteroid receptor, which are closely associated with asthma, are also obesity candidate genes. Furthermore, asthma risk almost certainly begins in utero. Prenatal stress, smoke, and exposure to air pollutants all increase asthma risk.[110–112] Asthma is defined by symptoms, and patients who have poor perception of the severity of their disease (under-perceivers) and patients who suffer overt symptoms but show only small changes in lung function (over-perceivers) have been described. Because dyspnoea is perceived in the anterior insula and amygdala,[113] perception modifying neuronal mechanisms will probably emerge in coming years.

Asthma–COPD overlap

Many asthmatics smoke, which worsens their disease and impairs steroids responses. Lapperre and colleagues[114] used factor analysis statistics in 117 patients with COPD by measuring lung function, DLCO (the single-breath diffusing lung capacity for carbon monoxide), PC_{20} MeCh (the concentration of inhaled methacholine causing a 20% fall in FEV_1), total IgE, exhaled nitric oxide, and differential cell counts in induced sputum. They noted

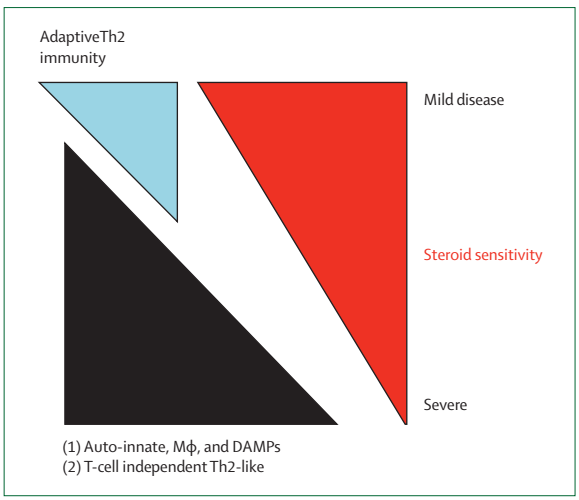

Figure 4: **The unexpected importance of innate immunity in asthma severity and reduced steroid sensitivity**
Innate immunity is associated with more severe disease and steroid insensitivity. The diagram shows that as disease severity worsens and becomes less steroid sensitive (red), the contribution of innate immunity increases (black). Classic Th2 immunity is shown in blue. Two novel processes are represented: (1) Auto-innate immunity is shown activating macrophages (tissue is also activated), particularly by factors released from damaged tissue (DAMPs) that trigger innate immunity locally; and (2) T-cell independent processes mediated by innate immunity that resemble Th2 immunity. MΦ=macrophage.

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.[114] 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.[115–118] Interleukin-13 promoter polymorphism is associated with adverse effects of smoke on lung function,[119] and smoking, airway hyper-responsiveness, and eosinophils interact positively for respiratory symptoms.[120] As in asthma, lung eosinophilia predicts smokers who will benefit from steroids; smoking greatly impedes the activity of steroids.[121,122] The newly discovered intermediate pendrin, like EGF, induces mucin in both asthma and COPD.[123] Furthermore, the interplay of interleukin 1β 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.[124] 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

very low attributable risk (<5%), with often poor replication[125] or lack of plausible biology or functional single nucleotide polymorphisms. Of this very large information set, two broad new notions are emerging: auto-innate immunity driven by tissue damage and Th2-like responses that occur without T cells (figure 4).

Until recently, innate immunity was thought of only as a rapid front-line defender against infections. It works by triggering host defence after recognition of pathogen associated molecular patterns (PAMPs) on invading pathogens. However, endogenous ligands released from damage associated molecular patterns (DAMPs—ie, ligands such as heat shock proteins, RAGE ligands, and HMGB1) are very able activators of innate immunity. Like PAMPs, DAMPS often signal via the toll-like receptor system and its characteristic MyD88 transduction pathway to directly promote inflammation. Innate immunity is intrinsically resistant to steroids,[40] which could help to explain why as asthma severity worsens, steroid sensitivity decreases (the molecular basis for this notion is an amalgam of processes including HDAC nitrosylation, MAP kinase phosphatase induction, and inhibitory signalling from altered matrix). This notion has a profound implication: wherever DAMPs and innate immunity contribute to disease, the process will be intrinsically steroid insensitive, which might be why more severe asthma endotypes with established tissue damage are steroid resistant. Furthermore, the main source of ligands to drive this auto-innate immunity is damaged tissue, which is normally removed by macrophages. However, as oxidative stress worsens (with severity or smoking) the ability of macrophages to recognise and remove effete cells decreases, leading to much greater so-called spill of DAMPs.[126,127]

A second major emerging idea is that there are mechanisms to induce Th2-like effects in the absence of Th2 cells, which is probably the reason why interleukin 13 is so widely associated with highly divergent asthma endotypes. In both cases, the existence of these pathways most probably is an indicator of phylogenetic evolution in that ancient innate immunity, and many of its effector cytokines, predate the evolution of lymphocytes and glucocorticosteroids by million of years. Molecular dissection of these processes is suggesting new intervention points for asthma endotypes.

Interleukin 17 came to attention as an indirect mediator of sustained neutrophilic inflammation.[128,129] Evidence suggests that interleukin 17 is a mediator of neutrophil variant and severe neutrophilic asthma endotypes.[130] A defined T-cell subset—Th17—has been identified, but interleukin-17 family cytokine production is not constrained to T cells. Th17 differentiation is dependent on the retinoid receptors RORα and RORγ (implicating diet and lipid metabolism),[131] and signalling intermediates STAT3 and STAT4.[132] Interleukin 17 is induced in human beings by interleukin 23, which is related to interleukin 12 (a negative regulator of Th2 immunity), especially in the context of interleukin 1β, interleukin 6, and tumour growth factor β. This mixture is important because tumour growth factor β and interleukin 6 exert counter-balancing control of the regulatory T cells (Tregs) that suppress inflammation, indicating a fine balance between suppression and disease.[130,133,134]

Dendritic cells are poised at the interface between innate and adaptive immunity, and are essential for both the induction and maintenance of allergic inflammation. Coactivation of dendritic cells by antigen in the presence of stem cell factor—a potent mast cell growth factor—induces sustained interleukin-6 production, triggering concurrent Th2 and Th17 induction via the c-kit receptor.[135] Because stem cell factor is also implicated in mast cell infiltration of airways smooth muscle, this axis predicts the usefulness of imatinib mesylate (Gleevec, or related compounds), which has potent c-kit receptor kinase blocking activity in some asthma endotypes, especially when mast cells are concurrently implicated.

Viral bronchiolitis in infancy, especially with respiratory syncytial virus, is a risk factor for persistent asthma. In rodents, similar exposure leads to life-long inflammation and phenotypic alteration of the lung. Kim and colleagues[136] have discovered that activation of macrophages by CD1d expressing natural killer T (NKT) cells induces production of interleukin 13 and its pathological sequelae entirely independently of adaptive immunity. This mechanism operates in both human asthma and COPD,[136] and defines an adaptive immunity (ie, T cell) independent endotype. Smoke directly and substantially worsens viral inflammation and remodelling.[137,138]

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.[139] 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 interleukins 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.[140]

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 colleagues[99] 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.[99] 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-tyrosone 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.[102]

The role of macrophages in asthma has probably been greatly underestimated, and this cell linage is increasingly researched as the role of innate immunity emerges. Macrophages do not always undergo classic activation, but might adopt alternative phenotypes[141,142] associated with hallmark features such as induction of AMCases (chitinases), which are linked to asthma severity.[143] Alternatively, specified 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,[144–146] including AMCases induction.[143] Similarly, mice with activated *Hck*—a *Src* family kinase—develop an aggressive T-cell- independent eosinophilic lung inflammation associated with progressive airway fibrosis.[145–147] *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.[148]

Biochemically, alternative macrophage activation has been linked to tumour growth factor β and interleukin 13.[141] 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 AstraZenceca 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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