

PROFESSOR DOUGLAS S ROBINSON (Orcid ID : 0000-0002-0578-740X)

DR PARAMESWARAN NAIR (Orcid ID : 0000-0002-1041-9492)

Received Date : 11-Oct-2016

Revised Date : 21-Dec-2016

Accepted Date : 27-Dec-2016

Article type : Unsolicited Review

Revisiting Type 2-high and Type 2-low airway inflammation in asthma: current knowledge and therapeutic implications

Authors: Douglas Robinson¹, Marc Humbert², Roland Buhl³, Alvaro A. Cruz⁴, Hiromasa Inoue⁵, Stephan Korom⁶, Nicola A. Hanania⁷, Parameswaran Nair⁸

Author affiliations

¹ Department of Respiratory Medicine, Severe Asthma Service, UCLH NHS Foundation Trust, London, UK

² Service de Pneumologie, Hôpital Bicêtre, Assistance Publique Hôpitaux de Paris; Univ. Paris-Sud, Université Paris-Saclay; INSERM U999, Le Kremlin-Bicêtre, France.

³ Pulmonary Department, Mainz University Hospital, Mainz, Germany

⁴ ProAR-Center of Excellence in Asthma, Federal University of Bahia School of Medicine, Salvador, Brazil

⁵ Department of Pulmonary Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

⁶ F. Hoffmann-La Roche Ltd, Basel, Switzerland

⁷ Section of Pulmonary and Critical Care Medicine, Baylor College of Medicine, Houston, Texas, USA

⁸ Division of Respiriology, Department of Medicine, McMaster University, Hamilton, Ontario, Canada

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cea.12880

This article is protected by copyright. All rights reserved.

Corresponding author

Douglas Robinson

Department of Respiratory Medicine,
Severe Asthma Service,
UCLH NHS Foundation Trust,
UCLH NHS Trust,
London, UK

Email: douglas.robinson@uclh.nhs.uk

Fax: +44 203 447 9476

Abstract

Asthma is a complex respiratory disorder characterized by marked heterogeneity in individual patient disease triggers and response to therapy. Several asthma phenotypes have now been identified, each defined by a unique interaction between genetic and environmental factors, including inflammatory, clinical and trigger-related phenotypes. Endotypes further describe the functional or pathophysiologic mechanisms underlying the patient's disease. Type 2-driven asthma is an emerging nomenclature for a common subtype of asthma and is characterized by the release of signature cytokines IL-4, IL-5 and IL-13 from cells of both the innate and adaptive immune systems. A number of well-recognized biomarkers have been linked to mechanisms involved in Type 2 airway inflammation, including fractional exhaled nitric oxide, serum IgE, periostin, and blood and sputum eosinophils. These Type 2 cytokines are targets for pharmaceutical intervention, and a number of therapeutic options are under clinical investigation for the management of patients with uncontrolled severe asthma. Anticipating and understanding the heterogeneity of asthma and subsequent improved characterization of different phenotypes and endotypes must guide the selection treatment to meet individual patients' needs.

Introduction

Asthma is a complex respiratory disorder with marked heterogeneity in etiology, symptom triggers, clinical characteristics and responses to therapy [1–3]. Research that embarked on better understanding this heterogeneity has led to increasing recognition and improved characterization of various asthma phenotypes that describe the outward manifestation of an individual's airway inflammation and that may be useful for predicting responsiveness to specific treatments [4]. In contrast to these recognizable clinical features in patients, the term 'endotype' has recently been introduced in relation to asthma heterogeneity to describe "a subtype of a condition defined by a unique or distinctive functional or pathophysiological mechanism" [5,6]. As opposed to phenotypes, which may change with time or in response to treatment, endotypes are relatively stable subgroups defined by underlying distinct genetic or molecular characteristics [7].

Biologic heterogeneity is present throughout the entire asthma spectrum, but is most evident in severe disease [8], which is defined by the International ERS/ATS Guidelines on Definition, Evaluation and Treatment of Severe Asthma as asthma "which requires treatment with high-dose inhaled corticosteroids [ICS] plus a second controller [and/or systemic corticosteroids] to prevent it from becoming "uncontrolled" or which remains "uncontrolled" despite this therapy" [9]. The current "one-size-fits-all" approach to asthma treatment does not target specific mechanisms that can drive an individual patient's disease and, therefore, has varying degrees of success.

The emerging knowledge of the pathophysiology of asthma

Historically, the earliest applied approach to classify asthma has been the assessment of the atopic status of an individual, with atopy referring to the patient's sensitization to allergens and consequent predisposition to developing asthma symptoms (extrinsic or allergic asthma) upon exposure [10].

Allergic asthma is diagnosed based on symptoms triggered by allergen exposure, and atopy can be confirmed by testing skin prick reactivity to common or specific allergens and/or by measuring the serum levels of specific immunoglobulin E (IgE) [1,10], which plays a major role in sustaining the allergic cascade in asthma [11]. Although thought as being rather distinct forms of asthma, atopic and non-atopic (non-allergic or intrinsic) asthma phenotypes are highly overlapping in their clinical

presentation and in the underlying inflammatory processes, including increased T-helper type 2 (Th2) cells, mast cell activation and eosinophilic airway infiltration [12–14].

Another approach to classify the disease pathophysiology has focused on the patterns of cellular inflammation and sequestration, particularly eosinophils and neutrophils, in the asthmatic lung. Four distinct subtypes of asthma based on the inflammatory cell count in induced sputum have been proposed, namely, eosinophilic asthma (eosinophils > 1.9%–3%), mixed eosinophilic and neutrophilic asthma (mixed granulocytic asthma; neutrophils and eosinophils both increased), neutrophilic asthma (neutrophils > 61% and total cell count greater than 10 million cells/g) and paucigranulocytic asthma (neutrophils and eosinophils both within normal range) [15,16]. Eosinophilic asthma is characterized by a higher number of eosinophils measured in sputum, bronchoalveolar lavage or blood. In patients with symptomatic asthma, eosinophilic inflammation (which was defined as > 2.5% eosinophils in sputum) has been reported in 70% to 80% of corticosteroid-naïve patients and 50% of corticosteroid-treated asthmatics [17], can occur in both allergic and non-allergic patients [14,18], and presence of eosinophils in sputum or lung biopsies is observed across the severity spectrum [15,19].

Further heterogeneity exists among patients with non-eosinophilic asthma, with some of these having also increased numbers of neutrophils in the airways [16]. Neutrophilic asthma is rare, but may be seen in patients with severe disease and it may be less responsive to corticosteroid treatment than eosinophilic asthma [3,20]. It is not clear if the neutrophil involvement of this phenotype is contributing to asthma pathobiology or severity or if it is simply a reflection of the level of corticosteroid therapy used to suppress the poorly corticosteroid-responsive non-neutrophilic inflammation or of an altered airway microbiome perhaps secondary to abnormalities of innate immunity [21–24]. Strategies such as inhibiting the CXCR2 receptor to decrease airway neutrophils are not associated with improved asthma control [25,26], bringing the role of neutrophils in severe asthma into question. When associated with an eosinophilic bronchitis (i.e. mixed granulocytic), this may indicate airway infections that may be contributing to relative steroid insensitivity [27].

By discovering two distinct types of T helper clones in mice in 1986, which could be distinguished based on their cytokine secretion profile, Mosmann *et al.* [28] not only advanced the understanding of adaptive immunity, but also provided the foundation for a new classification of asthma [29]. The Type 1 and Type 2 helper cell immune response paradigm describes defined immune responses that are

mediated by subpopulations of CD4⁺ T-cells. Th1 cells secrete mainly interleukin (IL)-2, interferon- γ and lymphotoxin- α [30], which orchestrate the defence against intracellular pathogens (e.g. bacteria and viruses). Th2 cells predominantly secrete cytokines IL-4, IL-5, IL-9 and IL-13, which play a central role in the inflammatory process directed against helminths and extracellular bacteria, and also in the pathophysiology of asthma [29,30]. In 1992, the predominance of a Th2-population of cells in the bronchoalveolar lavage from atopic asthmatics was shown [31]. Subsequent gene expression analyses (using epithelial [32] or sputum signatures [33]) suggest asthma can also be divided into at least two distinct molecular phenotypes based on the degree of Th2 inflammation; these have been described as Th2 “high” and Th2 “low”. Whether the Th2 low phenotype is a consistent and different type of asthma from Th2 high, or results from suppression of Th-2 inflammation by corticosteroids, remains to be established. These Th2 high and low phenotypes may show differential responses to available therapies, whereby patients with non-Th2-driven asthma may be less responsive to steroids compared with a predominantly Th2-high disease [32].

Recently, the nomenclature of asthma has evolved beyond the original Th2 concept to acknowledge that the cytokines associated with Type 2 helper lymphocyte polarization are broadly secreted by numerous cell types beyond the originally described Type 2 helper cell population. These include invariant T-cells, natural killer (NK) cells, eosinophil/basophil progenitor cells and Th1 cells under certain conditions [34], and Type 2 innate lymphoid cells (ILC2s) (for review please see [35]). ILC2s are part of the innate immune system, where antigen interaction and recognition are not required to elicit Type 2 cytokine secretion. Tightly regulated, they are an integral part of epithelial barrier immunity, involved in the pathophysiology of asthma [36], atopic dermatitis [37] and food allergy [38].

Type 2 inflammation has emerged as a pivotal disease mechanism that allows identifying patients with a distinct, Type 2-high endotype [29,32]. Type 2-low asthma, on the other hand, probably comprises several disease endotypes, each affecting relatively small subgroups of patients. For instance, recently a pathway that involves Th17 cells and that promotes, via the release of IL-17, neutrophil recruitment for the clearance of bacterial and fungal infections [39–41], has been described. The relative importance of this different inflammatory pathway may vary depending on the severity of asthma.

This review aims to summarize the current knowledge regarding Type 2 asthma, including the relevant cytokine pathways and biomarkers, and to identify areas of gaps in knowledge requiring future research. The focus on 'Type 2' rather than 'Th2' asthma reflects the important contribution of ILC2s and other of non-classic Th2 cells (CD4+ cells), in addition to Th2 cells, to the Type 2 cytokine milieu. We will consider how information on Type 2-high and Type 2-low involvement may be utilized by physicians to guide treatment decisions and what the relative contribution of the different inflammatory pathways might mean to patients in terms of the clinical endpoints.

Type 2 pathway of airway inflammation

For many years, asthma was considered to be mediated predominantly by an adaptive immune response; however, there is increasing recognition that innate immunity also plays an important role in asthma pathophysiology, with cross-talk between the innate and adaptive immune systems [42,43].

Type 2 immune responses in asthma are initiated by cytokines such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), termed 'alarmins', all of which are released following exposure to an insult (e.g. pollutants, viral infection and allergens). These epithelial-cell-derived cytokines activate antigen-presenting cells (dendritic cells and NK cells) to induce a Th2 adaptive immune response. Naïve T cells (Th0) are activated through antigen exposure and subsequently differentiate into a Th1 or Th2 phenotype, depending on a range of co-stimulatory factors, including the dose of antigen presented and the presence of local cytokines (particularly IL-4) [43,44]. The expression of IL-4 is key to Th2 polarization *in vitro*, although it has been demonstrated that Th2 cell differentiation can occur in the absence of IL-4 *in vivo* [44,45]. Th2 cells migrate to the airway epithelium and to the subepithelial mucosa, where they secrete the Type 2 cytokines IL-5 and IL-13, which play a central role in the disease pathophysiology and contribute to many of the hallmarks of asthma, including mucus production, IgE synthesis, subepithelial fibrosis, bronchial remodelling and airway hyperresponsiveness (AHR) [46,47]. In addition to this adaptive response leading to T helper cell polarization, IL-25, IL-33 and TSLP also directly activate ILC2s to secrete large amounts of IL-5 and IL-13 [48]. Interestingly, pulmonary ILC2s may also produce small amounts of IL-4 following *in vivo*

stimulation with IL-25 and IL-33 [49], but it is yet unclear whether this represents an active interface between innate and adaptive immune mechanisms.

Type 2 cytokines drive the recruitment of effector cells (mast cells, basophils and eosinophils) and mediate isotype switching of B-cell-secreted Igs to IgE upon exposure to antigens (Figure 1) [29,50,51]. Eosinophilic inflammation and IgE synthesis are, therefore, encompassed in Type 2 asthma. The precise effector functions of the various Type 2 cytokines and their involvement in different disease endpoints are subjects of considerable interest and clinical relevance.

IL-4, which acts as a regulatory cytokine upstream from Type 2 effector cytokines [77], binds to the IL-4R α receptor [78] that is broadly expressed on T helper cells, eosinophils, mast cells, B cells, bronchial epithelium, endothelium and airway smooth muscle cells. For signalling, the IL-4R α receptor needs to form a heterodimer with one of two additional surface receptors: IL-2 γ c or IL-13R α 1 [79]. By engaging with either one of these complexes to form a heterodimer, the type of the signal is defined [52]. Heterodimerization with the IL-2 γ c complex triggers a regulatory signal (Type I signal), modulating Th0 differentiation to Th2 and T_{reg} proliferation. In contrast, the heterodimerization between IL-4R α and the IL-13 α 1 receptor elicits the same signal as IL-13 binding this ligand, and inducing heterodimerization with IL-4R α (Type II signal).

IL-13 has been described as a key effector cytokine [53] due to the multifunctional role that it plays in many aspects of asthma pathogenesis, including B-cell isotype switching, mucus hypersecretion, goblet cell hyperplasia, subepithelial fibrosis and AHR [54,55]. IL-13 and IL-4 mediate subepithelial fibrosis and airway remodelling [51,56,57]. Exogenous administration of IL-13 in T-cell-deficient mice induces AHR and airway inflammation, and anti-IL-13 treatments block the late asthmatic response in allergen challenge models [46,54,58,59]. IL-13 signals through the IL4R α /IL13R α 1 heterodimer, triggering overlapping responses to those initiated by IL-4 binding IL13R α 1, and inducing the clinical hallmarks of asthma across a range of functional cells [54]. IL-13 also binds to a second receptor, IL13R α 2, which is thought to function predominantly as a decoy receptor [54] for excess IL-13. However, IL13R α 2 may have the capacity to mediate IL-13 signalling under certain circumstances [56,60]. By binding to IL-13 α 1 as a common receptor component, IL-13 and IL-4 utilize shared signalling pathways, including Janus kinases (JAKs) 1 and 3, to initiate signalling and activate signal

transducer and activator of transcription-6 (STAT6), which is an essential transcription factor for many of their biologic functions, and a variety of other signalling molecules [61].

IL-13 receptors are expressed on airway smooth muscle cells and airway epithelial cells, and IL-13 has been shown to enhance cholinergic-induced contractions of smooth muscle cells *in vitro* [62,63] and to promote goblet cell hyperplasia and mucus hypersecretion [64,65]. IL-13 induces a number of chemokines, leading to the recruitment and retention of eosinophils in inflamed airway tissue [54,66]. Thus, IL-4 and IL-13 share many activities: a key difference is that T cells do not bind IL-13 so that only IL-4 drives Th2 development [67].

IL-5 is predominantly produced by Th2 cells, ILC2 cells, mast cells, NK cells, NK T cells and eosinophils [68–70]. It exerts its effects by binding specifically to the alpha chain of the IL-5 receptor (IL-5R) and, once complexed to the β chain shared with IL-3 and granulocyte macrophage colony-stimulating factor, signals via the JAK-2/STAT-5 pathway [71]. IL-5 mediates its effects mostly via controlling eosinophil development, maturation and activation in the bone marrow, as well as subsequent mobilization and survival [68,72–77]. IL-5 also modulates the development and function of mast cells and basophils [51].

IL-9, secreted by mast cells, Th2 cells and ILC2 cells, stimulates the proliferation of activated T cells and promotes the proliferation and differentiation of mast cells [35,78,79]. IL-9 also increases production of IgE by B cells and seems to prime mast cells to respond to allergens via increased cell surface expression of Fc ϵ R1 receptors [80,81]. With regard to clinical manifestations, there is evidence from both human and murine studies that IL-9 is associated with increased predisposition to AHR [78,82,83]. In addition, IL-9 induces mucus hypersecretion and IL-13-dependent regulation of epithelial cell genes [78,80,84].

Type 2 inflammatory biomarkers

Biomarkers can be employed in several ways to aid treatment decisions: (i) identification of activated pathways or the pathogenesis of disease, (ii) prediction of response to specific treatments, (iii) monitoring success of a selected treatment option, or (iv) the assessment of the risk of disease progression.

Therefore, in parallel with ongoing research to better understand the Type 2 pathways in asthma pathogenesis, there is growing interest in a number of biomarkers of Type 2 inflammation that have potential utility for distinguishing Type 2-high and Type 2-low phenotypes and for predicting responsiveness to Type 2 cytokine-targeted therapy [29,85]. These include fractional exhaled nitric oxide (FeNO), serum IgE, blood or sputum eosinophils, and serum periostin [86].

Measuring FeNO in exhaled breath is a simple, non-invasive method that can be used as an indicator of IL-13 driven inflammation and of corticosteroid-responsive airway inflammation in symptomatic patients [87]. The release of IL-13 results in the activation of inducible nitric oxide synthase, leading to increased production of FeNO in the airway [88]. FeNO levels can be used for the assessment of asthma severity and to guide treatment [87,89]. FeNO actually represents a biomarker of Type 2 activity rather than asthma itself [90]; however, several other factors are thought to influence measurements, including age, ethnicity, diet, current smoking and concurrent viral infection [87,91].

Allergen-specific IgE antibodies are central to inflammatory processes in atopic asthma and are useful for the diagnosis of atopy. For the purpose of diagnosing allergies, measurements of specific IgE as opposed to total IgE are recommended [1,92]. The two main clinical applications of this biomarker are (i) to estimate the optimal dosage of the anti-IgE antibody omalizumab [93] for add-on treatment in cases of severe allergic asthma, when necessary, and (ii) in addition to other evaluation methods such as imaging, for screening for allergic bronchopulmonary aspergillosis, a condition associated with difficult-to-treat asthma that is often accompanied by very high serum total IgE [94].

Eosinophils are pivotal cellular effectors in the Type 2 inflammatory pathway and are thought to play a major role in maintaining long-term inflammation in asthma [95]. As mentioned above, there is no standardized cut-off for eosinophilic inflammation, but studies have used a sputum eosinophil cell count above 2–3% of the total cell count or blood eosinophil count of around 300 cells/ μ L [96–99].

Eosinophils can be measured in peripheral blood, lung tissue and airway lumen (sputum) using various techniques; however, invasive procedures such as tissue biopsies and assessment in induced sputum are not available for routine clinical practice except in very few academic centres [95].

In one study, eosinophilic airway infiltration, when defined as $\geq 4\%$ eosinophils in sputum, was shown to be associated with around 50% of severe asthma exacerbations [100]. Several studies have shown that sputum eosinophil-guided asthma management can reduce frequency of exacerbations [20,100–

102], with a cut-off of > 3% sputum eosinophils suggested as a sensitive and reliable biomarker to guide treatment [103]. In addition, increased eosinophil counts in sputum or peripheral blood in patients with severe asthma are associated with fixed airway obstruction [104–106] and peripheral blood eosinophil count may provide a mortality signal [107]. Eosinophils contribute to the modulation of the immune response and to the induction of AHR and remodelling, which are characteristic features of asthma, and can therefore be considered as key effector cells in asthma pathogenesis [108], with IL-5 as well as IL-4 and IL-13 and other inflammatory mediators – synergistically contributing to their accumulation in the lung.

The specific compartment in which eosinophils are measured may also be important as there can be discordance between sputum, tissue and systemic eosinophil count, whereby a high sputum eosinophil count does not necessarily correlate with elevated blood eosinophil numbers and *vice versa* [103,109,110]. Eosinophil dynamics across these compartments vary, and it is noteworthy that low numbers of blood and sputum eosinophils may be due to corticosteroid treatment rather than a non-eosinophilic phenotype [95,109]. Thus, there is greater chance for a discordance between blood and sputum eosinophils in patients with more severe asthma who are likely to be on higher doses of inhaled (or systemic) corticosteroids [111–113].

Periostin is an IL-13 and IL-4-inducible matricellular protein that is basolaterally secreted by bronchial epithelial cells and can be detected in peripheral blood [114–116]. Periostin has been implicated to play an integral role in several pathogenic processes in asthma, including airway remodelling, subepithelial fibrosis, eosinophil recruitment and regulation of mucus production from goblet cells [47,115–118]. Some reports have suggested that serum levels of periostin could be a clinically useful predictor of Type 2 eosinophilic airway inflammation in patients with uncontrolled, moderate-to-severe asthma [114]. Serum periostin is currently being evaluated as a predictive biomarker to identify patients who are most likely to respond to anti-IL-13 treatment (e.g. lebrikizumab) [119,120]. However, more needs to be known about serum periostin levels in healthy individuals and patients with asthma and other diseases. Whilst age beyond adolescence does not appear to affect the reference range of serum periostin, further research is needed to clarify the effect of ethnicity [121].

Given the complexity of inflammatory pathways in asthma, there is likely to be considerable overlap in these biomarkers in each of the asthma phenotypes and consequently, there be more than one dominant biomarker. Comparison of biomarkers used in different studies is also complicated by the use of varying techniques; for example, multiple enzyme-linked immunosorbent assays have been used in studies of periostin in asthma, and methods of sputum induction and processing to assess the contribution of eosinophils and different inflammatory cells are difficult to reproduce across laboratories [86,95,114,122].

Type 2-low asthma

There is evidence suggesting that, in addition to the Type 2 pathway, a distinct pathway driven by Th17 cells may form the basis of a clinically relevant asthma phenotype [123]. Th17 cells are characterized by secretion of IL-17A, IL-17F, IL-21 and IL-22 and have been identified in bronchial biopsies from patients with severe asthma [124–126]. Elevated levels of IL-17 are associated with corticosteroid resistance and are thought to contribute to AHR, mucus hypersecretion and airway obstruction [127]. Levels of Th17-derived IL-17 in the airway and peripheral blood are also positively correlated with disease severity [123,127,128]. A recent study showed an IL-4R α polymorphism associated with severe asthma drives conversion of regulatory T cells to Th17 cells [129]. Studies in mice and humans suggest that Th17 cells promote neutrophilic infiltration via the release of IL-17, particularly in severe asthma [130–132], although some evidence has shown that severe non-eosinophilic asthma does not respond to anti-IL-17 treatment [133]. A recent gene expression analysis using endobronchial tissue samples from patients with asthma of varying severity found a reciprocal relationship between Type 2 cytokines and Th17-related activity but that neutralization of both IL-13 and IL-17 protected mice from a range of inflammatory and airway responses [134].

To date, biomarkers of Type 2-low or neutrophilic asthma have not been described. However, as for Type 2 asthma, better understanding of the biological processes underlying this phenotype and subsequent identification of biomarkers will help guide treatment decisions and potentially develop novel targeted therapies [86]. Differential cell counts may be used to measure the relative numbers of eosinophils and neutrophils in the airway, although eosinophilic and neutrophilic asthma are not

mutually exclusive subtypes. The nature of inflammation may change in the same patient over time [135]. Several studies have found no inverse relationship between sputum eosinophil and neutrophil numbers [136], and eosinophils may be present in excess in addition to neutrophilic accumulation in the airways of patients with severe airway obstruction [136]. Inducing sputum is an invasive technique, so measuring sputum neutrophils may not be suitable for routine clinical practice [95]. However, unlike blood eosinophil counts and derived ratios, which can predict eosinophilic asthma, blood neutrophil parameters are poor surrogates for the proportion of neutrophils in the sputum [112,137].

One blood-based biomarker that could potentially be used to identify patients with neutrophilic asthma is the chitinase-like protein YKL-40, although conflicting reports exist. In one study, YKL-40 was shown to be increased in the serum and lungs of adult asthma patients and to be correlated with disease severity [138]. Serum levels of YKL-40 were higher in children with treatment-resistant asthma than in healthy children and were correlated with blood neutrophils [139]; however, this was challenged by another study [140]. An association between YKL-40 levels and blood eosinophils in asthmatic patients has also been described [77], meaning that the usefulness of YKL-40 as a biomarker for neutrophilic asthma is not confirmed. Clinical parameters that might suggest involvement of Type 2-low inflammation could include poor response to corticosteroid treatment. A combination of these approaches may provide the most reliable strategy for defining Type 2-low-driven asthma. One such study, the UK Refractory Asthma Stratification Programme (RASP-UK), aims to: identify Type 2-high patients when adherent to high-dose ICS, as candidates for Type 2-targeting biologic therapy; optimize corticosteroid treatment, avoiding excessive corticosteroid exposure; and will facilitate a better understanding of Type 2-low patients who are not responsive to corticosteroid therapy [141].

It is important to consider that the Type 2-high pathway and Type 2-low responses are likely to play a dynamic role in asthmatic inflammation, with the role of different T-cell subpopulations predominating in different phases of the disease [40]. The identification of a novel subset of T cells that can secrete both IL-4 and IL-17 (Th17/Th2 cells) also supports the hypothesis that different inflammatory phenotypes can co-exist in one patient [142].

Inflammation in childhood asthma

Special consideration needs to be given to childhood asthma. To adequately cover this topic is beyond the scope of the current review, but it should be noted that, although chronic atopic asthma does exist in childhood, the majority of children do not suffer from chronic airway inflammation between episodes of wheezing [143]. Biomarker studies have found that a heterogeneous pattern of airway inflammation, including increased cysteinyl leukotriene production, eosinophil activation, and neutrophil activation, exists during episodes of wheezing [143]. Whilst anti-inflammatory treatments have only limited effect in reducing preschool wheeze, azithromycin has been found to reduce the duration of wheeze episodes, probably due to a close association between viral and bacterial infections and childhood asthma-like symptoms [144]. Furthermore, non-inflammatory childhood asthma phenotypes have been described [145] and how similar these are to adult Type 2-low asthma requires further investigation. Unlike in adults, most of the exacerbations in children are not associated with a Type 2 inflammatory process and this may have relevance to selecting therapy for such exacerbations in severe asthma [146].

Therapeutic implications

Type 2 cytokines have a pivotal role in asthma pathogenesis, and, as such, a number of targeted agents have been investigated as potential treatments, including antibodies that target IL-4, IL-5 or IL-13 pathways. Along with clinical parameters, biomarkers that reflect Type 2 cytokine involvement are currently being used for patient selection or stratification in clinical trials of a number of these treatments. A summary of biomarker-guided investigative treatments is provided in Table 1.

Similar to the distinct Type 2 cytokine pathways, the relative importance of predominant biomarkers may vary depending on the clinical endpoint. High levels of FeNO or blood eosinophils may indicate the potential for corticosteroid responsiveness in asthmatic patients [87,147,148]. Total serum IgE may also be predictive of steroid responsiveness, although limited data are available [91,148]. The omalizumab EXTRA trial demonstrated that high blood eosinophil counts are associated with an increased rate of exacerbations. In this study, high levels of FeNO and periostin were also predictive of the risk of future exacerbations in the placebo arm [149]. Results from the LUTE/VERSE lebrikizumab studies indicated that higher periostin levels may also predict greater risk of future

exacerbations [120].

Several studies suggest that Type 2 biomarkers could be used to predict AHR in patients with asthma. FeNO has been shown to provide a sensitive biomarker for predicting AHR following mannitol challenge [150]. Serum periostin levels correlate with AHR to methacholine and mannitol in asthmatic children [151] and periostin is required for maximal AHR after allergic sensitization in mice [152]. A potential link between eosinophils and AHR is controversial, with mouse models suggesting AHR is dissociated from eosinophil infiltration [153,154]. In parallel, there was no improvement in AHR noted during a trial of mepolizumab, as measured with direct challenge after 50 weeks of treatment [155].

Ideally, classification of a Type 2-high asthma phenotype could be achieved using one simple biomarker test; however, in reality, determination of Type 2 involvement is likely to be based on a number of observations over time. These may include clinical parameters, such as responsiveness to steroids or the presence of early-onset disease, allergic phenotype, as well as Type 2 biomarkers (including sputum or blood eosinophils, serum periostin, FeNO and serum IgE) alone or in combination. FeNO and periostin may be employed to assess the response to biological agents inhibiting IL-13. Blood eosinophils can predict response to treatment with anti-IL-5 antibodies [149], and most recently an anti-IL-13 antibody [120]. Therapy with anti-IL-5 reduces blood eosinophils but not FeNO; anti-IL-13 therapy reduces FeNO and has been associated with raised blood eosinophils. An oral prostaglandin D2 receptor antagonist has recently been shown to reduce eosinophilic inflammation within the airways, but with no effect on peripheral blood eosinophil levels [156]. A panel of two or three biomarkers in combination may provide higher confidence in the identification of Type-2 driven asthma. In addition, other potential analytics for asthma classification are being evaluated and may become more widely available in the future. These include metabolomic approaches, such as nuclear magnetic resonance spectroscopy for measuring various urinary metabolites [157].

The underlying inflammatory pathways that contribute to asthma pathogenesis in individual patients have clear implications on the optimal choice of treatment. By establishing what is driving a patient's asthma it may be possible to identify patients most likely to be uncontrolled with existing treatments, and intercept with targeted therapies earlier. An improved understanding of the underlying mechanisms in asthma pathophysiology may also help in predicting clinical responses of different patient subgroups to new therapies, thereby providing more personalized treatment options and

potentially improving future asthma care [5].

In the future, biomarker tests such as differential blood cell counts and measures of serum periostin, FeNO and total and specific IgE may also be included in initial patient evaluations during routine practice, in addition to clinical asthma assessments. If there is a subgroup of asthmatics with evidence of predominantly Th1 or Th17-mediated inflammation, biomarkers and targeted therapeutics can potentially be developed for these patients [133]. For instance, there was no treatment effect with an antibody targeting IL-17 receptor signalling (brodalumab) in the full study population of subjects with inadequately controlled moderate-to-severe asthma taking regular ICS with asthma [133]. This is very likely because a good biomarker of IL-17 activity was not identified and bronchodilator reversibility, which was used as a selection criterion for patients entering the clinical trial, is not specific for IL-17 biology. In contrast, studies that selected patients based on persistent sputum or blood eosinophilia and prednisolone response consistently report a response to anti-IL5 therapy, indicating that persistent eosinophilia is a good biomarker for anti-IL-5 therapy [158].

Finally, given the crossover of T-cell-mediated inflammation to other inflammatory disorders and autoimmune diseases, the overarching concept of targeting inflammatory cytokines has implications for numerous non-asthma indications. The effects of Type 2 cytokine-targeted treatments are already being investigated in conditions such as eosinophilic oesophagitis and atopic dermatitis [159,160].

Conclusions and further research needs

Understanding what drives a patient's asthma is important for the patient and the treating physician. A crucial approach in this process may be to observe biomarkers to determine whether, or to what extent, their disease is mediated by Type 2 inflammatory pathways. The identification of different phenotypes and endotypes may help us to better understand the underlying mechanisms, inform studies to unveil endotype-specific causality, guide effective prevention and aid in routine clinical decision-making, ultimately improving the overall standard of asthma management.

To fully exploit the potential of predictive biomarkers, adaptive population-enrichment designs should be incorporated to validate novel therapies for asthma in addition to randomized controlled trials [161].

Such trial designs could allow for all participants to undergo randomization but for population enrichment to occur, eliminating non-performing subgroups (e.g. biomarker low) and enriching well-performing populations (e.g. biomarker high). With the help of an interim analysis, it could be established whether the biomarker-positive patients benefit differentially from the therapy compared with the biomarker-negative patients [162]. Alternatively, a broadening the population could occur by adding parallel groups of more 'real-world' patients following confirmation of the risk-benefit profile in a highly selected group. In both these approaches power calculations and biomarker cut off points would need careful consideration.

A number of unresolved issues related to asthma classification still remain. These include the need to understand what drives disease in those patients who do not show evidence of Type 2 inflammation and develop effective treatments. Even within Type 2-mediated asthma, there is uncertainty as to whether all cases of Type 2-high asthma are the same. The existence of atopic (IgE-driven) and non-atopic Type 2 asthma indicates they may not be. There could also be differences in the source and relative contribution of different cytokines in Type 2 asthma, so targeting one cytokine over another may affect more, or different, endpoints. In this respect, the potential use of one or more biomarkers might help physicians to select the most appropriate treatment. Together, improving understanding of Type 2-high and Type 2-low mechanisms and biomarkers may help to advance treatment options for many patients with asthma who remain uncontrolled despite use of current standard of care.

Acknowledgements

Support for third-party writing assistance for this manuscript, furnished by Kristina Standeven, PhD, of MediTech Media, was provided by F. Hoffmann-La Roche Ltd.

Conflicts of interest

RB reports personal fees from AstraZeneca, Chiesi, Roche and Teva, and grants and personal fees from Boehringer Ingelheim, GlaxoSmithKline, Novartis and Roche. **AAC** has acted as a paid consultant and/or speaker to Novartis, AstraZeneca, GlaxoSmithKline, Chiesi, Boehringer Ingelheim, MEDA and Roche and has received funding for research indirectly related to the subject of this work

from GlaxoSmithKline, AstraZeneca, Sanofi-Aventis and Merck Sharp & Dohme. **MH** reports personal fees from AstraZeneca, GlaxoSmithKline, Novartis, Roche and TEVA in the past five years. **NAH** has received honoraria for serving as consultant for Roche, AstraZeneca, Boehringer Ingelheim, Novartis, Mylan and research funding from Roche, GlaxoSmithKline, Boehringer Ingelheim, AstraZeneca and Chiesi. **HI** has acted as a paid consultant and/or speaker to Astellas, AstraZeneca, Boehringer Ingelheim, Roche-ChugaiPharm, GlaxoSmithKline, Kyorin, MerckSharp&Dohme, MeijiSeikaPharma, Novartis, Otsuka, and Pfizer and has received funding for research indirectly related to the subject of this work from Astellas, AstraZeneca, Boehringer-Ingelheim, ChugaiPharm, GlaxoSmithKline, Pfizer, MerckSharp&Dohme, Teijin-Pharma, and Torii. **SK** is an employee of F. Hoffmann-LaRoche. **PN** has received research grants from AZ, TEVA, Sanofi, Boehringer Ingelheim, Novartis and Roche and has provided consultancy and received honoraria from AstraZeneca, Teva, Sanofi, Novartis, Roche, Daiichi Sankyo and Knopp. **DSR** has acted as a paid consultant for Roche, Novartis, AstraZeneca and GlaxoSmithKline.

References

1. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention - updated 2016. 2016. Available at: <http://ginasthma.org/gina-reports/>. Accessed August 2016.
2. Reddel HK, Bateman ED, Becker A, et al. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J* 2015;46:622-39.
3. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet* 2006;368:804-13.
4. Corren J. Asthma phenotypes and endotypes: an evolving paradigm for classification. *Discov Med* 2013;15:243-9.
5. Lotvall J, Akdis CA, Bacharier LB, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011;127:355-60.
6. Wenzel S. Severe asthma: from characteristics to phenotypes to endotypes. *Clin Exp Allergy* 2012;42:650-8.
7. Green RH, Pavord I. Stability of inflammatory phenotypes in asthma. *Thorax* 2012;67:665-7.
8. Moore WC, Bleecker ER. Asthma heterogeneity and severity-why is comprehensive phenotyping important? *Lancet Respir Med* 2014;2:10-1.
9. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43:343-73.
10. Johansson SG, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report

of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004;113:832-6.

11. Busse W, Corren J, Lanier BQ, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 2001;108:184-90.
12. Barnes PJ. Intrinsic asthma: not so different from allergic asthma but driven by superantigens? *Clin Exp Allergy* 2009;39:1145-51.
13. Humbert M, Durham SR, Ying S, et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against "intrinsic" asthma being a distinct immunopathologic entity. *Am J Respir Crit Care Med* 1996;154:1497-504.
14. Humbert M, Menz G, Ying S, et al. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. *Immunol Today* 1999;20:528-33.
15. Haldar P, Pavord ID. Noneosinophilic asthma: a distinct clinical and pathologic phenotype. *J Allergy Clin Immunol* 2007;119:1043-52.
16. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006;11:54-61.
17. Gibson PG, Fujimura M, Niimi A. Eosinophilic bronchitis: clinical manifestations and implications for treatment. *Thorax* 2002;57:178-82.
18. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: Eosinophilic airway inflammation in nonallergic asthma. *Nat Med* 2013;19:977-9.
19. McGrath KW, Icitovic N, Boushey HA, et al. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med* 2012;185:612-9.
20. Green RH, Brightling CE, Woltmann G, et al. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002;57:875-9.
21. Green BJ, Wiriyaichai P, Grainge C, et al. Potentially pathogenic airway bacteria and neutrophilic inflammation in treatment resistant severe asthma. *PLoS One* 2014;9:e100645.
22. Nair P, Aziz-Ur-Rehman A, Radford K. Therapeutic implications of 'neutrophilic asthma'. *Curr Opin Pulm Med* 2015;21:33-8.
23. Gao P, Gibson PG, Baines KJ, et al. Anti-inflammatory deficiencies in neutrophilic asthma: reduced galectin-3 and IL-1RA/IL-1beta. *Respir Res* 2015;16:5.
24. Simpson JL, Daly J, Baines KJ, et al. Airway dysbiosis: *Haemophilus influenzae* and *Tropheryma* in poorly controlled asthma. *Eur Respir J* 2016;47:792-800.
25. Nair P, Gaga M, Zervas E, et al. Safety and efficacy of a CXCR2 antagonist in patients with severe asthma and sputum neutrophils: a randomized, placebo-controlled clinical trial. *Clin Exp Allergy* 2012;42:1097-103.
26. O'Byrne PM, Metev H, Puu M, et al. Efficacy and safety of a CXCR2 antagonist, AZD5069, in

patients with uncontrolled persistent asthma: a randomised, double-blind, placebo-controlled trial. *Lancet Respir Med* 2016;[http://dx.doi.org/10.1016/S2213-2600\(16\)30227-2](http://dx.doi.org/10.1016/S2213-2600(16)30227-2):

27. Mukherjee M, Svenningsen S, Nair P. Glucocorticosteroid subsensitivity and asthma severity. *Curr Opin Pulm Med* 2017;23:78-88.
28. Mosmann TR, Cherwinski H, Bond MW, et al. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-57.
29. Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. *Nat Rev Immunol* 2015;15:57-65.
30. Spellberg B, Edwards JE, Jr. Type 1/Type 2 immunity in infectious diseases. *Clin Infect Dis* 2001;32:76-102.
31. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
32. Woodruff PG, Modrek B, Choy DF, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;180:388-95.
33. Baines KJ, Simpson JL, Wood LG, et al. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol* 2011;127:153-60, 160.
34. Motomura Y, Kitamura H, Hijikata A, et al. The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in CD4+ T cells. *Nat Immunol* 2011;12:450-9.
35. Barlow JL, McKenzie AN. Type-2 innate lymphoid cells in human allergic disease. *Curr Opin Allergy Clin Immunol* 2014;14:397-403.
36. Christianson CA, Goplen NP, Zafar I, et al. Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33. *J Allergy Clin Immunol* 2015;136:59-68.
37. Kim BS, Siracusa MC, Saenz SA, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med* 2013;5:170ra16.
38. Lee JB, Chen CY, Liu B, et al. IL-25 and CD4(+) TH2 cells enhance type 2 innate lymphoid cell-derived IL-13 production, which promotes IgE-mediated experimental food allergy. *J Allergy Clin Immunol* 2016;137:1216-25.
39. Chen Y, Thai P, Zhao YH, et al. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* 2003;278:17036-43.
40. Cosmi L, Liotta F, Maggi E, et al. Th17 cells: new players in asthma pathogenesis. *Allergy* 2011;66:989-98.
41. Kao CY, Chen Y, Thai P, et al. IL-17 markedly up-regulates beta-defensin-2 expression in human airway epithelium via JAK and NF-kappaB signaling pathways. *J Immunol* 2004;173:3482-91.

- Accepted Article
42. Leite-de-Moraes M, Hammad H, Dy M. Crosstalk between Innate and Adaptive Cells on Allergic Process. *J Allergy (Cairo)* 2012;2012:720568.
 43. Oliphant CJ, Barlow JL, McKenzie AN. Insights into the initiation of type 2 immune responses. *Immunology* 2011;134:378-85.
 44. Noben-Trauth N, Hu-Li J, Paul WE. IL-4 secreted from individual naive CD4+ T cells acts in an autocrine manner to induce Th2 differentiation. *Eur J Immunol* 2002;32:1428-33.
 45. van Panhuys N, Tang SC, Prout M, et al. In vivo studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation. *Proc Natl Acad Sci U S A* 2008;105:12423-8.
 46. Rael EL, Lockey RF. Interleukin-13 signaling and its role in asthma. *World Allergy Organ J* 2011;4:54-64.
 47. Takayama G, Arima K, Kanaji T, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol* 2006;118:98-104.
 48. van Rijt L, von Richthofen H, Van Ree R. Type 2 innate lymphoid cells: at the cross-roads in allergic asthma. *Semin Immunopathol* 2016;38:483-96.
 49. Klein Wolterink RG, Kleinjan A, van Nimwegen M, et al. Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma. *Eur J Immunol* 2012;42:1106-16.
 50. Erazo A, Kutchukhidze N, Leung M, et al. Unique maturation program of the IgE response in vivo. *Immunity* 2007;26:191-203.
 51. Gibeon D, Menzies-Gow AN. Targeting interleukins to treat severe asthma. *Expert Rev Respir Med* 2012;6:423-39.
 52. Ramalingam TR, Pesce JT, Sheikh F, et al. Unique functions of the type II interleukin 4 receptor identified in mice lacking the interleukin 13 receptor alpha1 chain. *Nat Immunol* 2008;9:25-33.
 53. Cohn L, Elias JA, Chupp GL. Asthma: mechanisms of disease persistence and progression. *Annu Rev Immunol* 2004;22:789-815.
 54. Hershey GK. IL-13 receptors and signaling pathways: an evolving web. *J Allergy Clin Immunol* 2003;111:677-90.
 55. Leigh R, Ellis R, Wattie J, et al. Is interleukin-13 critical in maintaining airway hyperresponsiveness in allergen-challenged mice? *Am J Respir Crit Care Med* 2004;170:851-6.
 56. Fichtner-Feigl S, Strober W, Kawakami K, et al. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. *Nat Med* 2006;12:99-106.
 57. Komai M, Tanaka H, Masuda T, et al. Role of Th2 responses in the development of allergen-induced airway remodelling in a murine model of allergic asthma. *Br J Pharmacol* 2003;138:912-20.

- Accepted Article
58. Grunig G, Warnock M, Wakil AE, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
 59. Scheerens H, Arron J, Choy D, et al. Lebrikizumab treatment reduces serum periostin levels in asthma patients with elevated baseline levels of periostin,
 60. Chen W, Sivaprasad U, Gibson AM, et al. IL-13 receptor alpha2 contributes to development of experimental allergic asthma. *J Allergy Clin Immunol* 2013;132:951-8.
 61. Jiang H, Harris MB, Rothman P. IL-4/IL-13 signaling beyond JAK/STAT. *J Allergy Clin Immunol* 2000;105:1063-70.
 62. Grunstein MM, Hakonarson H, Leiter J, et al. IL-13-dependent autocrine signaling mediates altered responsiveness of IgE-sensitized airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L520-L528.
 63. Risse PA, Jo T, Suarez F, et al. Interleukin-13 inhibits proliferation and enhances contractility of human airway smooth muscle cells without change in contractile phenotype. *Am J Physiol Lung Cell Mol Physiol* 2011;300:L958-L966.
 64. Tanabe T, Fujimoto K, Yasuo M, et al. Modulation of mucus production by interleukin-13 receptor alpha2 in the human airway epithelium. *Clin Exp Allergy* 2008;38:122-34.
 65. Zhu Z, Homer RJ, Wang Z, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999;103:779-88.
 66. Pope SM, Brandt EB, Mishra A, et al. IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. *J Allergy Clin Immunol* 2001;108:594-601.
 67. de Waal MR, Abrams JS, Zurawski SM, et al. Differential regulation of IL-13 and IL-4 production by human CD8+ and CD4+ Th0, Th1 and Th2 T cell clones and EBV-transformed B cells. *Int Immunol* 1995;7:1405-16.
 68. Rosenberg HF, Phipps S, Foster PS. Eosinophil trafficking in allergy and asthma. *J Allergy Clin Immunol* 2007;119:1303-10.
 69. Sakuishi K, Oki S, Araki M, et al. Invariant NKT cells biased for IL-5 production act as crucial regulators of inflammation. *J Immunol* 2007;179:3452-62.
 70. Warren HS, Kinnear BF, Phillips JH, Lanier LL. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. *J Immunol* 1995;154:5144-52.
 71. Takatsu K. Interleukin-5 and IL-5 receptor in health and diseases. *Proc Jpn Acad Ser B Phys Biol Sci* 2011;87:463-85.
 72. Menzies-Gow A, Flood-Page P, Sehmi R, et al. Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol* 2003;111:714-9.
 73. Sanderson CJ. The biological role of interleukin 5. *Int J Cell Cloning* 1990;8 Suppl 1:147-53.
 74. Sitkauskienė B, Johansson AK, Sergejeva S, et al. Regulation of bone marrow and airway

CD34+ eosinophils by interleukin-5. *Am J Respir Cell Mol Biol* 2004;30:367-78.

75. Wardlaw AJ. Eosinophil trafficking in asthma. *Clin Med* 2001;1:214-8.
76. Kouro T, Takatsu K. IL-5- and eosinophil-mediated inflammation: from discovery to therapy. *Int Immunol* 2009;21:1303-9.
77. Tang Y, Zeng Y, Li Y. [Study on mutations of beta chain of high-affinity IgE receptor gene in people of Han nationality of southern China]. *Zhonghua Jie He He Hu Xi Za Zhi* 2001;24:142-4.
78. Oh CK, Raible D, Geba GP, Molfino NA. Biology of the interleukin-9 pathway and its therapeutic potential for the treatment of asthma. *Inflamm Allergy Drug Targets* 2011;10:180-6.
79. Parker JM, Oh CK, LaForce C, et al. Safety profile and clinical activity of multiple subcutaneous doses of MEDI-528, a humanized anti-interleukin-9 monoclonal antibody, in two randomized phase 2a studies in subjects with asthma. *BMC Pulm Med* 2011;11:14.
80. Louahed J, Kermouni A, Van Snick J, Renauld JC. IL-9 induces expression of granzymes and high-affinity IgE receptor in murine T helper clones. *J Immunol* 1995;154:5061-70.
81. Zhou Y, McLane M, Levitt RC. Th2 cytokines and asthma. Interleukin-9 as a therapeutic target for asthma. *Respir Res* 2001;2:80-4.
82. Cheng G, Arima M, Honda K, et al. Anti-interleukin-9 antibody treatment inhibits airway inflammation and hyperreactivity in mouse asthma model. *Am J Respir Crit Care Med* 2002;166:409-16.
83. Kearley J, Erjefalt JS, Andersson C, et al. IL-9 governs allergen-induced mast cell numbers in the lung and chronic remodeling of the airways. *Am J Respir Crit Care Med* 2011;183:865-75.
84. Steenwinckel V, Louahed J, Orabona C, et al. IL-13 mediates in vivo IL-9 activities on lung epithelial cells but not on hematopoietic cells. *J Immunol* 2007;178:3244-51.
85. Matsumoto H. Serum periostin: a novel biomarker for asthma management. *Allergol Int* 2014;63:153-60.
86. Kim MA, Shin YS, Pham ID, Park HS. Adult asthma biomarkers. *Curr Opin Allergy Clin Immunol* 2014;14:49-54.
87. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602-15.
88. Voraphani N, Gladwin MT, Contreras AU, et al. An airway epithelial iNOS-DUOX2-thyroid peroxidase metabolome drives Th1/Th2 nitrative stress in human severe asthma. *Mucosal Immunol* 2014;7:1175-85.
89. Berry MA, Shaw DE, Green RH, et al. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005;35:1175-9.

90. Scott M, Raza A, Karmaus W, et al. Influence of atopy and asthma on exhaled nitric oxide in an unselected birth cohort study. *Thorax* 2010;65:258-62.
91. Vijverberg SJ, Koenderman L, Koster ES, et al. Biomarkers of therapy responsiveness in asthma: pitfalls and promises. *Clin Exp Allergy* 2011;41:615-29.
92. National Heart Lung and Blood Institute. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. 2007. Available at: <http://www.nhlbi.nih.gov/guidelines/asthma/asthsumm.pdf>. Accessed August 2016.
93. Genentech I. Xolair Prescribing Information. 2010. Available at: <http://www.gene.com/gene/products/information/pdf/xolair-prescribing.pdf>.
94. Greenberger PA, Bush RK, Demain JG, et al. Allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol Pract* 2014;2:703-8.
95. Wadsworth S, Sin D, Dorscheid D. Clinical update on the use of biomarkers of airway inflammation in the management of asthma. *J Asthma Allergy* 2011;4:77-86.
96. Nair P. What is an "eosinophilic phenotype" of asthma? *J Allergy Clin Immunol* 2013;132:81-3.
97. National Institute for Health and Care Excellence. Mepolizumab for treating severe refractory eosinophilic asthma. 2016. Available at: <https://www.nice.org.uk/guidance/GID-TAG519/documents/html-content-3>
98. Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;380:651-9.
99. Schleich FN, Manise M, Sele J, et al. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. *BMC Pulm Med* 2013;13:11.
100. Turner MO, Hussack P, Sears MR, et al. Exacerbations of asthma without sputum eosinophilia. *Thorax* 1995;50:1057-61.
101. Jayaram L, Pizzichini MM, Cook RJ, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006;27:483-94.
102. O'Byrne PM. Therapeutic strategies to reduce asthma exacerbations. *J Allergy Clin Immunol* 2011;128:257-63.
103. Mukherjee M, Nair P. Blood or sputum eosinophils to guide asthma therapy? *Lancet Respir Med* 2015;3:824-5.
104. Guerra S, Sherrill DL, Kurzius-Spencer M, et al. The course of persistent airflow limitation in subjects with and without asthma. *Respir Med* 2008;102:1473-82.
105. ten Brinke A, Zwinderman AH, Sterk PJ, et al. Factors associated with persistent airflow limitation in severe asthma. *Am J Respir Crit Care Med* 2001;164:744-8.
106. Tubby C, Harrison T, Todd I, Fairclough L. Immunological basis of reversible and fixed airways disease. *Clin Sci (Lond)* 2011;121:285-96.

- Accepted Article
107. Ali Z, Dirks CG, Ulrik CS. Long-term mortality among adults with asthma: a 25-year follow-up of 1,075 outpatients with asthma. *Chest* 2013;143:1649-55.
 108. Possa SS, Leick EA, Prado CM, et al. Eosinophilic inflammation in allergic asthma. *Front Pharmacol* 2013;4:46.
 109. Bacci E, Cianchetti S, Ruocco L, et al. Comparison between eosinophilic markers in induced sputum and blood in asthmatic patients. *Clin Exp Allergy* 1998;28:1237-43.
 110. Schleich FN, Chevrement A, Paulus V, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J* 2014;44:97-108.
 111. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. *J Allergy Clin Immunol* 2015;135:822-4.
 112. Hastie AT, Moore WC, Li H, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. *J Allergy Clin Immunol* 2013;132:72-80.
 113. Wagener AH, de Nijs SB, Lutter R, et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 2015;70:115-20.
 114. Jia G, Erickson RW, Choy DF, et al. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012;130:647-54.
 115. Sidhu SS, Yuan S, Innes AL, et al. Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci U S A* 2010;107:14170-5.
 116. Woodruff PG, Boushey HA, Dolganov GM, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A* 2007;104:15858-63.
 117. Blanchard C, Mingler MK, McBride M, et al. Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. *Mucosal Immunol* 2008;1:289-96.
 118. Sehra S, Yao W, Nguyen ET, et al. Periostin regulates goblet cell metaplasia in a model of allergic airway inflammation. *J Immunol* 2011;186:4959-66.
 119. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088-98.
 120. Hanania NA, Noonan M, Corren J, et al. Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies. *Thorax* 2015;70:748-56.
 121. Caswell-Smith R, Hosking A, Cripps T, et al. Reference ranges for serum periostin in a population without asthma or chronic obstructive pulmonary disease. *Clin Exp Allergy* 2016;00:1-12.
 122. Kanemitsu Y, Matsumoto H, Izuhara K, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013;132:305-12.

123. Bhakta NR, Woodruff PG. Human asthma phenotypes: from the clinic, to cytokines, and back again. *Immunol Rev* 2011;242:220-32.
124. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005;6:1123-32.
125. Park H, Li Z, Yang XO, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005;6:1133-41.
126. Pene J, Chevalier S, Preisser L, et al. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J Immunol* 2008;180:7423-30.
127. McKinley L, Alcorn JF, Peterson A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* 2008;181:4089-97.
128. Agache I, Ciobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. *Respir Med* 2010;104:1131-7.
129. Massoud AH, Charbonnier LM, Lopez D, et al. An asthma-associated IL4R variant exacerbates airway inflammation by promoting conversion of regulatory T cells to TH17-like cells. *Nat Med* 2016;22:1013-22.
130. Al Ramli W, Prefontaine D, Chouiali F, et al. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol* 2009;123:1185-7.
131. Doe C, Bafadhel M, Siddiqui S, et al. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest* 2010;138:1140-7.
132. Wilson RH, Whitehead GS, Nakano H, et al. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. *Am J Respir Crit Care Med* 2009;180:720-30.
133. Busse WW, Holgate S, Kerwin E, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med* 2013;188:1294-302.
134. Choy DF, Hart KM, Borthwick LA, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. *Sci Transl Med* 2015;7:301ra129.
135. D'silva L, Cook RJ, Allen CJ, et al. Changing pattern of sputum cell counts during successive exacerbations of airway disease. *Respir Med* 2007;101:2217-20.
136. Fahy JV. Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. *Proc Am Thorac Soc* 2009;6:256-9.
137. Zhang XY, Simpson JL, Powell H, et al. Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clin Exp Allergy* 2014;44:1137-45.
138. Chupp GL, Lee CG, Jarjour N, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007;357:2016-27.
139. Konradsen JR, James A, Nordlund B, et al. The chitinase-like protein YKL-40: a possible

biomarker of inflammation and airway remodeling in severe pediatric asthma. *J Allergy Clin Immunol* 2013;132:328-35.

140. Santos CB, Davidson J, Covar RA, Spahn JD. The chitinase-like protein YKL-40 is not a useful biomarker for severe persistent asthma in children. *Ann Allergy Asthma Immunol* 2014;113:263-6.
141. Heaney LG, Djukanovic R, Woodcock A, et al. Research in progress: Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK). *Thorax* 2015;
142. Cosmi L, Maggi L, Santarlasci V, et al. Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4. *J Allergy Clin Immunol* 2010;125:222-30.
143. Grigg J. Antibiotics for preschool wheeze. *Lancet Respir Med* 2016;4:2-3.
144. Stokholm J, Chawes BL, Vissing NH, et al. Azithromycin for episodes with asthma-like symptoms in young children aged 1-3 years: a randomised, double-blind, placebo-controlled trial. *Lancet Respir Med* 2016;4:19-26.
145. Payne D, Bush A. Phenotype-specific treatment of difficult asthma in children. *Paediatr Respir Rev* 2004;5:116-23.
146. Jochmann A, Artusio L, Robson K, et al. Infection and inflammation in induced sputum from preschool children with chronic airways diseases. *Pediatr Pulmonol* 2016;51:778-86.
147. Meijer RJ, Postma DS, Kauffman HF, et al. Accuracy of eosinophils and eosinophil cationic protein to predict steroid improvement in asthma. *Clin Exp Allergy* 2002;32:1096-103.
148. Szeffler SJ, Phillips BR, Martinez FD, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 2005;115:233-42.
149. Hanania NA, Wenzel S, Rosen K, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013;187:804-11.
150. Sverrild A, Malinovschi A, Porsbjerg C, et al. Predicting airway hyperreactivity to mannitol using exhaled nitric oxide in an unselected sample of adolescents and young adults. *Respir Med* 2013;107:150-2.
151. Song JS, You JS, Jeong SI, et al. Serum periostin levels correlate with airway hyper-responsiveness to methacholine and mannitol in children with asthma. *Allergy* 2015;70:674-81.
152. Bentley JK, Chen Q, Hong JY, et al. Periostin is required for maximal airways inflammation and hyperresponsiveness in mice¹. *J Allergy Clin Immunol* 2014;134:1433-42.
153. Birrell MA, Battram CH, Woodman P, et al. Dissociation by steroids of eosinophilic inflammation from airway hyperresponsiveness in murine airways. *Respir Res* 2003;4:3.
154. Tournoy KG, Kips JC, Schou C, Pauwels RA. Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. *Clin Exp Allergy* 2000;30:79-85.
155. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory

eosinophilic asthma. *N Engl J Med* 2009;360:973-84.

156. Berair R, Gonem B, Singapuri A, et al. Effect of QAW039, an Oral Prostaglandin D2 Receptor (DP2/CRTh2) Antagonist, Upon Sputum and Bronchial Eosinophilic Inflammation and Clinical Outcomes in Treatment-Resistant Asthma: A Phase 2a Randomized Placebo-Controlled Trial.
157. Saude EJ, Skappak CD, Regush S, et al. Metabolomic profiling of asthma: diagnostic utility of urine nuclear magnetic resonance spectroscopy. *J Allergy Clin Immunol* 2011;127:757-64.
158. Hargreave FE, Nair P. Point: Is measuring sputum eosinophils useful in the management of severe asthma? Yes. *Chest* 2011;139:1270-3.
159. Corren J. Inhibition of interleukin-5 for the treatment of eosinophilic diseases. *Discov Med* 2012;13:305-12.
160. Hamilton JD, Suarez-Farinas M, Dhingra N, et al. Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol* 2014;134:1293-300.
161. Briasoulis O, Breckenridge R, Nunn A. External validity of trials should be taken into account before asthma drug candidates reach market authorisation. *Lancet Respir Med* 2016;4:601-3.
162. Bhatt DL, Mehta C. Adaptive Designs for Clinical Trials. *N Engl J Med* 2016;375:65-74.
163. Soler M, Matz J, Townley R, et al. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur Respir J* 2001;18:254-61.
164. Humbert M, Beasley R, Ayres J, et al. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy* 2005;60:309-16.
165. Hanania NA, Alpan O, Hamilos DL, et al. Omalizumab in severe allergic asthma inadequately controlled with standard therapy: a randomized trial. *Ann Intern Med* 2011;154:573-82.
166. Castro M, Zangrilli J, Wechsler ME, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med* 2015;3:355-66.
167. Bjermer L, Lemiere C, Maspero J, et al. Reslizumab for Inadequately Controlled Asthma with Elevated Blood Eosinophil Levels: a Randomized Phase 3 Study. *Chest* 2016;<http://dx.doi.org/10.1016/j.chest.2016.03.032>:
168. Corren J, Weinstein S, Janka L, et al. Phase 3 Study of Reslizumab in Patients with Poorly Controlled Asthma: Effects Across a Broad Range of Eosinophil Counts. *Chest* 2016;<http://dx.doi.org/10.1016/j.chest.2016.03.018>:
169. Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014;371:1198-207.
170. Bel EH, Wenzel SE, Thompson PJ, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014;371:1189-97.

171. Bleecker ER, FitzGerald JM, Chanez P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β_2 agonists: a randomised, multicentre, placebo-controlled Phase III trial. *Lancet* 2016;[http://dx.doi.org/10.1016/S0140-6736\(16\)31324-1](http://dx.doi.org/10.1016/S0140-6736(16)31324-1) :
172. Castro M, Wenzel SE, Bleecker ER, et al. Benralizumab, an anti-interleukin 5 receptor alpha monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. *Lancet Respir Med* 2014;2:879-90.
173. FitzGerald JM, Bleecker E, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2016;[http://dx.doi.org/10.1016/S0140-6736\(16\)31322-8](http://dx.doi.org/10.1016/S0140-6736(16)31322-8):
174. Hanania NA, Korenblat P, Chapman KR, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med* 2016;4:781-96.
175. Brightling CE, Chanez P, Leigh R, et al. Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. *Lancet Respir Med* 2015;3:692-701.
176. Wenzel S, Ford L, Pearlman D, et al. Dupilumab in Persistent Asthma with Elevated Eosinophil Levels. *N Engl J Med* 2013;368:2455-66.
177. Wenzel S, Castro M, Corren J, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting beta2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet* 2016;388:31-44.

Figure legends

Figure 1: Overview of a Type-2 driven asthma response: Type 2 cytokines drive the recruitment of effector cells (e.g. eosinophils) and mediate class switching of B cells to secrete IgE upon exposure to antigens. They are also central to many of the hallmarks of asthma, including mucus production, subepithelial fibrosis, bronchial remodelling and airway hyperresponsiveness

Table 1: Overview of biologic approaches currently in development for Type 2 asthma management

Target patient population	Studied/potential biomarker(s)	Biologic (drug name)	Drug type	Manufacturer	Development phase	Main effects	References
Moderate-to-severe, persistent; allergic asthma; GINA stage 5	Serum IgE	Omalizumab (Xolair®)	Anti-IgE mAb	Genentech/Novartis	Approved	Reduced exacerbation rates and OCS use	[1,11,163–165]
Moderate-to-severe, persistent asthma	Eosinophils > 400/μL (TBC)	Reslizumab	Anti-IL-5 mAb	Teva	Approved	Improved lung function (FEV ₁); Improved asthma control (ACQ score)	[166–168]
Severe, uncontrolled, refractory asthma	Blood eosinophil counts of ≥ 150 cells/μL at screening, or ≥ 300 cells/μL in the past year (TBC)	Mepolizumab	Anti-IL-5 mAb	GlaxoSmithKline	Approved	Reduced exacerbation rates Oral steroid reduction Improved lung function Improved QOL	[169,170]
Moderate-to-severe, uncontrolled asthma	Eosinophils > 400/μL (TBC)	Benralizumab	Anti-IL-5R mAb	AstraZeneca/MedImmune	III	Reduced exacerbation rates	[171–173]
Moderate-to-severe, uncontrolled asthma	Serum periostin ≥ 50 ng/mL (TBC)	Lebrikizumab	Anti-IL-13 mAb	Genentech/Roche	III	Improved lung function (FEV ₁); Trend for reduced exacerbation rates	[119,120,174]
Moderate-to-severe, persistent, uncontrolled asthma	Sputum IL-13, blood eosinophils, periostin, DPP-4 (TBC)	Tralokinumab	Anti-IL-13 mAb	AstraZeneca/MedImmune	IIb	Trend for reduced exacerbation rates	[175]
Moderate-to-severe asthma	Blood eosinophils ≥ 300 cells/μL or sputum eosinophils ≥ 3% (TBC)	Dupilumab	Anti-IL-4Rα mAb	Sanofi/Regeneron Pharmaceuticals	IIb	Reduced exacerbation rates; Improved lung function (FEV ₁); Improved asthma control (ACQ score)	[176,177]

ACQ, Asthma Control Questionnaire; FEV₁, forced expiratory volume in 1 second; mAb, monoclonal antibody; OCS, oral corticosteroid; TBC, to be confirmed, QOL quality of life scores

