

Emerging roles of pulmonary macrophages in driving the development of severe asthma

Ming Yang,^{*,1} Rakesh K. Kumar,[†] Philip M. Hansbro,^{*,2} and Paul S. Foster^{*,2}

^{*}Centre for Asthma and Respiratory Disease, School of Biomedical Sciences and Pharmacy, University of Newcastle and Hunter Medical Research Institute, Callaghan, Australia; and [†]Department of Pathology, School of Medical Sciences, University of New South Wales, Sydney, Australia

RECEIVED JULY 18, 2011; REVISED JANUARY 3, 2012; ACCEPTED JANUARY 10, 2012. DOI: 10.1189/jlb.0711357

ABSTRACT

Asthma is recognized as a heterogeneous disorder, although in most patients, the clinical manifestations are effectively managed with established combination therapies. However, 5–10% of asthmatics have severe asthma, which does not respond to treatment, and these patients account for >50% of asthma-related healthcare costs. New investigations into the pathogenesis of glucocorticoid resistance in severe asthma indicate that pulmonary macrophages may play central roles in promoting airway inflammation, particularly in asthma that is resistant to steroid therapy. Importantly, factors that are linked to the activation of pulmonary macrophages may contribute to glucocorticoid resistance and severe asthma. Here, we review recent advances in understanding the roles of pulmonary macrophages in the mechanisms of glucocorticoid resistance and the pathogenesis of severe asthma. We discuss the role of macrophage phenotype, infection, IFN- γ , LPS, associated signaling pathways, TNF- α , MIF, and other macrophage-associated factors. Understanding the pathogenesis of steroid-resistant severe asthma will contribute to the identification of optimal therapeutic strategies for the effective management of the disease. *J. Leukoc. Biol.* 91: 000–000; 2012.

Introduction

Allergic asthma is characterized by wheezing, cough, and dyspnea and is driven by the development and recruitment of CD4⁺ Th2 cells to the lung. These cells release cytokines (e.g., IL-4, -5, -9, and -13) that promote eosinophil and mast cell influx, mucus hypersecretion, airway wall remodeling,

and airway hyper-responsiveness (AHR), which underpin the clinical manifestations of mild to moderate disease (reviewed in refs. [1, 2]).

By contrast, severe asthmatics frequently have neutrophil-dominated airway inflammation rather than increased numbers of eosinophils in their sputum, a mixed Th2/Th1 and potentially Th17 phenotype, and chronic bacterial colonization of the airways (reviewed in ref. [2]). There is increasing evidence that Th2-mediated pathways may not primarily regulate disease in patients with severe asthma. Although the pathogenesis of severe asthma is complex and clearly multifactorial [3, 4], the prevalence and effects of respiratory tract infections and activated immune pathways involved in innate host defense strongly suggest a causal role [3, 5–12]. For example, activation of neutrophils and macrophages and elevated levels of CXCL8, IFN- γ , and LPS occur in the airways of severe asthmatics and are often linked to a decline in lung function. These inflammatory features are clearly distinct from those of allergic or eosinophilic asthma, where Th2 cell-mediated inflammation predominates [2, 13]. Thus, innate host defense pathways may contribute to the pathogenesis of severe asthma, as well as to the features of disease in individuals who are refractive to anti-inflammatory therapy with glucocorticoids [14, 15].

There is no universally accepted definition of severe asthma, although the recent World Health Organization statement provides a consensus definition in terms of current clinical control, with severe asthma having an increased risk of frequent, severe exacerbations and/or adverse reactions to medications and/or chronic morbidity. Thus, it includes untreated asthma, difficult-to-treat severe asthma, and treatment-resistant severe asthma [16]. A lack of improvement in FEV₁ after long-term glucocorticoid treatment is a characteristic feature. Indeed, failure to increase baseline FEV₁ by >15% after oral pred-

Abbreviations: AHR=airway hyper-responsiveness, BALF=BAL fluid, CHI3L1=chitinase 3-like 1, FEV₁=forced expiratory volume in 1 s, GCR=glucocorticoid receptor, IRF-1=IFN-regulatory factor 1, KC=keratinocyte-derived chemokine, MDSC=myeloid-derived suppressor cell, MIF=macrophage migration inhibitory factor, MMP-12=matrix metalloproteinase-12, Retn α =resistin-like molecule α , RSV=respiratory syncytial virus, RV=rhinoviruses, TAM=tumor-associated macrophage, TRIF=Toll-IL receptor domain-containing adaptor-inducing IFN- β

1. Correspondence: School of Biomedical Sciences and Pharmacy, University of Newcastle, 5th Floor David Maddison Clinical Sciences Bldg., cnr Watt and King Sts., Newcastle NSW 2300, Australia. E-mail: ming.yang@newcastle.edu.au
2. These authors contributed equally.

nisolone treatment of 20 mg daily in the 1st week and 40 mg daily for the 2nd week or variations thereof are reasonable, widely used measures [17]. Many clinical bodies, including the British Thoracic Society and the Severe Asthma Research Program, have developed working clinical definitions of severe asthma to promote unified approaches in clinical practice and research [18–20]. These programs define severe asthma based on response to glucocorticoids and clinical features of the disease.

PERSISTENT AHR

AHR is the exaggerated narrowing of the airways in response to nonspecific stimuli, and it is a major clinical manifestation of asthma. The degree of AHR often correlates with the severity of disease [13], and the titration of therapy based on control of AHR may also yield superior therapeutic outcomes [21]. The precise mechanisms that regulate the development of AHR remain unclear, but it has at least two components: one that is present at baseline and another that is related to environmental triggers, such as allergen exposure or infection [9, 22–24]. Allergen provocation of allergic asthmatics elicits an immediate bronchoconstriction and an acute inflammatory response [25]. This early-phase response, which is characterized by acute airway obstruction, can last from 30 min to several hours after allergen exposure [26] and is thought to be triggered by inflammatory mediators released from mast cells following cross-linking of surface-bound IgE by antigen [27]. Approximately 50% of individuals who experience an early-phase response may also exhibit a late-phase response that is associated with persistent AHR, lasting for at least 7 days [26]. Infiltrating inflammatory cells and their proinflammatory products have been linked to the development of persistent AHR [28–30]. However, persistent AHR may be dissociated from inflammatory cell recruitment [26], implying that resident pulmonary cells may contribute to this response.

GLUCOCORTICOID RESISTANCE

Chronic airway inflammation and AHR in the majority of asthmatics (mild to moderate, allergic) can be effectively managed by combination therapy with broad-spectrum, anti-inflammatory agents and bronchodilators, typically inhaled glucocorticoids, and long-acting β -agonists [31–33]. Glucocorticoids are used to suppress immune and inflammatory responses involving Th2 cells, eosinophils, and mast cells [31]. However, severe asthmatics remain refractory to steroid therapy [34].

Glucocorticoids are highly diffusible lipophilic molecules that bind to GCRs in the cytoplasm, and the resulting complex migrates to the nucleus [3]. In addition to inducing apoptosis in lymphocytes, activated GCR complexes inhibit the expression of proinflammatory genes (e.g., IL-2, PGs, and leukotrienes) and increase the expression of anti-inflammatory genes (e.g., annexin-1, secretory leukoprotease inhibitor 1, and IL-10) [3, 35]. This occurs through the regulation of gene transcription, including via the suppression of transcriptional signaling pathways, such as those activated by AP-1, MAPKs, NF-

κ B, and others. Furthermore, activated GCR complexes can modulate gene expression by reducing histone acetylation, destabilizing the mRNA of inflammatory factors (e.g., TNF- α), and decreasing the recruitment of transcriptional factors. Glucocorticoids also prevent proinflammatory cytokine (such as IL-1 β)-induced impairment of β 2R-mediated relaxation. This likely occurs through the restoration of adenylyl cyclase action and the suppression of the enhanced activity of cytosolic GPCR kinases [36].

The development of glucocorticoid resistance is multifactorial, and we have recently reviewed the mechanisms involved [4]. They include abnormal expression of GCRs, defects in transcriptional factor activity, epigenetic changes, and other environmental, physiological, and psychological factors. For example, phosphorylated STAT5 can directly bind the GCR protein, which may result in the loss of suppressive function of glucocorticoids in murine T cells [37]. Furthermore, excessive activation of NF- κ B overwhelms the suppressive responses induced by activated GCRs and leads to steroid resistance [38]. Epigenetic changes (e.g., histone acetylation and DNA methylation) critically modulate the expression of genes and determine whether target genes are expressed or suppressed [39]. Indeed, defective acetylation of histone acetylase activity and DNA methylation was linked to steroid resistance in a group of asthma patients [40, 41]. It is emerging that microRNAs, another class of epigenetic regulatory molecules, can induce steroid resistance by promoting the degradation of mRNA and suppressing the translation of GCR proteins [42–44]. Although evidence is limited, smoking and oxidative and chronic stress may also contribute to steroid resistance through persistent activation of PI3K, alterations in TLR activity, and impaired function of histone deacetylase [45–47].

PULMONARY MACROPHAGES AND ASTHMA

Macrophages have an indispensable role in host defense and contribute to almost every aspect of immunity, recognition, engulfment, and elimination of antigen from invading pathogen, cytokine release, and the induction of innate and adaptive immune responses [48, 49]. Depending on the tissue in which they reside, macrophages can develop distinct morphological features and functions. For example, macrophages in the liver and spleen, are important in defense against blood-borne pathogens, whereas peritoneal macrophages control bacteria of the enteric microflora [50, 51].

Pulmonary macrophages

Tissue macrophages in the lung (i.e., pulmonary macrophages) can be derived from peripheral blood monocytes or from a stable, self-sustaining population of resident lung macrophages. It has long been recognized that pulmonary macrophages modulate the contractility of airway smooth muscle by inducing histamine release from mast cells and basophils [52], by impairing airway β -adrenergic function through the production of oxygen radicals [53], and by augmenting cholinergic neurotransmission via the generation of thromboxane A2 [54].

These studies suggest that pulmonary macrophages may be involved directly in the development of airway hyperresponsiveness, which is a hallmark feature of asthma (**Fig. 1**).

Under resting conditions, pulmonary macrophages have a long half-life of 1–2 months, with a turnover rate of 40% in 1 year in mice [55, 56]. Like most macrophages, pulmonary macrophages express F4/80 and CD68, which are considered the classical macrophage markers [50]. Pulmonary macrophages also show unique properties compared with other tissue macrophages and express high levels of CD11c and DEC-205 [57, 58]. In other tissues, CD11c is generally only expressed by DCs, NK cells, and activated T cells subsets [59–61], whereas DEC-205 is normally only expressed by certain subpopulations of DCs [62]. In contrast, it is thought that DCs in the lung do not express F4/80 or CD68 [50]. These unique features of pulmonary macrophages may be determined by their tissue microenvironment (e.g., high oxygen tension), exposure to infectious agents, and immunoregulatory factors.

Although macrophages are the most abundant pulmonary innate immune cells, are crucial in modulating chronic inflammation [63], and have dysfunctional activation and phagocytosis in severe asthma [64], our understanding of the role of pulmonary macrophages in the pathogenesis of the disease remains rudimentary and controversial [65]. To date, asthma research has largely focused on the Th2/eosinophil axis, and the role of pulmonary macrophages has been neglected. Although it is true that Th2 cells and eosinophils are major contributors to the pathogenesis of allergic inflammation of the airways, nevertheless, studies in animal models of asthma have indicated a central role for pulmonary macrophages in the development of allergic airways disease.

In particular, activated pulmonary macrophages may be important effector cells in airway inflammation. In an experimental model of an acute exacerbation of chronic asthma, we have shown that pulmonary macrophages can stimulate Th2 cytokine secretion by primed CD4⁺ T cells via mechanisms involving the expression of CD80/86 costimulatory molecules [66].

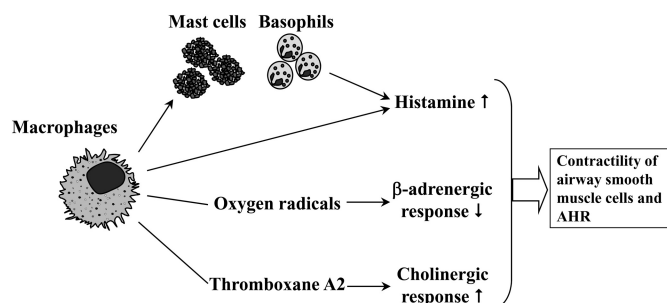


Figure 1. Macrophages regulate the contractility of airway smooth muscle through the production of histamine and by altering β -adrenergic and cholinergic responses. Macrophages produce oxygen radicals, which suppress the function of β -adrenergic receptors, and thromboxane A2, which increases the function of cholinergic receptors. Macrophages can also release histamine directly or stimulate basophils and mast cells to generate histamine. Each of these effects induces airway smooth muscle contraction and potentially, AHR.

M1 and M2 macrophages

Macrophages have been divided into M1 and M2a/b/c subtypes, based on their expression of cytokines, especially chemokines, and other specific markers: classically activated M1 macrophages and alternatively activated M2 macrophages [63]. It should be noted that the initial definition of these subtypes was largely on the basis of *in vitro* studies using bone marrow- or monocyte-derived macrophages [67, 68], although there is now increasing evidence that these phenotypes also exist *in vivo*.

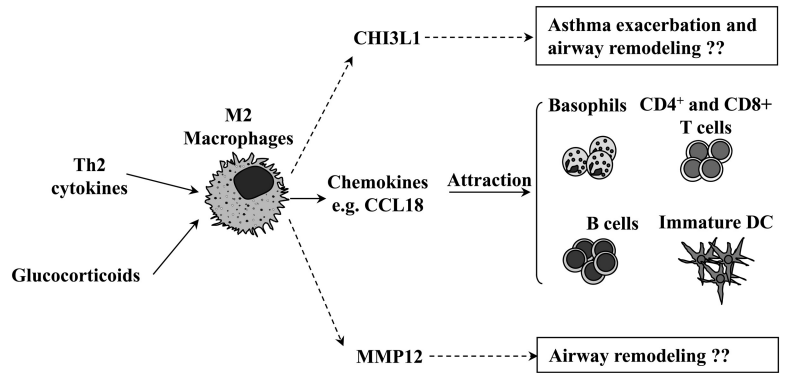
M1 macrophages are induced by Th1 cytokines, particularly IFN- γ and LPS, and characteristically produce CXCL9 and CXCL10 [67, 69]. M1 macrophages typically participate in Th1 responses and modulate host defense against intracellular pathogens, tumor cells, and tissue debris [63, 67].

By contrast, M2 macrophages are induced by IL-4 and IL-13 and other Th2 cytokines, and they typically produce chemokines such as CCL17, CCL22, and CCL24 [69–71]. Recent studies have identified roles for M2 macrophages in models of allergic inflammation of the airways [72, 73], and they are known to promote Th2 reactivity, including parasite elimination, suppression of inflammation, and tissue remodeling [74]. Moreover, their chemokine products are likely to be functionally relevant in asthma, as CCL24 cooperates with IL-13 in the recruitment of eosinophils [75].

M2 macrophages also secrete another relevant chemokine, CCL18, in response to stimulation with Th2 cytokines, and IL-4 driven CCL18 production is amplified further by glucocorticoids [76]. CCL18 is mainly produced by APCs, such as pulmonary macrophages and monocyte-derived DCs [76, 77]. As the mouse does not have a CCL18-equivalent gene, investigations of the effects of this chemokine have been performed using its overexpression by adenoviral vectors in the lungs of mice. Overexpression results in massive trafficking of T cells into the lung, pulmonary inflammation, and fibrosis [78, 79]. This suggests that CCL18 may contribute to the development of lung diseases by inducing the influx of T cells that have pathogenetic effects. CCL18 preferentially regulates the trafficking of Th2 cells and basophils and induces the release of histamine and intracellular calcium from basophils [80]. Further evidence for the involvement of CCL18 in asthma pathogenesis has been provided by the use of protein microarray analysis, which showed elevated levels of CCL18 in the sputum of asthma patients [81]. Together, these studies indicate an important role for CCL18 in the pathogenesis of asthma, through the recruitment of proasthmatic immune cells into the lung, although its exact mechanisms of action remain to be elucidated (**Fig. 2**).

Arginase I and Retn1 α are two well-known signature markers of M2 macrophages [68]. *In vivo* studies with Retn1 α -deficient mice have shown that Retn1 α reduces schistosomiasis-induced inflammation of the lung by suppressing Th2 responses [82], suggesting that M2 macrophages may have anti-inflammatory effects. However, increasing evidence indicates that they may be crucial in the elimination of parasites and in the development of airway inflammatory diseases [48]. Moreover, Retn1 α may contribute to the development of AHR

Figure 2. Mechanisms that underpin the contributions of human M2 macrophages to asthma exacerbations and airway remodeling. Th2 cytokines (e.g., IL-4 and -13) and glucocorticoids promote pulmonary macrophages to develop into M2 macrophages. Human M2 macrophages produce CHI3L1, MMP-12, and CCL18. CHI3L1 and MMP-12 may induce exacerbations of asthma and airway remodeling. CCL18 potentiates inflammatory responses by attracting CD4⁺ and CD8⁺ T cells, basophils, and B cells and immature DCs.



[83]. M2 macrophages have been shown to be more abundant in the BALF and airway wall tissue of asthmatic compared with healthy subjects [72, 84]. Investigations in children have revealed that during acute exacerbations of asthma, circulating monocyte populations up-regulate the expression of genes that are indicative of an alternatively activated phenotype [85]. Studies with animals have shown increases in the levels of M2 macrophages in the lung in models of asthma, and adoptive transfer of M2 macrophages during allergen challenge exacerbated the severity of allergen-induced disease [73]. Furthermore, enhanced levels of IL-13-producing macrophages have been found in the BALF from subjects with severe asthma [72], suggesting that M2 macrophages may contribute to reduced lung function in asthma patients. These studies demonstrate an association between M2 macrophages and asthma.

Emerging evidence also indicates a causative role for this macrophage phenotype in the pathogenesis of asthma, which likely involves their production of other inflammatory factors, such as CHI3L1 and MMP-12 [86, 87] (Fig. 2). The murine counterpart of CHI3L1 is Ym1, which is a very well-recognized marker of M2 macrophages and may be an eosinophil chemoattractant [88]. CHI3L1 participates in the proliferation, differentiation, and survival of cells and in the remodeling of ECM [89], and is closely linked to reduced lung function and AHR in asthmatic subjects [87]. Indeed, elevated levels of CHI3L1 were detected in the serum and lung tissue of patients with asthma and positively correlated with disease severity [90]. MMP-12 regulates the degradation of ECM and may contribute to airway remodeling in patients with asthma [91]. Recently, the gene variant of MMP-12 has also been linked to asthma exacerbations and increased severity of the disease [86, 92]. Mukhopadhyay and colleagues [92] have since shown that treatment with MMP-12 inhibitors significantly suppressed early and late airway responses to allergen in a sheep model of asthma. Together, these studies have indicated the possible mechanistic roles of pulmonary macrophages and the mediators they produce in the pathogenesis of severe asthma (Fig. 2).

Despite the evidence for different macrophage phenotypes, emerging studies have demonstrated the phenotypic plasticity of macrophages and the functional overlap between subtypes of these cells [68, 93]. For example, in an infectious model of *Listeria*, circulating monocytes can first have an M1 phenotype but subsequently develop an M2 phenotype.

Moreover, recent human studies suggest that M1 pulmonary macrophages play a key role in the development of severe asthma. Goleva et al. [8] showed that steroid-resistant asthmatic patients have increased expression of M1 and decreased expression of M2 markers on macrophages in BALF. These observations highlight the complex roles of pulmonary macrophages in the regulation of the pathogenesis of severe asthma.

Regulatory macrophages

There is also much evidence for the existence of other subtypes, e.g., regulatory macrophages [63]. A major feature of regulatory macrophages is the production of large amounts of IL-10, which is considered to be an anti-inflammatory cytokine [94]. These macrophages have been shown to reduce inflammation and pathological changes in acute and chronic models of inflammation [95, 96]. Thus, unlike the other two phenotypes, regulatory macrophages may be critical in modulating excessive immune responses and preventing inflammatory diseases.

In a mouse model of asthma, Tang and colleagues [97] have shown that pulmonary macrophages suppress Th2 cell-mediated airway inflammation and AHR through the up-regulation of Th1 responses. Depletion of pulmonary macrophages led to an enhanced Th2 phenotype and more severe airway inflammation and AHR after antigen challenge. This suppressive effect of pulmonary macrophages may be linked to the release of IFN- γ [97]. Furthermore, adoptive transfer of pulmonary macrophages from unsensitized rats protects sensitized rats from the development of AHR after OVA challenge [98]. There is likely bidirectional regulation between the Th2/eosinophil axis and pulmonary macrophages, as antigen challenge of sensitized rats suppresses the phagocytic function of pulmonary macrophages [99]. Moreover, recent studies have shown that adoptive transfer of activated eosinophils attenuates the suppressive role of pulmonary macrophages by increasing the differentiation of M2 macrophages [100]. As most of these studies do not directly determine by what mechanisms these macrophages might attenuate airway inflammation and AHR, it remains unclear how these cells exhibit their suppressive action, and if these macrophages are regulatory macrophages or other macrophage populations with suppressive roles. Additionally, these and several other populations of macrophages (i.e., regulatory macrophages, TAMs, and MDSCs) demon-

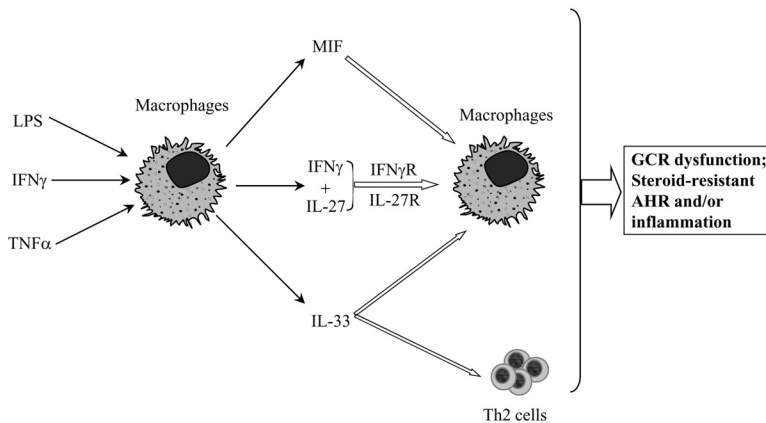


Figure 3. Mechanisms involved in the induction of AHR, airway inflammation, and glucocorticoid resistance by pulmonary macrophages and related factors. IFN- γ and LPS activate pulmonary macrophages to produce IL-27, which induces AHR and glucocorticoid resistance in synergy with IFN- γ . In addition, TNF- α may activate pulmonary macrophages in severe asthma. Activated pulmonary macrophages also produce MIF and IL-33, which are linked to AHR and glucocorticoid resistance. Furthermore, MIF and IL-33 potentiate the development of airway inflammation by activating macrophages and Th2 cells.

strate immune-suppressive activity; however, there are distinct differences among them [101]. Regulatory macrophages produce large amounts of IL-10 in response to Fc γ R stimulation [94]. TAMs are able to suppress anti-tumor immunity through the production of IL-10 and TGF- β , and MDSCs are thought to be the precursors of TAMs [51]. As the production of IL-10 is one of the significant features of regulatory macrophages and TAMs, another question remains: are these cells distinct populations of macrophages with suppressive roles, or are they the same cells that behave differently within a different tissue environment?

Interestingly, pulmonary macrophages from asthmatic subjects produce less IL-10 than those from healthy controls [102]. Furthermore, pulmonary macrophages from patients with severe compared with moderate asthma produce more IL-6, IL-8, and monocyte-derived chemokine, whereas IL-10 production is undetectable in these cells. This suggests that the inhibitory function of pulmonary macrophages is absent, whereas the inflammatory function of these cell is heightened in severe asthma [103]. Emerging evidence indicates that a group of macrophages—lung interstitial macrophages producing high levels of IL-10—prevents Th2 polarization and airway responses in an experimental model of asthma [104]. Lung interstitial macrophages are also crucial in maintaining immune homeostasis in the respiratory tract and preventing the development of airway allergic responses under normal conditions [104]. These observations may reflect the fact that the activation status of macrophages is a spectrum, as these are highly plastic immune cells that can respond differently to variable microenvironmental stimuli, rather than having discrete and stable subpopulations.

Thus, the roles of pulmonary macrophages in the regulation of the pathogenesis of asthma are complex, and much remains

unknown about the contributions of these cells to the disease. In particular, little is known about whether and how different populations of pulmonary macrophages may contribute to the pathogenesis of steroid resistance, AHR, and increasing the severity of asthma.

FACTORS INVOLVED IN THE PATHOGENESIS OF SEVERE ASTHMA

Pulmonary macrophages

In severe and difficult-to-manage forms of asthma, where steroids have limited efficacy, factors associated with the activation of host defense pathways are likely to contribute to pathogenesis. These include macrophage phenotype (see above); effect of infections on macrophages; IFN- γ , LPS, associated IFN- γ R, and MyD88 signaling; and the expression of cytokines, such as TNF- α , MIF, and IL-33 [3, 5, 7, 105–107]. The regulatory networks involving these factors are described in **Fig. 3** and **Table 1**.

Viral and bacterial infections

Viral and bacterial infections may synergize with allergens to contribute to the induction of asthma, enhance the severity of the disease, and alter the cellular profile of the inflammatory response in lungs [85, 108, 109]. We have reviewed the role of viruses and bacteria in the induction and exacerbation of asthma [6, 10]. RV, RSV, and influenza and *Chlamydia*, *Mycoplasma*, and *Haemophilus influenzae* are the most frequently associated viral and bacterial infections, respectively.

RV and RSV have been widely implicated in the development of asthma in childhood [6]. We have recently shown that production of cytokines, such as IL-25, by airway epithelial

TABLE 1. Summary of Cells, Factors, and Signaling Pathways That Contribute to the Pathogenesis of Severe Asthma

Cells	M1, M2, and regulatory macrophages; Th1, Th2, and Th17 lymphocytes; Eosinophils (?); Neutrophils (?); Airway epithelial cells (?); Fibroblasts (?)
Cytokines, chemokines, and other factors	IFN- γ , IL-27, TNF- α , MIF, IL-33, IL-4, IL-13, IL-17A/F, CCL18, CCL22/24 (?), IL-8, MMP12, CHI3L1
Signaling pathways	NF- κ B, MyD88, IRF-1, JAKs, STATs, MAPKs, ERK1/2, TRIF

cells may contribute to the induction of the allergic response [110–112]. There is mounting evidence that microbial infection of the airway early in life may also predispose to more severe forms of asthma. Neonatal infection of the airways with *Streptococcus pneumoniae*, *H. influenzae*, *Moraxella catarrhalis*, or combinations of these infections leads to recurrent wheeze and asthma [113]. Furthermore, *Chlamydia* respiratory infection in childhood is increasingly linked with severe asthma [9, 10, 24, 114, 115]. Infections in early life induce the release of macrophage chemoattractants from the airway epithelium, such as MCP-1 and -4 and GM-CSF [116]. Macrophages, IFNs, and TLRs play central roles in innate immune responses to infection, and these factors may contribute to inflammatory sequelae. Infectious agents are taken up by macrophages and monocytes and cause the release of IL-1, -6, -8, -10, and -12, platelet-activating factor, PGE₂, VEGF, and activate adaptive immune cells [117]. Furthermore, infections result in the increased expression of MHC-II on macrophages, which may initiate immune responses to allergens. Together, these infection-induced macrophage responses promote inflammation, which enhances allergic responses to allergens in early life and initiates or reinforces the development of asthma.

Respiratory infections are also the most frequent trigger of exacerbations of asthma, which are predominantly characterized by the activation of macrophages and neutrophils in sputum and BAL [118–120] and poor responsiveness to therapy [114, 115, 120]. Elevated levels of IFN- γ , a central factor involved in antiviral and antibacterial responses and detected clinically in association with many respiratory infections, are found in serum and sputum in atopic asthmatics [121, 122].

Infectious agents are also linked to more severe asthma in adulthood. Neutrophils migrate into the airways in response to these agents. Patients with more severe, steroid-resistant, neutrophilic forms of asthma have chronic airway colonization with bacteria, and *H. influenzae* is one of the more commonly isolated species [123]. We have also demonstrated recently that respiratory infection with *Chlamydia* in early life or in adulthood [9, 23, 24, 124] or with *H. influenzae* in adulthood [11, 12] increases the severity of allergic airway disease, which is steroid-resistant in mice.

Importantly, asthmatics may elicit a more intense response to infectious challenge, and an asthmatic phenotype (e.g., increased IL-13 levels) may lead to increased susceptibility to infection [6, 10, 125]. Treatment with macrolides as an add-on therapy, which has antimicrobial and anti-inflammatory effects, has been shown to significantly improve lung function and relief of symptoms in patients with severe asthma [126, 127]. The mechanisms of these associations are currently being elucidated.

IFN- γ and LPS

IFN- γ is implicated in the pathogenesis of severe asthma. An increased percentage of IFN- γ -expressing cells has been found in the airways of severe asthmatics, and this cytokine has been linked to the mechanisms regulating AHR [128, 129]. Recently IFN- γ has been implicated in airway remodeling and the induction of markers of alternatively activated macrophages [130] through the activation of the IFN- γ R on mast cells.

LPS is a major component of the cell walls of Gram-negative bacteria and of allergens that trigger asthma. Exposure to environmental LPS is thought to be a major risk factor for occupational asthma, and there is a strong relationship between levels of household LPS and exacerbations of asthma [113, 131]. High levels of LPS have been detected in BALF from steroid-resistant, severe asthmatics and are an important determinant of asthma severity [8, 132]. LPS drives macrophages toward classical activation and is associated with increased expression of the proinflammatory cytokines TNF- α , IL-1 β , and IL-6 [133]. Furthermore, the levels of neutrophils strongly correlate with the amount of LPS in BAL samples of subjects with severe asthma [8].

We and others have shown that IFN- γ -producing cells and LPS contribute to the induction of airway inflammation and AHR in mouse models and have demonstrated that IFN- γ /LPS-induced responses are steroid-resistant [134, 135]. Our investigations revealed a novel interaction between IFN- γ and LPS, which leads to the activation of pulmonary macrophages and the induction of steroid-resistant AHR [135]. These discoveries are supported by earlier in vitro findings, suggesting that “cross-talk” between IFN- γ - and TLR4-dependent pathways can regulate inflammatory networks in macrophages [136, 137], and block the anti-inflammatory effects of glucocorticoids in human monocytes [138].

IFN- γ R, MyD88, and IL-27 signaling pathways

The IFN- γ R pathway plays a key role in innate and adaptive immune responses by inducing the production of IRF-1 [139] and activating intracellular signaling pathways of JAK-STAT, MAPKs, and ERK1/2 [140, 141]. LPS-induced activation of TLR4 initiates a signaling cascade, which is mediated by the adaptor proteins MyD88 and TRIF. Nevertheless, the critical downstream molecules that regulate the inflammation mediated by IFN- γ /IFN- γ R and LPS-TLR4/MyD88, as well as the associated steroid-resistant AHR, remain unknown.

Recent studies suggest that IFN- γ and LPS-induced, steroid-resistant AHR may be mediated by IL-27, because of its potential role as a regulator of pulmonary macrophage function [142–145]. IL-27 is a heterodimeric cytokine that has two subunits, an EBV-induced gene 3 and a p28 chain [146]. It is produced by activated monocytes (or monocyte-derived DCs) and macrophages after microbial exposure [144, 146], and its expression is critically dependent on signaling through MyD88 [144]. IL-27 acts on a wide range of immune cells, including CD4⁺ and CD8⁺ T lymphocytes, NK cells, monocytes/macrophages, and activated DCs [143, 147–150]. The effect of IL-27 on monocytes/macrophages is complex, as it is able to induce or suppress the expression of surface activation molecules or cytokines by these cells [143, 149, 151–153]. The pleiotropic effects of IL-27 on monocytes/macrophages may depend on the timing and context of surrounding inflammatory signals and the nature of the local immune environment.

The contribution of IL-27 in different phenotypes of asthma has been little studied. However, it is known to promote IFN- γ production by Th1 cells, as well as innate host defense responses to infection [146, 154], both of which may contribute to severe forms of asthma and exacerbations. We have per-

formed investigations into the contribution of IL-27 to molecular and cellular mechanisms regulating steroid-resistant AHR [145]. We have demonstrated a critical role for IL-27 in the activation of pulmonary macrophages in allergic airways disease, which required synergy with IFN- γ . IL-27- and IFN- γ -induced AHR was dependent on MyD88 expression in macrophages, thus identifying a novel interaction among IL-27R, IFN- γ R, and MyD88 signaling pathways in this cell. We then showed that IL-27 and IFN- γ prevented the steroid-induced translocation of the GCR to the nucleus of pulmonary macrophages. Finally, we also observed that the expression of IL-27 and IFN- γ was significantly greater in the sputum of patients with neutrophilic asthma, compared with those with eosinophilic asthma.

These studies highlight the critical role of MyD88, not only in the development of inflammation but also in the pathogenesis of severe asthma. Although MyD88 was recognized initially as a key molecule in the transduction of TLR pathways, in vitro evidence also reveals a role for this adaptor protein in IFN- γ signaling [136]. Indeed, recent studies show that IFN- γ stimulation of macrophages induces a physical association between the IFN- γ R and MyD88 and the formation of a signaling complex, termed a signalosome, without affecting IFN- γ -induced JAK-STAT signaling/phosphorylation [137]. The signalosome may function to regulate specific aspects of the host-defense responses, as subsets of proinflammatory molecules are not transcribed in macrophages deficient in MyD88 when stimulated with IFN- γ [137]. Furthermore, the specific and critical role of MyD88 was supported further by the lack of effect of the inhibition of NF- κ B or other MAPK pathway intermediates (e.g., JNK and p38) on IFN- γ R/IL-27R pathway-regulated, steroid-resistant AHR [145].

TNF- α

TNF- α levels are also increased significantly in BALF from subjects with severe asthma [8, 155, 156]. It is produced by Th1 cells and macrophages, and its expression is steroid-resistant [156]. TNF- α mediates neutrophil and eosinophil recruitment, promotes T cell activation, and induces chronic airway remodeling and fibrosis [157–160]. As TNF- α is released by activated pulmonary macrophages, especially those with an M1 phenotype, these observations not only implicate M1 macrophages but also suggest a potential role for TNF- α in the mechanisms that lead to increased severity of asthma and steroid resistance.

MIF

MIF was first described in 1966 as a T cell factor that inhibits the random migration of macrophages [161]. Later, it was found that MIF can counteract the function of glucocorticoids and acts as an upstream activator of innate responses [162, 163]. MIF also promotes the expression of TLR4 on macrophages [164] and is released from innate-immune cells by proinflammatory mediators (e.g., LPS) [164, 165]. The production of MIF is also increased greatly in vitro by IFN- γ [166]. MIF acts through its receptor, CD74, but may also signal through the chemokine receptors CXCR2 and -4 [167]. Importantly, MIF has been recognized as a noncognate ligand of

CXCR2, playing a central role in the recruitment of monocytes/macrophages to sites of inflammation [167, 168]. The physiological actions of MIF strongly suggest that this cytokine may play a central role in regulating macrophage migration and function in severe asthma.

Increased levels of MIF have been found in mononuclear cells isolated from patients with glucocorticoid-resistant rheumatoid arthritis, systemic lupus erythematosus, and atherosclerosis, and treatment with the neutralizing antibody against MIF restores the anti-inflammatory effects of glucocorticoids in these cells [169]. High levels of MIF were also found in the BALF, serum, and sputum of asthmatics [170]. The importance of MIF in the pathogenesis of severe asthma is supported further by evidence that neutralization or deficiency of MIF significantly attenuates OVA-induced airway inflammation and AHR in animal models of asthma [171, 172]. It is now necessary to fully understand the contribution of MIF to the development of severe asthma, as this factor may have potential as a therapeutic target in difficult-to-control asthma.

IL-33

Recent studies have identified that IL-33 has an important role in Th2 cell-regulated airway inflammation and has a clear link to the pathogenesis of severe asthma, probably through the stimulation of macrophages and granulocytes [100, 173]. The IL-33R, ST2, has been detected on eosinophils, basophils, mast cells, NK cells, and CD4⁺ Th2 cells [174, 175]. Increased levels of IL-33 mRNA have been found in the serum and lungs of asthmatic patients [107, 176], and the production of this cytokine correlates with the development of severe asthma [107, 177]. Furthermore, intranasal instillation of IL-33 into naïve mice induces AHR, eosinophilic inflammation, and mucus hypersecretion and the production of Th2 cytokines in the lung [174, 178, 179]. When discovered, it was initially thought that IL-33 was released primarily by bronchial epithelial and airway smooth muscle cells [107, 177]. However, recent studies suggest that macrophages also produce large amounts of this cytokine, which further amplifies the activation of macrophages, in response to LPS stimulation [173]. Indeed further investigations have revealed that IL-33 exacerbates airway inflammation through the activation of alternatively activated (M2) macrophages [178]. We have shown recently that expression of IL-33 is up-regulated in a model of an acute exacerbation of asthma and that the development of exaggerated airway inflammation in this model can be prevented by blocking ST2 signaling (unpublished data).

Th17 cells and IL-17

IL-17A (often simply referred to as IL-17) and the related cytokine IL-17F are primarily secreted from Th17 cells [180] and have also been linked to the pathogenesis of severe asthma [7, 181]. These cytokines are also elevated in the sputum of asthmatics, which correlates with increased CXCL8 levels and neutrophil numbers [182]. IL-17A and -F drive neutrophilic inflammation [2, 180]. Recent evidence has also implicated IL-17A in the pathogenesis of severe asthma and in the development of fibrosis [9, 11]. This cytokine was shown to be

elevated in the airways of patients with severe asthma and to be associated with the elevated expression of type I and III collagens in these patients [181].

A role for Th17 cells in severe asthma is supported by the recent demonstration that these cells induced steroid-resistant, neutrophilic infiltration of the airways and AHR in a mouse model [183] (Fig. 4). In this model, adoptively transferred, transgenic Th17 cells increased the production IL-6, G-CSF, and the neutrophilic chemokine KC (the murine ortholog of CXCL8), which correlated with the induction of neutrophil influx and AHR. These inflammatory responses were dependent on IL-17 signaling through IL-17RA [183]. IL-17 also preferentially increases the production of IL-6 and factors that promote neutrophil survival (G-CSF) and migration (MIP-1 α and KC) from these cells in vitro [184]. Thus, collectively, emerging data indicate that Th17 cells may activate macrophages via the IL-17RA to coordinate pulmonary neutrophil influx and to regulate the production of IFN- γ and IL-6-type cytokines. However, their roles in glucocorticoid resistance have not been widely assessed. The precise mechanisms by which IL-17 regulates severe asthma and potentially steroid resistance need further investigation [7].

POTENTIAL TREATMENTS

Although there has been some progress in elucidating how innate-immune responses and respiratory tract infection contribute to steroid-resistant, severe asthma, the pathogenetic mechanisms remain poorly understood. Whereas it is clear that pulmonary macrophages may be activated in severe asthma, with significant alterations in the phenotype of these cells, only limited studies have been directed toward understanding their role in the pathogenesis of glucocorticoid resistance or severity of disease. Substantial, recent progress has been made in understanding the actions of glucocorticoids and the molecular mechanisms that lead to steroid resistance. Correspondingly, numerous therapies have been developed in attempts to treat steroid resistance in severe asthmatics. Immunosuppressants, antagonists against inflammatory factors (e.g., IL-2), and inhibitors of kinases (e.g., p38 MAPK and PI3K) may be effective in restoring the anti-in-

flammatory function of glucocorticoids in patients with severe asthma [185–187].

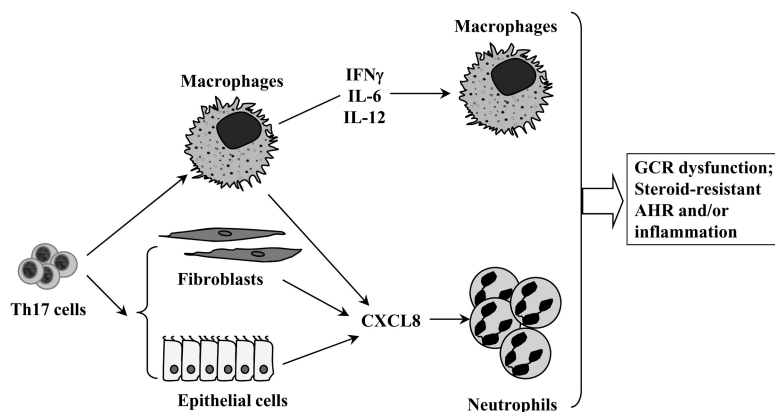
Anti-TNF- α -neutralizing antibodies and a soluble fusion protein have been trialed in moderate to severe asthma [2]. Treatment with antibody suppressed exacerbations in moderate asthmatics and improved some parameters of lung function [188, 189], but the largest trial was discontinued because of adverse events [190]. Treatment of severe asthmatics with the soluble fusion protein led to improvements in asthma symptoms, lung function, and AHR [155, 156]. However, the effects on steroid resistance were not assessed, as these studies defined their study population by the severity of disease but not by degree of reversibility after steroid administration [155, 156]. Further studies are eagerly awaited.

Anti-IL-13-neutralizing antibodies have also been tested as a therapy in steroid-resistant asthmatics with evidence of increased IL-13 activity [191]. Treatment significantly improved lung function (FEV₁) with the greatest effects in those with higher levels of indicators of IL-13 activity. Future studies could also be directed to targeting the other factors (IFN- γ and LPS signaling, IL-27, MIF, IL-33, and IL-17A/F), alone or in combination, which are associated with macrophage-induced, steroid-resistant, severe asthma. However, a cautious approach is necessary, as some of these treatments may lead to increased susceptibility to infection or have other unwanted side-effects.

CONCLUSIONS

Pulmonary macrophages and their phenotype, responses, and activating mediators (IFN- γ and LPS signaling pathways, TNF- α , MIF, IL-33, IL-17A/F, and other factors) potentially contribute to the pathogenesis of steroid-resistant asthma in a number of ways, and it is necessary to further elucidate the mechanisms involved. Improved understanding of how pulmonary macrophages regulate steroid-resistant airway inflammation and AHR may lead to the reversal of glucocorticoid resistance and more effective management of steroid-resistant asthma.

Figure 4. The contribution of Th17 cells, macrophages, and neutrophilic inflammation to glucocorticoid resistance. Th17 cells stimulate pulmonary macrophages, epithelial cells, and fibroblasts to release CXCL8 and induce neutrophil infiltration. Activated macrophages and neutrophils may then exhibit glucocorticoid resistance.



AUTHORSHIP

M.Y., R.K.K., P.M.H., and P.S.F. composed and edited the manuscript.

ACKNOWLEDGMENTS

The authors are supported by the National Health and Medical Research Council of Australia, the Australian Research Council, and the Cooperative Research Centre for Asthma and Airways.

DISCLOSURES

The authors have no financial conflict of interest.

REFERENCES

- Cohn, L., Elias, J. A., Chupp, G. L. (2004) Asthma: mechanisms of disease persistence and progression. *Annu. Rev. Immunol.* **22**, 789–815.
- Hansbro, P. M., Kaiko, G. E., Foster, P. S. (2011) Cytokine/anti-cytokine therapy—novel treatments for asthma? *Br. J. Pharmacol.* **163**, 81–95.
- Barnes, P. J., Adcock, I. M. (2009) Glucocorticoid resistance in inflammatory diseases. *Lancet* **373**, 1905–1917.
- Wang, W., Li, J. J., Foster, P. S., Hansbro, P. M., Yang, M. (2010) Potential therapeutic targets for steroid-resistant asthma. *Curr. Drug Targets* **11**, 957–970.
- Wenzel, S. E., Szeffler, S. J., Leung, D. Y., Sloan, S. I., Rex, M. D., Martin, R. J. (1997) Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. *Am. J. Respir. Crit. Care Med.* **156**, 737–743.
- Hansbro, N. G., Horvat, J. C., Wark, P. A., Hansbro, P. M. (2008) Understanding the mechanisms of viral induced asthma: new therapeutic directions. *Pharmacol. Ther.* **117**, 313–353.
- Al-Ramli, W., Prefontaine, D., Chouiali, F., Martin, J. G., Olivenstein, R., Lemiere, C., Hamid, Q. (2009) T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J. Allergy Clin. Immunol.* **123**, 1185–1187.
- Goleva, E., Hauk, P. J., Hall, C. F., Liu, A. H., Riches, D. W., Martin, R. J., Leung, D. Y. (2008) Corticosteroid-resistant asthma is associated with classical antimicrobial activation of airway macrophages. *J. Allergy Clin. Immunol.* **122**, 550–559.e3.
- Horvat, J. C., Starkey, M. R., Kim, R. Y., Beagley, K. W., Preston, J. A., Gibson, P. G., Foster, P. S., Hansbro, P. M. (2010) Chlamydial respiratory infection during allergen sensitization drives neutrophilic allergic airways disease. *J. Immunol.* **184**, 4159–4169.
- Hansbro, P. M., Beagley, K. W., Horvat, J. C., Gibson, P. G. (2004) Role of atypical bacterial infection of the lung in predisposition/protection of asthma. *Pharmacol. Ther.* **101**, 193–210.
- Essilfie, A. T., Simpson, J. L., Horvat, J. C., Preston, J. A., Dunkley, M. L., Foster, P. S., Gibson, P. G., Hansbro, P. M. (2011) Haemophilus influenzae infection drives IL-17-mediated neutrophilic allergic airways disease. *PLoS Pathog.* **7**, e1002244.
- Essilfie, A. T., Simpson, J. L., Dunkley, M. A., Morgan, L. C., Oliver, B. G., Gibson, P. G., Foster, P. S., Hansbro, P. M. (2012) Combined *Haemophilus influenzae* respiratory infection and allergic airways disease drives chronic infection and features of steroid-resistant neutrophilic asthma. *Thorax*, 2011, 7(1):e1002244.
- Eder, W., Ege, M. J., von Mutius, E. (2006) The asthma epidemic. *N. Engl. J. Med.* **355**, 2226–2235.
- Douwes, J., Gibson, P., Pekkanen, J., Pearce, N. (2002) Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* **57**, 643–648.
- Simpson, J. L., Scott, R. J., Boyle, M. J., Gibson, P. G. (2005) Differential proteolytic enzyme activity in eosinophilic and neutrophilic asthma. *Am. J. Respir. Crit. Care Med.* **172**, 559–565.
- Bousquet, J., Mantzouranis, E., Cruz, A. A., Ait-Khaled, N., Baena-Cagnani, C. E., Bleecker, E. R., Brightling, C. E., Burney, P., Bush, A., Busse, W. W., Casale, T. B., Chan-Yeung, M., Chen, R., Chowdhury, B., Chung, K. F., Dahl, R., Drazen, J. M., Fabbri, L. M., Holgate, S. T., Kauffmann, F., Haahtela, T., Khaltaev, N., Kiley, J. P., Masjedi, M. R., Mohammad, Y., O'Byrne, P., Partridge, M. R., Rabe, K. F., Togias, A., van Weel, C., Wenzel, S., Zhong, N., Zuberbier, T. (2010) Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. *J. Allergy Clin. Immunol.* **126**, 926–938.
- Corrigan, C. J., Kay, A. B. (1991) CD4 T lymphocyte activation in acute severe asthma. *Int. Arch. Allergy Appl. Immunol.* **94**, 270–271.
- European Network for Understanding Mechanisms of Severe Asthma. (2003) The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. *Eur. Respir. J.* **22**, 470–477.
- Wenzel, S. E., Busse, W. W. (2007) Severe asthma: lessons from the Severe Asthma Research Program. *J. Allergy Clin. Immunol.* **119**, 14–21.
- British Thoracic Society Scottish Intercollegiate Guidelines Network. (2008) British Guideline on the Management of Asthma. *Thorax* **63** (Suppl. 4), iv1–121.
- Sont, J. K., Willems, L. N., Bel, E. H., van Krieken, J. H., Vandenbroucke, J. P., Sterk, P. J. (1999) Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. *Am. J. Respir. Crit. Care Med.* **159**, 1043–1051.
- Meurs, H., Gosens, R., Zaagsma, J. (2008) Airway hyperresponsiveness in asthma: lessons from in vitro model systems and animal models. *Eur. Respir. J.* **32**, 487–502.
- Horvat, J. C., Starkey, M. R., Kim, R. Y., Phipps, S., Gibson, P. G., Beagley, K. W., Foster, P. S., Hansbro, P. M. (2010) Early-life chlamydial lung infection enhances allergic airways disease through age-dependent differences in immunopathology. *J. Allergy Clin. Immunol.* **125**, 617–625, 625.e1–625.e6.
- Horvat, J. C., Beagley, K. W., Wade, M. A., Preston, J. A., Hansbro, N. G., Hickey, D. K., Kaiko, G. E., Gibson, P. G., Foster, P. S., Hansbro, P. M. (2007) Neonatal chlamydial infection induces mixed T-cell responses that drive allergic airway disease. *Am. J. Respir. Crit. Care Med.* **176**, 556–564.
- Kay, A. B. (2001) Allergy and allergic diseases. First of two parts. *N. Engl. J. Med.* **344**, 30–37.
- Kariyawasam, H. H., Aizen, M., Barkans, J., Robinson, D. S., Kay, A. B. (2007) Remodeling and airway hyperresponsiveness but not cellular inflammation persist after allergen challenge in asthma. *Am. J. Respir. Crit. Care Med.* **175**, 896–904.
- Galli, S. J. (1993) New concepts about the mast cell. *N. Engl. J. Med.* **328**, 257–265.
- Frew, A. J., Moqbel, R., Azzawi, M., Hartnell, A., Barkans, J., Jeffery, P. K., Kay, A. B., Scheper, R. J., Varley, J., Church, M. K., et al. (1990) T lymphocytes and eosinophils in allergen-induced late-phase asthmatic reactions in the guinea pig. *Am. Rev. Respir. Dis.* **141**, 407–413.
- Broide, D. H., Gleich, G. J., Cuomo, A. J., Coburn, D. A., Federman, E. C., Schwartz, L. B., Wasserman, S. I. (1991) Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J. Allergy Clin. Immunol.* **88**, 637–648.
- Walker, C., Kaegi, M. K., Braun, P., Blaser, K. (1991) Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J. Allergy Clin. Immunol.* **88**, 935–942.
- Currie, G. P., Lee, D. K., Wilson, A. M. (2005) Effects of dual therapy with corticosteroids plus long acting β_2 -agonists in asthma. *Respir. Med.* **99**, 683–694.
- Fanta, C. H. (2009) Asthma. *N. Engl. J. Med.* **360**, 1002–1014.
- Anderson, G. P. (2008) Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* **372**, 1107–1119.
- Barnes, P. J., Woolcock, A. J. (1998) Difficult asthma. *Eur. Respir. J.* **12**, 1209–1218.
- Rhen, T., Cidlowski, J. A. (2005) Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N. Engl. J. Med.* **353**, 1711–1723.
- Mak, J. C., Hisada, T., Salmon, M., Barnes, P. J., Chung, K. F. (2002) Glucocorticoids reverse IL-1 β -induced impairment of β -adrenoceptor-mediated relaxation and up-regulation of G-protein-coupled receptor kinases. *Br. J. Pharmacol.* **135**, 987–996.
- Goleva, E., Kisich, K. O., Leung, D. Y. (2002) A role for STAT5 in the pathogenesis of IL-2-induced glucocorticoid resistance. *J. Immunol.* **169**, 5934–5940.
- Barnes, P. J. (2006) Corticosteroids: the drugs to beat. *Eur. J. Pharmacol.* **533**, 2–14.
- Peterson, C. L., Laniel, M. A. (2004) Histones and histone modifications. *Curr. Biol.* **14**, R546–R551.
- Kagoshima, M., Wilcke, T., Ito, K., Tsaprouni, L., Barnes, P. J., Panchard, N., Adcock, I. M. (2001) Glucocorticoid-mediated transrepression is regulated by histone acetylation and DNA methylation. *Eur. J. Pharmacol.* **429**, 327–334.
- Mathews, J. G., Ito, K., Barnes, P. J., Adcock, I. M. (2004) Defective glucocorticoid receptor nuclear translocation and altered histone acetylation patterns in glucocorticoid-resistant patients. *J. Allergy Clin. Immunol.* **113**, 1100–1108.
- Vreugdenhil, E., Verissimo, C. S., Mariman, R., Kamphorst, J. T., Barbosa, J. S., Zweers, T., Champagne, D. L., Schouten, T., Meijer, O. C., de Kloet, E. R., Fitzsimons, C. P. (2009) MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. *Endocrinology* **150**, 2220–2228.
- Uchida, S., Nishida, A., Hara, K., Kamemoto, T., Suetsugu, M., Fujimoto, M., Watanuki, T., Wakabayashi, Y., Otsuki, K., McEwen, B. S., Watanabe, Y. (2008) Characterization of the vulnerability to repeated stress in Fischer 344 rats: possible involvement of microRNA-mediated down-regulation of the glucocorticoid receptor. *Eur. J. Neurosci.* **27**, 2250–2261.

44. Kotani, A., Ha, D., Hsieh, J., Rao, P. K., Schotte, D., den Boer, M. L., Armstrong, S. A., Lodish, H. F. (2009) miR-128b is a potent glucocorticoid sensitizer in MLL-AF4 acute lymphocytic leukemia cells and exerts cooperative effects with miR-221. *Blood* **114**, 4169–4178.
45. Zhang, Y., Miao, J., Hanley, G., Stuart, C., Sun, X., Chen, T., Yin, D. (2008) Chronic restraint stress promotes immune suppression through Toll-like receptor 4-mediated phosphoinositide 3-kinase signaling. *J. Neuroimmunol.* **204**, 13–19.
46. Powell, N. D., Bailey, M. T., Mays, J. W., Stiner-Jones, L. M., Hanke, M. L., Padgett, D. A., Sheridan, J. F. (2009) Repeated social defeat activates dendritic cells and enhances Toll-like receptor dependent cytokine secretion. *Brain Behav. Immun.* **23**, 225–231.
47. Marwick, J. A., Caramori, G., Stevenson, C. S., Casolari, P., Jazrawi, E., Barnes, P. J., Ito, K., Adcock, I. M., Kirkham, P. A., Papi, A. (2009) Inhibition of PI3K restores glucocorticoid function in smoking-induced airway inflammation in mice. *Am. J. Respir. Crit. Care Med.* **179**, 542–548.
48. Gordon, S. (2003) Alternative activation of macrophages. *Nat. Rev. Immunol.* **3**, 23–35.
49. Kaiko, G. E., Horvat, J. C., Beagley, K. W., Hansbro, P. M. (2008) Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology* **123**, 326–338.
50. Taylor, P. R., Martinez-Pomares, L., Stacey, M., Lin, H. H., Brown, G. D., Gordon, S. (2005) Macrophage receptors and immune recognition. *Annu. Rev. Immunol.* **23**, 901–944.
51. Mosser, D. M., Edwards, J. P. (2008) Exploring the full spectrum of macrophage activation. *Nat. Rev. Immunol.* **8**, 958–969.
52. Schulman, E. S., Liu, M. C., Proud, D., MacGlashan Jr., D. W., Lichtenstein, L. M., Plaut, M. (1985) Human lung macrophages induce histamine release from basophils and mast cells. *Am. Rev. Respir. Dis.* **131**, 230–235.
53. Engels, F., Oosting, R. S., Nijkamp, F. P. (1985) Pulmonary macrophages induce deterioration of guinea-pig tracheal β -adrenergic function through release of oxygen radicals. *Eur. J. Pharmacol.* **111**, 143–144.
54. Tamaoki, J., Sekizawa, K., Ueki, I. F., Graf, P. D., Nadel, J. A., Bigby, T. D. (1987) Effect of macrophage stimulation on parasympathetic airway contraction in dogs. *Eur. J. Pharmacol.* **138**, 421–425.
55. Blussé van Oud Alblas, A., Mattie, H., van Furth, R. (1983) A quantitative evaluation of pulmonary macrophage kinetics. *Cell Tissue Kinet.* **16**, 211–219.
56. Bowden, D. H., Adamson, I. Y. (1980) Role of monocytes and interstitial cells in the generation of alveolar macrophages I. Kinetic studies of normal mice. *Lab. Invest.* **42**, 511–517.
57. Thepen, T., Van Rooijen, N., Kraal, G. (1989) Alveolar macrophage elimination in vivo is associated with an increase in pulmonary immune response in mice. *J. Exp. Med.* **170**, 499–509.
58. Van Rijt, L. S., Kuipers, H., Vos, N., Hijdra, D., Hoogsteden, H. C., Lambrecht, B. N. (2004) A rapid flow cytometric method for determining the cellular composition of bronchoalveolar lavage fluid cells in mouse models of asthma. *J. Immunol. Methods* **288**, 111–121.
59. Van Rijt, L. S., Jung, S., Kleinjan, A., Vos, N., Willart, M., Duez, C., Hoogsteden, H. C., Lambrecht, B. N. (2005) In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J. Exp. Med.* **201**, 981–991.
60. Grundy, M., Sentman, C. L. (2005) GFP transgenic mice show dynamics of lung macrophages. *Exp. Cell. Res.* **310**, 409–416.
61. Gonzalez-Juarrero, M., Shim, T. S., Kipnis, A., Junqueira-Kipnis, A. P., Orme, I. M. (2003) Dynamics of macrophage cell populations during murine pulmonary tuberculosis. *J. Immunol.* **171**, 3128–3135.
62. Bosio, C. M., Goodyear, A. W., Dow, S. W. (2005) Early interaction of *Yersinia pestis* with APCs in the lung. *J. Immunol.* **175**, 6750–6756.
63. Gordon, S., Taylor, P. R. (2005) Monocyte and macrophage heterogeneity. *Nat. Rev. Immunol.* **5**, 953–964.
64. Huynh, M. L., Malcom, K. C., Kotaru, C., Tilstra, J. A., Westcott, J. Y., Fadok, V. A., Wenzel, S. E. (2005) Defective apoptotic cell phagocytosis attenuates prostaglandin E2 and 15-hydroxyeicosatetraenoic acid in severe asthma alveolar macrophages. *Am. J. Respir. Crit. Care Med.* **172**, 972–979.
65. Peters-Golden, M. (2004) The alveolar macrophage: the forgotten cell in asthma. *Am. J. Respir. Cell Mol. Biol.* **31**, 3–7.
66. Herbert, C., Scott, M. M., Scruton, K. H., Keogh, R. P., Yuan, K. C., Hsu, K., Siegle, J. S., Tedla, N., Foster, P. S., Kumar, R. K. (2010) Alveolar macrophages stimulate enhanced cytokine production by pulmonary CD4+ T-lymphocytes in an exacerbation of murine chronic asthma. *Am. J. Pathol.* **177**, 1657–1664.
67. Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., Locati, M. (2004) The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* **25**, 677–686.
68. Murray, P. J., Wynn, T. A. (2011) Obstacles and opportunities for understanding macrophage polarization. *J. Leukoc. Biol.* **89**, 557–563.
69. Martinez, F. O., Gordon, S., Locati, M., Mantovani, A. (2006) Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J. Immunol.* **177**, 7303–7311.
70. Mantovani, A. (2008) From phagocyte diversity and activation to probiotics: back to Metchnikoff. *Eur. J. Immunol.* **38**, 3269–3273.
71. Stein, M., Keshav, S., Harris, N., Gordon, S. (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J. Exp. Med.* **176**, 287–292.
72. Kim, E. Y., Bataille, J. T., Patel, A. C., You, Y., Agapov, E., Grayson, M. H., Benoit, L. A., Byers, D. E., Alevis, Y., Tucker, J., Swanson, S., Tidwell, R., Tyner, J. W., Morton, J. D., Castro, M., Polineni, D., Patterson, G. A., Schwendener, R. A., Allard, J. D., Peltz, G., Holtzman, M. J. (2008) Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. *Nat. Med.* **14**, 633–640.
73. Melgert, B. N., Oriss, T. B., Qi, Z., Dixon-McCarthy, B., Geerlings, M., Hylkema, M. N., Ray, A. (2010) Macrophages: regulators of sex differences in asthma? *Am. J. Respir. Cell Mol. Biol.* **42**, 595–603.
74. Biswas, S. K., Mantovani, A. (2010) Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat. Immunol.* **11**, 889–896.
75. Pope, S. M., Fulkerson, P. C., Blanchard, C., Akei, H. S., Nikolaidis, N. M., Zimmermann, N., Molkentin, J. D., Rothberg, M. E. (2005) Identification of a cooperative mechanism involving interleukin-13 and eotaxin-2 in experimental allergic lung inflammation. *J. Biol. Chem.* **280**, 13952–13961.
76. Kodelja, V., Muller, C., Politz, O., Hakij, N., Orfanos, C. E., Goerdt, S. (1998) Alternative macrophage activation-associated CC-chemokine-1, a novel structural homologue of macrophage inflammatory protein-1 α with a Th2-associated expression pattern. *J. Immunol.* **160**, 1411–1418.
77. Adema, G. J., Hartgers, F., Verstraten, R., de Vries, E., Marland, G., Menon, S., Foster, J., Xu, Y., Nooyen, P., McClanahan, T., Bacon, K. B., Figdor, C. G. (1997) A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* **387**, 713–717.
78. Luzina, I. G., Papadimitriou, J. C., Anderson, R., Pochetuhin, K., Atamas, S. P. (2006) Induction of prolonged infiltration of T lymphocytes and transient T lymphocyte-dependent collagen deposition in mouse lungs following adenoviral gene transfer of CCL18. *Arthritis Rheum.* **54**, 2643–2655.
79. Pochetuhin, K., Luzina, I. G., Lockett, V., Choi, J., Todd, N. W., Atamas, S. P. (2007) Complex regulation of pulmonary inflammation and fibrosis by CCL18. *Am. J. Pathol.* **171**, 428–437.
80. De Nadai, P., Charbonnier, A. S., Chenivess, C., Senechal, S., Fournier, C., Gilet, J., Vorng, H., Chang, Y., Gosset, P., Wallaert, B., Tonnel, A. B., Lassalle, P., Tsicopoulos, A. (2006) Involvement of CCL18 in allergic asthma. *J. Immunol.* **176**, 6286–6293.
81. Kim, H. B., Kim, C. K., Iijima, K., Kobayashi, T., Kita, H. (2009) Protein microarray analysis in patients with asthma: elevation of the chemokine PARC/CCL18 in sputum. *Chest* **135**, 295–302.
82. Nair, M. G., Du, Y., Perrigoue, J. G., Zaph, C., Taylor, J. J., Goldschmidt, M., Swain, G. P., Yancopoulos, G. D., Valenzuela, D. M., Murphy, A., Karow, M., Stevens, S., Pearce, E. J., Artis, D. (2009) Alternatively activated macrophage-derived RELM(α) is a negative regulator of type 2 inflammation in the lung. *J. Exp. Med.* **206**, 937–952.
83. Chen, H., Jacobson, B. A., Mason, L., Wolf, S. F., Bowman, M. R. (2010) FIZZ1 potentiates the carbachol-induced tracheal smooth muscle contraction. *Eur. Respir. J.* **36**, 1165–1173.
84. Melgert, B. N., ten Hacken, N. H., Rutgers, B., Timens, W., Postma, D. S., Hylkema, M. N. (2011) More alternative activation of macrophages in lungs of asthmatic patients. *J. Allergy Clin. Immunol.* **127**, 831–833.
85. Subrata, L. S., Bizzintino, J., Mamessier, E., Bosco, A., McKenna, K. L., Wikstrom, M. E., Goldblatt, J., Sly, P. D., Hales, B. J., Thomas, W. R., Laing, I. A., LeSouef, P. N., Holt, P. G. (2009) Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. *J. Immunol.* **183**, 2793–2800.
86. Hunninghake, G. M., Cho, M. H., Tesfagzi, Y., Soto-Quiros, M. E., Avila, L., Lasky-Su, J., Stidley, C., Melen, E., Soderhall, C., Hallberg, J., Kull, I., Kere, J., Svartengren, M., Pershagen, G., Wickman, M., Lange, C., Demeo, D. L., Hersch, C. P., Klanderman, B. J., Raby, B. A., Sparrow, D., Shapiro, S. D., Silverman, E. K., Litonjua, A. A., Weiss, S. T., Celedon, J. C. (2009) MMP12, lung function, and COPD in high-risk populations. *N. Engl. J. Med.* **361**, 2599–2608.
87. Ober, C., Tan, Z., Sun, Y., Possick, J. D., Pan, L., Nicolae, R., Radford, S., Parry, R. R., Heinzmann, A., Deichmann, K. A., Lester, L. A., Gern, J. E., Lemanske Jr., R. F., Nicolae, D. L., Elias, J. A., Chupp, G. L. (2008) Effect of variation in CH13L1 on serum YKL-40 level, risk of asthma, and lung function. *N. Engl. J. Med.* **358**, 1682–1691.
88. Iwashita, H., Morita, S., Sagiya, Y., Nakanishi, A. (2006) Role of eosinophil chemotactic factor by T lymphocytes on airway hyperresponsiveness in a murine model of allergic asthma. *Am. J. Respir. Cell Mol. Biol.* **35**, 103–109.
89. Johansen, J. S., Jensen, B. V., Roslund, A., Price, P. A. (2007) Is YKL-40 a new therapeutic target in cancer? *Expert Opin. Ther. Targets* **11**, 219–234.
90. Chupp, G. L., Lee, C. G., Jarjour, N., Shim, Y. M., Holm, C. T., He, S., Dziura, J. D., Reed, J., Coyle, A. J., Kiener, P., Cullen, M., Grandsaigne, M., Dombret, M. C., Aubier, M., Pretolani, M., Elias, J. A. (2007) A chitinase-like protein in the lung and circulation of patients with severe asthma. *N. Engl. J. Med.* **357**, 2016–2027.

91. Parks, W. C., Shapiro, S. D. (2001) Matrix metalloproteinases in lung biology. *Respir. Res.* **2**, 10–19.
92. Mukhopadhyay, S., Sypek, J., Tavendale, R., Gartner, U., Winter, J., Li, W., Page, K., Fleming, M., Brady, J., O'Toole, M., Macgregor, D. F., Goldman, S., Tam, S., Abraham, W., Williams, C., Miller, D. K., Palmer, C. N. (2010) Matrix metalloproteinase-12 is a therapeutic target for asthma in children and young adults. *J. Allergy Clin. Immunol.* **126**, 70–76.e16.
93. Hume, D. A. (2008) Macrophages as APC and the dendritic cell myth. *J. Immunol.* **181**, 5829–5835.
94. Sutterwala, F. S., Noel, G. J., Salgame, P., Mosser, D. M. (1998) Reversal of proinflammatory responses by ligating the macrophage Fc γ receptor type I. *J. Exp. Med.* **188**, 217–222.
95. Gerber, J. S., Mosser, D. M. (2001) Reversing lipopolysaccharide toxicity by ligating the macrophage Fc γ receptors. *J. Immunol.* **166**, 6861–6868.
96. Fleming, B. D., Mosser, D. M. (2011) Regulatory macrophages: setting the threshold for therapy. *Eur. J. Immunol.* **41**, 2498–2502.
97. Tang, C., Inman, M. D., van Rooijen, N., Yang, P., Shen, H., Matsumoto, K., O'Byrne, P. M. (2001) Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN- γ -dependent mechanism. *J. Immunol.* **166**, 1471–1481.
98. Careau, E., Bissonnette, E. Y. (2004) Adoptive transfer of alveolar macrophages abrogates bronchial hyperresponsiveness. *Am. J. Respir. Cell Mol. Biol.* **31**, 22–27.
99. Careau, E., Proulx, L. I., Pouliot, P., Spahr, A., Turmel, V., Bissonnette, E. Y. (2006) Antigen sensitization modulates alveolar macrophage functions in an asthma model. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **290**, L871–L879.
100. Stolarski, B., Kurowska-Stolarska, M., Kewin, P., Xu, D., Liew, F. Y. (2010) IL-33 exacerbates eosinophil-mediated airway inflammation. *J. Immunol.* **185**, 3472–3480.
101. Murray, P. J., Wynn, T. A. (2011) Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* **11**, 723–737.
102. Manechotesuwan, K., Supawita, S., Kasetsinsombat, K., Wongkajornsilp, A., Barnes, P. J. (2008) Sputum indoleamine-2, 3-dioxygenase activity is increased in asthmatic airways by using inhaled corticosteroids. *J. Allergy Clin. Immunol.* **121**, 43–50.
103. Fitzpatrick, A. M., Higgins, M., Holguin, F., Brown, L. A., Teague, W. G. (2010) The molecular phenotype of severe asthma in children. *J. Allergy Clin. Immunol.* **125**, 851–857.e818.
104. Bedoret, D., Wallemacq, H., Marichal, T., Desmet, C., Quesada Calvo, F., Henry, E., Closset, R., Dewals, B., Thielen, C., Gustin, P., de Leval, L., Van Rooijen, N., Le Moine, A., Vanderplassen, A., Cataldo, D., Drion, P. V., Moser, M., Lekeux, P., Bureau, F. (2009) Lung interstitial macrophages alter dendritic cell functions to prevent airway allergy in mice. *J. Clin. Invest.* **119**, 3723–3738.
105. Simpson, J. L., Phipps, S., Gibson, P. G. (2009) Inflammatory mechanisms and treatment of obstructive airway diseases with neutrophilic bronchitis. *Pharmacol. Ther.* **124**, 86–95.
106. Wilson, R. H., Whitehead, G. S., Nakano, H., Free, M. E., Kolls, J. K., Cook, D. N. (2009) Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. *Am. J. Respir. Crit. Care Med.* **180**, 720–730.
107. Prefontaine, D., Lajoie-Kadoch, S., Foley, S., Audusseau, S., Olivenstein, R., Halayko, A. J., Lemiere, C., Martin, J. G., Hamid, Q. (2009) Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J. Immunol.* **183**, 5094–5103.
108. Murray, C. S., Poletti, G., Keadze, T., Morris, J., Woodcock, A., Johnston, S. L., Custovic, A. (2006) Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* **61**, 376–382.
109. Proud, D., Chow, C. W. (2006) Role of viral infections in asthma and chronic obstructive pulmonary disease. *Am. J. Respir. Cell Mol. Biol.* **35**, 513–518.
110. Siegle, J. S., Hansbro, N., Herbert, C., Rosenberg, H. F., Domachowske, J. B., Asquith, K. L., Foster, P. S., Kumar, R. K. (2010) Early-life viral infection and allergen exposure interact to induce an asthmatic phenotype in mice. *Respir. Res.* **11**, 14.
111. Kaiko, G. E., Phipps, S., Angkasekwina, P., Dong, C., Foster, P. S. (2010) NK cell deficiency predisposes to viral-induced Th2-type allergic inflammation via epithelial-derived IL-25. *J. Immunol.* **185**, 4681–4690.
112. Siegle, J. S., Hansbro, N., Dong, C., Angkasekwina, P., Foster, P. S., Kumar, R. K. (2011) Blocking induction of T helper type 2 responses prevents development of disease in a model of childhood asthma. *Clin. Exp. Immunol.* **165**, 19–28.
113. Thorne, P. S., Kulhankova, K., Yin, M., Cohn, R., Arbes Jr., S. J., Zeldin, D. C. (2005) Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in United States housing. *Am. J. Respir. Crit. Care Med.* **172**, 1371–1377.
114. Patel, K. K., Vicencio, A. C., Du, Z., Tsirilakis, K., Salva, P. S., Webley, W. C. (2010) Infectious *Chlamydia pneumoniae* is associated with elevated interleukin-8 and airway neutrophilia in children with refractory asthma. *Pediatr. Infect. Dis. J.* **29**, 1093–1098.
115. Webley, W. C., Tilahun, Y., Lay, K., Patel, K., Stuart, E. S., Andrzejewski, C., Salva, P. S. (2009) Occurrence of *Chlamydia trachomatis* and *Chlamydia pneumoniae* in paediatric respiratory infections. *Eur. Respir. J.* **33**, 360–367.
116. Dakhama, A., Lee, Y. M., Gelfand, E. W. (2005) Virus-induced airway dysfunction: pathogenesis and biomechanisms. *Pediatr. Infect. Dis. J.* **24**, S159–S169.
117. Schaller, M., Hogaboam, C. M., Lukacs, N., Kunkel, S. L. (2006) Respiratory viral infections drive chemokine expression and exacerbate the asthmatic response. *J. Allergy Clin. Immunol.* **118**, 295–302.
118. Bartlett, N. W., Walton, R. P., Edwards, M. R., Aniscenko, J., Caramori, G., Zhu, J., Glanville, N., Choy, K. J., Jourdan, P., Burnet, J., Tuthill, T. J., Pedrick, M. S., Hurle, M. J., Plumptre, C., Sharp, N. A., Bussell, J. N., Swallow, D. M., Schwarze, J., Guy, B., Almond, J. W., Jeffery, P. K., Lloyd, C. M., Papi, A., Killington, R. A., Rowlands, D. J., Blair, E. D., Clarke, N. J., Johnston, S. L. (2008) Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat. Med.* **14**, 199–204.
119. Contoli, M., Message, S. D., Laza-Stanca, V., Edwards, M. R., Wark, P. A., Bartlett, N. W., Keadze, T., Mallia, P., Stanciu, L. A., Parker, H. L., Slater, L., Lewis-Antes, A., Kon, O. M., Holgate, S. T., Davies, D. E., Kottenko, S. V., Papi, A., Johnston, S. L. (2006) Role of deficient type III interferon- λ production in asthma exacerbations. *Nat. Med.* **12**, 1023–1026.
120. Laza-Stanca, V., Stanciu, L. A., Message, S. D., Edwards, M. R., Gern, J. E., Johnston, S. L. (2006) Rhinovirus replication in human macrophages induces NF- κ B-dependent tumor necrosis factor α production. *J. Virol.* **80**, 8248–8258.
121. Ten Hacken, N. H., Oosterhoff, Y., Kauffman, H. F., Guevarra, L., Satoh, T., Tollerud, D. J., Postma, D. S. (1998) Elevated serum interferon- γ in atopic asthma correlates with increased airways responsiveness and circadian peak expiratory flow variation. *Eur. Respir. J.* **11**, 312–316.
122. Yoshida, M., Watson, R. M., Rerich, T., O'Byrne, P. M. (2005) Different profiles of T-cell IFN- γ and IL-12 in allergen-induced early and dual responders with asthma. *J. Allergy Clin. Immunol.* **115**, 1004–1009.
123. Wood, L. G., Simpson, J. L., Hansbro, P. M., Gibson, P. G. (2010) Potentially pathogenic bacteria cultured from the sputum of stable asthmatics are associated with increased 8-isoprostane and airway neutrophilia. *Free Radic. Res.* **44**, 146–154.
124. Kaiko, G. E., Phipps, S., Hickey, D. K., Lam, C. E., Hansbro, P. M., Foster, P. S., Beagley, K. W. (2008) *Chlamydia muridarum* infection subverts dendritic cell function to promote Th2 immunity and airways hyperreactivity. *J. Immunol.* **180**, 2225–2232.
125. Asquith, K. L., Horvat, J. C., Kaiko, G. E., Carey, A. J., Beagley, K. W., Hansbro, P. M., Foster, P. S. (2011) Interleukin-13 promotes susceptibility to chlamydial infection of the respiratory and genital tracts. *PLoS Pathog.* **7**, e1001339.
126. Gotfried, M. H. (2004) Macrolides for the treatment of chronic sinusitis, asthma, and COPD. *Chest* **125**, 52S–60S.
127. Simpson, J. L., Powell, H., Boyle, M. J., Scott, R. J., Gibson, P. G. (2008) Clarithromycin targets neutrophilic airway inflammation in refractory asthma. *Am. J. Respir. Crit. Care Med.* **177**, 148–155.
128. Shannon, J., Ernst, P., Yamauchi, Y., Olivenstein, R., Lemiere, C., Foley, S., Cicora, L., Ludwig, M., Hamid, Q., Martin, J. G. (2008) Differences in airway cytokine profile in severe asthma compared to moderate asthma. *Chest* **133**, 420–426.
129. Heaton, T., Rowe, J., Turner, S., Aalberse, R. C., de Klerk, N., Suriyaarachchi, D., Serralha, M., Holt, B. J., Hollams, E., Yerkovich, S., Holt, K., Sly, P. D., Goldblatt, J., Le Souef, P., Holt, P. G. (2005) An immunopidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. *Lancet* **365**, 142–149.
130. Yu, M., Eckart, M. R., Morgan, A. A., Mukai, K., Butte, A. J., Tsai, M., Galli, S. J. (2011) Identification of an IFN- γ /mast cell axis in a mouse model of chronic asthma. *J. Clin. Invest.* **121**, 3133–3143.
131. Hakonarson, H., Bjornsdottir, U. S., Halapi, E., Bradfield, J., Zink, F., Mouy, M., Helgadottir, H., Gudmundsdottir, A. S., Andrason, H., Adalsteinsson, A. E., Kristjansson, K., Birkenstein, I., Arnason, T., Andresdottir, M., Gislason, D., Gislason, T., Gulcher, J. R., Stefansson, K. (2005) Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients. *Proc. Natl. Acad. Sci. USA* **102**, 14789–14794.
132. Michel, O., Kips, J., Duchateau, J., Vertongen, F., Robert, L., Collet, H., Pauwels, R., Sergysels, R. (1996) Severity of asthma is related to endotoxin in house dust. *Am. J. Respir. Crit. Care Med.* **154**, 1641–1646.
133. Locati, M., Deuschle, U., Massardi, M. L., Martinez, F. O., Sironi, M., Sozzani, S., Bartfai, T., Mantovani, A. (2002) Analysis of the gene expression profile activated by the CC chemokine ligand 5/RANTES and by lipopolysaccharide in human monocytes. *J. Immunol.* **168**, 3557–3562.
134. Hayashi, N., Yoshimoto, T., Izuhara, K., Matsui, K., Tanaka, T., Nakaniishi, K. (2007) T helper 1 cells stimulated with ovalbumin and IL-18 induce airway hyperresponsiveness and lung fibrosis by IFN- γ and IL-13 production. *Proc. Natl. Acad. Sci. USA* **104**, 14765–14770.
135. Yang, M., Kumar, R. K., Foster, P. S. (2009) Pathogenesis of steroid-resistant airway hyperresponsiveness: interaction between IFN- γ and TLR4/MyD88 pathways. *J. Immunol.* **182**, 5107–5115.

136. Shi, S., Nathan, C., Schnappinger, D., Drenkow, J., Fuortes, M., Block, E., Ding, A., Gigeras, T. R., Schoolnik, G., Akira, S., Takeda, K., Ehrt, S. (2003) MyD88 primes macrophages for full-scale activation by interferon- γ yet mediates few responses to *Mycobacterium tuberculosis*. *J. Exp. Med.* **198**, 987–997.
137. Sun, D., Ding, A. (2006) MyD88-mediated stabilization of interferon- γ -induced cytokine and chemokine mRNA. *Nat. Immunol.* **7**, 375–381.
138. Szefer, S. J., Norton, C. E., Ball, B., Gross, J. M., Aida, Y., Pabst, M. J. (1989) IFN- γ and LPS overcome glucocorticoid inhibition of priming for superoxide release in human monocytes. Evidence that secretion of IL-1 and tumor necrosis factor- α is not essential for monocyte priming. *J. Immunol.* **142**, 3985–3992.
139. Kamijo, R., Harada, H., Matsuyama, T., Bosland, M., Gerecitano, J., Shapiro, D., Le, J., Koh, S. I., Kimura, T., Green, S. J., et al. (1994) Requirement for transcription factor IRF-1 in NO synthase induction in macrophages. *Science* **263**, 1612–1615.
140. Bach, E. A., Aguet, M., Schreiber, R. D. (1997) The IFN γ receptor: a paradigm for cytokine receptor signaling. *Annu. Rev. Immunol.* **15**, 563–591.
141. Ramana, C. V., Gil, M. P., Schreiber, R. D., Stark, G. R. (2002) Stat1-dependent and -independent pathways in IFN- γ -dependent signaling. *Trends Immunol.* **23**, 96–101.
142. Pflanz, S., Hibbert, L., Mattson, J., Rosales, R., Vaisberg, E., Bazan, J. F., Phillips, J. H., McClanahan, T. K., de Waal Malefyt, R., Kastelein, R. A. (2004) WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. *J. Immunol.* **172**, 2225–2231.
143. Ruckerl, D., Hessmann, M., Yoshimoto, T., Ehlers, S., Holscher, C. (2006) Alternatively activated macrophages express the IL-27 receptor α chain WSX-1. *Immunobiology* **211**, 427–436.
144. Liu, J., Guan, X., Ma, X. (2007) Regulation of IL-27 p28 gene expression in macrophages through MyD88- and interferon- γ -mediated pathways. *J. Exp. Med.* **204**, 141–152.
145. Li, J., Wang, W., Baines, K. J., Bowden, N. A., Hansbro, P. M., Gibson, P. G., Kumar, R. K., Foster, P. S., Yang, M. (2010) IL-27/IFN- γ induce MyD88-dependent steroid-resistant airway hyperresponsiveness by inhibiting glucocorticoid signaling in macrophages. *J. Immunol.* **185**, 4401–4409.
146. Pflanz, S., Timans, J. C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., Blumenschein, W. M., Mattson, J. D., Wagner, J. L., To, W., Zurawski, S., McClanahan, T. K., Gorman, D. M., Bazan, J. F., de Waal Malefyt, R., Rennick, D., Kastelein, R. A. (2002) IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naive CD4(+) T cells. *Immunity* **16**, 779–790.
147. Yoshida, H., Hamano, S., Senaldi, G., Covey, T., Faggioni, R., Mu, S., Xia, M., Wakeham, A. C., Nishina, H., Potter, J., Saris, C. J., Mak, T. W. (2001) WSX-1 is required for the initiation of Th1 responses and resistance to *L. major* infection. *Immunity* **15**, 569–578.
148. Morishima, N., Owaki, T., Asakawa, M., Kamiya, S., Mizuguchi, J., Yoshimoto, T. (2005) Augmentation of effector CD8+ T cell generation with enhanced granzyme B expression by IL-27. *J. Immunol.* **175**, 1686–1693.
149. Holscher, C., Holscher, A., Ruckerl, D., Yoshimoto, T., Yoshida, H., Mak, T., Saris, C., Ehlers, S. (2005) The IL-27 receptor chain WSX-1 differentially regulates antibacterial immunity and survival during experimental tuberculosis. *J. Immunol.* **174**, 3534–3544.
150. Wang, S., Miyazaki, Y., Shinozaki, Y., Yoshida, H. (2007) Augmentation of antigen-presenting and Th1-promoting functions of dendritic cells by WSX-1 (IL-27R) deficiency. *J. Immunol.* **179**, 6421–6428.
151. Imamichi, T., Yang, J., Huang, D. W., Brann, T. W., Fullmer, B. A., Adelsberger, J. W., Lempicki, R. A., Baseler, M. W., Lane, H. C. (2008) IL-27, a novel anti-HIV cytokine, activates multiple interferon-inducible genes in macrophages. *AIDS* **22**, 39–45.
152. Feng, X. M., Liu, N., Yang, S. G., Hu, L. Y., Chen, X. L., Fang, Z. H., Ren, Q., Lu, S. H., Liu, B., Han, Z. C. (2008) Regulation of the class II and class I MHC pathways in human THP-1 monocytic cells by interleukin-27. *Biochem. Biophys. Res. Commun.* **367**, 553–559.
153. Kalliolias, G. D., Ivashkiv, L. B. (2008) IL-27 activates human monocytes via STAT1 and suppresses IL-10 production but the inflammatory functions of IL-27 are abrogated by TLRs and p38. *J. Immunol.* **180**, 6325–6333.
154. Pearl, J. E., Khader, S. A., Solache, A., Gilmartin, L., Ghilardi, N., de Sauvage, F., Cooper, A. M. (2004) IL-27 signaling compromises control of bacterial growth in mycobacteria-infected mice. *J. Immunol.* **173**, 7490–7496.
155. Berry, M. A., Hargadon, B., Shelley, M., Parker, D., Shaw, D. E., Green, R. H., Bradding, P., Brightling, C. E., Wardlaw, A. J., Pavord, I. D. (2006) Evidence of a role of tumor necrosis factor α in refractory asthma. *N. Engl. J. Med.* **354**, 697–708.
156. Howarth, P. H., Babu, K. S., Arshad, H. S., Lau, L., Buckley, M., McConnell, W., Beckett, P., Al Ali, M., Chauhan, A., Wilson, S. J., Reynolds, A., Davies, D. E., Holgate, S. T. (2005) Tumor necrosis factor (TNF α) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. *Thorax* **60**, 1012–1018.
157. Lukacs, N. W., Strieter, R. M., Chensue, S. W., Widmer, M., Kunkel, S. L. (1995) TNF- α mediates recruitment of neutrophils and eosinophils during airway inflammation. *J. Immunol.* **154**, 5411–5417.
158. Scheurich, P., Thoma, B., Ucer, U., Pfizenmaier, K. (1987) Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)- α : induction of TNF receptors on human T cells and TNF- α -mediated enhancement of T cell responses. *J. Immunol.* **138**, 1786–1790.
159. Franchimont, D., Martens, H., Hagelstein, M. T., Louis, E., Dewe, W., Chrousos, G. P., Belaiche, J., Geenen, V. (1999) Tumor necrosis factor α decreases, and interleukin-10 increases, the sensitivity of human monocytes to dexamethasone: potential regulation of the glucocorticoid receptor. *J. Clin. Endocrinol. Metab.* **84**, 2834–2839.
160. Sullivan, D. E., Ferris, M., Pociask, D., Brody, A. R. (2005) Tumor necrosis factor- α induces transforming growth factor- β 1 expression in lung fibroblasts through the extracellular signal-regulated kinase pathway. *Am. J. Respir. Cell Mol. Biol.* **32**, 342–349.
161. Bloom, B. R., Bennett, B. (1966) Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science* **153**, 80–82.
162. Calandra, T., Bernhagen, J., Mitchell, R. A., Bucala, R. (1994) The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J. Exp. Med.* **179**, 1895–1902.
163. Calandra, T., Bernhagen, J., Metz, C. N., Spiegel, L. A., Bacher, M., Donnelly, T., Cerami, A., Bucala, R. (1995) MIF as a glucocorticoid-induced modulator of cytokine production. *Nature* **377**, 68–71.
164. Roger, T., David, J., Glauser, M. P., Calandra, T. (2001) MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature* **414**, 920–924.
165. Calandra, T., Roger, T. (2003) Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat. Rev. Immunol.* **3**, 791–800.
166. Busse, W. W., Lemanske Jr., R. F. (2001) Asthma. *N. Engl. J. Med.* **344**, 350–362.
167. Bernhagen, J., Krohn, R., Lue, H., Gregory, J. L., Zernecke, A., Koenen, R. R., Dewor, M., Georgiev, I., Schober, A., Leng, L., Kooistra, T., Fingerle-Rowson, G., Ghezzi, P., Kleemann, R., McColl, S. R., Bucala, R., Hickey, M. J., Weber, C. (2007) MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat. Med.* **13**, 587–596.
168. Pan, J. H., Sukhova, G. K., Yang, J. T., Wang, B., Xie, T., Fu, H., Zhang, Y., Satoskar, A. R., David, J. R., Metz, C. N., Bucala, R., Fang, K., Simon, D. I., Chapman, H. A., Libby, P., Shi, G. P. (2004) Macrophage migration inhibitory factor deficiency impairs atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation* **109**, 3149–3153.
169. Ayoub, S., Hickey, M. J., Morand, E. F. (2008) Mechanisms of disease: macrophage migration inhibitory factor in SLE, RA and atherosclerosis. *Nat. Clin. Pract. Rheumatol.* **4**, 98–105.
170. Rossi, A. G., Haslett, C., Hirani, N., Greening, A. P., Rahman, I., Metz, C. N., Bucala, R., Donnelly, S. C. (1998) Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma. *J. Clin. Invest.* **101**, 2869–2874.
171. Mizue, Y., Ghani, S., Leng, L., McDonald, C., Kong, P., Baugh, J., Lane, S. J., Craft, J., Nishihira, J., Donnelly, S. C., Zhu, Z., Bucala, R. (2005) Role for macrophage migration inhibitory factor in asthma. *Proc. Natl. Acad. Sci. USA* **102**, 14410–14415.
172. Kobayashi, M., Nasuhara, Y., Kamachi, A., Tanino, Y., Betsuyaku, T., Yamaguchi, E., Nishihira, J., Nishimura, M. (2006) Role of macrophage migration inhibitory factor in ovalbumin-induced airway inflammation in rats. *Eur. Respir. J.* **27**, 726–734.
173. Oboki, K., Ohno, T., Kajiura, N., Arae, K., Morita, H., Ishii, A., Nambu, A., Abe, T., Kiyonari, H., Matsumoto, K., Sudo, K., Okumura, K., Saito, H., Nakae, S. (2010) IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc. Natl. Acad. Sci. USA* **107**, 18581–18586.
174. Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., Zurawski, G., Moshrefi, M., Qin, J., Li, X., Gorman, D. M., Bazan, J. F., Kastelein, R. A. (2005) 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* **23**, 479–490.
175. Liew, F. Y., Pitman, N. I., McInnes, I. B. (2010) Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat. Rev. Immunol.* **10**, 103–110.
176. Sakashita, M., Yoshimoto, T., Hirota, T., Harada, M., Okubo, K., Osawa, Y., Fujieda, S., Nakamura, Y., Yasuda, K., Nakanishi, K., Tamari, M. (2008) Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. *Clin. Exp. Allergy* **38**, 1875–1881.
177. Prefontaine, D., Nadigel, J., Chouiali, F., Audusseau, S., Semlali, A., Chakir, J., Martin, J. G., Hamid, Q. (2010) Increased IL-33 expression by epithelial cells in bronchial asthma. *J. Allergy Clin. Immunol.* **125**, 752–754.
178. Kurowska-Stolarska, M., Stolarski, B., Kewin, P., Murphy, G., Corrigan, C. J., Ying, S., Pitman, N., Mirchandani, A., Rana, B., van Rooijen, N., Shepherd, M., McSharry, C., McInnes, I. B., Xu, D., Liew, F. Y. (2009) IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J. Immunol.* **183**, 6469–6477.

179. Kondo, Y., Yoshimoto, T., Yasuda, K., Futatsugi-Yumikura, S., Morimoto, M., Hayashi, N., Hoshino, T., Fujimoto, J., Nakanishi, K. (2008) Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. *Int. Immunol.* **20**, 791–800.
180. Ouyang, W., Kolls, J. K., Zheng, Y. (2008) The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* **28**, 454–467.
181. Chakir, J., Shannon, J., Molet, S., Fukakusa, M., Elias, J., Laviolette, M., Boulet, L. P., Hamid, Q. (2003) Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF- β , IL-11, IL-17, and type I and type III collagen expression. *J. Allergy Clin. Immunol.* **111**, 1293–1298.
182. Bullens, D. M., Truyen, E., Coteur, L., Dilissen, E., Hellings, P. W., Dupont, L. J., Ceuppens, J. L. (2006) IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir. Res.* **7**, 135.
183. McKinley, L., Alcorn, J. F., Peterson, A., Dupont, R. B., Kapadia, S., Logar, A., Henry, A., Irvin, C. G., Piganelli, J. D., Ray, A., Kolls, J. K. (2008) TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J. Immunol.* **181**, 4089–4097.
184. Lin, Y., Ritchie, S., Logar, A., Slight, S., Messmer, M., Rangel-Moreno, J., Gugliani, L., Alcorn, J. F., Strawbridge, H., Park, S. M., Onishi, R., Nyugen, N., Walter, M. J., Pociask, D., Randall, T. D., Gaffen, S. L., Iwakura, Y., Kolls, J. K., Khader, S. A. (2009) Interleukin-17 is required for T helper 1 cell immunity and host resistance to the intracellular pathogen *Francisella tularensis*. *Immunity* **31**, 799–810.
185. Irusen, E., Matthews, J. G., Takahashi, A., Barnes, P. J., Chung, K. F., Adcock, I. M. (2002) p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. *J. Allergy Clin. Immunol.* **109**, 649–657.
186. Rommel, C., Camps, M., Ji, H. (2007) PI3K δ and PI3K γ : partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat. Rev. Immunol.* **7**, 191–201.
187. Marone, R., Cmiljanovic, V., Giese, B., Wymann, M. P. (2008) Targeting phosphoinositide 3-kinase: moving towards therapy. *Biochim. Biophys. Acta* **1784**, 159–185.
188. Erin, E. M., Leaker, B. R., Nicholson, G. C., Tan, A. J., Green, L. M., Neighbour, H., Zacharasiewicz, A. S., Turner, J., Barnathan, E. S., Kon, O. M., Barnes, P. J., Hansel, T. T. (2006) The effects of a monoclonal antibody directed against tumor necrosis factor- α in asthma. *Am. J. Respir. Crit. Care Med.* **174**, 753–762.
189. Morjaria, J. B., Chauhan, A. J., Babu, K. S., Polosa, R., Davies, D. E., Holgate, S. T. (2008) The role of a soluble TNF α receptor fusion protein (etanercept) in corticosteroid refractory asthma: a double blind, randomised, placebo controlled trial. *Thorax* **63**, 584–591.
190. Wenzel, S. E., Barnes, P. J., Bleecker, E. R., Bousquet, J., Busse, W., Dahlen, S. E., Holgate, S. T., Meyers, D. A., Rabe, K. F., Antczak, A., Baker, J., Horvath, L., Mark, Z., Bernstein, D., Kerwin, E., Schlenker-Herzog, R., Lo, K. H., Watt, R., Barnathan, E. S., Chanez, P. (2009) A randomized, double-blind, placebo-controlled study of tumor necrosis factor- α blockade in severe persistent asthma. *Am. J. Respir. Crit. Care Med.* **179**, 549–558.
191. Corren, J., Lemanske, R. F., Hanania, N. A., Korenblat, P. E., Parsey, M. V., Arron, J. R., Harris, J. M., Scheerens, H., Wu, L. C., Su, Z., Mosesova, S., Eisner, M. D., Bohen, S. P., Matthews, J. G. (2011) Lebrikizumab treatment in adults with asthma. *N. Engl. J. Med.* **365**, 1088–1098.

KEY WORDS:

innate immunity · airway hyper-responsiveness · steroid resistance