

Short reviews

The allergic cascade: Review of the most important molecules in the asthmatic lung

Karolien Bloemen^{*,1}, Sandra Verstraelen^{*,1}, Rosette Van Den Heuvel,
Hilda Witters, Inge Nelissen, Greet Schoeters

*Centre of Expertise in Environmental Toxicology, Flemish Institute for Technological Research (VITO),
Boeretang 200, B-2400 Mol, Belgium*

Received 15 March 2007; received in revised form 17 July 2007; accepted 22 July 2007
Available online 15 August 2007

Abstract

Asthma is the most common chronic inflammatory disorder of the airways among children. It is a complex clinical disease characterized by airway obstruction, airway inflammation and airway hyperresponsiveness to a variety of stimuli. The development of allergic asthma exists of three phases, namely the induction phase, the early-phase asthmatic reaction (EAR) and the late-phase asthmatic reaction (LAR). Each phase is characterized by the production and interplay of various cell-derived mediators. In the induction phase, T helper cytokines are important in the development of asthma. Most important mediators in the EAR are preformed mediators, newly synthesized lipid mediators and cytokines that are produced by mast cells. During the LAR, inflammatory molecules are produced by various cell types, such as eosinophils, neutrophils, T cells, macrophages, dendritic cells, and structural cells. Chronical inflammation leads to structural changes of the airway architecture. In this review, the most important mediators involved in the induction phase, the early-phase and late-phase asthmatic reaction are discussed.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Airway hyperresponsiveness; Airway remodeling; Allergic cascade; Inflammation; Mediators

1. Introduction

The prevalence of asthma in children has dramatically increased in the last decades all over the world [1]. According to the World Health Organization (WHO), 300 million people suffer from asthma and 255,000 died of asthma in

2005. The exact cause of asthma remains uncertain. Several factors will influence the disease, for example genetic factors (e.g. a history of asthma in the family, atopy), environmental factors (e.g. air pollution, occupational exposure, viral infections, allergen exposure) and life style factors (e.g. smoking habits, food). In susceptible individuals, asthma causes recur-

Abbreviations: ADAM, a disintegrin and metalloproteinase; AHR, airway hyperresponsiveness; APCs, antigen-presenting cells; ASM, airway smooth muscle; BAL, bronchoalveolar lavage; BECs, bronchial epithelial cells; bFGF, basic fibroblast growth factor; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CRTH2, chemoattractant receptor homologous molecule expressed on Th2 cells; CXCL, CXC-chemokine ligand; Cys-LT, cysteinyl leukotriene; DCs, dendritic cells; EAR, early-phase asthmatic reaction; EBC, exhaled breath condensate; ECM, extracellular matrix; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EGF, epidermal growth factor; EP, eosinophil peroxidase; ET, endothelin; GCP, granulocyte chemotactic protein; GM-CSF, granulocyte macrophage colony stimulating factor; GRO, growth-regulated oncogene; ICAM, intercellular adhesion molecule; ICOS, inducible T cell costimulator; IFN, interferon; Ig, immunoglobulin; IGF, insulin-like growth factor; IL, interleukin; LAR, late-phase asthmatic reaction; LT, leukotriene; MBP, major basic protein; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; NO, nitric oxide; PAF, platelet activating factor; PDGF, platelet-derived growth factor; PECAM, platelet endothelial cell adhesion molecule; PG, prostaglandin; RANTES, regulated upon activation, normal T-cell expressed, and secreted; ROS, reactive oxygen species; TARC, thymus and activation-regulated chemokine; TGF, tumor growth factor; Th, T helper; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; Treg, regulated T cells; TX, thromboxane; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VLA, very late activation antigen

* Corresponding authors. Tel.: +32 14 33 51 07; fax: +32 14 58 26 57.

E-mail addresses: karolien.bloemen@vito.be (K. Bloemen), sandra.verstraelen@vito.be (S. Verstraelen).

¹ These authors contributed equally to this review.

rent episodes of wheezing, breathlessness, chest tightness and cough [2]. Crucial in the development of airway inflammation in allergic asthma is the allergic cascade. Inhaled allergens that escape the mucociliary clearance are taken up and processed by antigen presenting cells (APCs), which are distributed throughout the respiratory tract, from the nasal mucosa to the lung pleura. These APCs then migrate to the draining lymph nodes where the processed allergen is presented to allergen-specific T and B cells [3]. Interactions between those cells elicit responses that are characterized and influenced by secreted cytokines and the presence or absence of cell-bound costimulatory molecules. Activation of T helper (Th) cells by APCs leads to the production of cytokines that regulate the isotype switch of B cells in their production of immunoglobulin (Ig) E [4]. Once synthesized, IgE antibodies circulate in the blood before binding to the high-affinity IgE receptor FcεRI that is present on mast cells in tissue or on peripheral blood basophils. After re-exposure, allergens cross-link to mast cell-bound specific IgE, thus causing the activation of membrane and cytosolic pathways, which subsequently trigger the release of preformed mediators, such as histamine, the synthesis of prostaglandins (PGs) and leukotrienes (LTs), and the transcription of cytokines by mast cells [5]. These mediators cause the so-called early-phase asthmatic reaction (EAR), which is characterized by constriction of airway smooth muscle (ASM) cells, vascular leakage, mucus production, enhanced airway hyper-responsiveness (AHR) and recruitment of inflammatory cells [6]. This EAR is immediate, lasting 30–60 min and 4–6 h later followed by the late-phase asthmatic reaction (LAR) [7]. The late-phase is characterized by excessive inflammation of the airways, resulting in structural changes, including airway wall thickening, subepithelial fibrosis, goblet cell hyperplasia, myofibroblast hyperplasia, ASM cell hyperplasia and hypertrophy, and epithelial hypertrophy [8,9]. This is collectively known as airway remodeling.

Research has focused on mechanisms of airway inflammation, regulation of these processes, and translation of these events into altered lung function. The use of animal models, *in vitro* tests, human allergen challenge studies, and biopsy and lavage studies that generate human samples for *ex vivo* analysis, continues to expand our understanding of the role of various mediators, inflammatory molecules, and cellular and physiologic changes associated with allergic disorders [10]. Recent research focuses specifically on early markers for respiratory diseases using preferably non-invasive techniques such as exhaled breath condensate (EBC).

Molecular changes that occur in asthmatic lungs will be focussed on in this review. Special attention will be paid to cytokines and chemokines that are involved in asthma against the background of molecules that play an important role in normal lung physiology. A summary of the mediators discussed can be found in Table 1. These mediators offer the possibility to be used as diagnostic biomarkers. Potential markers measured in EBC are summarized in Table 2.

2. Induction of the allergic reaction

During the induction phase, allergens enter the airways, are processed by APCs, and are brought to the lymph nodes. Here, they are presented to T and B cells. Activation of Th cells leads to the production of various cytokines, such as interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, IL-18, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, TNF-β and granulocyte macrophage colony stimulating factor (GM-CSF). Of these, IL-4, IL-5, IL-9 and IL-13 are the most important in the development of asthma [11]. IL-4 and IL-13 play a role in isotope switching to IgE production. Together with IL-9, they are also important in mast cell development, mucus overproduction and AHR. IL-5 is the most important cytokine in eosinophil accumulation. In studies with EBC, sputum, bronchoalveolar

Table 1

Summary of mediators released by the various cell types that are involved in the early and late asthmatic reaction. Abbreviations are explained throughout the text

Cell source	Released mediators
<i>Induction phase</i>	
T cells	Cytokines (IL-4, IL-5, IL-9, IL-13)
<i>Early asthmatic reaction</i>	
Mast cells	Histamine; proteases (tryptase, chymase, carboxypeptidase); proteoglycans (heparin, chondroitin sulphate E); prostaglandins (PGD ₂); leukotrienes (LTC ₄); cytokines (TNF-α, IL-3, IL-4, IL-5, IL-6, IL-8, IL-16, GM-CSF); chemokines (CCL2, CCL3, CCL11)
Basophils	Histamine; leukotrienes (cys-LTs: LTC ₄ , LTD ₄ , LTE ₄); cytokines (IL-4, IL-13)
<i>Late asthmatic reaction</i>	
Eosinophils	MBP; ECP; EDN; EP; leukotrienes (cys-LTs: LTC ₄ , LTD ₄ , LTE ₄); cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-11, IL-12, TNF-α, TGF-α, TGF-β, GM-CSF); chemokines (CXCL8, CCL3, CCL5)
Neutrophils	Leukotrienes (LTA ₄ , LTB ₄); PAF; TXA ₂ ; cytokines (IL-1β, IL-6, TNF-α, TGF-β); chemokine (CXCL8); proteases (elastase, collagenase, gelatinase B); microbicidal products (lactoferrin, myeloperoxidase, lysozyme); reactive oxygen intermediates (superoxide, hydrogen peroxide); NO
T cells	Cytokines (IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, GM-CSF); chemokines (CCL1, CCL22)
Macrophages	Cytokines (IL-1, IL-6, IFN-γ, TNF-α); chemokines (CXCL8); lipids; PAF; ROS; NO
Dendritic cells	Chemokines (CCL2, CCL3, CCL4, CCL17, CCL22, CXCL8)
Endothelial cells	ICAM-1, ICAM-2; PECAM-1; VCAM-1; selectins (E-selectin, P-selectin)
Airway smooth muscle cells	Chemokines (CCL5, CCL7, CCL11, CCL13, CXCL8); cytokines (GM-CSF, IL-6); prostaglandins (PGE ₂); ECM proteins
Bronchial epithelial cells	Cytokines (IL-6, GM-CSF); chemokines (CCL11, CCL17, CCL22, CXCL1, CXCL6, CXCL8); ICAM-1

Table 2
Overview of molecules measured in EBC both in asthma and in healthy controls

Molecule	Asthma	References
IL-4	↑	[12–14]
IL-5	↑	[190]
IL-8	↑	[14]
IL-17	↑	[14]
TNF-α	↑	[14]
TGF-β	↑	[14]
IFN-γ	↓	[12]
CCL3	↑	[14]
CCL4	↑	[14]
CCL5	↑	[14]
CCL11	=	[191]
	↑	[192]
CCL17	↓	[191]
CCL22	↑	[191,192]
PDGF	↑	[13]
8-Isoprostane	↑	[174–176,184,185,193]
PGE2	↑	[173]
LTB4	↑	[173,181,184,185]
LTD4	↑	[181]
LTE4	↑	[173,181]
Cys-LT	↑	[174,176,193]

↑: A higher concentration of the molecule is present in EBC of asthmatic patients compared to controls; ↓: the concentration of this molecule is decreased in EBC from asthmatics; =: the molecule is present in equal amounts in the EBC of asthmatic patients and healthy controls.

lavage (BAL) fluid, and bronchial biopsy specimens, elevated levels of IL-4 [12–15], IL-5 [16], and IL-9 [17] were found in asthmatic airways, compared to control subjects.

3. Early-phase asthmatic reaction

3.1. Mast cells

The most crucial cell type in the EAR are mast cells [18]. Mast cells are involved in the pathophysiology of asthma through their capacity to secrete a wide variety of mediators after activation by allergens. Re-exposure to a previously met allergen leads to its binding on IgE antibodies that are attached to mast cell FcεRI receptors. This causes cross-linking of FcεRI receptors, whereupon mast cells degranulate and synthesize pro-inflammatory molecules [19,20]. Mediators produced by mast cells can be divided into preformed mediators (e.g. histamine), newly synthesized lipid mediators (e.g. PGs and LTs), and cytokines and growth factors (e.g. TNF-α, vascular endothelial growth factor (VEGF)) [21].

3.1.1. Preformed mediators

Preformed mediators are packaged within secretory granules in the mast cell and, on activation, are released into the extracellular environment within minutes. Principal granule constituents include histamine, proteases (tryptase, chymase, and carboxypeptidase) and proteoglycans (heparin and chondroitin sulphate E). Histamine exerts effects on smooth muscle cells (contraction), endothelial cells, venule permeability, nerve endings, and mucus secretion [21]. The function of tryptase *in vivo* is unknown, but *in vitro* it can cleave complement C3 and C3a,

activate fibroblasts, promote accumulation of inflammatory cells [21] and potentiate histamine-induced smooth muscle contraction [22]. Tryptase is used as a marker of mast cell degranulation. Increased concentrations of this protease have been found in BAL fluid in asthma patients [23]. Chymase has a procollagen proteinase activity and is probably directly toxic to the airway cells [24]. It can possibly also lead to mucus secretion. Other mast cell proteases likely contribute to activation of protein cascades and inflict local tissue damage [25].

3.1.2. Lipid mediators

Lipid mediators include PGs and LTs, inflammatory metabolites derived from the peroxidation of arachidonic acid. Some of these, such as PGD2 and LTC4, may be newly produced after mast cell activation. These molecules have various effects in the asthmatic airway, e.g. recruitment of inflammatory cells, bronchoconstriction and mucus secretion. These molecules are discussed in more detail later in this review.

3.1.3. Cytokines

Mast cells play a role in more persistent or chronic inflammatory responses through the release of multifunctional cytokines. TNF-α is a major cytokine produced by mast cells; it upregulates endothelial and epithelial adhesion molecules, increases AHR, and has antitumor effects. In an asthma model in mice, TNF-α contributes significantly to allergic inflammation and AHR, possibly by enhancing lymphocyte recruitment and T helper 2 (Th2) cytokine production [26]. Higher TNF-α concentrations were found in EBC from asthmatics compared to healthy controls [14]. Other cytokines produced by mast cells include IL-4, which is associated with Th2 cell differentiation and IgE synthesis, IL-3, GM-CSF, and IL-5, which are critical for eosinophil development and survival, and IL-6, CXC-chemokine ligand (CXCL) 8 (IL-8), and IL-16. Mast cells also secrete the profibrogenic cytokines tumor growth factor (TGF)-β and basic fibroblast growth factor (bFGF) 2 [27]. Moreover, they produce chemokines, such as CC-chemokine ligand (CCL) 3 (macrophage inflammatory protein (MIP)-1α) [28], which has the ability to stimulate histamine release from mast cells and basophils [29], and CCL2 (monocyte chemotactic protein (MCP)-1), and CCL11 (eotaxin-1), which are involved in leukocyte attraction during inflammation. Increased levels of CCL2 and CCL3 have been reported in BAL fluid of asthmatics [30–32]. This connects mast cell degranulation with the LAR in asthma.

3.2. Basophils

Beside mast cells, basophils also express the IgE receptor FcεRI and degranulate after re-exposure to allergen. Mediators, such as CC-chemokines (CCL11, CCL24 (eotaxin-2), CCL26 (eotaxin-3), CCL7 (MCP-3), CCL13 (MCP-4) and CCL5 (regulated upon activation, normal T-cell expressed, and secreted, RANTES)), formylmethionine-leucine-phenylalanine, IL-3, IL-5, GM-CSF, and histamine-releasing factor, potentiate FcεRI effects [28], leading to basophil mediator release. In addition to stimulating release of histamine and LTC4, CCL11 and CCL24

are also chemoattractant for basophils [33]. Complement C3a and C5a activate basophils through specific receptors. CXCL8 can induce a respiratory burst with the release of histamine and cysteinyl LTs (cys-LTs) [34] from human basophils, with enhanced release after IL-3, IL-5, or GM-CSF pretreatment [35,36]. Basophils produce a spectrum of mediators, including histamine, eicosanoids, LTs, IL-4, and IL-13 [37,38]. They are a dominant and rapid source of IL-4 in human systems [39] and express a variety of cytokine receptors (IL-3 receptor (IL-3R), IL-5R, and GM-CSFR), CC-chemokine receptors (CCR2 and CCR3), complement receptors (CD11b, CD11c, CD35, and CD88), Ig Fc-receptors (FcεRI and FcγRII), and chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2, a high affinity receptor for PGD2) [28,40]. The cytokines released are most important in the later phase of asthma.

4. Late-phase asthmatic reaction

The late-phase of the asthmatic reaction is characterized by excessive inflammation of the airways resulting in structural changes induced by various mediators derived from inflammatory cells, like eosinophils, neutrophils, T cells, macrophages, dendritic cells (DCs), endothelial cells, airway smooth muscle cells (ASM) and bronchial epithelial cells (BECs) [8]. The role of several chemokines is thereby firmly established [41–43].

4.1. Eosinophils

Eosinophils are the central effector cells in ongoing airway inflammation. They can be primed by a number of factors, including IL-3, IL-5, GM-CSF, CC-chemokines (CCL2, CCL3, CCL5, CCL7, CCL8 (MCP-2), CCL11, CCL13), and platelet activating factor (PAF), resulting in inflammatory mediator release [28,44], such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EP) [7,25]. Together, this quartet can inflict substantial damage to airway endothelial cells, extracellular matrix (ECM), and neurons: exposure of nerve endings to airway lumina may account for AHR, ASM cell contraction and increased vascular permeability on subsequent exposures to specific antigens or generalized irritants, ultimately leading to the recruitment of more eosinophils and Th2 cells to the airways [45]. Eosinophil mediator secretion in asthma has been confirmed by BAL fluid analysis, which shows increased concentrations of granule-derived basic proteins [23]. ECP release is higher in sputum obtained from untreated asthma compared to treated asthma patients [46]. ECP is presently the most widely used clinical marker of eosinophil activity in asthma. The mechanism of action of ECP is due to its cytotoxic capacity to create pores in cell membranes. It has been shown to have cytotoxic effects against bacteria, parasites, viruses and respiratory epithelial cells. ECP also stimulates mucus production in airways and histamine release by basophils and mast cells *in vitro* [47,48]. Eosinophils are also a major source of cys-LTs, particularly LTC₄ as a potent pro-inflammatory mediator, bronchoconstrictor, and inducer of glandular secretion.

Several cytokines are released by eosinophils, including some that are stimulatory to eosinophil proliferation and enhance their adhesion to endothelium, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, CXCL8, IL-10, IL-11, IL-12, GM-CSF, CCL3 and CCL5, TNF-α, TGF-β, TGF-α. Hence, they may promote eosinophil recruitment and activation through autocrine mechanisms. Eosinophils contribute to airway remodeling, possibly through the elaboration of TGF-β, and to mucus production and hyperreactivity [49,50].

Eosinophils involved in the inflammatory response produce large amounts of free radicals, such as superoxide and peroxynitrite [51]. They also express an array of cell-surface molecules, including cytokine receptors (IL-3R, IL-5R, and GM-CSFR), chemokine receptors (CCR1 and CCR3), FcγRII (CD32), FcαRI (secretory IgA), complement receptors (C3aR, CD88, and CD35), and adhesion molecules (very late antigen 4 and α4β7 integrin). Eosinophil expression of FcεRI is minimal and is of unclear functional significance [28]. Once established, the repetitive cycle of tissue damage and inflammatory cell recruitment becomes chronic. Even in the absence of sustained allergen, the chronic inflammation persists [51]. Eosinophil level in sputum is associated with the degree of chronic airway obstruction [52].

4.2. Neutrophils

Beside eosinophils, neutrophils also have an important role in the LAR. They can produce a wide range of products, including lipids (LTA₄, LTB₄, PAF, thromboxane (TX) A₂), cytokines (IL-1β, IL-6, TNF-α, TGF-β, CXCL8), proteases (elastase, collagenase, matrix metalloproteinase (MMP) 9), microbicidal products (lactoferrin, myeloperoxidase, lysozyme), reactive oxygen intermediates (superoxide, hydrogen peroxide, OH[•]) and nitric oxide (NO). Neutrophil products can cause airway narrowing, increased mucus secretion and increased ASM responsiveness [53]. Elastase in particular has an extensive range of actions. It breaks down not only elastin, but also collagen-3, promotes the formation of collagen-4, proteolyzes surfactant proteins and activates MMPs. On the epithelium, elastase enhances mucus secretion and reduces ciliary beat frequency. Elastase may also release CXCL8 and possibly other cytokines from the airway epithelium. Elastase damages the endothelium by increasing its permeability and promoting apoptosis, and it activates mast cells and eosinophils [54]. Although CXCL8 is produced by a large number of cells, the neutrophil is the main airway luminal cell containing CXCL8, suggesting autocrine secretion of CXCL8 in persistent asthma [53]. Levels of CXCL8 have been observed to be increased in non-eosinophilic asthma and correlate with neutrophil numbers. EBC and BAL concentrations of CXCL8 are higher in asthmatics compared to controls [14]. Presence of neutrophils and the neutrophil chemoattractant CXCL8 are associated with severe asthma [53,55].

4.3. T cells

T cells, especially Th2 lymphocytes, are important during the whole allergic cascade. However, a co-operative interaction

between Th2 and Th1 cytokines is involved in the pathogenesis of asthma. Th2 cells secrete cytokines, such as IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13 and GM-CSF, which exert a whole range of inflammatory effects. During the induction phase, Th2 cytokines are responsible for the production of IgE. In later stages of asthma, these cytokines are responsible for the ongoing inflammation. Th1 cells secrete cytokines, such as IFN- γ , IL-2, IL-12, IL-18, TNF- α , and TNF- β . Several studies show elevated levels of pro-inflammatory cytokines in EBC and BAL of asthmatic patients [12,14,16].

Also chemokine production is different in Th1 and Th2 lymphocytes. Th1 cells produce CCL5, and Th2 cells produce CCL1 (I-309) and CCL22 (macrophage-derived chemokine (MDC)) [56]. Certain chemokines like CCL1, CCL2, CCL11, CCL17 (thymus and activation-regulated chemokine (TARC)) and CCL22 get preferentially produced in the presence of Th2 cytokines, but also enhance and induce Th2 cytokine production by T cells [56]. CCL11, CCL17 and CCL22, which are Th2 cell-specific chemokines, further amplify the recruitment of CD4⁺ Th2 cells, thereby generating a self-sustaining pro-inflammatory loop [29,41,43,57]. In addition, Th1 cells express CCR5 and CXCR3 after activation and differentiation, whereas Th2 cells express CCR3, CCR4, CCR8 and CXCR4 [58]. Activated Th2 effector T cells express the costimulatory molecules, such as OX40 (CD134), inducible T cell costimulator (ICOS), CD28, and CD30 and the non-costimulatory molecule CD40L [59]. Regulated T cells (Treg) have the capacity to suppress Th2 responses to allergen. This process may be defective in those who develop allergic sensitization [60].

4.4. Macrophages

Macrophages play a dual role in allergic responses and inflammation in the airways. On the one hand, macrophages are recruited to the airways of allergic subjects following allergen challenge [61]. They secrete cytokines and chemokines, including IL-1, IFN- γ , TNF- α , IL-6, CCL2, CCL3, and CXCL8 [62,63], what results in the recruitment and activation of other inflammatory cells [64]. IL-6 is involved in macrophage differentiation [65]. Their ability to secrete bioactive lipids, PAFs, reactive oxygen species (ROS), NO and nitrates affects vascular smooth muscle tone and bronchial epithelial cells, and possibly bronchial smooth muscle tone [62]. On the other hand, macrophages are important immunoregulators by inducing a state of reversible T cell non-responsiveness during local antigenic stimulation, which subsequently affects the allergic response [66]. They may also inhibit allergic inflammation through the secretion of various inhibitory mediators, including PGE2 and IL-10. The secretion of these inhibitory mediators may be impaired in patients with asthma [23].

4.5. Dendritic cells

Dendritic cells are involved in both allergen presentation and the late-phase of the asthmatic cascade. Increased numbers of DCs in asthmatic airways could be an important factor in the persistence of chronic T cell-mediated allergic inflammation. Once

allergen-specific Th2 cells are drawn into the lung, repeated allergen presentation by lung DCs may drive the persistent stimulation of allergen-specific memory Th2 cells. This repeated Th2 stimulation would be expected to exacerbate acute asthma episodes and to perpetuate the state of chronic inflammation that contributes to the remodeling and AHR that characterize chronic asthma [67]. Depending on the stimulus, human DCs are able to produce CCL2, CCL3, CCL4 (MIP-1 β), CCL17, CCL22 and CXCL8 [68]. Interestingly, production of CCR4 ligands (CCL17, CCL22) by myeloid DCs suggests that these cells are capable of selective recruitment of Th2 cells and/or CD4⁺ CD25⁺ regulatory T cells at sites of inflammation [69]. In asthmatic patients, increased numbers of airway DCs expressing the high affinity IgE receptor Fc ϵ RI were observed [70].

4.6. Endothelial cells

Endothelial cells can express several adhesion molecules of the immunoglobulin superfamily, such as intercellular adhesion molecules (ICAM)-1 and -2, platelet endothelial cell adhesion molecule-1 (PECAM)-1, vascular adhesion molecule (VCAM)-1 and the selectin superfamily (E-selectin, P-selectin), which leads to the recruitment of more inflammatory cells to the site of inflammation. Corresponding ligands on leukocytes include more than a dozen integrins and L-selectin. Expression of most of the endothelial adhesion molecules is upregulated by certain cytokines, including TNF- α , IFN- γ , IL-1, IL-4 and IL-13, which are released during the acute phase, suggesting that endothelial changes enhance late-phase leukocyte infiltration of airway tissue, which is characteristic of allergic reactions [71–75]. In spite of extensive data about the existence of endothelial cell/leukocyte adhesion molecule pairs, the extent to which their expression has an impact on the course of asthma awaits confirmation. Currently, expression of VCAM-1 and ICAM-1 appears to be upregulated in persons with allergic inflammation after allergen challenge [76–78]. The affinity of VCAM-1 for very late activation antigen (VLA)-4, which is an integrin on eosinophils, basophils and lymphocytes, provides a theoretic mechanism explaining eosinophil influx in the late-phase response and an increase in basophils that might be responsible for a late-phase histamine release.

4.7. Airway smooth muscle cells

Human ASM cells are involved in the pathogenesis of asthma, because these cells contribute to AHR and airway obstruction. Several chemokines are produced by human ASM cells *in vitro* upon stimulation with cytokines (IL-4, IL-13, IL-1 β , TNF- α , TGF- β and PAF), including CCL5, CCL7, CCL11, CCL13 and CXCL8 [79–89]. For instance, a marked increase in CCL11 immunoreactivity in ASM was seen in an immunological study of the airways from patients with asthma [79,90]. These chemokines are involved in the recruitment of inflammatory cells into the lung and promote the upregulation of adhesion molecules on the cell surface. Furthermore, GM-CSF, which is an important stimulator of maturation, activation and survival of several inflammatory cells, is produced by ASM cells when

stimulated by a combination of IL-1 β and TNF- α [88,91,92]. Bradykinin can increase IL-6, CXCL8 and PGE2 release [93]. IL-6 is a pleiotropic cytokine that has both pro-inflammatory and anti-inflammatory properties in asthma [87,94–98]. Besides the release of inducible factors, ASM cells can generate extracellular matrix (ECM) proteins in patients with asthma. A selective profile of matrix proteins has the potential to cause cell proliferation and contribute to airway remodeling [99]. The regulation of ASM cell growth *in vitro* is controlled by growth-promoting (e.g. platelet-derived growth factor (PDGF), bFGF and thrombin) and growth-inhibitory (e.g. IL-4) substances [100]. IL-13 enhances proliferation and contraction of ASM [101–103]. ASM cells in culture obtained from asthmatic patients grow at twice the rate of those from non-asthmatic control individuals [90].

4.8. Epithelial cells

Bronchial epithelial cells play an active role in allergic inflammation by the secretion of pro-inflammatory mediators. CCL22 and CCL17 are strongly upregulated on airway epithelial cells, and CCL17 is increased in BAL fluid and sputum following allergen challenge in asthmatics [104,105]. CCL17 is also upregulated in the bronchial epithelium from asthmatic subjects compared with non-diseased subjects [106]. Epithelial cells express CCL11 while its receptor, CCR3, is highly expressed on eosinophils among others. Moreover, expression of both molecules is increased in asthmatics [107]. Besides chemokines, BECs from atopic asthmatics produce higher levels of cytokines, such as GM-CSF, IL-6 or CXCL8, and express more ICAM-1 (CD54), compared to non-asthmatics [108]. Free CXCL8 is present in the sera and bronchial tissue of subjects with severe atopic asthma, but not in samples from normal subjects or patients with mild atopic asthma, suggesting that CXCL8 may be a marker of severe asthma [35]. IL-17 induces the release of CXCL1 (growth-regulated oncogene (GRO- α)), CXCL6 (granulocyte chemotactic protein (GCP-2)) and CXCL8 from human BECs [109].

5. Airway remodeling

Ongoing inflammation may result in structural remodeling: wall thickening, subepithelial fibrosis, metaplasia, hypertrophy and hyperplasia of airway cells, cartilage breakdown, and angiogenesis [90,110,111]. Progress is being made in understanding the mechanisms of remodeling in which many asthma-related molecules are potentially involved. Below, the most prominent mediators of airway remodeling are summarized.

5.1. Matrix metalloproteinases

Connective tissue cells produce and secrete an array of macromolecules forming a complex network filling the extracellular space of the submucosa, called the ECM. ECM macromolecules are secreted locally and include fibrous proteins (collagen, elastin) and structural or adhesive proteins (fibronectin and laminin) embedded in a hydrated polysaccharide gel contain-

ing glycosaminoglycans. The ECM is a dynamic structure, and an equilibrium between synthesis and degradation of ECM components is required for the maintenance of its homeostasis. Although many proteases can cleave ECM molecules, Zn²⁺-dependent MMPs and their inhibitors (tissue inhibitor of metalloproteinases (TIMPs)), and the serum inhibitor α 2-macroglobulin are likely to be the normal physiologically relevant mediators of ECM degradation. They are mainly synthesized by connective tissue cells (especially fibroblasts), granulocytes, monocyte-derived macrophages, and structural cells. MMPs are thought to play a central role in development, morphogenesis, reproduction, and tissue resorption and remodeling [112]. The balance between MMPs and TIMPs is critical in tissue repair and remodeling, and its homeostasis plays an important role in the breakdown and deposition of ECM in the airway wall [113]. At least three subclasses of MMPs have been identified: collagenases, gelatinases and stromelysins. Especially MMP-3 (stromelysin-1), MMP-8 (interstitial collagenase), MMP-2 (72 kDa gelatinase) and MMP-9 (92 kDa gelatinase) are related to the pathogenesis of tissue remodeling and airway obstruction [113–115]. MMP-8 may initiate the cleavage of the triple helix of native collagen types I, II, III, VII and X, and MMP-9 may cleave native types IV and V collagens, and to a lesser extent fibronectin, laminin, entactin, plasminogen and insoluble elastin. Thus, MMP-9 has specific affinity for the subepithelial basal lamina, a specialized nonfibrillar connective tissue structure consisting of collagen type IV, laminin, entactin and fibronectin, that anchors epithelial cells to parenchymal surfaces. Inflammatory cells involved in asthma, such as macrophages, lymphocytes, mast cells, BECs and eosinophils, are able to express MMP-9. MMP-8 was previously considered to be derived only from degranulating neutrophils at the site of inflammation, but recent studies have provided evidence that certain non-polymorphonuclear lineage cells, such as human BECs, chondrocytes, synovial fibroblast, endothelial cells, and plasma cells can also express MMP-8 protein and mRNA. MMP-8 also proteolytically inactivates serine proteinase inhibitors α 1-antitrypsin and α 1-antichymotrypsin, and a general proteinase inhibitor α 2-macroglobulin [114–116]. MMPs are implicated in airway remodeling not only by matrix reorganization (ECM degradation), but also by alteration of angiogenesis [99] and smooth muscle hyperplasia processes [117]. Furthermore, they are involved in airway inflammation through their influence on inflammatory cell migration through the ECM, basement membrane and endothelial layer [116,118]. MMP-9 levels are significantly higher in the sputum and BAL fluid of asthmatic patients compared with control subjects. Levels of TIMP-1 are initially similar to those found in healthy control subjects, but increase gradually with ongoing disease [113,114,116,118–122]. One of the major cell types implicated in the production of TIMP is the alveolar macrophage, which produces more TIMP-1 both at protein and mRNA levels in asthmatic patients than alveolar macrophages from either non-asthmatic or steroid-treated asthmatic subjects [48,123]. TIMP-1 inhibits MMPs such as MMP-1, MMP-2, MMP-3 and MMP-9, all of which can degrade the ECM. TIMP-1 is synthesized and secreted by many connective tissue cells that also produce MMPs, including fibroblasts,

inflammatory cells, and endothelial cells [118,123]. Inflammatory mediators, cytokines and growth factors can modulate the production of both TIMPs and MMPs. TIMP-1 expression is upregulated by IL-6, whereas MMPs are modulated by IL-1 β and TNF- α , and increased levels of these cytokines have been detected in BAL fluid of asthmatic individuals after allergen challenge [123]. IL-10 downregulates macrophage expression of MMPs, including MMP-1 and MMP-9, while upregulating tissue inhibitors of metalloproteinases, specifically TIMP-1 [124,125]. IL-11 also induces the synthesis of TIMP-1 [35].

5.2. Cytokines

Tumor growth factor (TGF- β) is the most potent and widely studied profibrotic cytokine, because it is detected in exaggerated quantities in asthmatic BAL fluid and EBC before and after antigen challenge [14,126]. Moreover, TGF- β correlates with fibroblast number and disease severity [127–129]. TGF- β can be generated by many cells, such as macrophages, epithelial cells, fibroblasts, platelets, and eosinophils, and stimulates fibroblasts to produce and secrete many proteins of the ECM, like collagens I, II, IV and V, fibronectin, tenascin, elastin and biglycan. Furthermore, TGF- β downregulates the synthesis of MMPs and gene expression of decorin, a proteoglycan that regulates TGF- β activity, while upregulating the synthesis of MMPs and the synthesis of TIMPs [104,127,129–131]. In addition, TGF- β is an immunomodulatory cytokine and a potent chemoattractant for several cell types, including monocytes, fibroblasts, and mast cells. Due to its action in promoting growth and repair, TGF- β is thought to play an important role at sites of wound healing and tissue remodeling [104,131].

Besides TGF- β , other cytokines play an important role in the remodeling process. In *in vitro* and animal models, IL-4 and IL-13 have shown similar fibrotic properties mediated by the stimulation of TGF- β production by airway epithelial cells and lung macrophages [122,132–135]. Activation of lung fibroblasts is associated with an increased deposition of collagen and fibronectin, causing the thickening of the basal membrane and the appearance of diffuse subepithelial fibrosis [132]. IL-4 and IL-13, together with IL-5 and IL-9, also lead to goblet cell metaplasia in animal models [136] and IL-9 induces mucus gene expression in airway epithelial cells [137]. IL-11 is produced by various cell types, such as fibroblasts, osteoblasts, stromal, endothelial and epithelial cells and is found to be increasingly expressed in the airways of patients with severe asthma but not in those of mild asthmatic patients or healthy control subjects. Overexpression of IL-11 within the lungs results in subepithelial fibrosis and promotes the accumulation of myocytes and myofibroblasts [111,138]. IL-17 is produced by T cells and eosinophils, and induces bronchial fibroblasts to increase their expression of several mediators, such as IL-6, GM-CSF, CXCL8, PGE₂, which might contribute to the amplification, regulation, and/or perpetuation of local airway inflammation and remodeling [139]. A recently discovered cytokine, IL-25, triggers epithelial cell hyperplasia, cytokine production and AHR, increases mucus secretion, promotes the expression of Th2 genes such as IL-4, IL-5, IL-13 and CCL11, and produces

lung eosinophilia in experimental animal models of asthma following intranasal administration [140]. Various mediators, such as histamine, mast cell tryptase, fibronectin, TGF- β , IL-13, PDGF, bFGF, epidermal growth factor (EGF) and insulin-like growth factor (IGF)-1 and -3 have been implicated in smooth muscle cell proliferation [117,135,141]. GM-CSF and EGF are also found in exaggerated quantities in airway biopsies from patients with asthma, and the overexpression of GM-CSF causes pulmonary inflammation with eosinophilia and fibrosis. In limited investigations, however, neither GM-CSF, nor EGF has been demonstrated to correlate with parameters of airway fibrosis, raising the possibility that these mediators are involved in acute, but not chronic airway responses [129].

5.3. Chemokines

Several CC-chemokines responsible for initiating and maintaining airway allergic inflammation in asthma may also contribute to the pathogenesis of airway remodeling [142,143]. Among them, CCL2 is increased in asthma [144] and is a well-established profibrogenic mediator *in vitro* and *in vivo* by inducing TGF- β release and collagen deposition from lung fibroblasts [145,146], and by recruiting Th2 cells in the lung [147]. Other CC-chemokines, such as CCL7 and CCL22 also contribute to the development of pulmonary fibrosis [148,149].

5.4. Endothelin-1

Endothelin (ET)-1 may be involved in airway remodeling: it is mitogenic for ASM cells [150] and fibroblasts [151], and also stimulates collagen synthesis by the latter cells. There are a number of potential sources of ET-1 within the airways, including BECs, vascular endothelial cells, macrophages and mast cells [151]. Increased levels of ET-1 were found in BAL fluid from asthmatics [126] and immunoreactivity for ET-1 is raised in the epithelium of asthmatic subjects [152].

5.5. Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) may contribute to the pathophysiology of asthma in several ways. First, there is increasing evidence to support a role for VEGF, a major regulator of angiogenesis and enhancer of vascular permeability [153], as a mediator of structural changes in the airway wall of asthmatics. Greater expression of VEGF, angiogenin and bFGF leading to angiogenesis [154], may explain the increased airway vascularity observed in asthma [155,156]. *In vitro* studies suggest that VEGF expression can induce fibrosis associated with neovascularization [157]. Second, the ability to induce vascular permeability can induce extravasation of plasma proteins and subsequent tissue edema. VEGF may also indirectly recruit inflammatory leukocytes by inducing expression of CXCL8 and ICAM-1, and by enhancing transendothelial migration. Finally, other biological effects of VEGF are likely to be important in the pathological changes associated with airway remodeling, such as the ability to induce expression of MMPs in both endothelial cells and vascular ASM cells, and the ability

to induce expression of connective tissue growth factor and collagen [141]. Macrophages, eosinophils and CD34⁺ cells are the major source of VEGF [154], although *in vitro* studies suggest that lung fibroblasts derived from subjects with asthma also secrete VEGF in response to TGF- β . The expression of VEGF is upregulated in the bronchial mucosa of mild to moderate asthmatic patients, compared with that of control subjects, and is related to the number of vessels and mast cells, as well as to the basement membrane thickness [141].

5.6. Lipid mediators

5.6.1. Prostaglandin D₂

Prostaglandins appear to have several effects on the airways, including bronchoconstriction, plasma exudation, sensitization of nerve endings, and effects on inflammatory cells [35]. PGD₂ elicits biological responses by activating the D-prostanoid receptor DP and the CRTH2 receptor, which are linked to different signaling pathways. PGD₂ transmits pro-inflammatory stimuli to various immune cells by its specific receptor CRTH2 on T cells, basophils, and eosinophils. The role of DP is complex and depends of the type of inflammation and the nature of the target cell [158]. PGD₂ is a key mediator in inflammatory diseases in which it is likely to have a dual role, both promoting and inhibiting inflammatory processes depending on the animal species, the organ and the inflammatory stimulus [158]. Recently, evidence for a pro-inflammatory role of PGD₂ in the airways has been provided: overproduction of PGD₂ in mice results in enhanced pulmonary inflammation following allergen challenge [159], and inhalation of aerosolized PGD₂ enhances eosinophilic and lymphocytic airway inflammation in mice upon allergen exposure [160]. Furthermore, PGD₂ is involved in the recruitment of inflammatory cells because it stimulates the chemotaxis of Th2 cells, eosinophils, neutrophils and basophils *in vitro* and *in vivo* [161–165]. Also, the observation that PGD₂ enhances LTC₄ synthesis by eosinophils during allergic inflammation *in vivo* underscores a pro-inflammatory role of PGD₂ [166]. PGD₂ and PGF₂ α cause bronchoconstriction in asthmatic patients, but not in healthy subjects [35]. Higher levels of PGD₂ have been detected in asthmatic patients in BAL fluid upon allergen challenge [158].

5.6.2. Prostaglandin E₂

Prostaglandin E₂ is also an important PG produced in inflammatory processes [167]. It is the most important bronchoprotective metabolite yet identified in the airways [22]. The effects of PGE₂ on target cells may be complex, depending on the numbers and activity of their individual E-prostanoid receptors [167]. Inhaled PGE₂ causes bronchodilation in normal subjects [168], but may cause constriction in patients with asthma because of activation of reflex cholinergic bronchoconstriction [169,170]. Another study shows that inhalation of PGE₂ ameliorates the abnormal airway physiology and inflammation that characterize asthma [167]. PGE₂ inhibits the release of mediators from mast cells, monocytes, neutrophils and eosinophils [171,172]. PGE₂ and PGI₂ are vasodilators and therefore should theoretically increase leakage in asthmatic

airways [35]. PGE₂ was measured in EBC [173,174], but no difference was found between asthmatic patients and controls.

5.6.3. 8-Isoprostane

Isoprostanes are inflammatory metabolites derived from arachidonic acid. They are specific for lipid peroxidation and are related to oxidative stress [175]. 8-Isoprostane has important biological effects on airway and pulmonary vascular smooth muscles, epithelium, endothelium and sensory nerves. It plays a role in non-specific smooth muscle hyperresponsiveness, bronchoconstriction and edema [176]. The concentration of 8-isoprostane is increased in EBC of patients with asthma [174,175].

5.6.4. Cysteinyl leukotrienes

There is substantial evidence that cys-LTs (LTC₄, LTD₄ and LTE₄) play an important role in asthma. Cys-LTs mediate several steps in airway inflammation, including inflammatory cell recruitment, vascular leakage and possibly also airway remodeling. They decrease mucociliary clearance and are potent mediators of bronchoconstriction, plasma exudation and mucus secretion. There is a growing body of evidence that they may also increase eosinophilic inflammation. Cys-LTs are very potent contractile agents for human bronchi *in vitro*, being approximately a thousand times more potent than histamine [21,35]. The ability of cys-LTs to act as potent bronchoconstricting agents has also been demonstrated *in vivo*, both in normal subjects and in patients with asthma [172]. Inhaled LTD₄ also increases the maximal airway narrowing [177], and LTE₄ induces AHR to inhaled histamine, an effect that may persist for several days [178,179]. LTC₄ increases collagenase expression by lung fibroblasts [111]. Cys-LTs potentially elicit increased vascular permeability in airways, leading to airway edema [21,180]. They increase mucus secretion both directly via effects on goblet cells and submucosal gland cells [172], and indirectly via the activation of airway nerves, leading to reflex secretion from submucosal glands [172]. Asthmatic patients have higher cys-LTs concentrations in their EBC than healthy subjects [173,174,176,181].

5.6.5. Leukotriene B₄

Leukotriene B₄ is a potent neutrophil chemoattractant that enhances neutrophil-endothelial interactions and stimulates neutrophil activation. This leads to degranulation and the release of mediators, enzymes and superoxides. It has weak effects on smooth muscle, but may contribute to airway narrowing by producing local edema and by increasing mucus secretion [182,183]. LTB₄ levels are higher in asthma compared to controls [173,181,184,185].

5.7. ADAM33

Besides the pathologic and physiologic aspects of asthma, the remodeling process may potentially be influenced by genetic determinants. Genome screening has led to identification of genes or cluster of genes relevant to asthma and atopy [186]. In an expression microarray study using tissue obtained from

bronchial biopsies of healthy control subjects and subjects with allergic asthma, 79 genes were found to show significant differences in expression in asthmatic compared to control subjects [187]. A disintegrin and metalloproteinase (ADAM) 33 has been a focus of interest in the last few years. ADAM33 has been linked to asthma in a study of 460 white families [188]. Abnormal activity of this gene can lead to altered airway function, inflammation, and remodeling. Alterations in ADAM33 activity may underlie abnormalities in the function of ASM cells and fibroblasts linked to airway remodeling and AHR [189].

6. Conclusion

All processes that occur in the asthmatic lung are mediated by a broad network of various molecules. In this review, mediators secreted by the different cell types that play a role in the allergic cascade are discussed. Moreover, the effects of the most important mediators of airway remodeling are summarized. However, further research is still needed for a better understanding of the complex interactions between molecules and cells involved in the processes leading to asthma.

Acknowledgements

K. Bloemen and S. Verstraelen received a PhD-grant from VITO.

References

- [1] Ronchetti R, Villa MP, Barreto M, Rota R, Pagani J, Martella S, et al. Is the increase in childhood asthma coming to an end? Findings from three surveys of schoolchildren in Rome, Italy. *Eur Respir J* 2001;17(5):881–6.
- [2] Bharadwaj A, Agrawal DK. Immunomodulation in asthma: a distant dream or a close reality? *Int Immunopharmacol* 2004;4(4):495–511.
- [3] Holt PG. Antigen presentation in the lung. *Am J Respir Crit Care Med* 2000;162(4 Pt 2):S151–6.
- [4] Corry DB, Kheradmand F. Induction and regulation of the IgE response. *Nature* 1999;402(6760 Suppl):B18–23.
- [5] Turner H, Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature* 1999;402(6760 Suppl):B24–30.
- [6] Bradding P, Holgate ST. Immunopathology and human mast cell cytokines. *Crit Rev Oncol Hematol* 1999;31(2):119–33.
- [7] Baraniuk JN. Pathogenesis of allergic rhinitis. *J Allergy Clin Immunol* 1997;99(2):S763–72.
- [8] Elias JA. Airway remodeling in asthma. Unanswered questions. *Am J Respir Crit Care Med* 2000;161(3 Pt 2):S168–71.
- [9] Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. *J Clin Invest* 1999;104(8):1001–6.
- [10] Bochner BS, Busse WW. Advances in mechanisms of allergy. *J Allergy Clin Immunol* 2004;113(5):868.
- [11] Kay AB. Immunomodulation in asthma: mechanisms and possible pitfalls. *Curr Opin Pharmacol* 2003;3(3):220.
- [12] Shahid SK, Kharitonov SA, Wilson NM, Bush A, Barnes PJ. Increased interleukin-4 and decreased interferon-gamma in exhaled breath condensate of children with asthma. *Am J Respir Crit Care Med* 2002;165(9):1290–3.
- [13] Leung T-F, Wong GWK, Ko FWS, Li C-Y, Yung E, Lam CWK, et al. Analysis of growth factors and inflammatory cytokines in exhaled breath condensate from asthmatic children. *Int Arch Allergy Immunol* 2005;137(1):66.
- [14] Matsunaga K, Yanagisawa S, Ichikawa T, Ueshima K, Akamatsu K, Hirano T, et al. Airway cytokine expression measured by means of protein array in exhaled breath condensate: correlation with physiologic properties in asthmatic patients. *J Allergy Clin Immunol* 2006;118(1):84.
- [15] Quaedvlieg V, Henket M, Sele J, Louis R. Cytokine production from sputum cells in eosinophilic versus non-eosinophilic asthmatics. *Clin Exp Immunol* 2006;143(1):161–6.
- [16] Kim CK, Kim SW, Park CS, Kim BI, Kang H, Koh YY. Bronchoalveolar lavage cytokine profiles in acute asthma and acute bronchiolitis. *J Allergy Clin Immunol* 2003;112(1):64.
- [17] Shimbara A, Christodoulou P, Soussi-Gounni A, Olivenstein R, Nakamura Y, Levitt RC, et al. IL-9 and its receptor in allergic and nonallergic lung disease: increased expression in asthma. *J Allergy Clin Immunol* 2000;105(1 Part 1):108.
- [18] Venkatesha RT, Berla Thangam E, Zaidi AK, Ali H. Distinct regulation of C3a-induced MCP-1/CCL2 and RANTES/CCL5 production in human mast cells by extracellular signal regulated kinase and PI3 kinase. *Mol Immunol* 2005;42(5):581–7.
- [19] Hart PH. Regulation of the inflammatory response in asthma by mast cell products. *Immunol Cell Biol* 2001;79(2):149–53.
- [20] Holsapple MP, Jones D, Kawabata TT, Kimber I, Sarlo K, Selgrade MK, et al. Assessing the potential to induce respiratory hypersensitivity. *Toxicol Sci* 2006;91(1):4–13.
- [21] Galli SJ, Metcalfe DD, Dvorak AM. Basophils and mast cells and their disorders. In: Beutler E, Lichtman MA, Collier BS, Kipps TJ, Seligsohn U, editors. *Williams Haematology*, vol. 15. New York: McGraw-Hill; 2001. p. 801–15.
- [22] Johnson SR, Knox AJ. Synthetic functions of airway smooth muscle in asthma. *Trends Pharmacol Sci* 1997;18(8):288–92.
- [23] Barnes PJ. The role of inflammation and anti-inflammatory medication in asthma. *Respir Med* 2002;96(Suppl A):S9–15.
- [24] Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000;161(5):1720–45.
- [25] Pearlman DS. Pathophysiology of the inflammatory response. *J Allergy Clin Immunol* 1999;104(4 Pt 1):S132–7.
- [26] Nakae S, Ho LH, Yu M, Monteforte R, Iikura M, Suto H, et al. Mast cell-derived TNF contributes to airway hyperreactivity, inflammation, and T(H)2 cytokine production in an asthma model in mice. *J Allergy Clin Immunol* 2007;120(1):48–55.
- [27] Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006;117(6):1277.
- [28] Prussin C, Metcalfe DD. 5. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2006;117(2 Suppl Mini-Primer):S450–6.
- [29] Teran LM. CCL chemokines and asthma. *Immunol Today* 2000;21(5):235–42.
- [30] Holgate ST, Bodey KS, Janezic A, Frew AJ, Kaplan AP, Teran LM. Release of RANTES, MIP-1alpha, and MCP-1 into asthmatic airways following endobronchial allergen challenge. *Am J Respir Crit Care Med* 1997;156(5):1377–83.
- [31] Cruikshank WW, Long A, Tarpy RE, Kornfeld H, Carroll MP, Teran L, et al. Early identification of interleukin-16 (lymphocyte chemoattractant factor) and macrophage inflammatory protein 1 alpha (MIP1 alpha) in bronchoalveolar lavage fluid of antigen-challenged asthmatics. *Am J Respir Cell Mol Biol* 1995;13(6):738–47.
- [32] Alam R. Chemokines in allergic inflammation. *J Allergy Clin Immunol* 1997;99(3):273–7.
- [33] Forssmann U, Ugucioni M, Loetscher P, Dahinden CA, Langen H, Thelen M, et al. Eotaxin-2, a novel CC chemokine that is selective for the chemokine receptor CCR3, and acts like eotaxin on human eosinophil and basophil leukocytes. *J Exp Med* 1997;185(12):2171–6.
- [34] Dahinden CA, Kurimoto Y, De Weck AL, Lindley I, Dewald B, Baggiolini M. The neutrophil-activating peptide NAF/NAP-1 induces histamine and leukotriene release by interleukin 3-primed basophils. *J Exp Med* 1989;170(5):1787–92.
- [35] Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. *Pharmacol Rev* 1998;50(4):515–96.
- [36] Bischoff SC, Baggiolini M, de Weck AL, Dahinden CA. Interleukin 8-inhibitor and inducer of histamine and leukotriene release in human basophils. *Biochem Biophys Res Commun* 1991;179(1):628.

- [37] Schroeder JT, MacGlashan Jr DW, Lichtenstein LM. Human basophils: mediator release and cytokine production. *Adv Immunol* 2001;77:93–122.
- [38] Marone G, Triggiani M, de Paulis A. Mast cells and basophils: friends as well as foes in bronchial asthma? *Trends Immunol* 2005;26(1):25–31.
- [39] Devouassoux G, Foster B, Scott LM, Metcalfe DD, Prussin C. Frequency and characterization of antigen-specific IL-4- and IL-13-producing basophils and T cells in peripheral blood of healthy and asthmatic subjects. *J Allergy Clin Immunol* 1999;104(4 Pt 1):811–9.
- [40] Bochner BS, Schleimer RP. Mast cells, basophils, and eosinophils: distinct but overlapping pathways for recruitment. *Immunol Rev* 2001;179:5–15.
- [41] Nickel R, Beck LA, Stellato C, Schleimer RP. Chemokines and allergic disease. *J Allergy Clin Immunol* 1999;104(4 Pt 1):723–42.
- [42] Zimmermann N, Hershey GK, Foster PS, Rothenberg ME. Chemokines in asthma: cooperative interaction between chemokines and IL-13. *J Allergy Clin Immunol* 2003;111(2):227.
- [43] Lukacs NW. Role of chemokines in the pathogenesis of asthma. *Nat Rev Immunol* 2001;1(2):108–16.
- [44] Humbert M, Ying S, Corrigan C, Menz G, Barkans J, Pfister R, et al. Bronchial mucosal expression of the genes encoding chemokines RANTES and MCP-3 in symptomatic atopic and nonatopic asthmatics: relationship to the eosinophil-active cytokines interleukin (IL)-5, granulocyte macrophage-colony-stimulating factor, and IL-3. *Am J Respir Cell Mol Biol* 1997;16(1):1–8.
- [45] Kay AB, Klion AD. Anti-interleukin-5 therapy for asthma and hypereosinophilic syndrome. *Immunol Allergy Clin North Am* 2004;24(4):645–66, vii.
- [46] Zimmerman B, Silverman FS, Tarlo SM, Chapman KR, Kubay JM, Urch B. Induced sputum: comparison of postinfectious cough with allergic asthma in children. *J Allergy Clin Immunol* 2000;105(3):495.
- [47] Koh GCH, Shek LPC, Goh DYT, Van Bever H, Koh DSQ. Eosinophil cationic protein: is it useful in asthma? A systematic review. *Respir Med* 2007;101(4):696–705.
- [48] Hamid QA, Minshall EM. Molecular pathology of allergic disease I: lower airway disease. *J Allergy Clin Immunol* 2000;105(1 Part 1):20.
- [49] Lee JJ, Dimina D, Macias MP, Ochkur SI, McGarry MP, O'Neill KR, et al. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004;305(5691):1773–6.
- [50] Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, et al. A critical role for eosinophils in allergic airways remodeling. *Science* 2004;305(5691):1776–9.
- [51] Maddox L, Schwartz DA. The pathophysiology of asthma. *Annu Rev Med* 2002;53:477–98.
- [52] Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA, et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. *J Allergy Clin Immunol* 2001;108(5):753–8.
- [53] Gibson PG, Simpson JL, Salto N. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. *Chest* 2001;119(5):1329–36.
- [54] Sampson AP. The role of eosinophils and neutrophils in inflammation. *Clin Exp Allergy* 2000;30(Suppl 1):22–7.
- [55] Shute JK, Vrugt B, Lindley IJ, Holgate ST, Bron A, Aalbers R, et al. Free and complexed interleukin-8 in blood and bronchial mucosa in asthma. *Am J Respir Crit Care Med* 1997;155(6):1877–83.
- [56] Smit JJ, Lukacs NW. A closer look at chemokines and their role in asthmatic responses. *Eur J Pharmacol* 2006;533:277–88.
- [57] Luster AD. The role of chemokines in linking innate and adaptive immunity. *Curr Opin Immunol* 2002;14(1):129.
- [58] Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokine grammar for immune cells. *Annu Rev Immunol* 2004;22:891–928.
- [59] Krocze R, Hamelmann E. T-cell costimulatory molecules: optimal targets for the treatment of allergic airway disease with monoclonal antibodies. *J Allergy Clin Immunol* 2005;116(4):906–9.
- [60] Robinson DS, Larche M, Durham SR. Tregs and allergic disease. *J Clin Invest* 2004;114(10):1389–97.
- [61] Chanez P, Bousquet J, Couret I, Cornillac L, Barneon G, Vic P, et al. Increased numbers of hypodense alveolar macrophages in patients with bronchial asthma. *Am Rev Respir Dis* 1991;144(4):923–30.
- [62] Rosenwasser LJ. New immunopharmacologic approaches to asthma: role of cytokine antagonism. *J Allergy Clin Immunol* 2000;105(2 Pt 2):S586–92.
- [63] Gosset P, Tillie-Leblond I, Oudin S, Parmentier O, Wallaert B, Joseph M, et al. Production of chemokines and proinflammatory and antiinflammatory cytokines by human alveolar macrophages activated by IgE receptors. *J Allergy Clin Immunol* 1999;103(2 Pt 1):289–97.
- [64] Vissers JLM, van Esch BCAM, Hofman GA, van Oosterhout AJM. Macrophages induce an allergen-specific and long-term suppression in a mouse asthma model. *Eur Respir J* 2005;26(6):1040–6.
- [65] Diehl S, Rincon M. The two faces of IL-6 on Th1/Th2 differentiation. *Mol Immunol* 2002;39(9):531.
- [66] Thepen T, Van Rooijen N, Kraal G. Alveolar macrophage elimination in vivo is associated with an increase in pulmonary immune response in mice. *J Exp Med* 1989;170(2):499–509.
- [67] Lipscomb MF, Masten BJPR. Dendritic cells: immune regulators in health and disease. *J Physiol Rev* 2002;82(1):97–130.
- [68] Penna G, Vulcano M, Sozzani S, Adorini L. Differential migration behavior and chemokine production by myeloid and plasmacytoid dendritic cells. *Hum Immunol* 2002;63(12):1164–71.
- [69] Hammad H, Smits HH, Ratajczak C, Nithiananthan A, Wierenga EA, Stewart GA, et al. Monocyte-derived dendritic cells exposed to Der p 1 allergen enhance the recruitment of Th2 cells: major involvement of the chemokines TARC/CCL17 and MDC/CCL22. *Eur Cytokine Netw* 2003;14(4):219–28.
- [70] Tunon-De-Lara JM, Redington AE, Bradding P, Church MK, Hartley JA, Semper AE, et al. Dendritic cells in normal and asthmatic airways: expression of the alpha subunit of the high affinity immunoglobulin E receptor (Fc epsilon RI-alpha). *Clin Exp Allergy* 1996;26(6):648–55.
- [71] Pober JS, Lapierre LA, Stolpen AH, Brock TA, Springer TA, Fiers W, et al. Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and interleukin 1 species. *J Immunol* 1987;138(10):3319–24.
- [72] Godding V, Stark JM, Sedgwick JB, Busse WW. Adhesion of activated eosinophils to respiratory epithelial cells is enhanced by tumor necrosis factor-alpha and interleukin-1 beta. *Am J Respir Cell Mol Biol* 1995;13(5):555–62.
- [73] Pober JS, Gimbrone Jr MA, Lapierre LA, Mendrick DL, Fiers W, Rothlein R, et al. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J Immunol* 1986;137(6):1893–6.
- [74] Moser R, Fehr J, Bruijnzel PL. IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. *J Immunol* 1992;149(4):1432–8.
- [75] Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol* 1995;154(2):799–803.
- [76] Manolitsas ND, Trigg CJ, McAulay AE, Wang JH, Jordan SE, D'Ardenne AJ, et al. The expression of intercellular adhesion molecule-1 and the beta 1-integrins in asthma. *Eur Respir J* 1994;7(8):1439–44.
- [77] Lee BJ, Naclerio RM, Bochner BS, Taylor RM, Lim MC, Baroody FM. Nasal challenge with allergen upregulates the local expression of vascular endothelial adhesion molecules. *J Allergy Clin Immunol* 1994;94(6 Pt 1):1006–16.
- [78] Montefort S, Feather IH, Wilson SJ, Haskard DO, Lee TH, Holgate ST, et al. The expression of leukocyte-endothelial adhesion molecules is increased in perennial allergic rhinitis. *Am J Respir Cell Mol Biol* 1992;7(4):393–8.
- [79] Ghaffar O, Hamid Q, Renzi PM, Allakhverdi Z, Molet S, Hogg JC, et al. Constitutive and cytokine-stimulated expression of eotaxin by human airway smooth muscle cells. *Am J Respir Crit Care Med* 1999;159(6):1933–42.
- [80] Chung KF, Patel HJ, Fadlon EJ, Rousell J, Haddad EB, Jose PJ, et al. Induction of eotaxin expression and release from human airway smooth

- muscle cells by IL-1beta and TNFalpha: effects of IL-10 and corticosteroids. *Br J Pharmacol* 1999;127(5):1145–50.
- [81] Zuyderduyn S, Hiemstra PS, Rabe KF. TGF-beta differentially regulates TH2 cytokine-induced eotaxin and eotaxin-3 release by human airway smooth muscle cells. *J Allergy Clin Immunol* 2004;114(4):791–8.
- [82] Moore PE, Church TL, Chism DD, Panettieri Jr RA, Shore SA. IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK. *Am J Physiol Lung Cell Mol Physiol* 2002;282(4):L847–53.
- [83] Pang L, Knox AJ. Regulation of TNF-alpha-induced eotaxin release from cultured human airway smooth muscle cells by beta2-agonists and corticosteroids. *FASEB J* 2001;15(1):261–9.
- [84] Lazzeri N, Belvisi MG, Patel HJ, Chung KF, Yacoub MH, Mitchell JA. RANTES release by human airway smooth muscle: effects of prostaglandin E(2) and fenoterol. *Eur J Pharmacol* 2001;433(2/3):231–5.
- [85] Watson ML, Grix SP, Jordan NJ, Place GA, Dodd S, Leithead J, et al. Interleukin 8 and monocyte chemoattractant protein 1 production by cultured human airway smooth muscle cells. *Cytokine* 1998;10(5):346–52.
- [86] John M, Au BT, Jose PJ, Lim S, Saunders M, Barnes PJ, et al. Expression and release of interleukin-8 by human airway smooth muscle cells: inhibition by Th-2 cytokines and corticosteroids. *Am J Respir Cell Mol Biol* 1998;18(1):84–90.
- [87] Ammit AJ, Hoffman RK, Amrani Y, Lazaar AL, Hay DW, Torphy TJ, et al. Tumor necrosis factor-alpha-induced secretion of RANTES and interleukin-6 from human airway smooth-muscle cells. Modulation by cyclic adenosine monophosphate. *Am J Respir Cell Mol Biol* 2000;23(6):794–802.
- [88] Oltmanns U, Issa R, Sukkar MB, John M, Chung KF. Role of c-jun N-terminal kinase in the induced release of GM-CSF, RANTES and IL-8 from human airway smooth muscle cells. *Br J Pharmacol* 2003;139(6):1228–34.
- [89] Maruoka S, Hashimoto S, Gon Y, Takeshita I, Horie T. PAF-induced RANTES production by human airway smooth muscle cells requires both p38 MAP kinase and Erk. *Am J Respir Crit Care Med* 2000;161(3 Pt 1):922–9.
- [90] Black JL, Johnson PR. Factors controlling smooth muscle proliferation and airway remodelling. *Curr Opin Allergy Clin Immunol* 2002;2(1):47–51.
- [91] Saunders MA, Mitchell JA, Seldon PM, Yacoub MH, Barnes PJ, Gienbycz MA, et al. Release of granulocyte-macrophage colony stimulating factor by human cultured airway smooth muscle cells: suppression by dexamethasone. *Br J Pharmacol* 1997;120(4):545–6.
- [92] Song R, Ning W, Liu F, Ameredes BT, Calhoun WJ, Otterbein LE, et al. Regulation of IL-1beta-induced GM-CSF production in human airway smooth muscle cells by carbon monoxide. *Am J Physiol Lung Cell Mol Physiol* 2003;284(1):L50–6.
- [93] Pang L, Knox AJ. Bradykinin stimulates IL-8 production in cultured human airway smooth muscle cells: role of cyclooxygenase products. *J Immunol* 1998;161(5):2509–15.
- [94] Huang CD, Tliba O, Panettieri Jr RA, Amrani Y. Bradykinin induces interleukin-6 production in human airway smooth muscle cells: modulation by Th2 cytokines and dexamethasone. *Am J Respir Cell Mol Biol* 2003;28(3):330–8.
- [95] Elias JA, Wu Y, Zheng T, Panettieri R. Cytokine- and virus-stimulated airway smooth muscle cells produce IL-11 and other IL-6-type cytokines. *Am J Physiol* 1997;273(3 Pt 1):L648–55.
- [96] Ammit AJ, Lazaar AL, Irani C, O'Neill GM, Gordon ND, Amrani Y, et al. Tumor necrosis factor-alpha-induced secretion of RANTES and interleukin-6 from human airway smooth muscle cells: modulation by glucocorticoids and beta-agonists. *Am J Respir Cell Mol Biol* 2002;26(4):465–74.
- [97] Amrani Y, Ammit AJ, Panettieri Jr RA. Tumor necrosis factor receptor (TNFR)1, but not TNFR2, mediates tumor necrosis factor-alpha-induced interleukin-6 and RANTES in human airway smooth muscle cells: role of p38 and p42/44 mitogen-activated protein kinases. *Mol Pharmacol* 2001;60(4):646–55.
- [98] McKay S, Bromhaar MM, de Jongste JC, Hoogsteden HC, Saxena PR, Sharma HS. Pro-inflammatory cytokines induce c-fos expression followed by IL-6 release in human airway smooth muscle cells. *Mediators Inflamm* 2001;10(3):135–42.
- [99] Johnson C, Galis ZS. Matrix metalloproteinase-2 and -9 differentially regulate smooth muscle cell migration and cell-mediated collagen organization. *Arterioscler Thromb Vasc Biol* 2004;24(1):54–60.
- [100] Hawker KM, Johnson PRA, Hughes JM, Black JL. Interleukin-4 inhibits mitogen-induced proliferation of human airway smooth muscle cells in culture. *Am J Physiol Lung Cell Mol Physiol* 1998;275(3):L469–77.
- [101] Wills-Karp M. IL-12/IL-13 axis in allergic asthma. *J Allergy Clin Immunol* 2001;107(1):9–18.
- [102] Grunstein MM, Hakonarson H, Leiter J, Chen M, Whelan R, Grunstein JS, et al. IL-13-dependent autocrine signaling mediates altered responsiveness of IgE-sensitized airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002;282(3):L520–8.
- [103] Tliba O, Deshpande D, Chen H, Van Besien C, Kannan M, Panettieri Jr RA, et al. IL-13 enhances agonist-evoked calcium signals and contractile responses in airway smooth muscle. *Br J Pharmacol* 2003;140(7):1159–62.
- [104] Howarth PH, Knox AJ, Amrani Y, Tliba O, Panettieri Jr RA, Johnson M. Synthetic responses in airway smooth muscle. *J Allergy Clin Immunol* 2004;114(2 Suppl):S32–50.
- [105] Panina-Bordignon P, Papi A, Mariani M, Di Lucia P, Casoni G, Bellettato C, et al. The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001;107(11):1357–64.
- [106] Sekiya T, Miyamasu M, Imanishi M, Yamada H, Nakajima T, Yamaguchi M, et al. Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells. *J Immunol* 2000;165(4):2205–13.
- [107] Ying S, Robinson DS, Meng Q, Rottman J, Kennedy R, Ringler DJ, et al. Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur J Immunol* 1997;27(12):3507–16.
- [108] Marini M, Vittori E, Hollemborg J, Mattoli S. Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 1992;89(5):1001–9.
- [109] Prause O, Laan M, Lotvall J, Linden A. Pharmacological modulation of interleukin-17-induced GCP-2-, GRO-alpha- and interleukin-8 release in human bronchial epithelial cells. *Eur J Pharmacol* 2003;462(1–3):193–8.
- [110] Jenkins HA, Cool C, Szeffler SJ, Covar R, Brugman S, Gelfand EW, et al. Histopathology of severe childhood asthma: a case series. *Chest* 2003;124(1):32–41.
- [111] Bergeron C, Boulet LP. Structural changes in airway diseases: characteristics, mechanisms, consequences, and pharmacologic modulation. *Chest* 2006;129(4):1068–87.
- [112] Nagase H, Woessner Jr JF. Matrix metalloproteinases. *J Biol Chem* 1999;274(31):21491–4.
- [113] Vignola AM, Riccobono L, Mirabella A, Profita M, Chanez P, Bellia V, et al. Sputum metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio correlates with airflow obstruction in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1998;158(6):1945–50.
- [114] Lemjabbar H, Gosset P, Lamblin C, Tillie I, Hartmann D, Wallaert B, et al. Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. *Am J Respir Crit Care Med* 1999;159(4 Pt 1):1298–307.
- [115] Prikk K, Maisi P, Pirila E, Reintam MA, Salo T, Sorsa T, et al. Airway obstruction correlates with collagenase-2 (MMP-8) expression and activation in bronchial asthma. *Lab Invest* 2002;82(11):1535–45.
- [116] Wenzel SE, Balzar S, Cundall M, Chu HW. Subepithelial basement membrane immunoreactivity for matrix metalloproteinase 9: association with asthma severity, neutrophilic inflammation, and wound repair. *J Allergy Clin Immunol* 2003;111(6):1345–52.
- [117] Johnson S, Knox A. Autocrine production of matrix metalloproteinase-2 is required for human airway smooth muscle proliferation. *Am J Physiol* 1999;277(6 Pt 1):L1109–17.

- [118] Tanaka H, Miyazaki N, Oashi K, Tanaka S, Ohmichi M, Abe S. Sputum matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio in acute asthma. *J Allergy Clin Immunol* 2000;105(5):900–5.
- [119] Kelly EA, Busse WW, Jarjour NN. Increased matrix metalloproteinase-9 in the airway after allergen challenge. *Am J Respir Crit Care Med* 2000;162(3 Pt 1):1157–61.
- [120] Lee YC, Lee HB, Rhee YK, Song CH. The involvement of matrix metalloproteinase-9 in airway inflammation of patients with acute asthma. *Clin Exp Allergy* 2001;31(10):1623–30.
- [121] Cataldo DD, Bettiol J, Noel A, Bartsch P, Foidart JM, Louis R. Matrix metalloproteinase-9, but not tissue inhibitor of matrix metalloproteinase-1, increases in the sputum from allergic asthmatic patients after allergen challenge. *Chest* 2002;122(5):1553–9.
- [122] Bergeron C, Page N, Joubert P, Barbeau B, Hamid Q, Chakir J. Regulation of procollagen I (alpha1) by interleukin-4 in human bronchial fibroblasts: a possible role in airway remodelling in asthma. *Clin Exp Allergy* 2003;33(10):1389–97.
- [123] Mautino G, Henriquet C, Jaffuel D, Bousquet J, Capony F. Tissue inhibitor of metalloproteinase-1 levels in bronchoalveolar lavage fluid from asthmatic subjects. *Am J Respir Crit Care Med* 1999;160(1):324–30.
- [124] Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *J Clin Invest* 1995;96(5):2304–10.
- [125] Stearns ME, Rhim J, Wang M. Interleukin 10 (IL-10) inhibition of primary human prostate cell-induced angiogenesis: IL-10 stimulation of tissue inhibitor of metalloproteinase-1 and inhibition of matrix metalloproteinase (MMP)-2/MMP-9 secretion. *Clin Cancer Res* 1999;5(1):189–96.
- [126] Djukanovic R. Asthma: a disease of inflammation and repair. *J Allergy Clin Immunol* 2000;105(2 Pt 2):S522–6.
- [127] Ignatz RA, Massague J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem* 1986;261(9):4337–45.
- [128] Fine A, Goldstein RH. The effect of transforming growth factor-beta on cell proliferation and collagen formation by lung fibroblasts. *J Biol Chem* 1987;262(8):3897–902.
- [129] Vignola AM, Chanez P, Chiappara G, Merendino A, Pace E, Rizzo A, et al. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997;156(2 Pt 1):591–9.
- [130] Panettieri RA, Tan EM, Ciocca V, Luttmann MA, Leonard TB, Hay DW. Effects of LTD4 on human airway smooth muscle cell proliferation, matrix expression, and contraction in vitro: differential sensitivity to cysteinyl leukotriene receptor antagonists. *Am J Respir Cell Mol Biol* 1998;19(3):453–61.
- [131] Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17(3):326–33.
- [132] Doucet C, Brouty-Boye D, Pottin-Clemenceau C, Canonica GW, Jasmin C, Azzarone B. Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in asthma. *J Clin Invest* 1998;101(10):2129–39.
- [133] Doucet C, Brouty-Boye D, Pottin-Clemenceau C, Jasmin C, Canonica GW, Azzarone B. IL-4, IL-13 specifically increase adhesion molecule and inflammatory cytokine expression in human lung fibroblasts. *Int Immunol* 1998;10(10):1421–33.
- [134] Wen FQ, Kohyama T, Liu X, Zhu YK, Wang H, Kim HJ, et al. Interleukin-4- and interleukin-13-enhanced transforming growth factor-beta2 production in cultured human bronchial epithelial cells is attenuated by interferon-gamma. *Am J Respir Cell Mol Biol* 2002;26(4):484–90.
- [135] Espinosa K, Bosse Y, Stankova J, Rola-Pleszczynski M. CysLT1 receptor upregulation by TGF-beta and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD4. *J Allergy Clin Immunol* 2003;111(5):1032–40.
- [136] Fahy JV. Remodeling of the airway epithelium in asthma. *Am J Respir Crit Care Med* 2001;164(10 Pt 2):S46–51.
- [137] Longphre M, Li D, Gallup M, Drori E, Ordonez CL, Redman T, et al. Allergen-induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells. *J Clin Invest* 1999;104(10):1375–82.
- [138] Minshall E, Chakir J, Laviolette M, Molet S, Zhu Z, Olivenstein R, et al. IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. *J Allergy Clin Immunol* 2000;105(2 Pt 1):232–8.
- [139] Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Page N, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001;108(3):430.
- [140] Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S, et al. New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002;169(1):443–53.
- [141] Kazi AS, Lotfi S, Goncharova EA, Tliba O, Amrani Y, Krymskaya VP, et al. Vascular endothelial growth factor-induced secretion of fibronectin is ERK dependent. *Am J Physiol Lung Cell Mol Physiol* 2004;286(3):L539–45.
- [142] Keane MP. Chemokine profiling in pulmonary fibrosis: ready for prime time? *Am J Respir Crit Care Med* 2004;170(5):475–6.
- [143] Puxeddu I, Bader R, Piliponsky AM, Reich R, Levi-Schaffer F, Berkman N. The CC chemokine eotaxin/CCL11 has a selective profibrogenic effect on human lung fibroblasts. *J Allergy Clin Immunol* 2006;117(1):103–10.
- [144] Rozyk KJ, Plusa T, Kuna P, Pirozynska E. Monocyte chemotactic and activating factor/monocyte chemoattractant protein in bronchoalveolar lavage fluid from patients with atopic asthma and chronic bronchitis. Relationship to lung function tests, bronchial hyper-responsiveness and cytology of bronchoalveolar lavage fluid. *Immunol Lett* 1997;58(1):47–52.
- [145] Gharae-Kermani M, Denholm EM, Phan SH. Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. *J Biol Chem* 1996;271(30):17779–84.
- [146] Yamamoto T, Eckes B, Mauch C, Hartmann K, Krieg T. Monocyte chemoattractant protein-1 enhances gene expression and synthesis of matrix metalloproteinase-1 in human fibroblasts by an autocrine IL-1 alpha loop. *J Immunol* 2000;164(12):6174–9.
- [147] Carr MW, Roth SJ, Luther E, Rose SS, Springer TA. Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proc Natl Acad Sci USA* 1994;91(9):3652–6.
- [148] Choi ES, Jakubzick C, Carpenter KJ, Kunkel SL, Evanoff H, Martinez FJ, et al. Enhanced monocyte chemoattractant protein-3/CC chemokine ligand-7 in usual interstitial pneumonia. *Am J Respir Crit Care Med* 2004;170(5):508–15.
- [149] Belperio JA, Dy M, Murray L, Burdick MD, Xue YY, Strieter RM, et al. The role of the Th2 CC chemokine ligand CCL17 in pulmonary fibrosis. *J Immunol* 2004;173(7):4692–8.
- [150] Glassberg MK, Ergul A, Wanner A, Puett D. Endothelin-1 promotes mitogenesis in airway smooth muscle cells. *Am J Respir Cell Mol Biol* 1994;10(3):316–21.
- [151] Takuwa N, Takuwa Y, Yanagisawa M, Yamashita K, Masaki T. A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. *J Biol Chem* 1989;264(14):7856–61.
- [152] Springall DR, Howarth PH, Counihan H, Djukanovic R, Holgate ST, Polak JM. Endothelin immunoreactivity of airway epithelium in asthmatic patients. *Lancet* 1991;337(8743):697–701.
- [153] Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 2000;407(6801):242–8.
- [154] Hoshino M, Takahashi M, Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol* 2001;107(2):295–301.
- [155] Li X, Wilson JW. Increased vascularity of the bronchial mucosa in mild asthma. *Am J Respir Crit Care Med* 1997;156(1):229–33.
- [156] Tanaka H, Yamada G, Saikai T, Hashimoto M, Tanaka S, Suzuki K, et al. Increased airway vascularity in newly diagnosed asthma using a high-magnification bronchovideoscope. *Am J Respir Crit Care Med* 2003;168(12):1495–9.

- [157] Chetta A, Zanini A, Foresi A, D'Ippolito R, Tipa A, Castagnaro A, et al. Vascular endothelial growth factor up-regulation and bronchial wall remodelling in asthma. *Clin Exp Allergy* 2005;35(11):1437–42.
- [158] Kostenis E, Ulven T. Emerging roles of DP and CRTH2 in allergic inflammation. *Trends Mol Med* 2006;12(4):148–58.
- [159] Fujitani Y, Kanaoka Y, Aritake K, Uodome N, Okazaki-Hatake K, Urade Y. Pronounced eosinophilic lung inflammation and Th2 cytokine release in human lipocalin-type prostaglandin D synthase transgenic mice. *J Immunol* 2002;168(1):443–9.
- [160] Honda K, Arima M, Cheng G, Taki S, Hirata H, Eda F, et al. Prostaglandin D2 reinforces Th2 type inflammatory responses of airways to low-dose antigen through bronchial expression of macrophage-derived chemokine. *J Exp Med* 2003;198(4):533–43.
- [161] Monneret G, Gravel S, Diamond M, Rokach J, Powell WS. Prostaglandin D2 is a potent chemoattractant for human eosinophils that acts via a novel DP receptor. *Blood* 2001;98(6):1942–8.
- [162] Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 2001;193(2):255–61.
- [163] Shiraishi Y, Asano K, Nakajima T, Oguma T, Suzuki Y, Shiomi T, et al. Prostaglandin D2-induced eosinophilic airway inflammation is mediated by CRTH2 receptor. *J Pharmacol Exp Ther* 2005;312(3):954–60.
- [164] Almishri W, Cossette C, Rokach J, Martin JG, Hamid Q, Powell WS. Effects of prostaglandin D2, 15-deoxy-Delta12,14-prostaglandin J2, and selective DP1 and DP2 receptor agonists on pulmonary infiltration of eosinophils in Brown Norway rats. *J Pharmacol Exp Ther* 2005;313(1):64–9.
- [165] Yoshimura-Uchiyama C, Iikura M, Yamaguchi M, Nagase H, Ishii A, Matsushima K, et al. Differential modulation of human basophil functions through prostaglandin D2 receptors DP and chemoattractant receptor-homologous molecule expressed on Th2 cells/DP2. *Clin Exp Allergy* 2004;34(8):1283–90.
- [166] Mesquita-Santos FP, Vieira-de-Abreu A, Calheiros AS, Figueiredo IH, Castro-Faria-Neto HC, Weller PF, et al. Cutting edge: prostaglandin D2 enhances leukotriene C4 synthesis by eosinophils during allergic inflammation: synergistic in vivo role of endogenous eotaxin. *J Immunol* 2006;176(3):1326–30.
- [167] Ying S, O'Connor BJ, Meng Q, Woodman N, Greenaway S, Wong H, et al. Expression of prostaglandin E(2) receptor subtypes on cells in sputum from patients with asthma and controls: effect of allergen inhalational challenge. *J Allergy Clin Immunol* 2004;114(6):1309–16.
- [168] Walters EH, Davies BH. Dual effect of prostaglandin E2 on normal airways smooth muscle in vivo. *Thorax* 1982;37(12):918–22.
- [169] Pavord ID, Tattersfield AE. Bronchoprotective role for endogenous prostaglandin E2. *Lancet* 1995;345(8947):436–8.
- [170] Tilley SL, Hartney JM, Erikson CJ, Jania C, Nguyen M, Stock J, et al. Receptors and pathways mediating the effects of prostaglandin E2 on airway tone. *Am J Physiol Lung Cell Mol Physiol* 2003;284(4):L599–606.
- [171] Meja KK, Barnes PJ, Gienbycz MA. Characterization of the prostanoid receptor(s) on human blood monocytes at which prostaglandin E2 inhibits lipopolysaccharide-induced tumour necrosis factor-alpha generation. *Br J Pharmacol* 1997;122(1):149–57.
- [172] Barnes PJ. The mode of action of corticosteroids in asthma. *Res Immunol* 1998;149(3):225.
- [173] Montuschi P, Barnes PJ. Exhaled leukotrienes and prostaglandins in asthma. *J Allergy Clin Immunol* 2002;109(4):615–20.
- [174] Antczak A, Montuschi P, Kharitonov S, Gorski P, Barnes PJ. Increased exhaled cysteinyl-leukotrienes and 8-isoprostane in aspirin-induced asthma. *Am J Respir Crit Care Med* 2002;166(3):301–6.
- [175] Montuschi P, Corradi M, Ciabattini G, Nightingale J, Kharitonov SA, Barnes PJ. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 1999;160(1):216–20.
- [176] Zanonato S, Carraro S, Corradi M, Alinovi R, Pasquale MF, Piacentini G, et al. Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma. *J Allergy Clin Immunol* 2004;113(2):257.
- [177] Bel EH, van der Veen H, Kramps JA, Dijkman JH, Sterk PJ. Maximal airway narrowing to inhaled leukotriene D4 in normal subjects. Comparison and interaction with methacholine. *Am Rev Respir Dis* 1987;136(4):979–84.
- [178] Arm JP, Spur BW, Lee TH. The effects of inhaled leukotriene E4 on the airway responsiveness to histamine in subjects with asthma and normal subjects. *J Allergy Clin Immunol* 1988;82(4):654–60.
- [179] O'Hickey SP, Hawksworth RJ, Fong CY, Arm JP, Spur BW, Lee TH. Leukotrienes C4, D4, and E4 enhance histamine responsiveness in asthmatic airways. *Am Rev Respir Dis* 1991;144(5):1053–7.
- [180] Henderson Jr WR, Lewis DB, Albert RK, Zhang Y, Lamm WJ, Chiang GK, et al. The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J Exp Med* 1996;184(4):1483–94.
- [181] Cap P, Chladek J, Pehal F, Maly M, Petru V, Barnes PJ, et al. Gas chromatography/mass spectrometry analysis of exhaled leukotrienes in asthmatic patients. *Thorax* 2004;59(6):465–70.
- [182] Busse WW. Inflammation in asthma: the cornerstone of the disease and target of therapy. *J Allergy Clin Immunol* 1998;102(4 Suppl 1):S17.
- [183] Black PN, Fuller RW, Taylor GW, Barnes PJ, Dollery CT. Effect of inhaled leukotriene B4 alone and in combination with prostaglandin D2 on bronchial responsiveness to histamine in normal subjects. *Thorax* 1989;44(6):491–5.
- [184] Carpagnano GE, Barnes PJ, Francis J, Wilson N, Bush A, Kharitonov SA. Breath condensate pH in children with cystic fibrosis and asthma: a new noninvasive marker of airway inflammation? *Chest* 2004;125(6):2005–10.
- [185] Tufvesson E, Björner L. Methodological improvements for measuring eicosanoids and cytokines in exhaled breath condensate. *Respir Med* 2006;100(1):34–8.
- [186] Beghe B, Barton S, Rorke S, Peng Q, Sayers I, Gaunt T, et al. Polymorphisms in the interleukin-4 and interleukin-4 receptor alpha chain genes confer susceptibility to asthma and atopy in a Caucasian population. *Clin Exp Allergy* 2003;33(8):1111–7.
- [187] Laprise C, Sladek R, Ponton A, Bernier MC, Hudson TJ, Laviolette M. Functional classes of bronchial mucosa genes that are differentially expressed in asthma. *BMC Genomics* 2004;5(1):21.
- [188] Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002;418(6896):426–30.
- [189] Holgate ST, Davies DE, Powell RM, Holloway JW. ADAM33: a newly identified protease involved in airway remodelling. *Pulm Pharmacol Ther* 2006;19(1):3.
- [190] Profita M, La Grutta S, Carpagnano E, Riccobono L, Di Giorgi R, Bonanno A, et al. Noninvasive methods for the detection of upper and lower airway inflammation in atopic children. *J Allergy Clin Immunol* 2006;118:1068–74.
- [191] Leung TF, Wong GW, Ko FW, Lam CW, Fok TF. Increased macrophage-derived chemokine in exhaled breath condensate and plasma from children with asthma. *Clin Exp Allergy* 2004;34(5):786–91.
- [192] Ko FWS, Lau CYK, Leung TF, Wong GWK, Lam CWK, Lai CKW, et al. Exhaled breath condensate levels of eotaxin and macrophage-derived chemokine in stable adult asthma patients. *Clin Exp Allergy* 2006;36(1):44–51.
- [193] Baraldi E, Ghirio L, Piovan V, Carraro S, Ciabattini G, Barnes PJ, et al. Increased exhaled 8-isoprostane in childhood asthma. *Chest* 2003;124(1):25–31.