

REVIEW

Pathogenesis of severe asthma

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Clinical & Experimental Allergy

Summary

Patients with severe asthma have asthma symptoms which are difficult to control, require high dosages of medication, and continue to experience persistent symptoms, asthma exacerbations or airflow obstruction. Epidemiological and clinical evidences point to the fact that severe asthma is not a single phenotype. Cluster analyses have identified subclasses of severe asthma using parameters such as patient characteristics, and cytokine profiles have also been useful in classifying moderate and severe asthma. The IL-4/IL-13 signalling pathway accounts for the symptoms experienced by a subset of severe asthmatics with allergen-associated symptoms and high serum immunoglobulin E (IgE) levels, and these patients are generally responsive to anti-IgE treatment. The IL-5/IL-33 signalling pathway is likely to play a key role in the disease pathogenesis of those who are resistant to high doses of inhaled corticosteroid but responsive to systemic corticosteroids and anti-IL5 therapy. The IL-17 signalling pathway is thought to contribute to 'neutrophilic asthma'. Although traditionally viewed as players in the defence mechanism against viral and intracellular bacterial infection, mounting evidence supports a role for Th1 cytokines such as IL-18 and IFN- γ in severe asthma pathogenesis. Furthermore, these cytokine signalling pathways interact to contribute to the spectrum of clinical pathological outcomes in severe asthma. To date, glucocorticoids are the most effective anti-asthma drugs available, yet severe asthma patients are typically resistant to the effects of glucocorticoids. Glucocorticoid receptor dysfunction and histone deacetylase activity reduction are likely to contribute to glucocorticoid resistance in severe asthma patients. This review discusses recent development in different cytokine signalling pathways, their interactions and steroid resistance, in the context of severe asthma pathogenesis.

Keywords airway hyperresponsiveness, airway inflammation, allergic airway responses, animal models, animal models, asthma, candidate genes for asthma, cysteinyl leukotrienes, IL17, polymorphisms

Submitted 17 November 2011; revised 08 February 2012; accepted 13 February 2012

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Cite this as: A. H. Poon,

D. H. Eidelman, J. G. Martin, C. Laprise and Q. Hamid, *Clinical & Experimental Allergy*, 2012 (42) 625–637.

Introduction

It is increasingly clear that severe asthma is not a single phenotype. This has prompted attempts to subtype severe asthma using a variety of clinical parameters and mathematical tools. One such tool is cluster analysis, which groups observations into subsets based on likeness. Studies using cluster analysis to identify subclasses of 'severe asthma' have demonstrated that patient characteristics such as pulmonary function, reversibility of airway obstruction, age of onset, co-morbidities and prior hospitalization for asthma can be used as parameters to further subdivide severe asthma [1, 2]. In addition, cytokine profiles can be used

to classify moderate and severe asthma [3, 4]. In this review, we will discuss recent developments in the pathogenesis of severe asthma in the context of these recent reports and speculatively link specific cytokine signalling pathways to subclasses of severe asthma.

Asthma is traditionally viewed as an eosinophilic airway inflammatory disorder associated with bronchial hyperresponsiveness (BHR) [5, 6]. Indeed, the number of eosinophils in the lung is associated with disease severity [5, 7] and has been used to assign clinical phenotypes and to guide therapy in severe asthma [7, 8]. Patients with severe asthma have asthma symptoms which are difficult to control, require high dosages of medication, and continue to experience persistent

symptoms, asthma exacerbations or airflow obstruction even with aggressive therapy. It has become apparent that severe asthma is a different disease than mild and moderate asthma. Dysregulation of the Th1/Th2 cytokines production in severe asthma have been shown to differ from mild asthma [9–12]. Steroid treatment is effective in reducing the number of cells which expressed Th2 cytokines and increased those which expressed Th1 cytokine (IFN- γ) in steroid sensitive asthma, yet it fails to do so for the more severe steroid resistant asthma [9, 10]. Eosinophils do not appear to be the only important effector cells in severe asthma, however, as increases in neutrophils have also been found in patients with severe, persistent asthma [13–17]. Moreover, in some patients, mast cells are associated with disease severity [18–20]. Pathologically, hallmarks of asthma severity are inflammation and in particular airway remodelling [21] (Fig. 1). The focus on eosinophilia in part reflects a view of asthma as being associated with an immune response biased towards that associated with type 2 helper T cells (Th2), which are characterized by their production and secretion of Th2 cytokines such as interleukin (IL)-4, 5, 9, 13 and 33. These cytokines in turn drive eosinophilic inflammation and tissue damage, leading to BHR and additional mediator release. However, immune regulation in severe asthma is different from mild and moderate asthma. Increased expression of other cytokines including IFN- γ , IL-8, IL-18 and IL-17 has been found in bronchial biopsies of severe asthmatics [22–25] and genetic studies have found non-Th2 genes to be associated with severe asthma [26–28]. This paper will explore the contributions of different cytokine

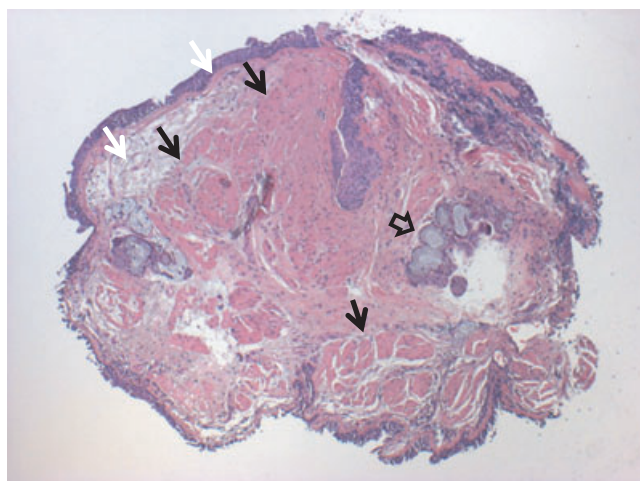


Fig. 1. Bronchial airway remodelling in severe asthma. Bronchial biopsy tissue from a severe asthma subject. Severe airway remodelling is evident by the presence of subepithelial fibrosis (white arrow), mucus gland hyperplasia (open arrow) and increased airway smooth muscle mass (black arrows).

signalling pathways, their interactions and the phenomenon of steroid resistance to the pathogenesis of severe asthma.

Cytokine signalling pathways

IL-4/IL-13 pathway

The IL-4/IL-13 signalling system is very important in severe asthma [29], as has recently been reviewed [30]. In particular, the IL-4/IL-13 signalling pathway accounts for the symptoms experienced by a subset of severe asthmatics with allergen-associated symptoms, eosinophilic and high serum IgE levels, and who generally benefit from anti-IgE treatment in terms of reducing symptoms and IgE levels [2, 31]. In this pathway, IL-4 binds to receptor IL-4R α , followed by heterodimerization with a second receptor chain, either IL-2R gamma component (IL-2R γ_c) or IL-13-bound IL-13 receptor alpha 1 (IL-13R α_1) chain [32]. Both IL-4–IL-4R α –IL-2R γ_c and IL-4–IL-4R α –IL-13R–IL-13R α_1 complex lead to eventual activation of signal transducer and activator of transcription 6 (STAT6), as summarized elsewhere [29]. Activated STAT6 binds to a special DNA element of target genes and modulates their expression [33–35]. IL-13 binds to either IL-13R α_1 or the decoy receptor IL-13R α_2 [36]. Unlike IL-4R α , IL-2R γ_c and IL-13R α_1 are not ubiquitously expressed allowing for IL-4 and IL-13 cell-specific downstream effects. The best evidence suggests that IL-13 is more closely related to manifestations of asthma, while IL-4 is involved in differentiation and stimulation of Th2 cells, synthesis of immunoglobulin E (IgE) and activation of macrophages [37]. This may relate to the effects of IL-13 on structural cells such as airway smooth muscle (ASM); IL-13 enhances the contractility of this tissue [38, 39].

Various lines of evidence demonstrate the importance of IL-4 and IL-13 in allergic asthma [29, 40]. In animals, a chronic house dust mite (HDM) exposure model demonstrated elevated IL-4, IL-13 and TGF β 1 levels in bronchoalveolar lavage (BAL), accompanied by goblet cell hyperplasia, subepithelial fibrosis and increased ASM area [41]. In another HDM model, IL-4 deficient mice failed to exhibit airway remodelling despite the presence of airways inflammation [42]. Of note, with chronic exposure to ovalbumin (OVA) rather than HDM, IL-4 deficient mice were similar to wild-type mice in regard to airway inflammation, epithelial hyperplasia, subepithelial fibrosis and airway hyperreactivity, suggesting that the development of airway remodelling may be dependent on antigen type [43]. Mice deficient in IL-13 shows decreased STAT6 phosphorylation and TGF β activity after chronic OVA exposure [44] as well as attenuated BHR, ASM hyperplasia and IgE synthesis [45]. In contrast, over-expression of IL-13 leads

to increased recruitment of pulmonary neutrophils, lymphocytes and eosinophils, increased mucus producing goblet cells, BHR and resistance to glucocorticoid treatment [46]. Similarly, suppression of IL-13 by anti-IL-13 antibody reduced airway hyperresponsiveness, eosinophil infiltration and mucus production [47].

In humans, genetic, immunohistochemical and clinical studies provide strong support for the importance of IL-4/IL-13 signalling pathway in severe asthma *per se*. In a genome-wide association study, variants in the region of *IL-4* and *IL-13* were associated with susceptibility to severe asthma [26]. Genetic variants in this region were also associated with asthma severity, lung function and total IgE [26, 48, 49]. Severe asthmatics expressed increased numbers of IL-13 mRNA positive cells in the large airways [10] and greater numbers of IL-13 protein positive cells in the ASM bundles compared with mild and moderate asthmatics. The number of IL-13 expressing cells roughly correlated with sputum and submucosal eosinophil counts [10]. Sputum IL-13 protein has been reported to be elevated in severe asthma [50], although this was not seen in another, smaller study [51]. In a study aimed at classifying severe asthma based on cytokines in BAL, IL-4 level, when combined with nine other mediators, discriminated one subclass from three others, each with distinct clinical and cellular characteristics [3]. IL-13 was not analysed since it was not usually detectable in BAL [3]. Other evidence comes from clinical trials. A phase II clinical trial of lebrikizumab, an anti-IL-13 monoclonal antibody for the treatment of severe asthma reported improved lung function particularly in those with high IL-13 activity as suggested by high levels of periostin [52], an epithelial product responsive to IL-13. In contrast, AMG317, an anti-IL-4R α monoclonal antibody, failed to improve symptom scores overall; however, in a subgroup of severe asthma patients with the highest baseline Asthma Control Questionnaire (ACQ) scores, improvements were achieved, suggesting that IL-4/IL-13 antagonism was most effective in the most severe patients [53].

Tumour necrosis factor receptor super family member 6 (TNFRS6), chitinase and periostin are downstream mediators of the IL-4/IL-13 signalling pathway with potential importance in asthma [54–56]. TNFRS6 is also known as apoptosis stimulating fragment ligand (FasL) and is associated with programmed cell death [57, 58]. In allergic mice, stimulation of TNFRS6 by anti-Fas antibody resulted in epithelial shedding, increased mucus producing cells, mucus plug formation and neutrophilia [59]. In human epithelial cells, incubation with IL-13, IL-4 and IL-9 increased the expression of matrix metalloproteinase 7 (MMP7) and FasL release. Moreover, the expression of MMP7 was increased in primary epithelial cells from severe asthmatics [55].

Chitin, the main component of arthropod exoskeletons, plays an important role in protecting invading pathogens against harsh conditions inside the host [60]. The host uses the enzyme chitinase to degrade chitin as part of its antiparasite response [61]. The expression of acidic mammalian chitinase (AMCase) and chitinase 3-like protein 1 (CHI3L1) proteins are elevated in asthmatic lungs [54, 62]. Serum CHI3L1 level correlates with airway remodelling and asthma severity [62]. Genetic variants of the CHI3L1 gene are associated with asthma risk and lung function [63]. In children, joint genetic effects were reported for AMCase, IL-4, IL-13 and toll-like receptor 10 (TLR 10) in asthma risk [64].

Lastly, periostin, an extracellular matrix protein associated with subepithelial fibrosis in asthma [56, 65] has been shown to induce epithelial to mesenchymal transition (EMT). During EMT, epithelial cells become invasive and migrate away from the basement membrane [66]. As noted above, anti-IL-13 antibody has been reported to improve FEV₁, particularly in those with high serum periostin [52].

At this point the association of the IL-4/IL-13 signalling pathway with allergic, eosinophilic asthma is mostly supported by animal studies. In the majority of human studies on severe asthma, subject enrolment procedures emphasized on asthma severity but overlooked severe asthma subtypes.

IL-5/IL-33 pathway

Some asthmatic patients fail to exhibit allergen-associated symptoms. Typically they are non-atopic asthmatics with late onset disease often associated with chronic sinusitis and nasal polyps. They have severe exacerbations associated with eosinophilia and high levels of cysteinyl leukotrienes and are resistant to high doses of inhaled corticosteroid but responsive to systemic corticosteroids and anti-IL-5 therapy [2]. Speculatively, the IL-5/IL-33 signalling pathway may contribute to the pathogenesis of this subgroup of 'non-atopic late onset eosinophilic severe asthma'.

The IL-5 signalling pathway has been reviewed elsewhere [67, 68]. Briefly, IL-5 binds to its receptor, IL-5R α , leading to recruitment of the β c chain to form the IL-5–IL-5R α – β c complex. Through at least three different signalling pathways IL-5 alters gene expression, notably inducing genes involved in the growth, survival and activation of eosinophils [67]. In a chronic allergen exposure model, IL-5 deficient mice demonstrated reduced intraepithelial eosinophils, airway inflammatory cells and BHR, but epithelial hypertrophy and subepithelial fibrosis were similar to wild-type mice [43]. In another chronic exposure model, BAL eosinophils were undetectable, and BAL TGF- β 1 levels, collagen

content and fibrotic changes were all decreased in IL-5R α deficient mice [69]. Similar changes in wild-type mice were observed after anti-IL-5 antibody administration. Conversely, over-expression of IL-5 resulted in greater BAL eosinophils, BAL TGF- β 1, higher collagen content and more fibrotic changes [69]. Therefore, it appears that eosinophils may drive certain aspects of airway remodelling that may contribute to the severity of disease, a view supported by observations from human asthmatic biopsy specimens [70].

In humans, sputum IL-5 protein levels correlate with frequent asthma exacerbations [71, 72]. Anti-IL-5 treatment reduced blood and sputum eosinophil levels, asthma exacerbation frequency and airway wall area [7, 73]. The IL-5 gene resides in the same region as *IL-4* and *IL-13*, hence the associations described above between genetic variants in the region with severe asthma and asthma severity may reflect a contribution by *IL-5* [26, 48, 49]. Furthermore, the association of IL-5 level with chronic sinusitis and with nasal polyps further supports a role of IL-5 in the pathogenesis of this subset of severe asthma [74].

The prominence of IL-5 in the pathogenesis of 'non-atopic late onset eosinophilic severe asthma' suggests the possibility of an IL-4 independent Th2 response [75], suggesting IL-33 involvement. IL-33 is able to direct naïve T cells to develop into Th2 cells that produce mainly IL-5 [75]. IL-33 appears to have a role in the enhancement of chronic inflammation and airway remodelling [76], mediating its effects both through receptor- and non-receptor mediated mechanisms. When IL-33 binds to its receptor, interleukin 1 receptor like-1 (IL-1RL1), followed by recruitment of a co-receptor, the interleukin-1 receptor accessory protein (IL-1RAcP) to form the IL-33-IL-1RL1-IL-1RAcP complex [77], gene expression is altered through at least two different pathways. Alternatively, IL-33 translocates to the nucleus and modulates gene expression through chromatin binding [78, 79], regulating Th2-associated cytokine genes such as IL-4, IL-5 and IL-13 [80]. In addition, mast cells, basophils, eosinophils, macrophages, natural killer cells and T cells are all targets of IL-33 [81, 82].

In a murine model of repeated IL-33 administration, blood eosinophilia and high serum levels of IgE and IgA were associated with Th2 cytokines production (IL-4, IL-5 and IL-13) without a change in Th1 cytokines [80]. The airway epithelial lining of these mice was hypertrophied and contained large amounts of mucus [80]. In humans, three large-scale genome-wide association studies detected genetic variants at the IL-33 and IL-1RL1 gene regions to be associated with asthma and/or blood eosinophil count [83–85]. IL-33 protein was increased in the BAL of moderate asthmatics compared to mild and normal subjects [76] and

increased airway epithelial IL-33 protein expression was found in severe asthma [76]. In human lungs, IL-33 leads to increase production of IL-6, IL-8, IL-13 and monocyte chemotactic protein 1 (MCP-1) in a cell-specific manner [86]. Furthermore, IL-33 induces contraction of human fibroblast and ASM cells [87]. These findings suggest that IL-33 may contribute to airway remodelling by inducing Th2 cytokine production, by promoting morphological changes in asthma relevant cells and by inducing chemoattractant production (e.g. IL-8 and MCP-1).

Here, we speculate the association between the IL-5/IL-33 signalling pathway and non-atopic late onset severe asthma based on disease history, symptoms, the effectiveness of anti-IL-5 treatment and the upregulation of IL-5 production by IL-33. Further studies enrolling specifically non-atopic late onset severe asthma subjects are warranted to substantiate the association claimed here.

IL-17

Some severe asthma patients exhibit sputum neutrophilia without eosinophilia, suggesting the possible involvement of IL-17 [22, 23, 88–90]. This subset of patients often have variable airflow obstruction, resistant to corticosteroid and are responsive to macrolide antibiotics, as reviewed recently [91, 92]. A separate CD4⁺ T cell lineage, Th17 cells are driven by IL-6 and maintained by IL-23. They are the main source of IL-17 production [93–96]. Within the IL-17 family [97–100], IL-17A and IL-17F are most closely associated with asthma severity and pulmonary neutrophil recruitment [22, 23, 88, 90]. Forming homodimers and heterodimers (IL-17A/A, IL-17F/F and IL-17A/F), these cytokines bind to their receptors, IL-17RA and IL-17RC [101], triggering intracellular signalling that can modulate gene expression by directly initiating transcription or by promoting mRNA stability of target genes [101, 102]. Among the target genes are chemokines (e.g. IL-8) and transcription factors associated with inflammatory gene expression [103].

Support for a role of IL-17 in neutrophilia [104–108] comes from a series of murine experiments. In a chronic allergen exposure model, the lungs of mice exposed to OVA had increased BAL neutrophils and IL-17 mRNA, in addition to Th2 cytokines (IL-4, IL-5, IL-10 and IL-13) [104]. In OVA exposed mice [104], prolonged anti-IL-17 monoclonal antibody treatment decreased neutrophil count in BAL, bone marrow and blood, increased eosinophil count in BAL and blood, and elevated IL-5 protein level, without effect on BHR [104]. In another chronic OVA exposure model, OVA exposed mice had increased BAL IL-17 and more pulmonary Th17 cells [109], which correlated with the

duration of OVA exposure and airway remodelling [109].

Various studies have reported elevated level of IL-17 mRNA and protein in the lung tissues of severe asthma patients compared to milder patients or healthy subjects [22, 23, 88, 90, 110]. Correlations in sputum between IL-17A and IL-8 mRNA level, IL-17A and IL-5 mRNA level, IL-17A mRNA level and neutrophil count, and IL-8 mRNA level and neutrophil count have been reported [90]. These associations are consistent with *in vitro* findings that IL-17A can promote neutrophil migration through IL-8 stimulation by facilitating neutrophil transmigration [89, 111]. Various studies have identified genetic variants of IL-17A and IL-17F to be associated with asthma risk but none has dealt with severe asthma [112–115]. Hence, IL-17 signalling pathway may be associated with a subset of patients characterized as having 'neutrophilic' asthma.

IL-18/IFN- γ

There is mounting evidence supporting a role for Th1 cells in some severe asthma patients. Th1 cells are differentiated from Th0 in the presence of IL-12, IL-18 and IFN- γ , and secrete the signature cytokine IFN- γ [116, 117]. After binding to its receptors, IFN- γ receptor 1 (IFNGR1) and IFNGR2 [118, 119], IFN- γ induces phosphorylation and dimerization of the signal transducer and activator of transcription 1 (STAT1) protein, leading to modulation of target gene transcription [120, 121]. Although IFN- γ has been traditionally associated with defence against viral and intracellular bacteria, studies in humans and mice have implicated IFN- γ in the pathogenesis of severe asthma. In a chronic OVA exposure model, mice deficient in IFN- γ and IFN- γ receptor 1 (IFN- γ R1) showed diminished BAL leukocytosis and failed to develop increased airway resistance [122]. The chronic effects of IFN- γ deficiency contrast with findings in acute models [123]. Similarly, the transfer of IFN- γ producing Th1 cells to unsensitized mice induced BHR and neutrophilia in BAL after acute challenge by lipopolysaccharide (LPS) and OVA [124]. Neither LPS nor OVA alone induced BHR and neutrophilia, and depletion of IFN- γ by antibody abolished BHR but not neutrophilia [124]. In humans, a greater number of IFN- γ expressing cells have been found in the subepithelium of severe asthma patients compared to moderate asthma patients [25]. Furthermore, the percentage of stimulated IFN- γ producing CD8⁺ T cells from blood of asthmatic subjects is correlated with asthma severity, eosinophil counts and BHR [125, 126]. Nevertheless, despite a strong association with asthma phenotype, no genetic association of IFN- γ with severe asthma or asthma severity has yet been found.

IFN- γ can be induced by IL-18, known as interferon-gamma inducing factor. Expressed by a wide range of immune and non-immune cells, IL-18 is up-regulated by LPS [127, 128]. After binding to its receptors (IL-18R α and IL-18R β) on Th1 cells, IL-18 activates transcription factors such as NF- κ B, subsequently inducing production of both Th1 and Th2 cytokines by Th1 cells [129]. IL-18 deficient mice chronically exposed to OVA had lower IFN- γ , TGF- β 1 and IL-13 level in BAL, less airway inflammation and less airway remodelling [130]. In another study, exposure to IL-18 and OVA in naïve mice to which Th1 memory cells had been adoptively transferred, induced changes in BAL and airways similar to those seen in their Th2 counterparts exposed to OVA or OVA and IL-18 but not either OVA or IL-18 alone [131, 132]. Given that LPS and IL-18 are potent drivers of Th1 cell differentiation, these findings underscore a role in asthma for the Th1 response, via IFN- γ . Interestingly, poorly controlled asthma is associated with higher serum IL-18 levels [133], and in a Japanese population, a genetic variant of *IL-18* with greater transcriptional activity was found to be associated with asthma severity [134]. Furthermore, variants of IL-18R α and IL-18R β have been found to be associated with severity of BHR, total serum IgE and serum eosinophils [135]. Hence, the IL-18/IFN- γ signalling pathway appears to be associated with disease severity instead of any subsets of severe asthma.

Steroid resistance

To date, glucocorticoids are the most effective anti-asthma drugs available, yet severe asthma patients are typically resistant to the effects of glucocorticoids [2, 136]. Glucocorticoids act by binding to glucocorticoid receptors (GR) in the cytoplasm and translocate to the nucleus, where their receptors dimerize. The resulting complex binds to DNA, suppressing inflammation by regulating the expression of several immune genes [137]. Two forms of GR exist, GR α and GR β [138]. GR β binds to DNA but not glucocorticoid; hence, GR β inhibits GR α -mediated gene expression by forming transcriptionally inactive GR α / β heterodimers in the nucleus [139–141].

Steroid resistance in asthma largely relates to GR dysfunction either through altered GR-binding affinity or GR β overexpression [142–149]. Steroid resistant asthma patients have elevated IL-2 and IL-4 in BAL [9], and a combination of IL-2 and IL-4 has been shown to reduce GR-binding affinity in the nucleus of T cell without affecting cytosolic GR [143, 147], similar to the affinity reduction observed in severe asthmatics [147]. The administration of IFN- γ was able to restore the glucocorticoid effects in reducing T cell proliferation. Fur-

thermore, the removal of IL-2 and IL-4 from the growth medium augmented the GR-binding affinity in T cells from steroid resistant asthmatics to a comparable level to that of normal subjects [143].

GR β expression is elevated in individuals with severe asthma, fatal asthma and glucocorticoid-insensitive asthma compared to those with moderate asthma, mild asthma and glucocorticoid-sensitive asthma, respectively [144–146, 148]. In steroid resistant asthma patients with elevated BAL IL-2 and IL-4, corticosteroids failed to reduce the expression of these cytokines or to augment IFN- γ expression [9]. IL-2, IL-4 and IL-17 have been shown to increase GR β expression in epithelial cells and peripheral blood mononuclear cells (PBMCs) from steroid resistant asthmatic subjects [148, 150]. Furthermore, animal studies have demonstrated Th17 cells to be resistant to corticosteroid treatment, with no effect on IL-17 and chemokine reduction, neutrophil influx and BHR [151].

Histone acetylation and deacetylation abnormalities

Another major mechanism of steroid resistance relates to reduction in histone deacetylase (HDAC) activity. Nuclear DNA is wrapped around core histones. Inflammatory signals lead to their acetylation, followed by the opening up of the DNA for chromatin remodelling, RNA polymerase II association and initiation of gene transcription [152]. Binding of glucocorticoid to GR leads to acetylation of the glucocorticoid-GR complex by histone acetyltransferases (HAT), and subsequent translocation to the nucleus where it forms dimers at its target genes and modulates gene expression. Alternatively, HDAC deacetylates the glucocorticoid-GR complex to repress gene transcription by interacting with HAT and transcription factors such as NF- κ B [137].

Several lines of evidence support a pivotal role for histone acetylation and deacetylation in steroid resistance in severe asthma. Deletion of the HDAC1 gene in T cells resulted in an increased Th2 associated inflammatory response in OVA exposed mice [153]. Compared to wild-type T cells, HDAC1 deficient Th2 cells produced higher levels of IL-4, IL-10 and IL-13, and HDAC1 deficient Th1 cells produce higher IFN- γ and lower levels of IL-13 [153]. Furthermore, HDAC1 has been reported to interact with DNA at the IL-4 locus [153], directly interfering with IL-4 gene expression and suppressing Th2 response. Additionally, HAT and HDAC activities in PBMCs are reported to be lower in severe asthma [154]. HAT activity was correlated with various LPS-induced pro-inflammatory cytokines, while HDAC activity was negatively correlated with inflammatory cytokines such as granulocyte-macrophage-colony stimulating factor (GM-CSF) and IFN- γ [154]. While glucocorticoid increased HDAC protein expression and

activity in PBMCs from mild asthmatics, no effect was detected in severe asthmatics [155]. Similarly, glucocorticoid had no effect in increasing HDAC1 and HDAC2 protein expression in the epithelium and submucosa of moderate and severe asthmatics [144]. There is increasing evidence suggesting that oxidative stress contributes to HDAC activity reduction and glucocorticoid insensitivity in severe asthma [156, 157]. Oxidative stress reduced HDAC2 activity in human bronchial epithelial cells [158] and reduced the potency of steroid and HDAC activity in alveolar macrophages [159]. Although no polymorphisms of the GR or HDAC genes have been reported to be associated with glucocorticoid insensitivity in adult severe asthmatics, there is genetic support for the notion that glucocorticoid insensitivity in severe asthma resides in their target genes [160].

Recently, a genome-wide association study has identified a variant in the glucocorticoid-induced transcript 1 gene (*GLCCI1*) to be associated with reduced lung function in response to inhaled glucocorticoid [161]. Improvement in FEV₁ after inhaled glucocorticoid treatment was the lowest for individuals who carried two copies mutant allele when compared to those with two copies of the common allele, accompanied by a higher risk of poor response. In the same study the mutant allele has been shown to have a weaker promoter activity compared to the common allele. The encoded protein is involved in glucocorticoid signalling and has been suggested as a marker of glucocorticoid-induced apoptosis [162].

Cytokine signalling pathway interactions

Interactions of signalling pathways further complicate the understanding and treatment of severe asthma and contribute to a spectrum of clinical and pathological outcomes observed. The cytokine production pattern by Th cells is malleable and is influenced by the surrounding cytokine milieu. For example, Th-17 cells express the signature cytokines IL-17 and IL-22, but can exhibit either Th1 or Th2 characteristics under the influence of other cytokines [163, 164]. Similarly, Th1 cells can also be conditioned to express Th2 cytokines (IL-4, IL-9, IL-13 and GM-CSF), in addition to IFN- γ [131, 132]. Figure 2 schematically illustrates the malleability of cytokine productions by various lineages of T cells and their downstream effects on the pathologies of severe asthma.

Th17 cells express their signature cytokines IL-17 and IL-22 in a pro-Th17 environment (e.g. in the presence of IL-6 and IL-23), however, they can also be conditioned to exhibit either Th1 or Th2 characteristics. In mice, Th0 cells polarized to Th17 expressed IL-4/IL-13 complex receptors (IL-4R α , common- γ -chain and IL-13R α 1) [163]. After stimulation of Th17 cells with

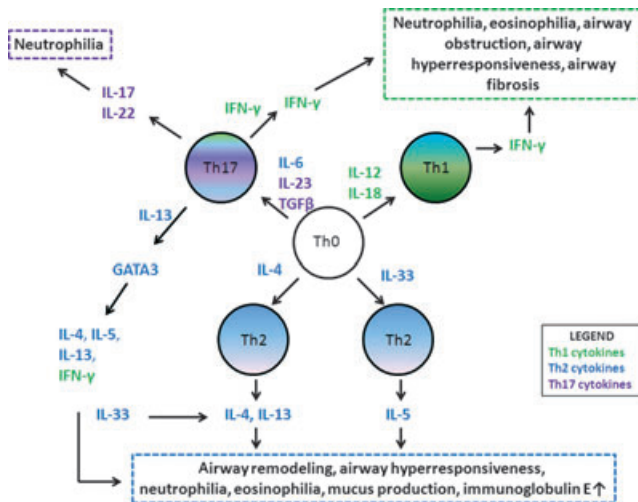


Fig. 2. Cytokine pathway interactions. Th1, Th2 and Th17 cells can be conditioned by cytokines to exhibit non-classical cytokine production patterns and contribute to the different pathologies of severe asthma. Cytokines and downstream effects are colour coded based on the associated Th response: Th1(green), Th2 (blue) and Th17 (purple). Secreted cytokines are depicted at the end of the arrows and inducing cytokines are at the side of the arrows.

IL-13, the mRNA expression of IL-13R α 1 and GATA-binding protein 3 (GATA3) was increased and the number of IL-17A producing Th17 cells was decreased, compared to unstimulated Th0 cells [163].

In Th17 cells, GATA3 is critical to the development of non-Th17 characteristics. Acting as a master switch for Th2 differentiation [165], GATA3 binds to the DNA sequence 'GATA' to modulate expression of target genes [166] including IL-4, IL-5, IL-13 and IFN- γ [165, 167]. GATA3 expression can be induced via IL-4, IL-33 and other mediators, underscoring the ability of GATA3 to promote Th2 responses even in the absence of allergen mediated IL-4 exposure [168]. In mice, over-expression of GATA3 resulted in Th17 cells exhibiting Th2 characteristics with the production of IL-4, IL-5 and IL-13 and decreased IL-17 [169]. The production of IL-4 and IL-13 by Th17 cells further induced expression of GATA3 in Th17 cells [163]. In contrast, in the absence of GATA3 over-expression, Th17 cells produce IL-17 rather than Th2 cytokines [169]. Similarly, in the presence of IFN- γ , Th17 cells can exhibit a Th1 phenotype. In mice, the majority of Th17 cells found in the

spleen do not express a functional IL-12 receptor (IL-12R β 2); however, in *ex vivo* isolated Th17 cells, IFN- γ induced the expression of the IL-12R β 2 gene [164], rendering these Th17 cells responsive to IL-12 and to upregulation of IFN- γ [164]. Likewise, as noted above, Th1 cells can be conditioned to express Th2 cytokines (IL-4, IL-9, IL-13 and GM-CSF), in addition to IFN- γ [131, 132]. Therefore, Th1 memory cells can manifest Th2-associated pathologies under the influence of IL-18 and allergen exposure.

The close proximity of cytokine and cytokine receptor genes introduces another level of interactions. The Th2 cytokine genes of IL-4, IL-5 and IL-13 located side by side on chromosome 5q31 and the interleukin-1 receptor genes [IL-1R1, IL-1R2, IL-1RL1 (also known as IL-33R), IL-1RL2, IL-18R1 and IL-18RAP] reside on chromosome 2q12 [170, 171]. The proximity of these genes suggests the possibility of coordinated gene modulations [172, 173].

Conclusions

There are substantial differences in cytokine production between severe asthma and mild to moderate disease, particularly with regard to the importance of Th1 and Th17 cells. The cytokine expression patterns of these cells activate a variety of downstream signalling pathways, triggering interactions among pathways and effects like glucocorticoid resistance. This complexity often leads to inconsistent conclusions from animal and human studies using different models and populations underscoring the complexity of severe asthma. Further work is necessary to sort out the mechanisms by which cytokine networks lead to severe asthma in some individuals.

Acknowledgements

This review included results obtained from the Difficult Asthma Program. We thank Dr Ronald Olivenstein for their involvement in the programme. We also thank the Richard and Edith Strauss Foundation of Canada for supporting this programme including the support of Dr Audrey Poon in the form of fellowship.

Conflict of interest: All authors declared no conflict of interest in regards to the publication of this article.

References

- 1 Fitzpatrick AM, Teague WG, Meyers
DA *et al.* Heterogeneity of severe
asthma in childhood: confirmation
by cluster analysis of children in the
National Institutes of Health/National

- Heart, Lung, and Blood Institute Severe Asthma Research Program. *J Allergy Clin Immunol* 2011; **127**:382–9.
- 2 Moore WC, Meyers DA, Wenzel SE *et al*. Identification of asthma phenotypes using cluster analysis in the

- Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010; **181**:315–23.
- 3 Brasier AR, Victor S, Boetticher G *et al*. Molecular phenotyping of severe asthma using pattern recognition of bronchoalveolar lavage-derived cyto-

- kines. *J Allergy Clin Immunol* 2008; 121:30–7.
- 4 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.
 - 5 Fahy JV. Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. *Proc Am Thorac Soc* 2009; 6:256–9.
 - 6 Hogan SP, Rosenberg HF, Moqbel R *et al*. Eosinophils: biological properties and role in health and disease. *Clin Exp Allergy* 2008; 38:709–50.
 - 7 Haldar P, Brightling CE, Hargadon B *et al*. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009; 360:973–84.
 - 8 Haldar P, Pavord ID, Shaw DE *et al*. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008; 178:218–24.
 - 9 Leung DY, Martin RJ, Szeffler SJ *et al*. Dysregulation of interleukin 4, interleukin 5, and interferon gamma gene expression in steroid-resistant asthma. *J Exp Med* 1995; 181:33–40.
 - 10 Naseer T, Minshall EM, Leung DY *et al*. Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy. *Am J Respir Crit Care Med* 1997; 155:845–51.
 - 11 Bentley AM, Hamid Q, Robinson DS *et al*. Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. *Am J Respir Crit Care Med* 1996; 153:551–6.
 - 12 Robinson D, Hamid Q, Ying S *et al*. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5, and interferon-gamma cytokine gene expression. *Am Rev Respir Dis* 1993; 148:401–6.
 - 13 Hastie AT, Moore WC, Meyers DA *et al*. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *J Allergy Clin Immunol* 2010; 125:1028–36.
 - 14 Ordóñez CL, Shaughnessy TE, Matthay MA, Fahy JV. Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma: clinical and biologic significance. *Am J Respir Crit Care Med* 2000; 161 (4 Pt 1):1185–90.
 - 15 Shaw DE, Berry MA, Hargadon B *et al*. Association between neutrophilic airway inflammation and airflow limitation in adults with asthma. *Chest* 2007; 132:1871–5.
 - 16 Wenzel SE, Schwartz LB, Langmack EL *et al*. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999; 160:1001–8.
 - 17 Woodruff PG, Khashayar R, Lazarus SC *et al*. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. *J Allergy Clin Immunol* 2001; 108:753–8.
 - 18 Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. *J Allergy Clin Immunol* 2004; 113:101–8.
 - 19 Balzar S, Fajt ML, Comhair SA *et al*. Mast cell phenotype, location, and activation in severe asthma: data from the severe asthma research program. *Am J Respir Crit Care Med* 2011; 183:299–309.
 - 20 Balzar S, Chu HW, Strand M, Wenzel S. Relationship of small airway chymase-positive mast cells and lung function in severe asthma. *Am J Respir Crit Care Med* 2005; 171:431–9.
 - 21 Al-Muhsen S, Johnson JR, Hamid Q. Remodeling in asthma. *J Allergy Clin Immunol* 2011; 128:451–62.
 - 22 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.
 - 23 Chakir J, Shannon J, Molet S *et al*. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 2003; 111:1293–8.
 - 24 Pepe C, Foley S, Shannon J *et al*. Differences in airway remodeling between subjects with severe and moderate asthma. *J Allergy Clin Immunol* 2005; 116:544–9.
 - 25 Shannon J, Ernst P, Yamauchi Y *et al*. Differences in airway cytokine profile in severe asthma compared to moderate asthma. *Chest* 2008; 133:420–6.
 - 26 Li X, Howard TD, Zheng SL *et al*. Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. *J Allergy Clin Immunol* 2010; 125:328–35.
 - 27 Wenzel SE, Balzar S, Ampleford E *et al*. IL4R alpha mutations are associated with asthma exacerbations and mast cell/IgE expression. *Am J Respir Crit Care Med* 2007; 175:570–6.
 - 28 Wu AC, Lasky-Su J, Rogers CA, Klanderma BJ, Litonjua AA. Fungal exposure modulates the effect of polymorphisms of chitinases on emergency department visits and hospitalizations. *Am J Respir Crit Care Med* 2010; 182:884–9.
 - 29 Wills-Karp M, Finkelman FD. Untangling the complex web of IL-4- and IL-13-mediated signaling pathways. *Sci Signal* 2008; 1:e55.
 - 30 Oh CK, Geba GP, Molino N. Investigational therapeutics targeting the IL-4/IL-13/STAT-6 pathway for the treatment of asthma. *Eur Respir Rev* 2010; 19:46–54.
 - 31 Humbert M, Berger W, Rapatz G, Turk F. Add-on omalizumab improves day-to-day symptoms in inadequately controlled severe persistent allergic asthma. *Allergy* 2008; 63:592–6.
 - 32 LaPorte SL, Juo ZS, Vaclavikova J *et al*. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* 2008; 132:259–72.
 - 33 Elo LL, Jarvenpaa H, Tuomela S *et al*. Genome-wide profiling of interleukin-4 and STAT6 transcription factor regulation of human Th2 cell programming. *Immunity* 2010; 32:852–62.
 - 34 Chapoval SP, Dasgupta P, Smith EP *et al*. STAT6 expression in multiple cell types mediates the cooperative development of allergic airway disease. *J Immunol* 2011; 186:2571–83.
 - 35 Nofziger C, Vezzoli V, Dossena S *et al*. STAT6 links IL-4/IL-13 stimulation with pendrin expression in asthma and chronic obstructive pulmonary disease. *Clin Pharmacol Ther* 2011; 90:399–405.
 - 36 Wood N, Whitters MJ, Jacobson BA *et al*. Enhanced interleukin (IL)-13 responses in mice lacking IL-13 receptor alpha 2. *J Exp Med* 2003; 197:703–9.

- 37 Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and IL4 locus accessibility. *Annu Rev Immunol* 2006; 24:607–56.
- 38 Deshpande DA, Dogan S, Walseth TF *et al.* Modulation of calcium signaling by interleukin-13 in human airway smooth muscle: role of CD38/cyclic adenosine diphosphate ribose pathway. *Am J Respir Cell Mol Biol* 2004; 31:36–42.
- 39 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.
- 40 Finkelman FD, Hogan SP, Hershey GK, Rothenberg ME, Wills-Karp M. Importance of cytokines in murine allergic airway disease and human asthma. *J Immunol* 2010; 184: 1663–74.
- 41 Ahn JH, Kim CH, Kim YH *et al.* Inflammatory and remodeling events in asthma with chronic exposure to house dust mites: a murine model. *J Korean Med Sci* 2007; 22:1026–33.
- 42 Johnson JR, Swirski FK, Gajewska BU *et al.* Divergent immune responses to house dust mite lead to distinct structural-functional phenotypes. *Am J Physiol Lung Cell Mol Physiol* 2007; 293:L730–L739.
- 43 Foster PS, Ming Y, Matthei KI, Young IG, Temelkovski J, Kumar RK. Dissociation of inflammatory and epithelial responses in a murine model of chronic asthma. *Lab Invest* 2000; 80:655–62.
- 44 Hirota JA, Ask K, Fritz D *et al.* Role of STAT6 and SMAD2 in a model of chronic allergen exposure: a mouse strain comparison study. *Clin Exp Allergy* 2009; 39:147–58.
- 45 Nath P, Leung SY, Williams AS *et al.* Complete inhibition of allergic airway inflammation and remodelling in quadruple IL-4/5/9/13/-/- mice. *Clin Exp Allergy* 2007; 37:1427–35.
- 46 Therien AG, Bernier V, Weicker S *et al.* Adenovirus IL-13-induced airway disease in mice: a corticosteroid-resistant model of severe asthma. *Am J Respir Cell Mol Biol* 2008; 39: 26–35.
- 47 Yang G, Volk A, Petley T *et al.* Anti-IL-13 monoclonal antibody inhibits airway hyperresponsiveness, inflammation and airway remodeling. *Cytokine* 2004; 28:224–32.
- 48 Graves PE, Kabesch M, Halonen M *et al.* A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J Allergy Clin Immunol* 2000; 105:506–13.
- 49 Liu X, Beaty TH, Deindl P *et al.* Associations between total serum IgE levels and the 6 potentially functional variants within the genes IL4, IL13, and IL4RA in German children: the German Multicenter Atopy Study. *J Allergy Clin Immunol* 2003; 112:382–8.
- 50 Delimpoura V, Bakakos P, Tseliou E *et al.* Increased levels of osteopontin in sputum supernatant in severe refractory asthma. *Thorax* 2010; 65:782–6.
- 51 Macedo P, Hew M, Torrego A *et al.* Inflammatory biomarkers in airways of patients with severe asthma compared with non-severe asthma. *Clin Exp Allergy* 2009; 39:1668–76.
- 52 Corren J, Lemanske RF, Hanania NA *et al.* Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011; 365:1088–98.
- 53 Corren J, Busse W, Meltzer EO *et al.* A randomized, controlled, phase 2 study of AMG 317, an IL-4R α antagonist, in patients with asthma. *Am J Respir Crit Care Med* 2010; 181:788–96.
- 54 Zhu Z, Zheng T, Homer RJ *et al.* Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 2004; 304:1678–82.
- 55 Wadsworth SJ, Atsuta R, McIntyre JO, Hackett TL, Singhera GK, Dorscheid DR. IL-13 and TH2 cytokine exposure triggers matrix metalloproteinase 7-mediated Fas ligand cleavage from bronchial epithelial cells. *J Allergy Clin Immunol* 2010; 126:366–74.
- 56 Sidhu SS, Yuan S, Innes AL *et al.* Roles of epithelial cell-derived periostin in TGF- β activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci U S A* 2010; 107:14170–5.
- 57 Itoh N, Yonehara S, Ishii A *et al.* The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 1991; 66: 233–43.
- 58 Suda T, Takahashi T, Golstein P, Nagata S. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 1993; 75:1169–78.
- 59 Uller L, Rydell-Tormanen K, Persson CG, Erjefalt JS. Anti-Fas mAb-induced apoptosis and cytolysis of airway tissue eosinophils aggravates rather than resolves established inflammation. *Respir Res* 2005; 6:90.
- 60 Shahabuddin M, Vinetz JM. Chitinases of human parasites and their implications as antiparasitic targets. *EXS* 1999; 87:223–34.
- 61 Shibata Y, Foster LA, Bradfield JF, Myrvik QN. Oral administration of chitin down-regulates serum IgE levels and lung eosinophilia in the allergic mouse. *J Immunol* 2000; 164:1314–21.
- 62 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.
- 63 Ober C, Tan Z, Sun Y *et al.* Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function. *N Engl J Med* 2008; 358: 1682–91.
- 64 Heinzmann A, Brugger M, Bierbaum S, Mailaparambil B, Kopp MV, Strauch K. Joint influences of Acidic-Mammalian-Chitinase with interleukin-4 and toll-like receptor-10 with interleukin-13 in the genetics of asthma. *Pediatr Allergy Immunol* 2010; 21(4 Pt 2):e679–e686.
- 65 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.
- 66 Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009; 119:1420–8.
- 67 Takatsu K, Nakajima H. IL-5 and eosinophilia. *Curr Opin Immunol* 2008; 20:288–94.
- 68 Takatsu K, Kouro T, Nagai Y. Interleukin 5 in the link between the innate and acquired immune response. *Adv Immunol* 2009; 101:191–236.
- 69 Tanaka H, Komai M, Nagao K *et al.* Role of interleukin-5 and eosinophils in allergen-induced airway remodel-

- ing in mice. *Am J Respir Cell Mol Biol* 2004; **31**:62–8.
- 70 Flood-Page P, Menzies-Gow A, Phipps S *et al*. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003; **112**:1029–36.
 - 71 Xu J, Jiang F, Nayeri F, Zetterstrom O. Apoptotic eosinophils in sputum from asthmatic patients correlate negatively with levels of IL-5 and eotaxin. *Respir Med* 2007; **101**:1447–54.
 - 72 Dente FL, Carnevali S, Bartoli ML *et al*. Profiles of proinflammatory cytokines in sputum from different groups of severe asthmatic patients. *Ann Allergy Asthma Immunol* 2006; **97**:312–20.
 - 73 Nair P, Pizzichini MM, Kjarsgaard M *et al*. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009; **360**:985–93.
 - 74 Riechelmann H, Deutschle T, Rozsasi A, Keck T, Polzehl D, Burner H. Nasal biomarker profiles in acute and chronic rhinosinusitis. *Clin Exp Allergy* 2005; **35**:1186–91.
 - 75 Kurowska-Stolarska M, Kewin P, Murphy G *et al*. IL-33 induces antigen-specific IL-5 + T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol* 2008; **181**:4780–90.
 - 76 Prefontaine D, Nadigel J, Chouiali F *et al*. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010; **125**:752–4.
 - 77 Chackerian AA, Oldham ER, Murphy EE, Schmitz J, Pflanz S, Kastelein RA. IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex. *J Immunol* 2007; **179**:2551–5.
 - 78 Ali S, Mohs A, Thomas M *et al*. The dual function cytokine IL-33 interacts with the transcription factor NF- κ B to dampen NF- κ B-stimulated gene transcription. *J Immunol* 2011; **187**:1609–16.
 - 79 Roussel L, Erard M, Cayrol C, Girard JP. Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket. *EMBO Rep* 2008; **9**:1006–12.
 - 80 Schmitz J, Owyang A, Oldham E *et al*. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005; **23**:479–90.
 - 81 Lloyd CM. IL-33 family members and asthma - bridging innate and adaptive immune responses. *Curr Opin Immunol* 2010; **22**:800–6.
 - 82 Oboki K, Ohno T, Kajiwara N, Saito H, Nakae S. IL-33 and IL-33 receptors in host defense and diseases. *Allergol Int* 2010; **59**:143–60.
 - 83 Gudbjartsson DF, Bjornsdottir US *et al*. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009; **41**:342–7.
 - 84 Moffatt MF, Gut IG, Demenais F *et al*. A large-scale, consortium-based genome-wide association study of asthma. *N Engl J Med* 2010; **363**:1211–21.
 - 85 Torgerson DG, Ampleford EJ, Chiu GY *et al*. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011; **43**:887–92.
 - 86 Yagami A, Orihara K, Morita H *et al*. IL-33 mediates inflammatory responses in human lung tissue cells. *J Immunol* 2010; **185**:5743–50.
 - 87 Silver MR, Margulis A, Wood N, Goldman SJ, Kasaian M, Chaudhary D. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res* 2010; **59**:207–18.
 - 88 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.
 - 89 Roussel L, Houle F, Chan C *et al*. IL-17 promotes p38 MAPK-dependent endothelial activation enhancing neutrophil recruitment to sites of inflammation. *J Immunol* 2010; **184**:4531–7.
 - 90 Bullens DM, Truyen E, Coteur L *et al*. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res* 2006; **7**:135.
 - 91 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.
 - 92 Wenzel S. Severe asthma: from characteristics to phenotypes to endotypes. *Clin Exp Allergy* 2012; **42**:650–8.
 - 93 Aujla SJ, Alcorn JF. T(H)17 cells in asthma and inflammation. *Biochim Biophys Acta* 2011; **1810**:1066–79.
 - 94 Chen Z, Tato CM, Muul L, Laurence A, O'Shea JJ. Distinct regulation of interleukin-17 in human T helper lymphocytes. *Arthritis Rheum* 2007; **56**:2936–46.
 - 95 Wilson NJ, Boniface K, Chan JR *et al*. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* 2007; **8**:950–7.
 - 96 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.
 - 97 Lee J, Ho WH, Maruoka M *et al*. IL-17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL-17Rh1. *J Biol Chem* 2001; **276**:1660–4.
 - 98 Starnes T, Broxmeyer HE, Robertson MJ, Hromas R. Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. *J Immunol* 2002; **169**:642–6.
 - 99 Li H, Chen J, Huang A *et al*. Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. *Proc Natl Acad Sci U S A* 2000; **97**:773–8.
 - 100 Yao Z, Painter SL, Fanslow WC *et al*. Human IL-17: a novel cytokine derived from T cells. *J Immunol* 1995; **155**:5483–6.
 - 101 Gaffen SL. Structure and signalling in the IL-17 receptor family. *Nat Rev Immunol* 2009; **9**:556–67.
 - 102 Shen F, Gaffen SL. Structure-function relationships in the IL-17 receptor: implications for signal transduction and therapy. *Cytokine* 2008; **41**:92–104.
 - 103 Shen F, Ruddy MJ, Plamondon P, Gaffen SL. Cytokines link osteoblasts and inflammation: microarray analysis of interleukin-17- and TNF-alpha-induced genes in bone cells. *J Leukoc Biol* 2005; **77**:388–99.
 - 104 Hellings PW, Kasran A, Liu Z *et al*. Interleukin-17 orchestrates the granulocyte influx into airways after aller-

- gen inhalation in a mouse model of allergic asthma. *Am J Respir Cell Mol Biol* 2003; 28:42–50.
- 105 Oda N, Canelos PB, Essayan DM, Plunkett BA, Myers AC, Huang SK. Interleukin-17F induces pulmonary neutrophilia and amplifies antigen-induced allergic response. *Am J Respir Crit Care Med* 2005; 171:12–8.
 - 106 Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, Cook DN. Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. *Am J Respir Crit Care Med* 2009; 180:720–30.
 - 107 Sergejeva S, Ivanov S, Lotvall J, Linden A. Interleukin-17 as a recruitment and survival factor for airway macrophages in allergic airway inflammation. *Am J Respir Cell Mol Biol* 2005; 33:248–53.
 - 108 Suzuki S, Kokubu F, Kawaguchi M *et al.* Expression of interleukin-17F in a mouse model of allergic asthma. *Int Arch Allergy Immunol* 2007; 143 (Suppl. 1):89–94.
 - 109 Wang Q, Li H, Yao Y, Xia D, Zhou J. The overexpression of heparin-binding epidermal growth factor is responsible for Th17-induced airway remodeling in an experimental asthma model. *J Immunol* 2010; 185: 834–41.
 - 110 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.
 - 111 Letuve S, Lajoie-Kadoch S, Audusseau S *et al.* IL-17E upregulates the expression of proinflammatory cytokines in lung fibroblasts. *J Allergy Clin Immunol* 2006; 117:590–6.
 - 112 Chen J, Deng Y, Zhao J *et al.* The polymorphism of IL-17 G-152A was associated with childhood asthma and bacterial colonization of the hypopharynx in bronchiolitis. *J Clin Immunol* 2010; 30:539–45.
 - 113 Wang JY, Shyr SD, Wang WH *et al.* The polymorphisms of interleukin 17A (IL17A) gene and its association with pediatric asthma in Taiwanese population. *Allergy* 2009; 64: 1056–60.
 - 114 Jin EH, Choi EY, Yang JY, Chung HT, Yang YS. Significant association between IL-17F promoter region polymorphism and susceptibility to asthma in a Korean population. *Int Arch Allergy Immunol* 2011; 155: 106–10.
 - 115 Kawaguchi M, Takahashi D, Hizawa N *et al.* IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. *J Allergy Clin Immunol* 2006; 117:795–801.
 - 116 Lazarevic V, Glimcher LH. T-bet in disease. *Nat Immunol* 2011; 12: 597–606.
 - 117 Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. *Adv Immunol* 2007; 96:41–101.
 - 118 Celada A, Allen R, Esparza I, Gray PW, Schreiber RD. Demonstration and partial characterization of the interferon-gamma receptor on human mononuclear phagocytes. *J Clin Invest* 1985; 76:2196–205.
 - 119 Jung V, Rashidbaigi A, Jones C, Tischfield JA, Shows TB, Pestka S. Human chromosomes 6 and 21 are required for sensitivity to human interferon gamma. *Proc Natl Acad Sci U S A* 1987; 84:4151–5.
 - 120 Hu X, Ivashkiv LB. Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases. *Immunity* 2009; 31:539–50.
 - 121 Gough DJ, Levy DE, Johnstone RW, Clarke CJ. IFN-gamma signaling-does it mean JAK-STAT? *Cytokine Growth Factor Rev* 2008; 19:383–94.
 - 122 Yu M, Eckart MR, Morgan AA *et al.* Identification of an IFN-gamma/mast cell axis in a mouse model of chronic asthma. *J Clin Invest* 2011; 121: 3133–43.
 - 123 Yoshida M, Leigh R, Matsumoto K *et al.* Effect of interferon-gamma on allergic airway responses in interferon-gamma-deficient mice. *Am J Respir Crit Care Med* 2002; 166: 451–6.
 - 124 Yang M, Kumar RK, Foster PS. Pathogenesis of steroid-resistant airway hyperresponsiveness: interaction between IFN-gamma and TLR4/MyD88 pathways. *J Immunol* 2009; 182:5107–15.
 - 125 Magnan AO, Mely LG, Camilla CA *et al.* Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma. Increased IFN-gamma-producing CD8(+) T cells in asthma. *Am J Respir Crit Care Med* 2000; 161:1790–6.
 - 126 Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4 + and CD8 + T cells in atopic asthma. *Am J Respir Crit Care Med* 2005; 171:224–30.
 - 127 Kelly DM, Greene CM, Meachery G *et al.* Endotoxin up-regulates interleukin-18: potential role for gram-negative colonization in sarcoidosis. *Am J Respir Crit Care Med* 2005; 172:1299–307.
 - 128 Lapa e Silva JR, Possebon da Silva MD, Lefort J, Vargaftig BB. Endotoxins, asthma, and allergic immune responses. *Toxicology* 2000; 152:31–5.
 - 129 Nakanishi K, Tsutsui H, Yoshimoto T. Importance of IL-18-induced super Th1 cells for the development of allergic inflammation. *Allergol Int* 2010; 59:137–41.
 - 130 Yamagata S, Tomita K, Sato R, Niwa A, Higashino H, Tohda Y. Interleukin-18-deficient mice exhibit diminished chronic inflammation and airway remodelling in ovalbumin-induced asthma model. *Clin Exp Immunol* 2008; 154:295–304.
 - 131 Hayashi N, Yoshimoto T, Izuhara K, Matsui K, Tanaka T, Nakanishi K. T helper 1 cells stimulated with ovalbumin and IL-18 induce airway hyperresponsiveness and lung fibrosis by IFN-gamma and IL-13 production. *Proc Natl Acad Sci U S A* 2007; 104:14765–70.
 - 132 Sugimoto T, Ishikawa Y, Yoshimoto T, Hayashi N, Fujimoto J, Nakanishi K. Interleukin 18 acts on memory T helper cells type 1 to induce airway inflammation and hyperresponsiveness in a naive host mouse. *J Exp Med* 2004; 199:535–45.
 - 133 Patil SP, Wisnivesky JP, Busse PJ, Halm EA, Li XM. Detection of immunological biomarkers correlated with asthma control and quality of life measurements in sera from chronic asthmatic patients. *Ann Allergy Asthma Immunol* 2011; 106:205–13.
 - 134 Harada M, Obara K, Hirota T *et al.* A functional polymorphism in IL-18 is associated with severity of bronchial asthma. *Am J Respir Crit Care Med* 2009; 180:1048–55.
 - 135 Reijmerink NE, Postma DS, Bruinenberg M *et al.* Association of IL1RL1, IL18R1, and IL18RAP gene cluster polymorphisms with asthma and

- atopy. *J Allergy Clin Immunol* 2008; 122:651–4.
- 136 Bhavsar PK, Levy BD, Hew MJ *et al*. Corticosteroid suppression of lipoxin A4 and leukotriene B4 from alveolar macrophages in severe asthma. *Respir Res* 2010; 11:71.
 - 137 Barnes PJ. Mechanisms and resistance in glucocorticoid control of inflammation. *J Steroid Biochem Mol Biol* 2010; 120:76–85.
 - 138 Hollenberg SM, Weinberger C, Ong ES *et al*. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* 1985; 318:635–41.
 - 139 Bamberger CM, Bamberger AM, de CM, Chrousos GP. Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. *J Clin Invest* 1995; 95: 2435–41.
 - 140 Oakley RH, Sar M, Cidlowski JA. The human glucocorticoid receptor beta isoform. Expression, biochemical properties, and putative function. *J Biol Chem* 1996; 271:9550–9.
 - 141 Oakley RH, Jewell CM, Yudit MR, Bofetado DM, Cidlowski JA. The dominant negative activity of the human glucocorticoid receptor beta isoform. Specificity and mechanisms of action. *J Biol Chem* 1999; 274: 27857–66.
 - 142 Sher ER, Leung DY, Surs W *et al*. Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy. *J Clin Invest* 1994; 93:33–9.
 - 143 Kam JC, Szeffler SJ, Surs W, Sher ER, Leung DY. Combination IL-2 and IL-4 reduces glucocorticoid receptor-binding affinity and T cell response to glucocorticoids. *J Immunol* 1993; 151:3460–6.
 - 144 Bergeron C, Fukakusa M, Olivenstein R *et al*. Increased glucocorticoid receptor-beta expression, but not decreased histone deacetylase 2, in severe asthma. *J Allergy Clin Immunol* 2006; 117:703–5.
 - 145 Christodoulopoulos P, Leung DY, Elliott MW *et al*. Increased number of glucocorticoid receptor-beta-expressing cells in the airways in fatal asthma. *J Allergy Clin Immunol* 2000; 106:479–84.
 - 146 Hamid QA, Wenzel SE, Hauk PJ *et al*. Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma. *Am J Respir Crit Care Med* 1999; 159(5 Pt 1):1600–4.
 - 147 Irusen E, Matthews JG, Takahashi A, Barnes PJ, Chung KF, Adcock IM. p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. *J Allergy Clin Immunol* 2002; 109: 649–57.
 - 148 Leung DY, Hamid Q, Vottero A *et al*. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. *J Exp Med* 1997; 186:1567–74.
 - 149 Matthews JG, Ito K, Barnes PJ, Adcock IM. Defective glucocorticoid receptor nuclear translocation and altered histone acetylation patterns in glucocorticoid-resistant patients. *J Allergy Clin Immunol* 2004; 113:1100–8.
 - 150 Vazquez-Tello A, Semlali A, Chakir J *et al*. Induction of glucocorticoid receptor-beta expression in epithelial cells of asthmatic airways by T-helper type 17 cytokines. *Clin Exp Allergy* 2010; 40:1312–22.
 - 151 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.
 - 152 Ito K, Barnes PJ, Adcock IM. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol* 2000; 20:6891–903.
 - 153 Grausenburger R, Bilic I, Boucheron N *et al*. Conditional deletion of histone deacetylase 1 in T cells leads to enhanced airway inflammation and increased Th2 cytokine production. *J Immunol* 2010; 185:3489–97.
 - 154 Hew M, Bhavsar P, Torrego A *et al*. Relative corticosteroid insensitivity of peripheral blood mononuclear cells in severe asthma. *Am J Respir Crit Care Med* 2006; 174:134–41.
 - 155 Cosio BG, Mann B, Ito K *et al*. Histone acetylase and deacetylase activity in alveolar macrophages and blood monocytes in asthma. *Am J Respir Crit Care Med* 2004; 170:141–7.
 - 156 Kirkham P, Rahman I. Oxidative stress in asthma and COPD: antioxidants as a therapeutic strategy. *Pharmacol Ther* 2006; 111:476–94.
 - 157 Fitzpatrick AM, Teague WG, Burwell L, Brown MS, Brown LA. Glutathione oxidation is associated with airway macrophage functional impairment in children with severe asthma. *Pediatr Res* 2011; 69:154–9.
 - 158 Ito K, Hanazawa T, Tomita K, Barnes PJ, Adcock IM. Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. *Biochem Biophys Res Commun* 2004; 315:240–5.
 - 159 Milara J, Navarro A, Almudever P, Lluch J, Morcillo EJ, Cortijo J. Oxidative stress-induced glucocorticoid resistance is prevented by dual PDE3/PDE4 inhibition in human alveolar macrophages. *Clin Exp Allergy* 2011; 41:535–46.
 - 160 Maranville JC, Luca F, Richards AL *et al*. Interactions between glucocorticoid treatment and Cis-regulatory polymorphisms contribute to cellular response phenotypes. *PLoS Genet* 2011; 7:e1002162.
 - 161 Tantisira KG, Lasky-Su J, Harada M *et al*. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. *N Engl J Med* 2011; 365:1173–83.
 - 162 Chapman MS, Askew DJ, Kuscuglu U, Miesfeld RL. Transcriptional control of steroid-regulated apoptosis in murine thymoma cells. *Mol Endocrinol* 1996; 10:967–78.
 - 163 Newcomb DC, Zhou W, Moore ML *et al*. A functional IL-13 receptor is expressed on polarized murine CD4 + Th17 cells and IL-13 signaling attenuates Th17 cytokine production. *J Immunol* 2009; 182:5317–21.
 - 164 Lexberg MH, Taubner A, Albrecht I *et al*. IFN-gamma and IL-12 synergize to convert in vivo generated Th17 into Th1/Th17 cells. *Eur J Immunol* 2010; 40:3017–27.
 - 165 Hosoya T, Maillard I, Engel JD. From the cradle to the grave: activities of GATA-3 throughout T-cell development and differentiation. *Immunol Rev* 2010; 238:110–25.
 - 166 Marine J, Winoto A. The human enhancer-binding protein Gata3 binds to several T-cell receptor regulatory elements. *Proc Natl Acad Sci U S A* 1991; 88:7284–8.
 - 167 Ho IC, Tai TS, Pai SY. GATA3 and the T-cell lineage: essential functions before and after T-helper-2-cell differentiation. *Nat Rev Immunol* 2009; 9:125–35.

- 168 Fang TC, Yashiro-Ohtani Y, Del BC, Knoblock DM, Blacklow SC, Pear WS. Notch directly regulates Gata3 expression during T helper 2 cell differentiation. *Immunity* 2007; **27**:100–10.
- 169 van Hamburg JP, de Bruijn MJ, Ribeiro de AC *et al.* Enforced expression of GATA3 allows differentiation of IL-17-producing cells, but constrains Th17-mediated pathology. *Eur J Immunol* 2008; **38**:2573–86.
- 170 Morgan JG, Dolganov GM, Robbins SE, Hinton LM, Lovett M. The selective isolation of novel cDNAs encoded by the regions surrounding the human interleukin 4 and 5 genes. *Nucleic Acids Res* 1992; **20**:5173–9.
- 171 Dale M, Nicklin MJ. Interleukin-1 receptor cluster: gene organization of IL1R2, IL1R1, IL1RL2 (IL-1R_{p2}), IL1RL1 (T1/ST2), and IL18R1 (IL-1R_{p1}) on human chromosome 2q. *Genomics* 1999; **57**:177–9.
- 172 Rowell E, Merckenschlager M, Wilson CB. Long-range regulation of cytokine gene expression. *Curr Opin Immunol* 2008; **20**:272–80.
- 173 Miele A, Dekker J. Long-range chromosomal interactions and gene regulation. *Mol Biosyst* 2008; **4**: 1046–57.