

Pathogenesis of Asthma

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SUMMARY

While asthma is considered an inflammatory disorder of the conducting airways, it is becoming increasingly apparent that the disease is heterogeneous with respect to immunopathology, clinical phenotypes, response to therapies, and natural history. Once considered purely an allergic disorder dominated by Th2-type lymphocytes, IgE, mast cells, eosinophils, macrophages, and cytokines, the disease also involves local epithelial, mesenchymal, vascular and neurologic events that are involved in directing the Th2 phenotype to the lung and through aberrant injury-repair mechanisms to remodeling of the airway wall. Structural cells provide the necessary “soil” upon which the “seeds” of the inflammatory response are able to take root and maintain a chronic phenotype and upon which are superimposed acute and subacute episodes usually driven by environmental factors such as exposure to allergens, microorganisms, pollutants or caused by inadequate antiinflammatory treatment. Greater consideration of additional immunologic and inflammatory pathways are revealing new ways of intervening in the prevention and treatment of the disease. Thus increased focus on environmental factors beyond allergic exposure (such as virus infection, air pollution, and diet) are identifying targets in structural as well as immune and inflammatory cells at which to direct new interventions.

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INTRODUCTION

Asthma is a disorder of the conducting airways, which contract too much and too easily spontaneously and in response to a wide range of exogenous and endogenous stimuli. This airway hyperresponsiveness is accompanied by enhanced sensory irritability of the airways and increased mucus secretion. The different clinical expressions of asthma involve varying environmental factors that interact with the airways to cause acute and chronic inflammation, and the varying contributions of smooth muscle contraction, edema and remodeling of the formed elements of the airways. Heterogeneity of asthma also relates to the different response to therapies. Asthma is considered a good example of gene–environment interactions, although no single gene or environmental factor accounts for the disease. This chapter focuses on the pathophysiologic events underlying the inflammatory and remodeling response.

AIRWAY INFLAMMATION IS FUNDAMENTAL TO ASTHMA PATHOGENESIS

Airway inflammation in asthma is a multicellular process involving mainly eosinophils, neutrophils, CD4⁺T lymphocytes and mast cells, with eosinophilic infiltration being the most striking feature (Kay 2005) (Fig. 78.1). The inflammatory process is largely restricted to the conducting airways but as the disease becomes more severe and chronic the inflammatory infiltrate spreads both proximally and distally to include the small airways and in some cases adjacent alveoli (Kraft *et al.* 1999). The inflammatory response in the small airways appears to be predominantly outside the airway smooth muscle (Fig. 78.1), whereas in the large airways inflammation of the submucosa dominates (Haley *et al.* 1998). This Th2 type of inflammation is common to chronic allergic inflammatory responses at multiple tissue sites and indeed is seen at these sites in patients with asthma

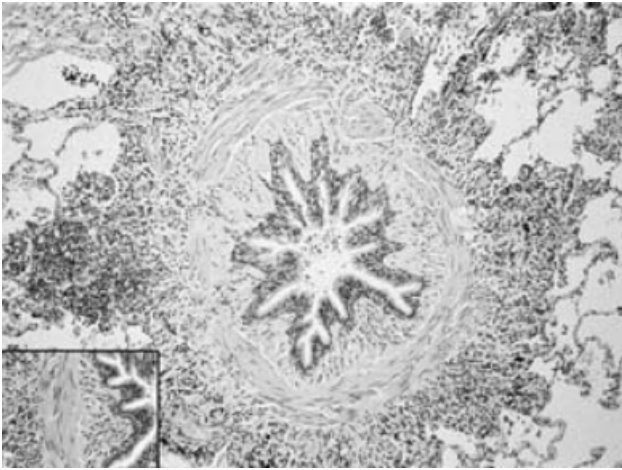


Fig. 78.1 Cross-section of a small asthmatic airway showing abundant inflammation both inside and outside the smooth muscle and remodeling as evidenced by the deposition of new matrix beneath the epithelium and in all layers of the airway. (Holgate & Polosa 2006).

who frequently express comorbidities such as chronic rhinitis, sinusitis, atopic dermatitis, and food allergy (Kay 2001). There continues to be debate about the specific contribution of individual cell types in these other diseases, but at least in asthma with an allergic component a common theme is now emerging. Other chapters have provided considerable detail of the potential individual roles of these cells and their mediators in the allergic tissue response, so only brief mention is made of them here with respect to their relevance to human asthma.

THE IMMUNE RESPONSE

A fundamental feature of asthma associated with allergic sensitization is the ability of the airway to recognize common environmental allergens and to generate a Th2 cytokine response to them. Recognizing that in excess of 40% of the Western population is atopic (i.e., have elevated IgE to common environmental allergens), only about 7% express their atopy in the form of asthma (Beasley *et al.* 2001). Therefore, a crucial question to ask is what mechanisms account for the specific expression of atopy in the conducting airways and why some patients despite being highly atopic have no evidence of asthma? One explanation is the way that the immune response to allergens is regulated at the surface of the airways.

A fundamental feature of allergen sensitization is the uptake and processing of inhaled allergens by dendritic cells situated in the airway epithelium and submucosa and which extend their processes to the airway surface

(von Garnier *et al.* 2005; Hammad & Lambrecht 2006). Uptake of allergen is enhanced by IgE bound to high-affinity receptors on dendritic cells that facilitate allergen internalization (Kitamura *et al.* 2007). Once inside the dendritic cell, processing of allergens by cathepsin S and the subsequent selection of peptides loaded onto and presented by HLA molecules (MHC class II) is fundamental to the ability of these cells to serve as antigen-presenting cells to T lymphocytes (Riese & Chapman 2000). Once the dendritic cell has engaged allergen, it receives signals to migrate to local lymphoid collections where antigen presentation takes place. Its specific chemokine receptors, including CCR7 and its ligands CCL19 and CCL21 (and to a lesser extent CXCR4 and its ligand CXCL12), are involved in this chemotactic migration to enable contact with naive T cells (Humrich *et al.* 2006; Pease & Williams 2006) (see Chapter 8). Presentation of a selected antigen peptide to the T-cell receptor initiates sensitization and the subsequent immune response to the specific allergen (Smit & Lukacs 2006). The nature of this immune response depends on whether engagement of selective costimulatory molecules occurs in parallel. For efficient antigen-dependent T-cell activation engagement of either CD80 (B7.1) or CD86 (B7.2) on the dendritic cells with CD28 on T cells leads to sensitization, whereas lack of, or inefficient, engagement of these costimulatory molecules may lead to anergy (Larche *et al.* 1998; van Rijk *et al.* 2004). An alternative method of preventing sensitization and rendering T cells anergic is engagement of a second costimulatory molecule, cytotoxic T-lymphocyte antigen (CTLA)4, which has a higher affinity than either CD80 or CD86 for CD28 and can therefore prevent CD80/CD86 costimulation (Jaffar *et al.* 1999a, b). This is the basis of the successful clinical application of the CTLA4-immunoglobulin fusion protein abatacept, used as an immunomodulatory agent in such diseases as rheumatoid arthritis and in an animal model of allergen-induced airway inflammation (Weinblatt *et al.* 2006).

In more severe asthma the relative importance of CD28 signaling in supporting the inflammatory response is reduced (Lordan *et al.* 2001). Under these circumstances other costimulatory pathways are thought to be engaged in T-cell activation, including ICOS and its multiple possible ligands B7-RP1, PD-L1 (B7-H1), PD-L2 (B7-DC), B7-H3 and B7-H4 (B7-S1) (Greenwald *et al.* 2005) as well as OX40 (CD134) and its ligand OX40L (CD252) (Salek-Ardakani *et al.* 2003; Burgess *et al.* 2005).

The capacity of dendritic cells to generate interleukin (IL)-12 determines the balance between Th1 and Th2

responses, IL-12 polarizing T-cell differentiation in favor of a Th1 response (Kuipers *et al.* 2004). However, while IL-12 is able to counteract Th2 sensitization, it is also able to contribute to maximal expression of allergic airway disease post sensitization (Meyts *et al.* 2006). Once sensitized, T cells not only migrate back to the airways to the site of antigen presentation under the influence of the chemokines CCL11, CCL24, CCL26, CCL7, CCL13, CCL17 and CCL22 (which interact with their reciprocal receptors including CCR3, CCR4, CCR5, CCR6, CCR7 and CCR8) (Garcia *et al.* 2005; Kallinich *et al.* 2005), but these cells also become potent producers of a range of cytokines, the majority of which are expressed on the long arm of chromosome 5, namely IL-3, IL-4, IL-5, IL-6, IL-9, IL-13 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Kay 2006; Ryu *et al.* 2006). IL-1 β produced by macrophages, monocytes, dendritic cells, and smooth muscle and epithelial cells in large amounts (Schmitz *et al.* 2003; Dragon *et al.* 2006) and IL-2 produced by T cells further enhance antigen-induced T-cell proliferation and maturation (Anderson 2002).

There is now persuasive evidence that at least in mild to moderate asthma Th2-type cells dominate the T-cell repertoire in the airways (Anderson 2002). Through cytokine production, they have the capacity to recruit secondary effector cells such as macrophages, basophils and eosinophils into the inflammatory zone where these cells become primed and subsequently activated for mediator secretion (Fig. 78.2). There has also been recent interest in the potential role of IL-4- and IL-13-secreting, CCR9⁺ natural killer (NK) T cells in orchestrating the inflammatory response in chronic asthma (Sen *et al.* 2005; Akbari *et al.* 2006; Umetsu & Dekruyff 2006) (see

Chapter 3) (Pham-Thi *et al.* 2006; Thomas *et al.* 2006), although initial findings of their primacy has been challenged (Ho 2007; Vijayanand *et al.* 2007). Overall, it is the Th2-type T cell bearing the CCR4 chemokine receptor that is the cell which dominates the allergic immune response and may be the cell most probably responsible for contributing to the ongoing chronic inflammatory response. Indeed, asthma severity is reported to be associated with an increase in CCR4⁺ T cells (Ishida *et al.* 2006). Thus, inhibitors of CCR4 (e.g., the antibody-dependent cell cytotoxic monoclonal antibody KM2760) could be highly effective in the treatment of asthma by inactivating or removing CCR4⁺ Th2 cells (Ishida *et al.* 2006).

While Th2-type T cells may be important in the pathogenesis of mild to moderate asthma, as the disease becomes more severe and chronic Th1-type T cells are recruited that have the capacity to secrete tumor necrosis factor (TNF)- α and interferon (IFN)- γ (Truyen *et al.* 2006). This more complicated T-cell profile may help to explain the aggressive and tissue-damaging aspects of the immune response in more severe disease. Although these Th1-type cells, as well as CD8⁺ T cells, have been incriminated in both more severe asthma (Hamzaoui *et al.* 2005) and during asthma exacerbations (especially following virus infection) (O'Sullivan 2005), the precise mechanisms whereby they achieve this is still largely unknown. Although the T lymphocyte has been given primacy with regard to the orchestration of the inflammatory response in asthma, there have been no studies using selective T-cell-depleting strategies with either monoclonal antibodies or drugs in mild to moderate asthma (Kon & Kay 1999; Bharadwaj & Agrawal 2004). However, in a small proof of concept study, a single

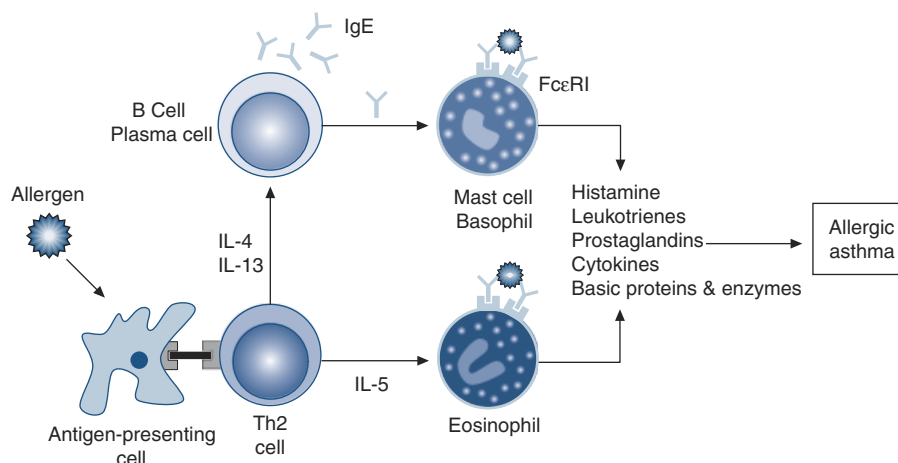


Fig. 78.2 Schematic representation of the inflammatory cascade in allergic asthma. (Bradding *et al.* 2006).

infusion of anti-CD3 improved lung function in patients with severe disease (Kon *et al.* 1998). The immunosuppressant approach to asthma treatment has not been pursued largely due to difficulties in developing calcineurin inhibitors such as cyclosporin A and tacrolimus in an inhaled form and problems with side effects.

MAST CELLS

The mast cell has long been associated with asthma. The early asthmatic reaction following inhaled allergen provocation is mast-cell dependent and drugs such as sodium cromoglycate and nedocromil sodium are believed to mediate their effects by inhibiting mast cell mediator secretion (Holgate 1996). For many years it was thought that the mast cells present in the airway epithelium and submucosa were fundamental to the contribution that these cells make to asthma (Shahana *et al.* 2005), but recent studies have indicated that mast cells deeper in the airway wall are also important. While undoubtedly mucosal-type mast cells (tryptase positive, chymase negative) under the control of T lymphocytes (specifically IL-3, IL-4 and IL-9) are highly responsive to inhaled allergens (and possibly other stimuli such as hypertonicity) in causing bronchoconstriction, there has been recent interest in mast cells present deeper in the airway wall and in the more peripheral airways as being more fundamental to some of the chronic inflammatory responses in asthma (Bradding *et al.* 2006). Of particular interest has been the discovery that in chronic asthma (but apparently not in eosinophilic bronchitis), mast cells are markedly increased in association with airway smooth muscle in both the large and the small airways (Brightling *et al.* 2002). It is at this site that mast cells interact with airway smooth muscle through the action of autacoid mediators such as leukotriene (LT)_{D4}, prostaglandin (PG)_{D2} and histamine, but also contributing to fibrogenesis and an increase in smooth muscle as part of the "remodeling" response (Kaur *et al.* 2006; Plante *et al.* 2006). Although many factors are involved in the regulation of mast cell function in the submucosa and deeper in the airway wall, mast cells in the airway smooth muscle differ from mucosal mast cells in the being of the connective tissue-type (tryptase positive, chymase positive, and carboxypeptidase positive) and in being more dependent on stem cell factor (SCF) for their survival. SCF (c-kit ligand) is produced by the epithelium, smooth muscle and fibroblasts and is upregulated in asthma (Al Muhsen *et al.* 2004; Plante *et al.* 2006). In addition, CXCL8 and CXCL10 produced by airway smooth muscle itself are not only

important in the recruitment of mast cells into this compartment by interacting with their receptors CXCR3 and CXCR2 respectively, but also in their priming for enhanced mediator secretion (Woodman *et al.* 2006; Scott & Bradding 2005). In the reverse direction, mast cells secrete CCL19 that, through its receptor CCR7, stimulates airway smooth muscle migration and probably contributes to the smooth muscle hyperplasia (Kaur *et al.* 2006). Thus, airway smooth muscle is partly dependent on mast cells for its survival and enhanced contractility, whereas mast cells are dependent on smooth muscle factors for their survival and activation.

On activation, irrespective of their subtype, mast cells release preformed granule-associated mediators such as histamine, tryptase and other proteases, heparin and some cytokines, as well as newly formed eicosanoids that include PGD₂, thromboxane (TX)A₂, and the cysteinyl leukotrienes (LTC₄ and LTD₄) (Bradding *et al.* 2006). These mediators are potent smooth muscle contractile agents and also increase microvascular permeability. Both PGD₂ and LTD₄ interact with cell-surface receptors on eosinophils, macrophages, basophils and mast cells where they serve as chemoattractant as well as priming agents (Ogawa & Calhoun 2006). Thus, cysteinyl leukotriene antagonists such as montelukast and pranlukast are not only able to block the acute effects of leukotrienes on the formed elements of the airway, but also exert some antiinflammatory action.

The mast cell is also well endowed with neutral proteases (Reed & Kita 2004). One that has attracted particular interest is tryptase, a tetrameric neutral protease that has a preferential action on G protein-coupled protease-activated receptor type 2 (PAR2), which is present on many cell types including epithelial cells, fibroblasts, smooth muscle cells, endothelial cells, and on a number of inflammatory cells (Cairns 2005; Hallgren & Pejler 2006). The exact role of the PAR2 receptor in asthma has yet to be clearly defined, but there is increasing evidence that its activation is involved in mesenchymal cell proliferation and airway wall remodeling (Berger *et al.* 2001). Mast cells and eosinophils are also an important source of the zinc-dependent matrix metalloproteinases (MMP)-3 and MMP-9, which through their interaction with matrix proteins and proteoglycans have also been incriminated in airway wall remodeling (Dahlen *et al.* 1999; Wenzel *et al.* 2003).

Activation of mast cells, particularly through the highaffinity IgE receptor (FcεRI), leads to the release of certain cytokines that are packaged within mast cell granules, including TNF-α, IL-4 and IL-5 (Bradding

et al. 1995; Wilson *et al.* 2000), but also induces transcription of these and other cytokines and chemokines that are then secreted over a period of up to 72 hours following cell perturbation (Okayama *et al.* 1995, 2003). These cytokines and chemokines undoubtedly contribute to the ongoing inflammatory response in asthma and may be partly responsible for the allergen-induced late-phase inflammatory response characteristic of allergen provocation. Blockade of IgE using the monoclonal antibody omalizumab leads to marked attenuation of both the early and late phase allergen-induced bronchoconstrictor and skin inflammatory responses, linking both to mast cell activation (Fahy *et al.* 1997; Ong *et al.* 2005). However, the precise mechanisms through which late-phase bronchoconstriction and the associated inflammatory cell influx with neutrophils and eosinophils lead to bronchoconstriction is not fully established, although its partial attenuation with antileukotriene drugs suggests at least some role for cysteinyl leukotrienes (Leigh *et al.* 2002).

EOSINOPHILS

A very prominent cell in the inflammation of allergic asthma is the eosinophil leukocyte, which is present not only in the airway wall but in uncontrolled asthma is also

found in large numbers in the sputum and bronchoalveolar lavage fluid (Kay 2005; Lemiere *et al.* 2006). These cells are in large part initially recruited from the bone marrow as CD34 precursors, following the release of PGD₂, cysteinyl leukotrienes, cytokines and chemokines from the asthmatic airway. The developing eosinophils then pass from the circulation via the microvascular compartment into the airway wall. There has been much research on the mechanisms involved in this recruitment, including the role of specific chemokines and adhesion molecules (reviewed in detail elsewhere in this book). Suffice to say that IL-3 and GM-CSF and eotaxins 1–3 are crucial to the early derivation of eosinophils from CD34⁺ bone marrow precursor cells, with IL-5 being responsible for their maturation and recruitment into the airways (Robinson *et al.* 1999; Sehmi *et al.* 2003) (Fig. 78.3). Eosinophils are a rich source of granule basic proteins, such as major basic protein, eosinophil peroxidase, and eosinophil cationic protein, and also have the capacity to generate eicosanoids such as prostacyclin (PGI₂) and cysteinyl leukotrienes and release potentially tissue-damaging superoxide and a range of cytokines and chemokines (Kariyawasam & Robinson 2006). The dramatic reduction in sputum and tissue eosinophils that occurs on treatment of asthma with inhaled or oral corticosteroids associated with clinical improvement has

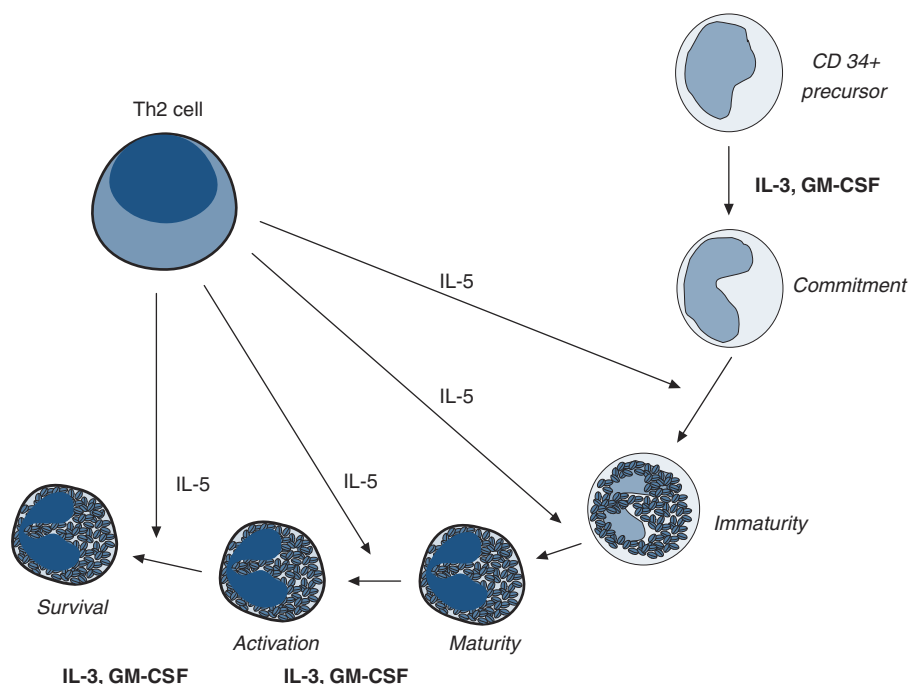


Fig. 78.3 Th2 cytokines involved in the maturation and recruitment of eosinophils from CD34⁺ precursors in the bone marrow and airway wall. Note the prominent contribution of IL-5. See text for definition of abbreviations.

led to the idea that eosinophils are fundamental to airway dysfunction in asthma and are the principal target for this drug class (Djukanovic *et al.* 1992, 1997).

Undoubtedly, eosinophils play an important role in the allergic inflammatory response, but recently their primacy in the inflammatory milieu of asthma has been challenged (Flood-Page *et al.* 2003a). Following allergen challenge in moderately severe asthma, the administration of a humanized blocking IgG monoclonal antibody directed to IL-5 resulted in a dramatic reduction (>80%) in circulating and sputum eosinophils. However, this was not accompanied by any inhibitory effect on the allergen-induced late-phase reaction in the airways or skin or acquired airway hyperreactivity (Leckie *et al.* 2000; Phipps *et al.* 2004). In a large clinical study three injections of anti-IL-5 given 2 weeks apart also had a dramatic effect on circulating and sputum eosinophils but paradoxically did not affect any of the clinical outcome measures of asthma including baseline lung function (Fig. 78.4). These studies questioned either the primacy given to the eosinophil as the dominant inflammatory cell of asthma or the effectiveness of anti-IL-5

to deplete eosinophils. Thus, in a series of subsequent bronchial biopsy studies, anti-IL-5 antibody therapy only reduced airway eosinophils by about 50% and it has been suggested that the remaining 50% of eosinophils could account for ongoing asthma symptomatology (Flood-Page *et al.* 2003a) (Fig. 78.4). Anti-IL-5 monoclonal antibody also induced bone marrow eosinophil maturation arrest and decreased CD34⁺ eosinophil progenitors in the bronchial mucosa of atopic asthmatics (Flood-Page *et al.* 2003b). Further studies have demonstrated that eosinophil depletion using this approach was able to modify certain matrix proteins in the subepithelial basement membrane such as tenascin C, lumican and procollagen 3 (Flood-Page *et al.* 2003b) (Fig. 78.4). Based on these findings, it has been suggested that the eosinophil may be playing a more important role in airway remodeling than has hitherto been recognized (Kay *et al.* 2004). Persistence of some eosinophils in asthmatic airway tissue in the presence of IL-5 blockade might be explained by some loss of the IL-5 receptor from such cells when eosinophils are recruited into the airways (Liu *et al.* 2002). A positive contribution of eosinophils to

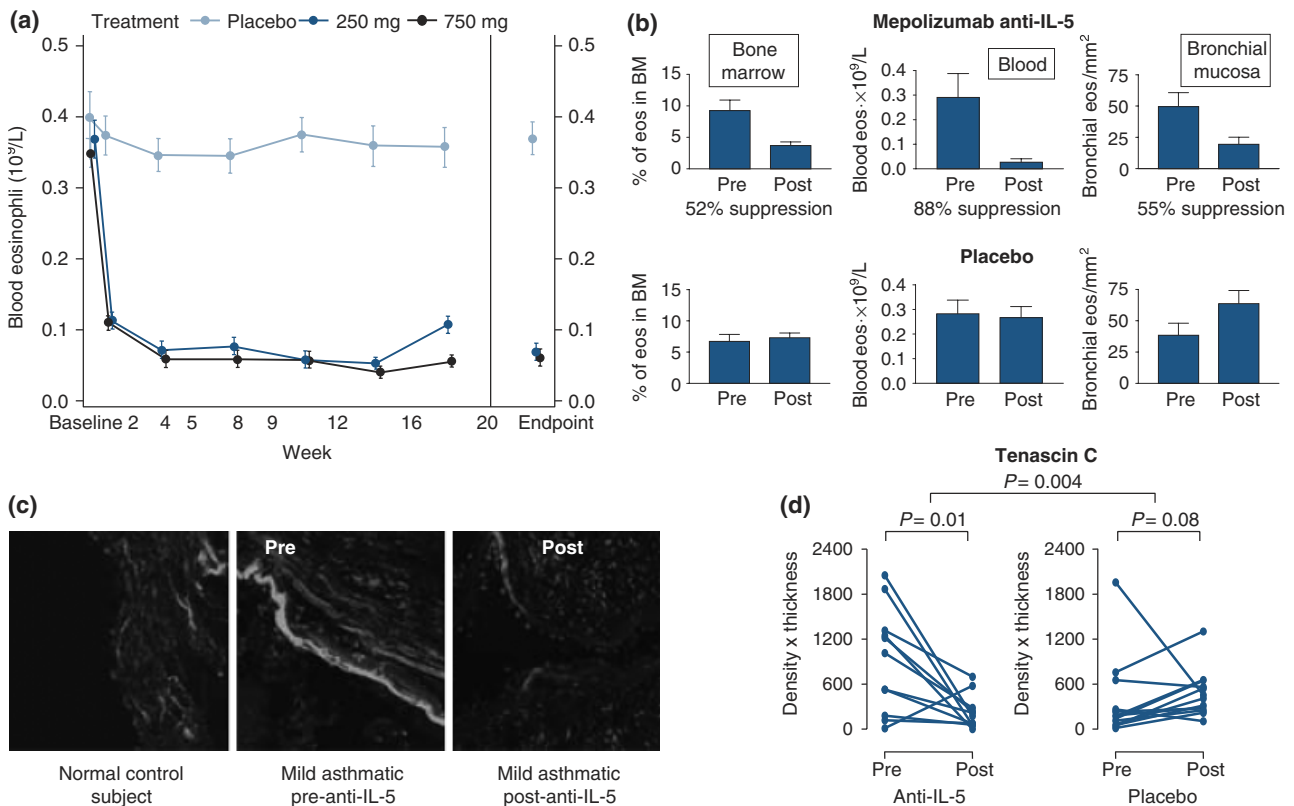


Fig. 78.4 Effect of intravenous injections of anti-IL-5 monoclonal antibody (mepolizumab) on (a) circulating eosinophils in over 100 moderately severe asthmatic patients; (b) comparative effect on eosinophils at different sites; (c) immunoreactivity to tenascin C; (d) tenascin C quantified by image analysis. (Reproduced with permission from (b & c) Flood-Page *et al.* 2003b (a & d) Flood-Page *et al.* 2007).

tissue remodeling is supported by their capacity to generate transforming growth factor (TGF)- β 1 and support fibroblast proliferation, collagen synthesis and myofibroblast maturation (Williams & Jose 2000).

Attraction of eosinophils to the site of inflammation is largely mediated through eotaxins 1, 2 and 3, macrophage chemotactic factor (MCP)-3, MCP-4, and RANTES (Williams & Jose 2000). These chemokines interact with the CCR3 and, to a lesser degree, CCR5 receptors on eosinophils and, as with other chemokines, not only provoke their directed migration but also their priming. Therefore, it is somewhat disappointing that despite showing activity in mouse models (Das *et al.* 2006), CCR3 receptor antagonists have so far proven disappointing when tested in the clinic.

There is also considerable debate about how eosinophils are cleared from the airways in asthma. At one time it was thought that programmed cell death, mediated by withdrawal of specific cytokines such as IL-5 and GM-CSF, resulted in accelerated programmed cell death (Meagher *et al.* 1996) and uptake by macrophages and epithelial cells (Sexton *et al.* 2004). Similarly, it was believed that an important component of corticosteroid action was through acceleration of this apoptotic response (Meagher *et al.* 1996). However, detailed examination of asthmatic tissue has demonstrated little evidence of eosinophil apoptosis; rather eosinophils display an unusual form of cytolysis that leaves their eosinophil granules intact within the airway wall even though the cell membrane and cytoplasm have disappeared (Uller *et al.* 2004). Corticosteroids have also been shown to increase clearance of airway eosinophils through egression into the airway lumen as well as by inhibiting chemokine production (Uller *et al.* 2006). In an animal model, activation of apoptosis through the Fas receptor has been shown to magnify rather than resolve asthmatic type inflammation (Uller *et al.* 2005). Thus, while much has been learnt about eosinophils in asthma, there still remain many unanswered questions. The role of neutrophils is discussed later in this chapter.

MONOCYTES AND MACROPHAGES

Monocytes are able to differentiate into macrophages and dendritic cells in the presence of GM-CSF (Gajewska *et al.* 2003), the latter requiring IL-4 (Webb *et al.* 2007). In chronic asthma both monocytes and macrophages are prominent cells in the airway mucosa and undoubtedly play an important role in disease pathogenesis. While these cells are an important source of cysteinyl leuko-

trienes, reactive oxygen and a variety of lysosomal enzymes, their precise role in mediating tissue damage and contributing to the overall airway pathology of asthma is largely unknown. In corticosteroid-refractory asthma, monocytes and macrophages are thought to play an increasingly important role and may well account for the ongoing chronic inflammation associated with their preferential infiltration into the airway wall in patients with longstanding corticosteroid-resistant disease (Sher *et al.* 1994; Loke *et al.* 2006). It is clear that more attention focused on these cells is required, especially how they fit into the overall Th2 paradigm and whether they represent a separate component of the immune and inflammatory response which has not yet been adequately explored.

BASOPHILS

Although basophils have largely been thought of as circulating IgE-triggered inflammatory cells, in certain types of immune response they do accumulate in tissues. The discovery of unique basophil-specific markers such as basogranulin (Mochizuki *et al.* 2003; Agis *et al.* 2006) has enabled their identification in the airways of subjects with asthma (Macfarlane *et al.* 2000; Kepley *et al.* 2001). However, at this time, it is not clear what their precise role is in either acute or chronic disease, although it is known that they share many of their recruitment mechanisms with eosinophils and are likely to be accompaniments of eosinophil infiltration (Gangur *et al.* 2003).

NONALLERGIC (LATE-ONSET OR INTRINSIC) ASTHMA

Although the majority of asthma is associated with atopy and has its onset in early childhood across all ages and especially after the age of 40 years, there are forms of asthma that appear to be independent of atopy (Humbert 2000). These nonallergic forms of the disease have been subject to careful comparative investigation but so far no clear pathogenic pathways have been identified (Humbert *et al.* 1999; Corrigan 2004). There is some evidence to suggest that local IgE mechanisms may be involved with the detection of IgE isotype switching in airway biopsies, but the clinical significance of this has yet to be determined (Ying *et al.* 2001; Jayaratnam *et al.* 2005). The immunopathology of late-onset non-allergic asthma appears to be very similar to that of allergic asthma, although there have been some differences reported in the relative proportion of the various

inflammatory cells present (Ying *et al.* 1997; Ying *et al.* 1999; Strek 2006). It is important to recognize that some forms of late-onset asthma have an occupational cause due to non-IgE-dependent sensitization to chemicals in the workplace (Malo 2005; Slavin 2005). In addition to immunologic sensitization to chemicals, both intrinsic asthma (Nahm *et al.* 2002) and asthma caused by diisocyanate exposure are associated with antibodies directed to epithelial components (Ye *et al.* 2006a). Other autoantibodies in asthma include those directed to heat-shock protein (HSP)-70 (Yang *et al.* 2005), CD28 (Neuber *et al.* 2006), and α -enolase (Nahm *et al.* 2006). Whether such autoantibodies are truly pathogenetic or related more to tissue damage and inflammation remains to be established (Ye *et al.* 2006b). Whatever the mechanisms, this type of asthma is likely to be highly heterogeneous, exhibiting overlap with chronic obstructive pulmonary disease. It is clearly an area where much more careful phenotyping is required as well as the application of novel cell and molecular methodology to determine underlying mechanisms.

THE AIRWAY EPITHELIUM IN ASTHMA

The airway epithelium, while playing an important role as a physical barrier, is now known to be fundamental to asthma pathogenesis. Bronchial biopsies in anything but the mildest forms of asthma show areas of epithelial metaplasia and damage, thickening of the subepithelial basal lamina, increased number of myofibroblasts, and other evidence of airway remodeling such as hypertrophy and hyperplasia of airway smooth muscle, mucous gland hyperplasia, angiogenesis and an altered deposition and composition of extracellular matrix proteins and proteoglycans (Knight & Holgate 2003; Holgate *et al.* 2004). While these pathologic features have been commonly reported in asthma deaths and in bronchial biopsies from patients with asthma of varying severity, more recently similar findings have been found in the airways of children in relation to the onset of asthma (Fedorov *et al.* 2005; Saglani *et al.* 2005; Barbato *et al.* 2006). Evidence of epithelial damage with upregulation of epidermal growth factor receptors (EGFRs) and features of impaired proliferation, e.g., reduced expression of proliferative markers such as Ki67 and proliferating cell nuclear antigen (PCNA) and upregulation of the cell cyclin inhibitor, nuclear p21^{wt}, suggest that, as in adult asthma (Puddicombe *et al.* 2003), the epithelium is chronically injured and unable to repair properly (Bucchieri *et al.* 2002; Kicic *et al.* 2006) (Fig. 78.5). One

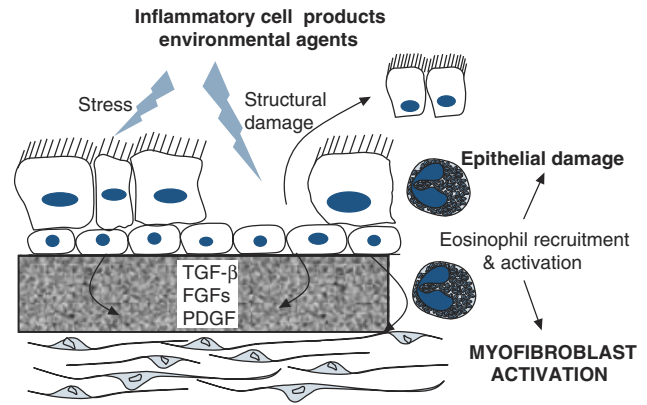


Fig. 78.5 Schematic diagram of epithelial injury and activation of subepithelial mesenchymal cells through the release of growth factors creating a chronic wound scenario. See text for definition of abbreviations.

important feature of the asthmatic epithelium is its capacity to defend itself against oxidant injury (Bucchieri *et al.* 2002; Comhair *et al.* 2005), a feature that may partly explain why asthmatic subjects are so sensitive to oxidant pollutants such as ozone, environmental tobacco smoke and ambient air particulates (Truong-Tran *et al.* 2003).

Under normal circumstances the epithelium forms a highly regulated and almost impermeable barrier through the formation of tight junctions (Godfrey 1997). These protein complexes at the apex of the columnar cells (Mullin *et al.* 2005) comprise a series of proteins that includes claudins and transmembrane adhesion proteins that connect adjacent cells. Structural integrity is also maintained through cell-cell and cell-extracellular matrix interactions that includes involvement of E-cadherin, desmosomes and hemidesmosomes (Roche *et al.* 1993). Although at one time thought to be an artifact of sample handling (Ordonez *et al.* 2000), it is now clear in asthma that the epithelium is more fragile with easy loss of the columnar cells due to disruption of both tight junctions and desmosomal attachments (Montefort *et al.* 1992; Barbato *et al.* 2006; Shahana *et al.* 2006) (Fig. 78.6). This abnormality is also apparent in the epithelium of nasal polyps from asthmatics compared with those from nonasthmatic subjects (Shahana *et al.* 2005). Using differentiated epithelial cells in culture brushed from normal and asthmatic airways, it has been shown that the permeability (leakiness) of the asthmatic epithelium is greatly increased, leading to greater access of inhaled allergens, pollutants and other irritants to basal cells and the underlying airway tissue. This increase in epithelial permeability has also been observed

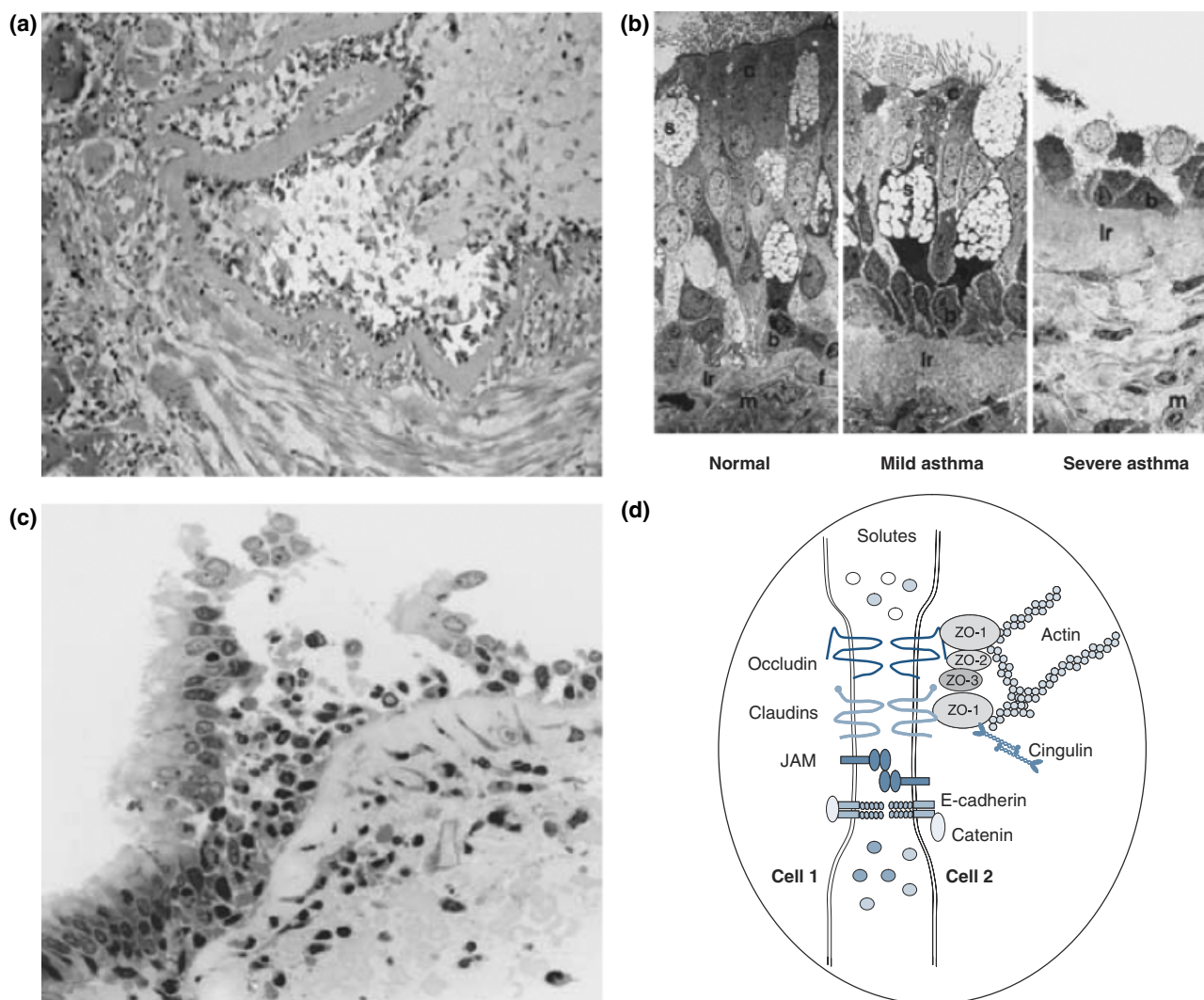


Fig. 78.6 Role of the epithelium in chronic asthma. (a) Extensive epithelial damage and mucus plugging of an airway from a patient who has died from asthma. (b) Progressive epithelial damage and disposition of matrix in asthma relative to disease severity. (c) Infiltration of the asthmatic epithelium with eosinophils associated with damage. (d) Molecular components of tight junctions that control intracellular permeability.

in vivo using inhaled radiolabeled impermanent probes, such as technetium labeled DTPA (Ilowitz *et al.* 1989). A reduction in the ability of the airway epithelium to exclude inhaled environmental agents may partly explain why certain atopic individuals go on to develop asthma whereas those with good barrier function do not. This loss of barrier function may reflect a broader abnormality in affecting other organs such as the skin, conjunctiva and gut that are foci for other atopic disorders (Hijazi *et al.* 2004; Liu *et al.* 2005; Hughes *et al.* 2006; Proksch *et al.* 2006).

A recent development has been the discovery of a number of genes encoded on chromosome 1q, including filaggrin and the S100 proteins, that are involved in

maintaining epithelial integrity in both the skin and the airways (Marshall *et al.* 2001) (Fig. 78.7). The association of genetic polymorphism of the pro-filaggrin gene on chromosome 1q13 with atopic dermatitis and asthma (Palmer *et al.* 2006; Ying *et al.* 2006) is of considerable interest and refocuses the possible origins of asthma more toward the epithelium and formed elements of the airways, rather than the immune response alone (Hudson 2006).

Another example of an environmental injury targeting the airway epithelium in asthma is the effect of respiratory virus infections. It has long been known that common cold viruses such as rhinovirus are associated with exacerbations of asthma in both children and adults

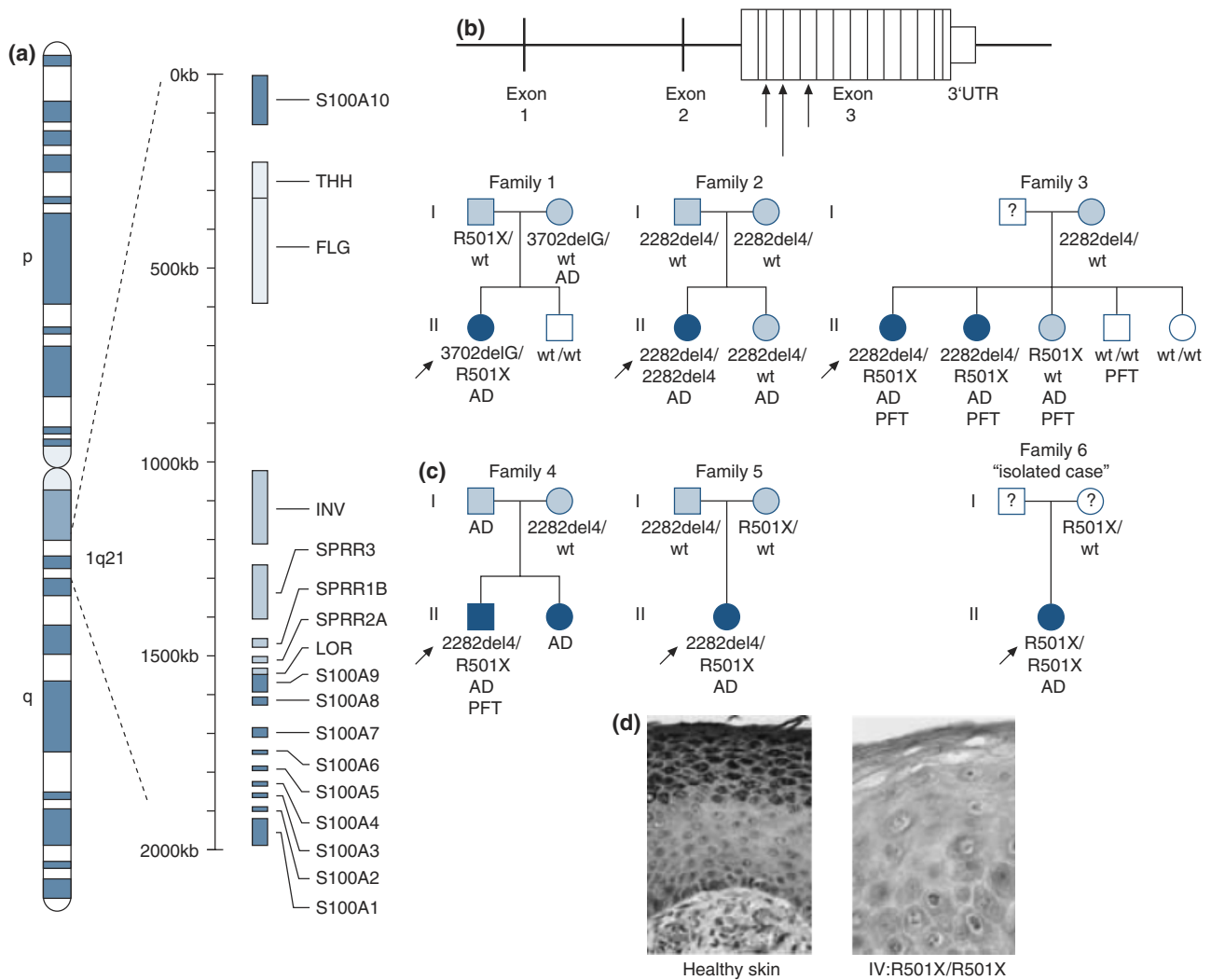


Fig. 78.7 Molecules involved in regulating epithelial integrity. (a) Gene cluster on chromosome 1q encoding a series of genes involved in maintaining epithelial integrity, including filaggrin (FLG), involucrin (INV), lorin (LOR), and the S100 proteins. (b) Genomic architecture of the filaggrin gene. The arrows denote single nucleotide polymorphisms in exon 3 associated with loss of function. (c) Six family pedigrees with ichthyosis vulgaris (homozygotes shown as symbols and heterozygotes as symbols); note the strong association with atopic dermatitis (AD). (d) Immunohistochemical localization of filaggrin to superficial layer of the skin and its absence in a patient homozygous for the R501X genotype of ichthyosis vulgaris. (Reproduced from Palmer *et al.* 2006, with permission).

(Johnston *et al.* 1995; Corne *et al.* 2002). Bronchial biopsy studies have shown that the airway epithelium is the preferential site for these viruses to enter the airway tissue (Papadopoulos *et al.* 2000). Infection of asthmatic epithelial cells compared with normal epithelial cells *in vitro* has revealed that the former lack the ability to generate IFN- β and IFN- λ , cytokines essential for eliminating viruses partly through induction of apoptosis (Wark *et al.* 2005; Contoli *et al.* 2006). In asthma the viruses continue to replicate until they kill the epithelial cells cytotoxically, leading to massive virus shedding and infection of adjacent cells, as well as the release of

mediators from the damaged cells. By adding IFN- β back into the cell cultures, resistance to rhinovirus infection is restored (Wark *et al.* 2005), suggesting that this may be a new approach to the prevention and treatment of acute asthma exacerbations (Holgate 2005).

A second type of injury that targets the epithelium is air pollution, asthma worsening at times of air pollution episodes. A number of studies have shown that the antioxidant defenses mounted by the airway epithelium in asthma are markedly reduced and are associated with reductions in superoxide dismutase (Comhair *et al.* 2005) and glutathione peroxidase (Qujeq *et al.* 2003; Misso &

Thompson 2005). In not being able to defend itself adequately against oxidant damage, the airway epithelium is damaged more easily (Rahman *et al.* 2006). The association of asthma with ozone and particle pollution episodes can be explained on this basis (Morrison *et al.* 2006).

A third example is the inflammatory and immune cascade initiated by activation of Toll-like receptors (TLRs). The recent discovery that a novel cytokine, thymic stromal lymphopoietin (TSLP), is generated by airway and skin epithelial cells following activation of TLR3, TLR6, TLR7, TLR8 and TLR9 and the capacity of this cytokine to generate Th2-polarized and mast cell activation responses places this cytokine in a unique position to orchestrate the airway inflammatory response of asthma (Ying *et al.* 2005; Liu 2006).

When damaged, the airway epithelium needs to repair but, as referred to above, the repair process is compromised in asthma. The airway epithelium enters into a chronic "wound scenario" with consequent production of a variety

of cytokines and growth factors in an attempt to repair the "wound" (Fig. 78.8). One group of growth factors central to epithelial repair and its altered phenotype in asthma is epidermal growth factor (EGF) and related molecules (HB-EGF, amphiregulin) (Puddicombe *et al.* 2000) that, by interacting through their tyrosine kinase receptors, promote repair. EGFR stimulation of the damaged epithelium also generates a mucus-secretory phenotype (Polosa *et al.* 2002; Hamilton *et al.* 2005; Casalino-Matsuda *et al.* 2006) and an altered inflammatory response involving neutrophils (Hamilton *et al.* 2003), which are all characteristic of more chronic and severe asthma. Other epithelial-derived factors include chemokines attracting neutrophils and other inflammatory cells, as well as a range of growth factors including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF)-1, FGF-2 and TGF- β that are active on fibroblasts and smooth muscle (Fig. 78.5). The secretion of latent TGF- β and its activation by proteolytic enzymes are fundamental to the subsequent

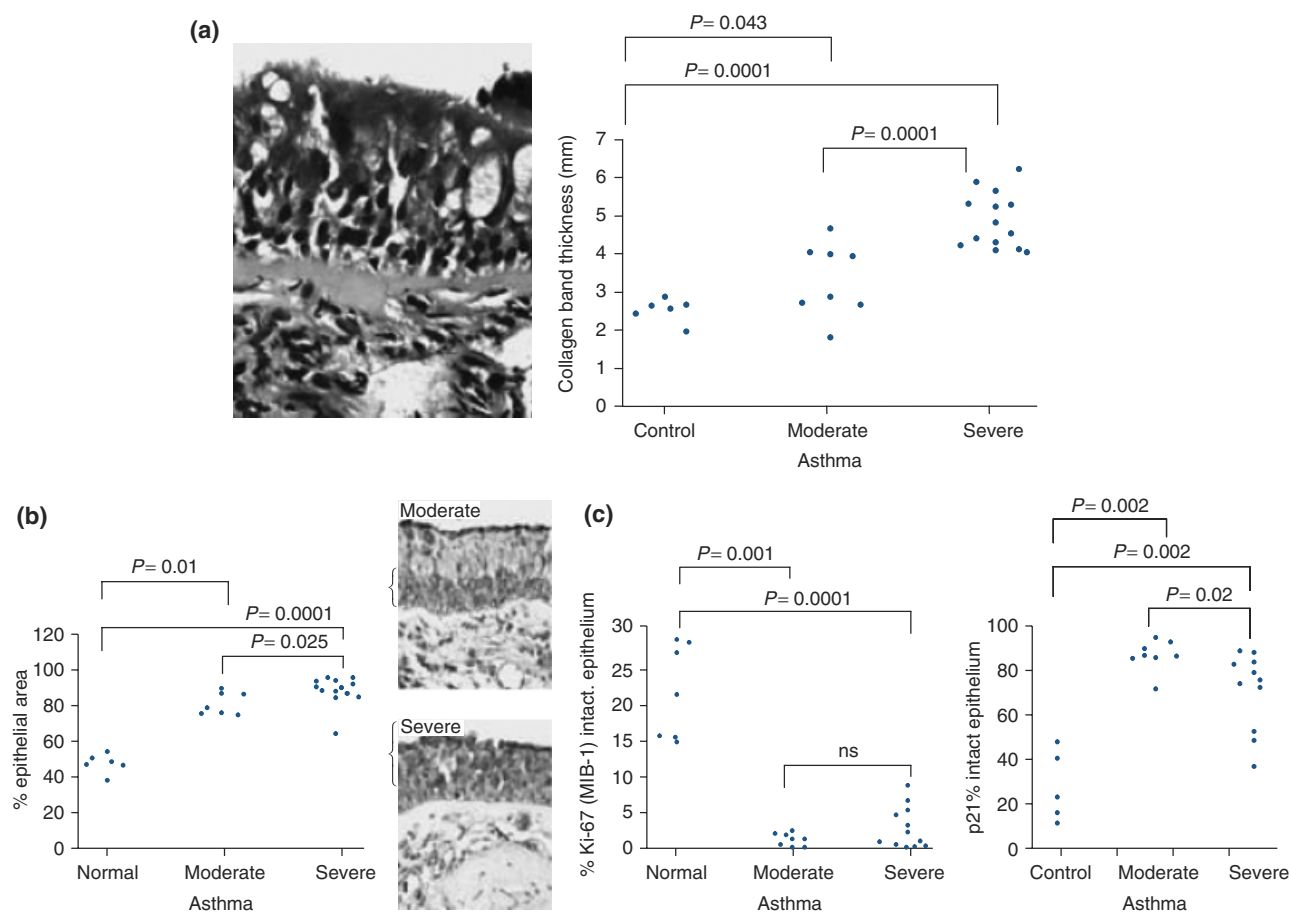


Fig. 78.8 Evidence for epithelial injury and impaired repair in childhood asthma. Bronchial biopsies in asthma show increased thickening of the lamina reticularis (a), increased expression of the epidermal growth factor receptor (b), reduced biomarkers of cell proliferation and increased nuclear translocation of the cell cycle inhibitor p21 (c), reproduced with permission of Fedorov *et al.* *Thorax* 2005.

airway remodeling that accompanies all but the most mild asthma (Boxall *et al.* 2006; Howell & McAnulty 2006). TGF- β itself is able to further impair epithelial repair responses while at the same time promoting the differentiation of fibroblasts into myofibroblasts (Leung *et al.* 2006). Bronchial biopsies from asthma have revealed that myofibroblasts are present in increased numbers in the subepithelial and submucosal region of asthmatic patients and increase in proportion to disease chronicity and severity (Choe *et al.* 2006; Wicks *et al.* 2006). The precise role of these cells in airway wall remodeling has yet to be proven, but it is highly likely that they do play a key role, both with respect to making good the damage caused to the epithelium through the deposition of matrix in the lamina reticulosa of the basement membrane and also as a result of interactions with recruited inflammatory cells such as eosinophils and mast cells, thereby helping sustain the chronic inflammatory response (Fig. 78.9).

The conducting airways contain an epithelium that is stratified, the upper layer containing a mixture of ciliated, goblet and Clara cells. In chronic asthma the

number of goblet cells that secrete viscous mucus increases, with a parallel reduction in ciliated cells. Since mucus production is fundamental to the pathogenesis of chronic asthma, this metaplastic change in the airway epithelium is of great importance, particularly since this has been shown to occur in the more peripheral airways which are normally devoid of goblet cells (Ordonez *et al.* 2001; Perez-Vilar 2006). The factors responsible for goblet cell metaplasia include the Th2 cytokines IL-4, IL-9 and IL-13 as well as TNF- α , which are all capable of directing differentiation to a mucus-secreting phenotype and interact by inducing EGFR-mediated goblet cell metaplasia through receptor transduction and induction of EGFR ligands such as HB-EGF and amphiregulin (Cohn 2006; Temann *et al.* 2007). In asthma it is mucin 5AC that dominates the mucin glycoproteins that are secreted and it is this mucin which is largely responsible for the unusual viscoelastic properties observed in asthmatic sputum that lead to difficulties in its expectoration (Morcillo & Cortijo 2006; Voynow *et al.* 2006). Recently IL-13 has been shown to regulate a chloride channel (GOB5; CLCA3), which is

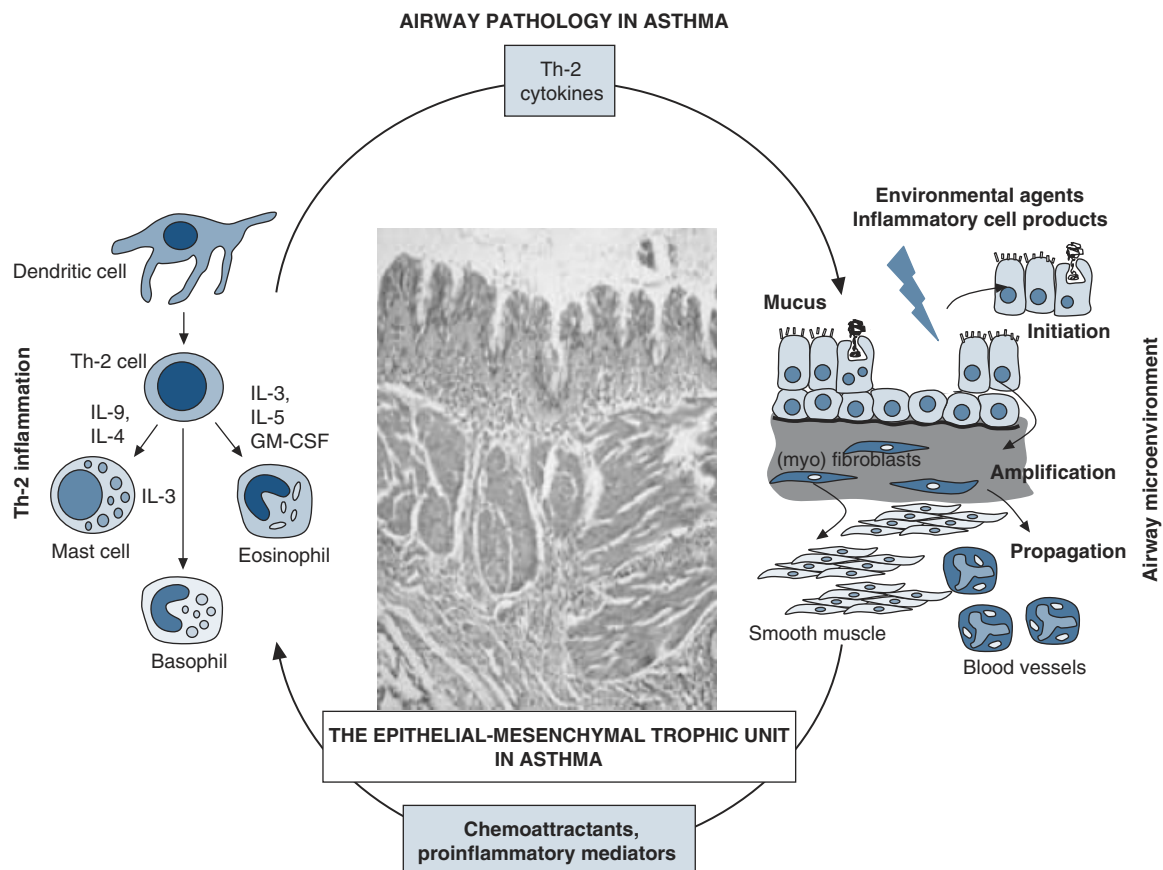


Fig. 78.9 Schematic representation of the interactions between airway inflammation and remodeling in clinical asthma involving the epithelial-mesenchymal trophic unit. (Reproduced with permission of Holgate *et al.* 2004).

intimately involved in the regulation and secretion of mucin from goblet cells (Thai *et al.* 2005; Long *et al.* 2006). However, the identification of multiple forms of GOB5 and its precise role in asthma has yet to be fully understood, though it does represent a therapeutic target.

One interesting idea that may partly account for the onset of asthma in genetically susceptible children is the fact that the airway epithelium is fundamentally abnormal both in its response to environmental injury and its repair. A recent study (COAST) in children born of asthmatic and atopic parents has shown that those who develop more persistent wheezing at the age of 4 years are those who had frequent symptomatic virus infections in early infancy, particularly rhinovirus (Lemanske *et al.* 2005). Impaired innate immunity, possible due to a genetic defect in interferon production in response to virus infection, may initiate the chronic injury–repair cycle associated with the onset of chronic disease. Longitudinal cohort studies have also demonstrated that while atopy failed to predict persistent wheezing up to the age of 5 years, beyond this age those who persistently wheeze are the atopic children whereas those who lose their wheezing (“grow out of” asthma) are the non-atopics (Illi *et al.* 2006) (Fig. 78.10). Together, these observations point to environmental factors operating independently at different stages during the evolution of asthma and again places the airway epithelium at the boundary where environmental interactions take place to generate the asthma phenotype.

AIRWAY WALL REMODELING

Although difficult to define functionally, from a structural standpoint there is ample evidence that, beyond airway inflammation, changes to the formed elements of the airway contribute significantly to the pathophysiol-

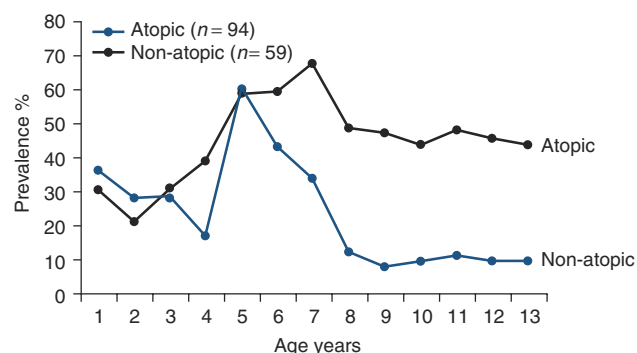


Fig. 78.10 Influence of atopy on the persistence of asthma after 5 years of age. (Reproduced from Illi *et al.* 2006).

ogy of asthma (James & Carroll 2000) (Figs 78.1 and 78.9). The most obvious change is in the airway smooth muscle, which not only increases in amount due to hypertrophy and hyperplasia, but also spreads both up and down the airways (Fig. 78.1). In asthma the increase in muscle and its altered function most likely underlies an important component of hyperresponsiveness that characterizes this disease. However, intensive studies have tried to identify cell and molecular abnormalities in the airway smooth muscle that may account for its abnormal behavior in this disease, but as yet there are no clear mechanisms that explain this (James 2005). What has emerged is that in chronic asthma the airways become thickened, not only due to an increase in airway smooth muscle, but also as a consequence of the laying down of new matrix proteins including collagen fibers, increased proliferation of microvessels along with vascular leakage and deposition of proteoglycans, with their ability to sequester water. In both children and adults, high-resolution computed tomography (HRCT) has revealed an association between airway wall thickness and disease chronicity and severity that can best be explained on the basis of remodeling (Bumbacea *et al.* 2004; Jain *et al.* 2005). Paradoxically, however, airway wall thickness is inversely correlated with airway hyperresponsiveness, suggesting that this thickening with deposition of matrix proteins may be a protective response against frequent smooth muscle contraction (Shaw *et al.* 2004). Thus in patients who are highly hyperreactive with brittle asthma, the airway remodeling response is minimal, whereas in those with chronic disease who exhibit some degree of fixed airflow obstruction, remodeling is more prominent (Paganin *et al.* 1996). Remodeling as a series of interacting processes is complex. For example, while immunostaining for proteoglycans such as biglycan, lumican, versican and decorin is increased in both moderate and severe asthma, with no differences in the amount present in the subepithelial layer, deposition of biglycan and lumican is significantly greater in the smooth muscle in moderate compared with severe asthma, suggesting a compensatory role (Pini *et al.* 2007).

One molecule that has emerged as involved in airway remodeling is ADAM33. This susceptibility gene for asthma was first identified by positional cloning (Van Eerdewegh *et al.* 2002) and has been replicated in a number of studies (Blakey *et al.* 2005). In being preferentially expressed in airway mesenchymal cells (fibroblasts and smooth muscle), it has been shown to be involved in the pathogenesis of airway hyperresponsiveness and decline in lung function over time (Jongepier

et al. 2004; van Diemen *et al.* 2005). ADAM33 has metalloproteinase, fusogenic, adhesion and intracellular signaling activities and exist in at least six alternatively spliced isoforms (Van Eerdewegh *et al.* 2002). Although the full-length molecule (120 kDa) is expressed as a transmembrane protein, a soluble form (~55 kDa) has also recently been identified whose levels increase in proportion to disease severity (Lee *et al.* 2006; Foley *et al.* 2007). Of the many potential biological actions that ADAM33 possesses, its proteolytic activity, which is also present in the soluble form, is likely to be important in generating growth factors that influence mesenchymal cell number and or maturation (Holgate *et al.* 2006).

Persistent airway inflammation may be an important factor that contributes to airway wall remodeling, including the secretion of mediators and growth factors such as TGF- β 1 from eosinophils. Kariyawasam *et al.* (2007) have recently shown that, in those with both early and late asthmatic responses to allergen challenge, markers of airway wall remodeling such as tenascin, procollagens 1 and 3, HSP-47 and α -smooth muscle actin (marker of myofibroblasts) were all elevated beyond the 7-day time point when the inflammatory response had resolved. However, as discussed above, epithelial injury, impaired repair, and the secretion of growth factors from this structure may also contribute to the ongoing repair response characteristic of remodeling. Since these changes have been described in the absence of eosinophil infiltration, it may be that epithelial injury and remodeling is a necessary precursor to the onset of asthma, the altered microenvironment in the airway then providing the opportunity for inhaled environmental insults to gain access and generate the characteristic chronic inflammatory response involving atopy. The various growth factors involved in airway wall remodeling are similar to those involved in branching morphogenesis of the lung in the developing fetus (Bousquet *et al.* 2004; Holgate *et al.* 2006) and this has led to use of the term "epithelial mesenchymal trophic unit" to describe the interaction between the epithelium and the underlying mesenchymal cells in generating this altered structural response in chronic disease (Holgate *et al.* 2001; Knight *et al.* 2004) (Fig. 78.9). Regarding asthma not only as an inflammatory disease but also one in which there is abnormal signaling between the epithelium and mesenchymal cells has created an opportunity to search for novel therapeutics that act on this aspect of the disease. If epithelial injury and impaired repair are important as pathophysiologic mechanisms, then new treatments targeted to these pathways will represent a

real advance. When applied to a damaged asthmatic epithelium *in vitro*, both EGF and keratinocyte growth factor are able to restore full barrier function as well reestablishing the protection of the epithelium against environmental injuries (Berlanga *et al.* 2002; Basuroy *et al.* 2006). Clinical trials are also currently in progress to investigate the effect of surfactant as a potential "barrier treatment" for chronic asthma, with the idea that by excluding environmental agents from penetrating the airway wall, continued aggravation of the underlying airway inflammation may be reduced (Babu *et al.* 2003).

The relative importance of airway inflammation and epithelial mesenchymal signaling in asthma pathogenesis has recently been highlighted by three studies that have demonstrated that inhaled corticosteroids, when administered to children born of asthmatic and atopic parents for 1–4 years, has no effect on the natural history of asthma (Bisgaard *et al.* 2006; Guilbert *et al.* 2006; Murray *et al.* 2006). Two previous trials of inhaled corticosteroids administered over prolonged periods to older children also failed to influence the course of the disease (Childhood Asthma Management Program Research Group 2000; Pauwels *et al.* 2003). Thus, if airway inflammation (and by implication Th2 driven-immunity) was the *cause* of the asthmatic process and the airway dysfunction that is associated with it, then its prevention at the time of disease onset should influence the natural cause, whereas in these trials it did not.

VASCULAR REMODELING

One aspect of airway remodeling that is frequently neglected is the role of the vasculature. In both adults and children recent biopsy studies have demonstrated a large increase in the number of microvessels present in the airways of patients with chronic asthma, with some evidence that the endothelium of these vessels is undergoing proliferation (Vrugt *et al.* 2000; Wilson & Robertson 2002; Barbato *et al.* 2006). Vascular remodeling has also been shown to occur in a chronic allergen exposure rat model (Tigani *et al.* 2006). Since microvascular leakage as well as recruitment of inflammatory cells is fundamental to asthma pathogenesis, a greater understanding of the cellular and molecular mechanisms involved in new vessel formation in the airways is important. The discovery of vascular endothelial growth factor (VEGF) in the damaged airway epithelium in asthma as an important mediator contributing to microvascular leak, proliferation, and vascular remodeling is being pursued with much interest (Chetta *et al.* 2005;

Abdel-Rahman *et al.* 2006; Bhandari *et al.* 2006). An additional mediator that has recently been discovered in association with the T-cell-dependent component of the allergen-induced late asthmatic response is calcitonin gene-related peptide, a potent vasodilator (Kay *et al.* 2007). It is likely that, as with fibroblasts and smooth muscle, this aspect of the remodeling response involves interaction of a wide range of different mediators including factors that act simultaneously on all three cell types (e.g., TGF- β).

NEURAL REMODELING

An increase in neural networks in the asthmatic airway is also key to disease pathogenesis. Nerve growth factors or neurotrophins are clearly of importance in promoting the altered neural regulation that undoubtedly occurs in asthma (Nassenstein *et al.* 2006) but the precise mechanisms have yet to be defined. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) as well as other members of this family are produced by epithelial cells and smooth muscle as well as a range of inflammatory cells including mast cells and eosinophils (Olgart *et al.* 2002). Circulating levels of NGF correlate with asthma severity (Bonini *et al.* 1996). The neurotrophins activate two receptor subtypes: TrkA (tropomyosin receptor kinase A) and p75 NTR (neurotrophin receptor) in the death receptor family (Frossard *et al.* 2004). The neurotrophins not only promote neural growth and maturation but are also important mast cell growth factors, enhance eosinophil survival, and augment human airway smooth muscle responsiveness (Kassel *et al.* 2001; Nassenstein *et al.* 2003; Frossard *et al.* 2005; Hahn *et al.* 2006) and therefore have the capacity to enhance asthmatic airway inflammation (Quarcoo *et al.* 2004). Bronchoconstriction provoked by irritants such as sulfur dioxide and ozone are also likely to operate via this abnormal neural network, with the release of acetylcholine and neuropeptides that have multiple effects on inflammatory cells, smooth muscle, blood vessels, and mucus-secreting cells. Although neuropeptide antagonists have so far been disappointing in asthma, the pleiotropic effects of the neurotrophins and their receptors make them more attractive therapeutic targets (de Vries *et al.* 2006; Watson *et al.* 2006).

HETEROGENITY OF ASTHMA

Asthma is a heterogeneous disorder (Wenzel 2006). Although different types of asthma have long been re-

cognized, such as that triggered by exposure to allergens, occupational chemicals, nonsteroidal antiinflammatory drugs (NSAIDs) and nonallergic "intrinsic" asthma, there has never been a cellular or molecular basis for these different manifestations of intermittent airflow obstruction other than exposure to a particular environmental stimulus. With the advent of fiberoptic bronchoscopy and the ability to biopsy different parts of the bronchial tree, noninvasive lung imaging such as HRCT and the development of therapeutics that target single cells or pathways, it is now clear that asthma is not a single disease but a wide range of different disorders that share some common phenotypes such as reversible airflow obstruction and associated symptoms. For the last 50 years we have only had corticosteroids, β_2 -adrenoceptor agonists and other bronchodilators (such as xanthines), and cromone-like drugs to treat asthma and this has led to the reinforcement of asthma as a "single disease." Using noninvasive markers of airway inflammation suggests the presence of at least four distinct "phenotypes": eosinophilic, neutrophilic, mixed inflammatory, and paucigranulocytic asthma (Wenzel 2006).

Eosinophilic asthma

Bronchial biopsy studies of patients with mild asthma have shown that eosinophilic airway inflammation is highly characteristic regardless of whether patients were atopic, nonatopic, aspirin-sensitive, or had occupational asthma (Brightling *et al.* 2003; Bochner & Busse 2005). A broad correlation between clinical asthma severity and the degree of airway eosinophilia has been recorded, especially when eosinophils appear to be activated. With the advent of sputum induction it appears that there is a complex relationship between eosinophilic inflammation and other markers of asthma, including lung function and airway hyperresponsiveness. In contrast eosinophilic airway inflammation appears to be much more closely related to the risk of severe asthma exacerbation (Green *et al.* 2002). This particular phenotype would be worth investigating for a potential therapeutic effect of anti-IL-5 therapy which, as referred to earlier, has failed to modify baseline asthma even though it has modifying effects on indices of remodeling.

Noneosinophilic asthma

The use of induced sputum as well as lavage and fiberoptic bronchoscopy has revealed that some patients with asthma have a sputum neutrophilia in the absence

of eosinophils (Wenzel *et al.* 1999; Tsoumakidou *et al.* 2006). Other studies have noted neutrophilic inflammation in some patients with severe asthma and during virus-induced exacerbations (Wark *et al.* 2002). In addition, patients with severe asthma treated with oral (but not inhaled) corticosteroids also exhibit a predominantly neutrophilic airway inflammation and absence of eosinophils (Nguyen *et al.* 2005). Intense neutrophilic inflammation has also been reported in patients ventilated for acute severe asthma (Tonnel *et al.* 2001) and in those who died suddenly of asthma (Carroll *et al.* 1996). In general, asthma associated with neutrophils tends to be a more aggressive disease possibly with more tissue destruction and airway remodeling (Holgate & Polosa 2006). Intervention with the anti-TNF p75 fusion protein etanercept over a period of 10–12 weeks has shown clinical benefit in such patients, suggesting that as the disease becomes more chronic and severe the inflammatory phenotype changes from Th2 more toward a Th1 type (Kumar *et al.* 2006) (Fig. 78.11).

Neutrophilic and mixed eosinophilic and neutrophilic asthma has also been associated with *Mycoplasma pneumoniae* and/or *Chlamydia pneumoniae* infection, with a beneficial response to macrolide antimicrobials (Johnston *et al.* 2006; Kraft & Hamid 2006). Whether the

chronic bacterial infection is primary or secondary to the underlying asthmatic response in such patients is not known, but since asthmatic airway epithelial cells are known to be deficient in their ability to mount a primary interferon response following infection with common respiratory viruses such as rhinoviruses (Wark *et al.* 2005; Contoli *et al.* 2006), it is possible that defective innate immunity may be fundamental to the origins and progression of chronic disease. Indeed, longitudinal cohort studies have shown that children with early onset and persistent wheezing in the first 3–5 years of life and classified as severe childhood asthma tend to maintain this phenotype throughout life (Stein & Martinez 2004) and it is these individuals who appear to have increased susceptibility to virus infection early in life (Lemanske *et al.* 2005) and possibly are those who are also susceptible to chronic bacterial infection (Chaudhuri *et al.* 2005, 2006; Singh *et al.* 2007).

Tobacco smoking is also associated with a greater neutrophil component and, importantly, corticosteroid refractoriness in both the airways (Chaudhuri *et al.* 2003; Tomlinson *et al.* 2005) and systemically (Livingston *et al.* 2007). One possible explanation for this is the effect of smoking and oxidative stress in reducing histone

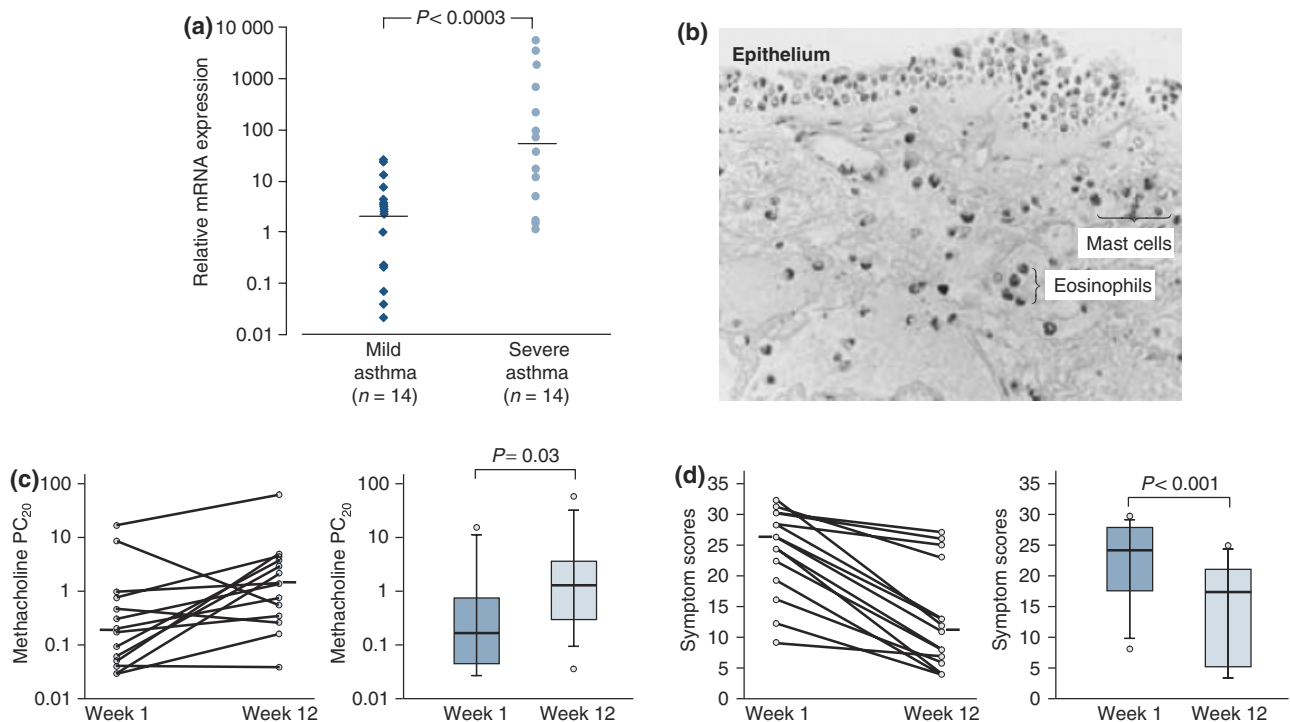


Fig. 78.11 Role of tumor necrosis factor (TNF)- α in severe asthma. (a) Relative expression of TNF- α mRNA in bronchial biopsies from patients with mild and severe corticosteroid-refractory asthma. (b) Immunohistochemical staining for TNF- α in severe asthmatic biopsy. (c) Effect of etanercept twice weekly for 12 weeks on methacholine airway responsiveness. (d) Effect of etanercept on asthma symptoms in severe asthma. (Reproduced with permission of Howarth *et al.* 2005).

deacetylase activity in the nuclear chromatin, thereby diminishing the opportunity for corticosteroids to access antiinflammatory genes (Adcock *et al.* 2005).

Paucigranulocytic asthma

Although sudden asthma death has been recorded in the absence of airway inflammation, this is highly unusual. Asthma in the absence of either neutrophils or eosinophils (paucigranulocytic asthma) has been described in which MMP-9 levels in sputum disease were normal (as opposed to elevated levels in patients with eosinophilic asthma; Simpson *et al.* 2005), suggesting that an abnormal epithelium or underlying mesenchyme and/or smooth muscle may itself lead to an asthma phenotype without the presence of obvious inflammation.

Refractory asthma as a distinct inflammatory phenotype

Much recent attention has focused on patients with chronic asthma taking high doses of inhaled and oral corticosteroids, long-acting β_2 -adrenoceptor agonists, and other antiasthma therapies and yet who remain symptomatic. This refractory asthmatic phenotype is associated with upregulation of the TNF- α pathway with increased expression of membranebound TNF- α , TNF-receptor 1, and TNF- α converting enzyme by peripheral blood monocytes (Berry *et al.* 2005). This is consistent with the finding of increased mucosal TNF- α expression in severe asthma compared with patients with mild disease (Howarth *et al.* 2005; Silvestri *et al.* 2006) and the identification of a phenotype that is responsive to anti-TNF therapy (Russo & Polosa 2005) (Fig. 78.8).

A range of abnormalities has been described that are said to be associated with corticosteroid-refractory asthma including defects in nuclear histone acetylation (Barnes 2006), overexpression of the β -isoform of the corticosteroid receptor serving a dominant negative function in the presence of corticosteroids (Lewis-Tuffin & Cidlowski 2006), a defect in vitamin D signaling (Xystrakis *et al.* 2006), and increased airway wall remodeling leading to a degree of fixed airflow obstruction (Bai & Knight 2005).

A NEW APPROACH TO CATEGORIZING ASTHMA SUBPHENOTYPES

Recognizing that asthma is a complex disease involving varying components of airway inflammation, hyperresponsiveness, variable airflow obstruction, and a range

of symptoms it has been possible to apply statistical methods in an attempt to dissect this complexity into subphenotypes within a population of patients with severe asthma (Wardlaw *et al.* 2005). Cluster analysis is one such statistical tool that seeks to organize information about variables so that heterogeneous groups of subjects can be classified into relatively homogeneous "clusters." Haldar *et al.* (2005) have used this technique of cluster analysis to identify subphenotypes within a population of patients with severe disease. In an analysis of over 270 patients with severe asthma they identified four distinct phenotypes.

- 1 Patients with relatively well-controlled symptoms and minimal airway inflammation.
- 2 A group with early-onset atopic asthma with severe symptoms, persistent airway inflammation, and markedly variable airflow obstruction.
- 3 A group of mainly females who have late-onset asthma with marked symptoms but minimal eosinophilic inflammation, many of whom are obese.
- 4 A group with male predominance who have late-onset asthma characterized by persistent eosinophilic inflammation in the absence of symptoms.

It may be that these different subphenotypes have therapeutic implications but clearly much larger studies are needed to answer this question.

ASTHMA: A DISORDER INVOLVING VARIABLE AIRWAY INFLAMMATION AND REMODELING

As we learn more about asthma subphenotypes and the immunopathology of asthma it is becoming increasingly apparent that the disorder is a spectrum of pathophysiologic processes that to some degree map onto the different therapeutic responses (Fig. 78.9). At the mildest end of the spectrum, i.e., allergic asthma associated with other allergic comorbidities such as rhinitis and atopic dermatitis, the disease is dominated by Th2-mediated inflammatory responses that are usually (but not always) responsive to corticosteroids. Also in this category is intermittent asthma associated with seasonal changes in aeroallergens, which in addition to being responsive to inhaled corticosteroids may also benefit from allergen-specific immunotherapy. In patients with anything more than mild disease evidence of altered epithelial-mesenchymal communication is found that might explain the more chronic nature of asthma as it evolves from childhood into adult disease and also its variable nature in response to a variety of environmental factors

in addition to allergens such as air pollutants, infectious agents and certain drugs. Alterations in the epithelium or underlying mesenchyme leading to the generation of growth factors and cytokines that sustain inflammation can be thought of as generating a “fertile soil” in which the “seeds” of inflammation are able to take hold and persist (Davies *et al.* 2003; Holgate *et al.* 2004). The third type of asthma is one in which the epithelial–mesenchymal component becomes dominant, leading to progressive airflow obstruction, a component of which is irreversible with β_2 -adrenoceptor agonists and corticosteroids (James *et al.* 2005; Bergeron & Boulet 2006). It is in these patients that tissue damage and remodeling becomes prominent along with increased mucus secretion and the “plugging” of peripheral airways. Superimposed on these three broad pathophysiologic phenotypes are a range of environmental factors that individuals with asthma will respond to. Thus, asthma associated with NSAIDs is characterized by increased cysteinyl leukotriene production and in general a more beneficial response to antileukotriene therapy (Kumlin *et al.* 1992; Dahlen *et al.* 2002). Patients with severe allergic asthma in which IgE plays a predominant role in driving the disease are responsive to the IgE-blocking monoclonal antibody omalizumab, with attendant reductions in IgE-associated airway inflammation (Holgate *et al.* 2005). The more aggressive disease asso-

ciated with increasing neutrophilic inflammation may respond to macrolide antimicrobials, but it is in this group that anti-TNF therapy may be beneficial. Additional factors that can influence the clinical expression of asthma include female hormone status (Beck 2001; Stanford *et al.* 2006), obesity (Appleton *et al.* 2006; McLachlan *et al.* 2006), infection with *Aspergillus* (bronchopulmonary allergic aspergillosis) (Gibson 2006), and in rare cases pulmonary vasculitis (Churg–Strauss syndrome) (Keogh & Specks 2006).

IDENTIFICATION OF NOVEL MECHANISMS THROUGH GENETICS

With increased knowledge asthma appears more and more complex in its pathophysiology, although genetic studies are beginning to reveal molecules and pathways that may underlie the origins of this disease (Holloway & Holgate 2004; Meurer *et al.* 2006). It is of particular interest that the majority of the novel genes identified through positional cloning that increase susceptibility to asthma are preferentially expressed in either the epithelium or the mesenchyme and smooth muscle rather than the immune or inflammatory pathways (Cookson 2004; Vendelin *et al.* 2005; Holgate *et al.* 2006) (Fig. 78.12). It is also of note that many of the molecules that appear to be

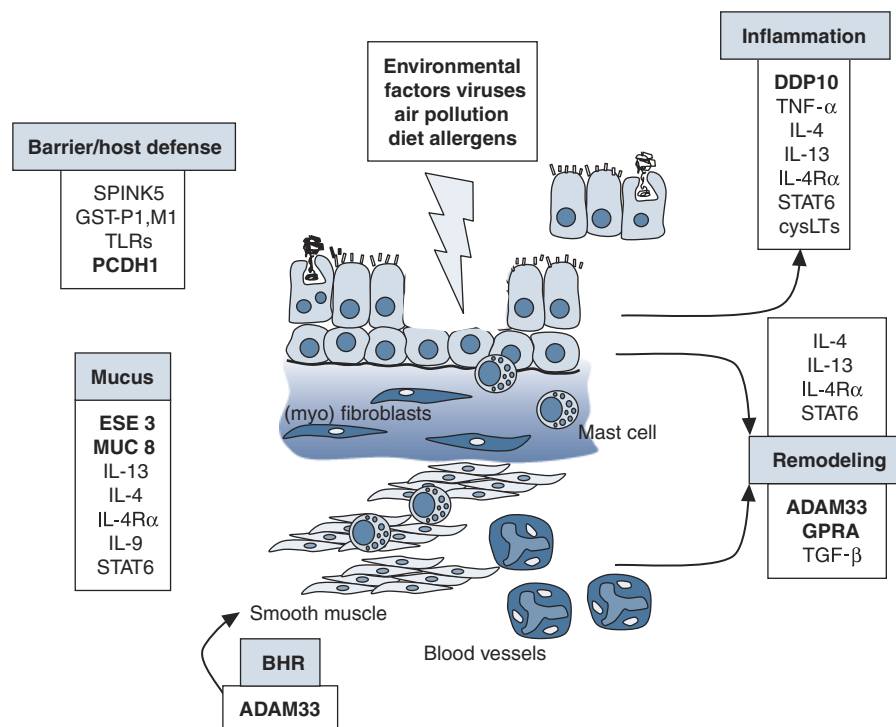


Fig. 78.12 Asthma susceptibility genes expressed in the epithelium and mesenchyme.

involved in disordered epithelial–mesenchymal signaling in asthma are also utilized in fetal branching morphogenesis of the lung, suggesting that asthma at least in some of its manifestations has morphogenetic origins (Vendelin *et al.* 2005; Holgate *et al.* 2006). Babies whose airways are unable to adequately resist the early-life environmental insults such as environmental tobacco smoke, air pollutants, infections and allergens may be the children who go on to develop asthma, not because he or she has any fundamental abnormality in their adaptive immune system, but because their airways are inadequately equipped to defend themselves against environmental insults (Eder *et al.* 2006). Thus, in planning new therapeutic strategies it is interesting to speculate whether a novel approach to asthma might be to increase the intrinsic resistance of the airways to the environment rather than concentrating all our efforts in suppressing inflammation or manipulating the immune response.

CONCLUDING COMMENTS

The recognition that asthma is a highly heterogeneous disorder in terms of associated environmental factors,

clinical expression, response to different therapies, and natural history has opened up the disease beyond allergic sensitization. This has important implications, moving us back to considering asthma as a disease whose origins lie in the airways. Human genetics linked to environmental epidemiology through epigenetics is revealing entirely novel mechanisms and approaches to treatment. The application of cell and molecular biology to different phenotypes of asthma at different points in their natural history will eventually reveal why atopy in some individuals evolves into asthma while in others it does not. An important aspect of this relates to factors that down-regulate immune and inflammatory reactions such as regulatory T cells. These are considered in detail in Chapter 4.

Although at one time asthma was considered a relatively simple disease, treated with a limited range of drugs, it is now realized that all of these suppress rather than cure the disease. What is now needed is a concerted effort to understand why certain people develop asthma and others do not. It is only with this increased understanding that a real chance of cure and prevention will occur.

REFERENCES

- Abdel-Rahman, A.M., el Sahrigy, S.A. & Bakr, S.I. (2006) A comparative study of two angiogenic factors: vascular endothelial growth factor and angiogenin in induced sputum from asthmatic children in acute attack. *Chest* **129**, 266–71.
- Adcock, I.M., Ito, K. & Barnes, P.J. (2005) Histone deacetylation: an important mechanism in inflammatory lung diseases. *COPD* **2**, 445–55.
- Agis, H., Krauth, M.T., Bohm, A. *et al.* (2006) Identification of basogranulin (BB1) as a novel immunohistochemical marker of basophils in normal bone marrow and patients with myeloproliferative disorders. *Am J Clin Pathol* **125**, 273–81.
- Akbari, O., Faul, J.L., Hoyte, E.G. *et al.* (2006) CD4⁺ invariant T-cell-receptor⁺ natural killer T cells in bronchial asthma. *N Engl J Med* **354**, 1117–29.
- Al Muhsen, S.Z., Shablovsky, G., Olivenstein, R., Mazer, B. & Hamid, Q. (2004) The expression of stem cell factor and c-kit receptor in human asthmatic airways. *Clin Exp Allergy* **34**, 911–16.
- Anderson, G.P. (2002) The immunobiology of early asthma. *Med J Aust* **177** (suppl.):S47–S49.
- Appleton, S.L., Adams, R.J., Wilson, D.H., Taylor, A.W. & Ruffin, R.E. (2006) Central obesity is associated with nonatopic but not atopic asthma in a representative population sample. *J Allergy Clin Immunol* **118**, 1284–91.
- Babu, K.S., Woodcock, D.A., Smith, S.E., Staniforth, J.N., Holgate, S.T. & Conway, J.H. (2003) Inhaled synthetic surfactant abolishes the early allergen-induced response in asthma. *Eur Respir J* **21**, 1046–9.
- Bai, T.R. & Knight, D.A. (2005) Structural changes in the airways in asthma: observations and consequences. *Clin Sci* **108**, 463–77.
- Barbato, A., Turato, G., Baraldo, S. *et al.* (2006) Epithelial damage and angiogenesis in the airways of children with asthma. *Am J Respir Crit Care Med* **174**, 975–81.
- Barnes, P.J. (2006) How corticosteroids control inflammation: Quintiles Prize Lecture 2005. *Br J Pharmacol* **148**, 245–54.
- Basuroy, S., Seth, A., Elias, B., Naren, A.P. & Rao, R. (2006) MAPK interacts with occludin and mediates EGF-induced prevention of tight junction disruption by hydrogen peroxide. *Biochem J* **393**, 69–77.
- Beasley, R., Pekkanen, J. & Pearce, N. (2001) Has the role of atopy in the development of asthma been over-emphasized? *Pediatr Pulmonol Suppl.* **23**, 149–50.
- Beck, S.A. (2001) Asthma in the female: hormonal effect and pregnancy. *Allergy Asthma Proc* **22**, 1–4.
- Berger, P., Perng, D.W., Thabrew, H. *et al.* (2001) Tryptase and agonists of PAR-2 induce the proliferation of human airway smooth muscle cells. *J Appl Physiol* **91**, 1372–9.
- Bergeron, C. & Boulet, L.P. (2006) Structural changes in airway diseases: characteristics, mechanisms, consequences, and pharmacologic modulation. *Chest* **129**, 1068–87.
- Berlanga, J., Lodos, J. & Lopez-Saura, P. (2002) Attenuation of internal organ damages by exogenously administered epidermal growth factor (EGF) in burned rodents. *Burns* **28**, 435–42.

- Berry, M.A., Hargadon, B., Shelley, M. *et al.* (2006) Evidence of a role for TNF α in refractory asthma. *N Engl J Med* **354**, 697.
- Bhandari, V., Choo-Wing, R., Chapoval, S.P. *et al.* (2006) Essential role of nitric oxide in VEGF-induced, asthma-like angiogenic, inflammatory, mucus, and physiologic responses in the lung. *Proc Natl Acad Sci USA* **103**, 11021–6.
- Bharadwaj, A. & Agrawal, D.K. (2004) Immunomodulation in asthma: a distant dream or a close reality? *Int Immunopharmacol* **4**, 495–511.
- Bisgaard, H., Hermansen, M.N., Loland, L., Halkjaer, L.B. & Buchvald, F. (2006) Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med* **354**, 1998–2005.
- Blakey, J., Halapi, E., Bjornsdottir, U.S. *et al.* (2005) The contribution of ADAM 33 polymorphisms to the population risk of asthma. *Thorax* **60**, 274–6.
- Bochner, B.S. & Busse, W.W. (2005) Allergy and asthma. *J Allergy Clin Immunol* **115**, 953–9.
- Bonini, S., Lambiase, A., Bonini, S. *et al.* (1996) Circulating nerve growth factor levels are increased in humans with allergic diseases and asthma. *Proc Natl Acad Sci USA* **93**, 10955–60.
- Bousquet, J., Jacot, W., Yssel, H., Vignola, A.M. & Humbert, M. (2004) Epigenetic inheritance of fetal genes in allergic asthma. *Allergy* **59**, 138–47.
- Boxall, C., Holgate, S.T. & Davies, D.E. (2006) The contribution of transforming growth factor- β and epidermal growth factor signalling to airway remodelling in chronic asthma. *Eur Respir J* **27**, 208–29.
- Bradding, P., Okayama, Y., Howarth, P.H., Church, M.K. & Holgate, S.T. (1995) Heterogeneity of human mast cells based on cytokine content. *J Immunol* **155**, 297–307.
- Bradding, P., Walls, A.F. & Holgate, S.T. (2006) The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* **117**, 1277–84.
- Brightling, C.E., Bradding, P., Symon, F.A., Holgate, S.T., Wardlaw, A.J. & Pavord, I.D. (2002) Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* **346**, 1699–705.
- Brightling, C.E., Symon, F.A., Birring, S.S., Bradding, P., Wardlaw, A.J. & Pavord, I.D. (2003) Comparison of airway immunopathology of eosinophilic bronchitis and asthma. *Thorax* **58**, 528–32.
- Buccheri, F., Puddicombe, S.M., Lordan, J.L. *et al.* (2002) Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. *Am J Respir Cell Mol Biol* **27**, 179–85.
- Bumbacea, D., Campbell, D., Nguyen, L. *et al.* (2004) Parameters associated with persistent airflow obstruction in chronic severe asthma. *Eur Respir J* **24**, 122–8.
- Burgess, J.K., Blake, A.E., Boustany, S. *et al.* (2005) CD40 and OX40 ligand are increased on stimulated asthmatic airway smooth muscle. *J Allergy Clin Immunol* **115**, 302–8.
- Cairns, J.A. (2005) Inhibitors of mast cell tryptase β as therapeutics for the treatment of asthma and inflammatory disorders. *Pulm Pharmacol Ther* **18**, 55–66.
- Carroll, N., Carello, S., Cooke, C. & James, A. (1996) Airway structure and inflammatory cells in fatal attacks of asthma. *Eur Respir J* **9**, 709–15.
- Casalino-Matsuda, S.M., Monzon, M.E. & Forteza, R.M. (2006) Epidermal growth factor receptor activation by epidermal growth factor mediates oxidant-induced goblet cell metaplasia in human airway epithelium. *Am J Respir Cell Mol Biol* **34**, 581–91.
- Chaudhuri, R., Livingston, E., McMahon, A.D., Thomson, L., Borland, W. & Thomson, N.C. (2003) Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med* **168**, 1308–11.
- Chaudhuri, R., McMahon, A.D., McSharry, C.P. *et al.* (2005) Serum and sputum neurotrophin levels in chronic persistent cough. *Clin Exp Allergy* **35**, 949–53.
- Chaudhuri, R., Livingston, E., McMahon, A.D. *et al.* (2006) Effects of smoking cessation on lung function and airway inflammation in smokers with asthma. *Am J Respir Crit Care Med* **174**, 127–33.
- Chetta, A., Zanini, A., Foresi, A. *et al.* (2005) Vascular endothelial growth factor up-regulation and bronchial wall remodeling in asthma. *Clin Exp Allergy* **35**, 1437–42.
- Childhood Asthma Management Program Research Group (2000) Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med* **343**, 1054–63.
- Choe, M.M., Sporn, P.H. & Swartz, M.A. (2006) Extracellular matrix remodeling by dynamic strain in a three-dimensional tissue-engineered human airway wall model. *Am J Respir Cell Mol Biol* **35**, 306–13.
- Cohn, L. (2006) Mucus in chronic airway diseases: sorting out the sticky details. *J Clin Invest* **116**, 306–8.
- Comhair, S.A., Xu, W., Ghosh, S. *et al.* (2005) Superoxide dismutase inactivation in pathophysiology of asthmatic airway remodeling and reactivity. *Am J Pathol* **166**, 663–74.
- Contoli, M., Message, S.D., Laza-Stanca, V. *et al.* (2006) Role of deficient type III interferon- λ production in asthma exacerbations. *Nat Med* **12**, 1023–6.
- Cookson, W. (2004) The immunogenetics of asthma and eczema: a new focus on the epithelium. *Nat Rev Immunol* **4**, 978–88.
- Corne, J.M., Marshall, C., Smith, S. *et al.* (2002) Frequency, severity, and duration of rhinovirus infections in asthmatic and nonasthmatic individuals: a longitudinal cohort study. *Lancet* **359**, 831–4.
- Corrigan, C. (2004) Mechanisms of intrinsic asthma. *Curr Opin Allergy Clin Immunol* **4**, 53–6.
- Dahlen, B., Shute, J. & Howarth, P. (1999) Immunohistochemical localisation of the matrix metalloproteinases MMP-3 and MMP-9 within the airways in asthma. *Thorax* **54**, 590–6.
- Dahlen, S.E., Malmstrom, K., Nizankowska, E. *et al.* (2002) Improvement of aspirin-intolerant asthma by montelukast, a leukotriene antagonist: a randomized, double-blind, placebo-controlled trial. *Am J Respir Crit Care Med* **165**, 9–14.
- Das, A.M., Vaddi, K.G., Solomon, K.A. *et al.* (2006) Selective inhibition of eosinophil influx into the lung by small molecule CC chemokine receptor 3 antagonists in mouse models of allergic inflammation. *J Pharmacol Exp Ther* **318**, 411–17.
- Davies, D.E., Wicks, J., Powell, R.M., Puddicombe, S.M. & Holgate, S.T. (2003) Airway remodelling in asthma: new insights. *J Allergy Clin Immunol* **111**, 215–25.
- de Vries, A., Engels, F., Henricks, P.A. *et al.* (2006) Airway hyperresponsiveness in allergic asthma in guinea-pigs is mediated by nerve growth factor via the induction of substance P: a potential role for trkA. *Clin Exp Allergy* **36**, 1192–200.

- Djukanovic, R., Wilson, J.W., Britten, K.M. *et al.* (1992) Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* **145**, 669–74.
- Djukanovic, R., Homeyard, S., Gratziau, C. *et al.* (1997) The effect of treatment with oral corticosteroids on asthma symptoms and airway inflammation. *Am J Respir Crit Care Med* **155**, 826–32.
- Dragon, S., Rahman, M.S., Yang, J., Unruh, H., Halayko, A.J. & Soussi-Gounni, A. (2006) IL-17 enhances IL-1 β mediated CXCL-8 release from human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* **292**, L1023–9.
- Eder, W., Ege, M.J. & von Mutius, E. (2006) The asthma epidemic. *N Engl J Med* **355**, 2226–35.
- Fahy, J.V., Fleming, H.E., Wong, H.H. *et al.* (1997) The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* **155**, 1828–34.
- Fedorov, I.A., Wilson, S.J., Davies, D.E. & Holgate, S.T. (2005) Epithelial stress and structural remodelling in childhood asthma. *Thorax* **60**, 389–94.
- Flood-Page, P.T., Menzies-Gow, A.N., Kay, A.B. & Robinson, D.S. (2003a) Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* **167**, 199–204.
- Flood-Page, P., Menzies-Gow, A., Phipps, S. *et al.* (2003b) Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* **112**, 1029–36.
- Foley, S.C., Mogas, A.K., Olivenstein, R. *et al.* (2007) Increased expression of ADAM33 and ADAM8 with disease progression in asthma. *J Allergy Clin Immunol* **119**, 863–71.
- Frossard, N., Freund, V. & Advenier, C. (2004) Nerve growth factor and its receptors in asthma and inflammation. *Eur J Pharmacol* **500**, 453–65.
- Frossard, N., Naline, E., Olgart, H.C., Georges, O. & Advenier, C. (2005) Nerve growth factor is released by IL-1 β and induces hyperresponsiveness of the human isolated bronchus. *Eur Respir J* **26**, 15–20.
- Gajewska, B.U., Wiley, R.E. & Jordana, M. (2003) GM-CSF and dendritic cells in allergic airway inflammation: basic mechanisms and prospects for therapeutic intervention. *Curr Drug Targets Inflamm Allergy* **2**, 279–92.
- Gangur, V., Birmingham, N.P., Thanesvorakul, S. & Joseph, S. (2003) CCR3 and CXCR3 as drug targets for allergy: principles and potential. *Curr Drug Targets Inflamm Allergy* **2**, 53–62.
- Garcia, G., Godot, V. & Humbert, M. (2005) New chemokine targets for asthma therapy. *Curr Allergy Asthma Rep* **5**, 155–60.
- Gibson, P.G. (2006) Allergic bronchopulmonary aspergillosis. *Semin Respir Crit Care Med* **27**, 185–91.
- Godfrey, R.W. (1997) Human airway epithelial tight junctions. *Microsc Res Tech* **38**, 488–99.
- Green, R.H., Brightling, C.E., McKenna, S. *et al.* (2002) Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* **360**, 1715–21.
- Greenwald, R.J., Freeman, G.J. & Sharpe, A.H. (2005) The B7 family revisited. *Annu Rev Immunol* **23**, 515–48.
- Guilbert, T.W., Morgan, W.J., Zeiger, R.S. *et al.* (2006) Long-term inhaled corticosteroids in preschool children at high risk for asthma. *N Engl J Med* **354**, 1985–97.
- Hahn, C., Islamian, A.P., Renz, H. & Nockher, W.A. (2006) Airway epithelial cells produce neurotrophins and promote the survival of eosinophils during allergic airway inflammation. *J Allergy Clin Immunol* **117**, 787–94.
- Halder, P., Green, R.H., Berry, M.A., Wardlaw, A.J. & Pavord, I.D. (2005) Heterogeneity in refractory asthma: the use of cluster analysis to identify distinct cohorts. *Thorax* **60**, ii33.
- Haley, K.J., Sunday, M.E., Wiggs, B.R. *et al.* (1998) Inflammatory cell distribution within and along asthmatic airways. *Am J Respir Crit Care Med* **158**, 565–72.
- Hallgren, J. & Pejler, G. (2006) Biology of mast cell tryptase. An inflammatory mediator. *FEBS J* **273**, 1871–95.
- Hamilton, L.M., Torres-Lozano, C., Puddicombe, S.M. *et al.* (2003) The role of the epidermal growth factor receptor in sustaining neutrophil inflammation in severe asthma. *Clin Exp Allergy* **33**, 233–40.
- Hamilton, L.M., Puddicombe, S.M., Dearman, R.J. *et al.* (2005) Altered protein tyrosine phosphorylation in asthmatic bronchial epithelium. *Eur Respir J* **25**, 978–85.
- Hammad, H. & Lambrecht, B.N. (2006) Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. *J Allergy Clin Immunol* **118**, 331–6.
- Hamzaoui, A., Chaouch, N., Grairi, H., Ammar, J. & Hamzaoui, K. (2005) Inflammatory process of CD8⁺. *Mediat Inflamm* **2005**(3), 160–6.
- Hijazi, Z., Molla, A.M., Al Habashi, H., Muawad, W.M., Molla, A.M. & Sharma, P.N. (2004) Intestinal permeability is increased in bronchial asthma. *Arch Dis Child* **89**, 227–9.
- Ho, L.P. (2007) Natural killer T cells in asthma: toward increased understanding. *N Engl J Med* **356**, 1466–8.
- Holgate, S.T. (1996) The immunopharmacology of mild asthma. *J Allergy Clin Immunol* **98**, S7–S16.
- Holgate, S.T. (2005) Exacerbations: the asthma paradox. *Am J Respir Crit Care Med* **172**, 941–3.
- Holgate, S.T. & Polosa, R. (2006) The mechanisms, diagnosis, and management of severe asthma in adults. *Lancet* **368**, 780–93.
- Holgate, S.T., Lackie, P.M., Howarth, P.H. *et al.* (2001) Invited lecture: activation of the epithelial mesenchymal trophic unit in the pathogenesis of asthma. *Int Arch Allergy Immunol* **124**, 253–8.
- Holgate, S.T., Holloway, J., Wilson, S., Bucchieri, F., Puddicombe, S. & Davies, D.E. (2004) Epithelial–mesenchymal communication in the pathogenesis of chronic asthma. *Proc Am Thorac Soc* **1**, 93–8.
- Holgate, S.T., Casale, T., Wenzel, S., Bousquet, J., Deniz, Y. & Reisner, C. (2005) The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. *J Allergy Clin Immunol* **115**, 459–65.
- Holgate, S.T., Yang, Y., Haitchi, H.M. *et al.* (2006) The genetics of asthma: ADAM33 as an example of a susceptibility gene. *Proc Am Thorac Soc* **3**, 440–3.
- Holloway, J.W. & Holgate, S.T. (2004) Genetics. *Chem Immunol Allergy* **84**, 1–35.
- Howarth, P.H., Babu, K.S., Arshad, H. *et al.* (2005) Tumour necrosis factor (TNF- α) as a novel therapeutic target in symptomatic corticosteroid-dependent asthma. *Thorax* **60**, 1012–18.
- Howell, J.E. & McAnulty, R.J. (2006) TGF- β : its role in asthma and therapeutic potential. *Curr Drug Targets* **7**, 547–65.

- Hudson, T.J. (2006) Skin barrier function and allergic risk. *Nat Genet* 38, 399–400.
- Hughes, J.L., Lackie, P.M., Wilson, S.J., Church, M.K. & McGill, J.I. (2006) Reduced structural proteins in the conjunctival epithelium in allergic eye disease. *Allergy* 61, 1268–74.
- Humbert, M. (2000) [Does intrinsic asthma exist?]. *Rev Mal Respir* 17, 245–54.
- Humbert, M., Menz, G., Ying, S. *et al.* (1999) The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. *Immunol Today* 20, 528–33.
- Humrich, J.Y., Humrich, J.H., Averbeck, M. *et al.* (2006) Mature monocyte-derived dendritic cells respond more strongly to CCL19 than to CXCL12: consequences for directional migration. *Immunology* 117, 238–47.
- Illi, S., von Mutius, E., Lau, S., Niggemann, B., Gruber, C. & Wahn, U. (2006) Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 368, 763–70.
- Ilowite, J.S., Bennett, W.D., Sheetz, M.S., Groth, M.L. & Nierman, D.M. (1989) Permeability of the bronchial mucosa to 99mTc-DTPA in asthma. *Am Rev Respir Dis* 139, 1139–43.
- Ishida, T., Ishii, T., Inagaki, A. *et al.* (2006) The CCR4 as a novel-specific molecular target for immunotherapy in Hodgkin lymphoma. *Leukemia* 20, 2162–8.
- Jaffar, Z., Roberts, K., Pandit, A., Linsley, P., Djukanovic, R. & Holgate, S. (1999a) B7 costimulation is required for IL-5 and IL-13 secretion by bronchial biopsy tissue of atopic asthmatic subjects in response to allergen stimulation. *Am J Respir Cell Mol Biol* 20, 153–62.
- Jaffar, Z.H., Stanciu, L., Pandit, A., Lordan, J., Holgate, S.T. & Roberts, K. (1999b) Essential role for both CD80 and CD86 costimulation, but not CD40 interactions, in allergen-induced Th2 cytokine production from asthmatic bronchial tissue: role for alphabeta, but not gammadelta, T cells. *J Immunol* 163, 6283–91.
- Jain, N., Covar, R.A., Gleason, M.C., Newell, J.D. Jr, Gelfand, E.W. & Spahn, J.D. (2005) Quantitative computed tomography detects peripheral airway disease in asthmatic children. *Pediatr Pulmonol* 40, 211–18.
- James, A. (2005) Remodelling of airway smooth muscle in asthma: what sort do you have? *Clin Exp Allergy* 35, 703–7.
- James, A. & Carroll, N. (2000) Airway smooth muscle in health and disease; methods of measurement and relation to function. *Eur Respir J* 15, 782–9.
- James, A.L., Elliot, J.G., Abramson, M.J. & Walters, E.H. (2005) Time to death, airway wall inflammation and remodelling in fatal asthma. *Eur Respir J* 26, 429–34.
- Jayaratham, A., Corrigan, C.J. & Lee, T.H. (2005) The continuing enigma of non-atopic asthma. *Clin Exp Allergy* 35, 835–7.
- Johnston, S.L., Pattemore, P.K., Sanderson, G. *et al.* (1995) Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 310, 1225–9.
- Johnston, S.L., Blasi, F., Black, P.N., Martin, R.J., Farrell, D.J., Nieman, R.B. (2006) The effect of telithromycin in acute exacerbations of asthma. *N Engl J Med* 354, 1589–600.
- Jongepier, H., Boezen, H.M., Dijkstra, A. *et al.* (2004) Polymorphisms of the ADAM33 gene are associated with accelerated lung function decline in asthma. *Clin Exp Allergy* 34, 757–60.
- Kallinich, T., Schmidt, S., Hamelmann, E. *et al.* (2005) Chemokine-receptor expression on T cells in lung compartments of challenged asthmatic patients. *Clin Exp Allergy* 35, 26–33.
- Kariyawasam, H.H. & Robinson, D.S. (2006) The eosinophil: the cell and its weapons, the cytokines, its locations. *Semin Respir Crit Care Med* 27, 117–27.
- 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.
- Kassel, O., de Blay, F., Duvernelle, C. *et al.* (2001) Local increase in the number of mast cells and expression of nerve growth factor in the bronchus of asthmatic patients after repeated inhalation of allergen at low-dose. *Clin Exp Allergy* 31, 1432–40.
- Kaur, D., Saunders, R., Berger, P. *et al.* (2006) Airway smooth muscle and mast cell-derived CC chemokine ligand 19 mediate airway smooth muscle migration in asthma. *Am J Respir Crit Care Med* 174, 1179–88.
- Kay, A.B. (2001) Allergy and allergic diseases. Second of two parts. *N Engl J Med* 344, 109–13.
- Kay, A.B. (2005) The role of eosinophils in the pathogenesis of asthma. *Trends Mol Med* 11, 148–52.
- Kay, A.B. (2006) The role of T lymphocytes in asthma. *Chem Immunol Allergy* 91, 59–75.
- Kay, A.B., Phipps, S. & Robinson, D.S. (2004) A role for eosinophils in airway remodelling in asthma. *Trends Immunol* 25, 477–82.
- Kay, A.B., Ali, F.R., Heaney, L.G. *et al.* (2007) Airway expression of calcitonin gene-related peptide in T-cell peptide-induced late asthmatic reactions in atopics. *Allergy* 62, 495–503.
- Keogh, K.A. & Specks, U. (2006) Churg–Strauss syndrome. *Semin Respir Crit Care Med* 27, 148–57.
- Kepley, C.L., McFeeley, P.J., Oliver, J.M. & Lipscomb, M.F. (2001) Immunohistochemical detection of human basophils in postmortem cases of fatal asthma. *Am J Respir Crit Care Med* 164, 1053–8.
- Kicic, A., Sutanto, E.N., Stevens, P.T., Knight, D.A. & Stick, S.M. (2006) Intrinsic biochemical and functional differences in bronchial epithelial cells of children with asthma. *Am J Respir Crit Care Med* 174, 1110–18.
- Kitamura, K., Takeda, K., Koya, T. *et al.* (2007) Critical role of the Fc receptor {gamma}-chain on APCs in the development of allergen-induced airway hyperresponsiveness and inflammation. *J Immunol* 178, 480–8.
- Knight, D.A. & Holgate, S.T. (2003) The airway epithelium: structural and functional properties in health and disease. *Respirology* 8, 432–46.
- Knight, D.A., Lane, C.L. & Stick, S.M. (2004) Does aberrant activation of the epithelial-mesenchymal trophic unit play a key role in asthma or is it an unimportant sideshow? *Curr Opin Pharmacol* 4, 251–6.
- Kon, O.M. & Kay, A.B. (1999) Anti-T cell strategies in asthma. *Inflamm Res* 48, 516–23.
- Kon, O.M., Sihra, B.S., Compton, C.H., Leonard, T.B., Kay, A.B. & Barnes, N.C. (1998) Randomised, dose-ranging, placebo-controlled study of chimeric antibody to CD4 (keliximab) in chronic severe asthma. *Lancet* 352, 1109–13.
- Kraft, M. & Hamid, Q. (2006) Mycoplasma in severe asthma. *J Allergy Clin Immunol* 117, 1197–8.
- Kraft, M., Martin, R.J., Wilson, S., Djukanovic, R. & Holgate, S.T. (1999) Lympho-

- cyte and eosinophil influx into alveolar tissue in nocturnal asthma. *Am J Respir Crit Care Med* 159, 228–34.
- Kuipers, H., Heirman, C., Hijdra, D. *et al.* (2004) Dendritic cells retrovirally over-expressing IL-12 induce strong Th1 responses to inhaled antigen in the lung but fail to revert established Th2 sensitization. *J Leukoc Biol* 76, 1028–38.
- Kumar, R.K., Webb, D.C., Herbert, C. & Foster, P.S. (2006) Interferongamma as a possible target in chronic asthma. *Inflamm Allergy Drug Targets* 5, 253–6.
- Kumlin, M., Dahlen, B., Björck, T., Zetterstrom, O., Granstrom, E. & Dahlen, S.E. (1992) Urinary excretion of leukotriene E4 and 11-dehydro-thromboxane B2 in response to bronchial provocations with allergen, aspirin, leukotriene D4, and histamine in asthmatics. *Am Rev Respir Dis* 146, 96–103.
- Larche, M., Till, S.J., Haselden, B.M. *et al.* (1998) Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. *J Immunol* 161, 6375–82.
- Leckie, M.J., Brinke, A.T., Khan, J. *et al.* (2000) Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airways hyper-responsiveness, and the late asthmatic response. *Lancet* 356, 2144–8.
- Lee, J.Y., Park, S.W., Chang, H.K. *et al.* (2006) A disintegrin and metalloproteinase 33 protein in patients with asthma: relevance to airflow limitation. *Am J Respir Crit Care Med* 173, 729–35.
- Leigh, R., Vethanayagam, D., Yoshida, M. *et al.* (2002) Effects of montelukast and budesonide on airway responses and airway inflammation in asthma. *Am J Respir Crit Care Med* 166, 1212–17.
- Lemanske, R.F. Jr, Jackson, D.J., Gangnon, R.E. *et al.* (2005) Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 116, 571–7.
- Lemiere, C., Ernst, P., Olivenstein, R. *et al.* (2006) Airway inflammation assessed by invasive and noninvasive means in severe asthma: eosinophilic and noneosinophilic phenotypes. *J Allergy Clin Immunol* 118, 1033–9.
- Leung, S.Y., Niimi, A., Noble, A. *et al.* (2006) Effect of transforming growth factor-beta receptor I kinase inhibitor 2,4-disubstituted pteridine (SD-208) in chronic allergic airway inflammation and remodeling. *J Pharmacol Exp Ther* 319, 586–94.
- Lewis-Tuffin, L.J. & Cidlowski, J.A. (2006) The physiology of human glucocorticoid receptor beta (hGRbeta) and glucocorticoid resistance. *Ann NY Acad Sci* 1069, 1–9.
- Liu, L.Y., Sedgwick, J.B., Bates, M.E. *et al.* (2002) Decreased expression of membrane IL-5 receptor alpha on human eosinophils: I. Loss of membrane IL-5 receptor alpha on airway eosinophils and increased soluble IL-5 receptor alpha in the airway after allergen challenge. *J Immunol* 169, 6452–8.
- Liu, Y.J. (2006) Thymic stromal lymphopoietin: master switch for allergic inflammation. *J Exp Med* 203, 269–73.
- Liu, Z., Li, N. & Neu, J. (2005) Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr* 94, 386–93.
- Livingston, E., Chaudhuri, R., McMahon, A.D., Fraser, I., McSharry, C.P. & Thomson, N.C. (2007) Systemic sensitivity to corticosteroids in smokers with asthma. *Eur Respir J* 29, 64–71.
- Loke, T.K., Mallett, K.H., Ratoff, J. *et al.* (2006) Systemic glucocorticoid reduces bronchial mucosal activation of activator protein 1 components in glucocorticoid-sensitive but not glucocorticoid-resistant asthmatic patients. *J Allergy Clin Immunol* 118, 368–75.
- Long, A.J., Sypek, J.P., Askew, R. *et al.* (2006) Gob-5 contributes to goblet cell hyperplasia and modulates pulmonary tissue inflammation. *Am J Respir Cell Mol Biol* 35, 357–65.
- Lordan, J.L., Davies, D.E., Wilson, S.J. *et al.* (2001) The role of CD28–B7 costimulation in allergen-induced cytokine release by bronchial mucosa from patients with moderately severe asthma. *J Allergy Clin Immunol* 108, 976–81.
- Macfarlane, A.J., Kon, O.M., Smith, S.J. *et al.* (2000) Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J Allergy Clin Immunol* 105, 99–107.
- McLachlan, C.R., Poulton, R., Car, G. *et al.* (2006) Adiposity, asthma, and airway inflammation. *J Allergy Clin Immunol* 119, 634–9.
- Malo, J.L. (2005) Future advances in work-related asthma and the impact on occupational health. *Occup Med* 55, 606–11.
- Marshall, D., Hardman, M.J., Nield, K.M. & Byrne, C. (2001) Differentially expressed late constituents of the epidermal cornified envelope. *Proc Natl Acad Sci USA* 98, 13031–6.
- Meagher, L.C., Cousin, J.M., Seckl, J.R. & Haslett, C. (1996) Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. *J Immunol* 156, 4422–8.
- Meurer, J.R., Lustig, J.V. & Jacob, H.J. (2006) Genetic aspects of the etiology and treatment of asthma. *Pediatr Clin North Am* 53, 715–25.
- Meyts, I., Hellings, P.W., Hens, G. *et al.* (2006) IL-12 contributes to allergen-induced airway inflammation in experimental asthma. *J Immunol* 177, 6460–70.
- Misso, N.L. & Thompson, P.J. (2005) Oxidative stress and antioxidant deficiencies in asthma: potential modification by diet. *Redox Rep* 10, 247–55.
- Mochizuki, A., McEuen, A.R., Buckley, M.G. & Walls, A.F. (2003) The release of basogranulin in response to IgE-dependent and IgE-independent stimuli: validity of basogranulin measurement as an indicator of basophil activation. *J Allergy Clin Immunol* 112, 102–8.
- Montefort, S., Roberts, J.A., Beasley, R., Holgate, S.T. & Roche, W.R. (1992) The site of disruption of the bronchial epithelium in asthmatic and non-asthmatic subjects. *Thorax* 47, 499–503.
- Morcillo, E.J. & Cortijo, J. (2006) Mucus and MUC in asthma. *Curr Opin Pulm Med* 12, 1–6.
- Morrison, D., Rahman, I. & MacNee, W. (2006) Permeability, inflammation and oxidant status in airspace epithelium exposed to ozone. *Respir Med* 100, 2227–34.
- Mullin, J.M., Agostino, N., Rendon-Huerta, E. & Thornton, J.J. (2005) Keynote review: epithelial and endothelial barriers in human disease. *Drug Discov Today* 10, 395–408.
- Murray, C.S., Woodcock, A., Langley, S.J., Morris, J. & Custovic, A. (2006) Secondary prevention of asthma by the use of Inhaled Fluticasone propionate in Wheezy Infants (IFWIN): double-blind, randomised, controlled study. *Lancet* 368, 754–62.
- Naum, D.H., Lee, Y.E., Yim, E.J. *et al.* (2002) Identification of cytokeratin 18 as a bronchial epithelial autoantigen asso-

- ciated with nonallergic asthma. *Am J Respir Crit Care Med* 165, 1536–9.
- Nahm, D.H., Lee, K.H., Shin, J.Y., Ye, Y.M., Kang, Y. & Park, H.S. (2006) Identification of alpha-enolase as an autoantigen associated with severe asthma. *J Allergy Clin Immunol* 118, 376–81.
- Nassenstein, C., Braun, A., Erpenbeck, V.J. *et al.* (2003) The neurotrophins nerve growth factor, brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4 are survival and activation factors for eosinophils in patients with allergic bronchial asthma. *J Exp Med* 198, 455–67.
- Nassenstein, C., Schulte-Herbruggen, O., Renz, H. & Braun, A. (2006) Nerve growth factor: the central hub in the development of allergic asthma? *Eur J Pharmacol* 533, 195–206.
- Neuber, K., Mahnss, B., Hubner, C., Gergely, H. & Weichenthal, M. (2006) Autoantibodies against CD28 are associated with atopic diseases. *Clin Exp Immunol* 146, 262–9.
- Nguyen, L.T., Lim, S., Oates, T. & Chung, K.F. (2005) Increase in airway neutrophils after oral but not inhaled corticosteroid therapy in mild asthma. *Respir Med* 99, 200–7.
- Ogawa, Y., Calhoun & W.J. (2006) The role of leukotrienes in airway inflammation. *J Allergy Clin Immunol* 118, 789–98.
- Okayama, Y., Petit-Frere, C., Kassel, O. *et al.* (1995) IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J Immunol* 155, 1796–808.
- Okayama, Y., Tkaczyk, C., Metcalfe, D.D. & Gilfillan, A.M. (2003) Comparison of Fc epsilon RI- and Fc gamma RI-mediated degranulation and TNF-alpha synthesis in human mast cells: selective utilization of phosphatidylinositol-3-kinase for Fc gamma RI-induced degranulation. *Eur J Immunol* 33, 1450–9.
- Olgart, H.C., de Blay, F., Oster, J.P. *et al.* (2002) Nerve growth factor levels and localisation in human asthmatic bronchi. *Eur Respir J* 20, 1110–16.
- Ong, Y.E., Menzies-Gow, A., Barkans, J. *et al.* (2005) Anti-IgE (omalizumab) inhibits late-phase reactions and inflammatory cells after repeat skin allergen challenge. *J Allergy Clin Immunol* 116, 558–64.
- Ordenez, C., Ferrando, R., Hyde, D.M., Wong, H.H. & Fahy, J.V. (2000) Epithelial desquamation in asthma: artifact or pathology? *Am J Respir Crit Care Med* 162, 2324–9.
- Ordenez, C.L., Khashayar, R., Wong, H.H. *et al.* (2001) Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 163, 517–23.
- O'Sullivan, S.M. (2005) Asthma death, CD8⁺T cells, and viruses. *Proc Am Thorac Soc* 2, 162–5.
- Paganin, F., Seneiter, E., Chanez, P. *et al.* (1996) Computed tomography of the lungs in asthma: influence of disease severity and etiology. *Am J Respir Crit Care Med* 153, 110–14.
- Palmer, C.N., Irvine, A.D., Terron-Kwiatkowski, A. *et al.* (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 38, 441–6.
- Papadopoulos, N.G., Bates, P.J., Bardin, P.G. *et al.* (2000) Rhinoviruses infect the lower airways. *J Infect Dis* 181, 1875–84.
- Pauwels, R.A., Pedersen, S., Busse, W.W. *et al.* (2003) Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial. *Lancet* 361, 1071–6.
- Pease, J.E. & Williams, T.J. (2006) Chemokines and their receptors in allergic disease. *J Allergy Clin Immunol* 118, 305–18.
- Perez-Vilar, J. (2006) Mucin granule intraluminal organization. *Am J Respir Cell Mol Biol* 36, 183–90.
- Pham-Thi, N., de Blic, J. & Leite-de-Moraes, M.C. (2006) Invariant natural killer T cells in bronchial asthma. *N Engl J Med* 354, 2613–16.
- Phipps, S., Flood-Page, P., Menzies-Gow, A., Ong, Y.E. & Kay, A.B. (2004) Intravenous anti-IL-5 monoclonal antibody reduces eosinophils and tenascin deposition in allergen-challenged human atopic skin. *J Invest Dermatol* 122, 1406–12.
- Pini, L., Hamid, Q., Shannon, J. *et al.* (2007) Differences in proteoglycan deposition in the airways of moderate and severe asthmatics. *Eur Respir J* 29, 71–7.
- Plante, S., Semlali, A., Joubert, P. *et al.* (2006) Mast cells regulate procollagen I (alpha 1) production by bronchial fibroblasts derived from subjects with asthma through IL-4/IL-4 delta 2 ratio. *J Allergy Clin Immunol* 117, 1321–7.
- Polosa, R., Puddicombe, S.M., Krishna, M.T. *et al.* (2002) Expression of c-erbB receptors and ligands in the bronchial epithelium of asthmatic subjects. *J Allergy Clin Immunol* 109, 75–81.
- Proksch, E., Folster-Holst, R. & Jensen, J.M. (2006) Skin barrier function, epidermal proliferation and differentiation in eczema. *J Dermatol Sci* 43, 159–69.
- Puddicombe, S.M., Polosa, R. & Richter, A. (2000) Involvement of the epidermal growth factor receptor in epithelial repair in asthma. *FASEB J* 14, 1362–74.
- Puddicombe, S.M., Torres-Lozano, C., Richter, A. *et al.* (2003) Increased expression of p21(waf) cyclin dependent kinase inhibitor in asthmatic bronchial epithelium. *Am J Respir Cell Mol Biol* 28, 61–8.
- Quarcoo, D., Schulte-Herbruggen, O., Lommatzsch, M. *et al.* (2004) Nerve growth factor induces increased airway inflammation via a neuropeptide-dependent mechanism in a transgenic animal model of allergic airway inflammation. *Clin Exp Allergy* 34, 1146–51.
- Qujeq, D., Hidari, B., Bijani, K. & Shirdel, H. (2003) Glutathione peroxidase activity and serum selenium concentration in intrinsic asthmatic patients. *Clin Chem Lab Med* 41, 200–2.
- Rahman, I., Biswas, S.K. & Kode, A. (2006) Oxidant and antioxidant balance in the airways and airway diseases. *Eur J Pharmacol* 533, 222–39.
- Reed, C.E. & Kita, H. (2004) The role of protease activation of inflammation in allergic respiratory diseases. *J Allergy Clin Immunol* 114, 997–1008.
- Riese, R.J. & Chapman, H.A. (2000) Cathepsins and compartmentalization in antigen presentation. *Curr Opin Immunol* 12, 107–13.
- Robinson, D.S., North, J., Zeibecoglou, K. *et al.* (1999) Eosinophil development and bone marrow and tissue eosinophils in atopic asthma. *Int Arch Allergy Immunol* 118, 98–100.
- Roche, W.R., Montefort, S., Baker, J. & Holgate, S.T. (1993) Cell adhesion molecules and the bronchial epithelium. *Am Rev Respir Dis* 148, S79–S82.
- Russo, C. & Polosa, R. (2005) TNF-alpha as a promising therapeutic target in chronic asthma: a lesson from rheumatoid arthritis. *Clin Sci* 109, 135–42.
- Ryu, H.J., Jung, H.Y., Park, J.S. *et al.* (2006) Gene-based single nucleotide polymorphisms and linkage disequilibrium

- patterns of 29 asthma candidate genes in the chromosome 5q31–33 region in Koreans. *Int Arch Allergy Immunol* 139, 209–16.
- Saglani, S., Malmstrom, K., Pelkonen, A.S. *et al.* (2005) Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. *Am J Respir Crit Care Med* 171, 722–7.
- Salek-Ardakani, S., Song, J., Halteman, B.S. *et al.* (2003) OX40 (CD134) controls memory T helper 2 cells that drive lung inflammation. *J Exp Med* 198, 315–24.
- Schmitz, N., Kurrer, M. & Kopf, M. (2003) The IL-1 receptor 1 is critical for Th2 cell type airway immune responses in a mild but not in a more severe asthma model. *Eur J Immunol* 33, 991–1000.
- Scott, K. & Bradding, P. (2005) Human mast cell chemokines receptors: implications for mast cell tissue localization in asthma. *Clin Exp Allergy* 35, 693–7.
- Sehmi, R., Dorman, S., Baatjes, A. *et al.* (2003) Allergen-induced fluctuation in CC chemokine receptor 3 expression on bone marrow CD34⁺ cells from asthmatic subjects: significance for mobilization of haemopoietic progenitor cells in allergic inflammation. *Immunology* 109, 536–46.
- Sen, Y., Yongyi, B., Yuling, H. *et al.* (2005) V alpha 24-invariant NKT cells from patients with allergic asthma express CCR9 at high frequency and induce Th2 bias of CD3⁺ T cells upon CD226 engagement. *J Immunol* 175, 4914–26.
- Sexton, D.W., Al Rabia, M., Blaylock, M.G. & Walsh, G.M. (2004) Phagocytosis of apoptotic eosinophils but not neutrophils by bronchial epithelial cells. *Clin Exp Allergy* 34, 1514–24.
- Shahana, S., Bjornsson, E., Ludviksdottir, D. *et al.* (2005) Ultrastructure of bronchial biopsies from patients with allergic and non-allergic asthma. *Respir Med* 99, 429–43.
- Shahana, S., Jaunmuktane, Z., Asplund, M.S. & Roomans, G.M. (2006) Ultrastructural investigation of epithelial damage in asthmatic and non-asthmatic nasal polyps. *Respir Med* 100, 2018–28.
- Shaw, T.J., Wakely, S.L., Peebles, C.R. *et al.* (2004) Endobronchial ultrasound to assess airway wall thickening: validation in vitro and in vivo. *Eur Respir J* 23, 813–17.
- Sher, E.R., Leung, D.Y., Surs, W. *et al.* (1994) Steroid-resistant asthma. Cellular mechanisms contributing to inadequate response to glucocorticoid therapy. *J Clin Invest* 93, 33–9.
- Silvestri, M., Bontempelli, M., Giacomelli, M. *et al.* (2006) High serum levels of tumour necrosis factor- α and interleukin-8 in severe asthma: markers of systemic inflammation? *Clin Exp Allergy* 36, 1373–81.
- 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, 559–65.
- Singh, A.M., Moore, P.E., Gern, J.E., Lemanske, R.F.Jr. & Hartert, T.V. (2007) Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. *Am J Respir Crit Care Med* 175, 108–19.
- Slavin, R.G. (2005) The allergist and the workplace: occupational asthma and rhinitis. *Allergy Asthma Proc* 26, 255–61.
- Smit, J.J. & Lukacs, N.W. (2006) A closer look at chemokines and their role in asthmatic responses. *Eur J Pharmacol* 533, 277–88.
- Stanford, K.L., Mickleborough, T.D., Ray, S., Lindley, M.R., Kocaja, D.M. & Stager, J.M. (2006) Influence of menstrual cycle phase on pulmonary function in asthmatic athletes. *Eur J Appl Physiol* 96, 703–10.
- Stein, R.T. & Martinez, F.D. (2004) Asthma phenotypes in childhood: lessons from an epidemiological approach. *Paediatr Respir Rev* 5, 155–61.
- Strek, M.E. (2006) Difficult asthma. *Proc Am Thorac Soc* 3, 116–23.
- Temann, U.A., Laouar, Y., Eynon, E.E., Homer, R. & Flavell, R.A. (2007) IL9 leads to airway inflammation by inducing IL13 expression in airway epithelial cells. *Int Immunol* 19, 1–10.
- Thai, P., Chen, Y., Dolganov, G. & Wu, R. (2005) Differential regulation of MUC5AC/Muc5ac and hCLCA-1/mGob-5 expression in airway epithelium. *Am J Respir Cell Mol Biol* 33, 523–30.
- Thomas, S.Y., Lilly, C.M. & Luster, A.D. (2006) Invariant natural killer T cells in bronchial asthma. *N Engl J Med* 354, 2613–16.
- Tigani, B., Cannet, C., Quintana, H.K. *et al.* (2006) Lung inflammation and vascular remodeling after repeated allergen challenge detected noninvasively by MRI. *Am J Physiol Lung Cell Mol Physiol* 292, 644–53.
- Tomlinson, J.E., McMahon, A.D., Chaudhuri, R., Thompson, J.M., Wood, S.F. & Thomson, N.C. (2005) Efficacy of low and high dose inhaled corticosteroid in smokers versus non-smokers with mild asthma. *Thorax* 60, 282–7.
- Tonnel, A.B., Gosset, P. & Tillie-Leblond, I. (2001) Characteristics of the Inflammatory response in bronchial lavage fluids from patients with status asthmaticus. *Int Arch Allergy Immunol* 124, 267–71.
- Truong-Tran, A.Q., Grosser, D., Ruffin, R.E., Murgia, C. & Zalewski, P.D. (2003) Apoptosis in the normal and inflamed airway epithelium: role of zinc in epithelial protection and procaspase-3 regulation. *Biochem Pharmacol* 66, 1459–68.
- Truyen, E., Coteur, L., Dilissen, E. *et al.* (2006) Evaluation of airway inflammation by quantitative Th1/Th2 cytokine mRNA measurement in sputum of asthma patients. *Thorax* 61, 202–8.
- Tsoumakidou, M., Papadopoulou, E., Tzanakis, N. & Siafakas, N.M. (2006) Airway inflammation and cellular stress in noneosinophilic atopic asthma. *Chest* 129, 1194–202.
- Uller, L., Andersson, M., Greiff, L., Persson, C.G. & Erjefalt, J.S. (2004) Occurrence of apoptosis, secondary necrosis, and cytolysis in eosinophilic nasal polyps. *Am J Respir Crit Care Med* 170, 742–7.
- Uller, L., Rydell-Tormanen, K., Persson, C.G. & Erjefalt, J.S. (2005) Anti-Fas mAb-induced apoptosis and cytolysis of airway tissue eosinophils aggravates rather than resolves established inflammation. *Respir Res* 6, 90.
- Uller, L., Lloyd, C.M., Rydell-Tormanen, K., Persson, C.G. & Erjefalt, J.S. (2006) Effects of steroid treatment on lung CC chemokines, apoptosis and transepithelial cell clearance during development and resolution of allergic airway inflammation. *Clin Exp Allergy* 36, 111–21.
- Umetsu, D.T., Dekruyff, R.H. (2006) A role for natural killer T cells in asthma. *Nat Rev Immunol* 6, 953–8.
- van Diemen, C.C., Postma, D.S., Vonk, J.M., Bruinenberg, M., Schouten, J.P., Boezen, H.M. (2005) A Disintegrin A Metalloprotease 33 polymorphisms and lung function decline in the general population. *Am J Respir Crit Care Med* 172, 329–33.

- Van Eerdewegh, P., Little, R.D., Dupuis, J. *et al.* (2002) Association of the ADAM-33 gene with asthma and bronchial hyper-responsiveness. *Nature* **418**, 426–30.
- van Rijt, L.S., Vos, N., Willart, M. *et al.* (2004) Essential role of dendritic cell CD80/CD86 costimulation in the induction, but not reactivation, of TH2 effector responses in a mouse model of asthma. *J Allergy Clin Immunol* **114**, 166–73.
- Vendelin, J., Pulkkinen, V., Rehn, M. *et al.* (2005) Characterization of GPRA, a novel G protein-coupled receptor related to asthma. *Am J Respir Cell Mol Biol* **33**, 262–70.
- Vijayanand, P., Seumois, G., Pickard, C. *et al.* (2007) Invariant natural killer T cells in asthma and chronic obstructive pulmonary disease. *N Engl J Med* **356**, 1410–22.
- von Garnier, C., Figueira, L., Wikstrom, M. *et al.* (2005) Anatomical location determines the distribution and function of dendritic cells and other APCs in the respiratory tract. *J Immunol* **175**, 1609–18.
- Voynow, J.A., Gendler, S.J. & Rose, M.C. (2006) Regulation of mucin genes in chronic inflammatory airway diseases. *Am J Respir Cell Mol Biol* **34**, 661–5.
- Vrugt, B., Wilson, S., Bron, A., Holgate, S.T., Djukanovic, R. & Aalbers, R. (2000) Bronchial angiogenesis in severe glucocorticoid-dependent asthma. *Eur Respir J* **15**, 1014–21.
- Wardlaw, A.J., Silverman, M., Siva, R., Pavord, I.D. & Green, R. (2005) Multi-dimensional phenotyping: towards a new taxonomy for airway disease. *Clin Exp Allergy* **35**, 1254–62.
- Wark, P.A., Johnston, S.L., Moric, I., Simpson, J.L., Hensley, M.J. & Gibson, P.G. (2002) Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur Respir J* **19**, 68–75.
- Wark, P.A., Johnston, S.L., Bucchieri, F. *et al.* (2005) Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* **201**, 937–47.
- Watson, J.J., Fahey, M.S., van den Worm, E. *et al.* (2006) TrkAd5: a novel therapeutic agent for treatment of inflammatory pain and asthma. *J Pharmacol Exp Ther* **316**, 1122–9.
- Webb, D.C., Cai, Y., Matthaei, K.I. & Foster, P.S. (2007) Comparative roles of IL-4, IL-13, and IL-4R α in dendritic cell maturation and CD4⁺ Th2 cell function. *J Immunol* **178**, 219–27.
- Weinblatt, M., Combe, B., Covucci, A., Aranda, R., Becker, J.C. & Keystone, E. (2006) Safety of the selective costimulation modulator abatacept in rheumatoid arthritis patients receiving background biologic and nonbiologic disease-modifying antirheumatic drugs: a one-year randomized, placebo-controlled study. *Arthritis Rheum* **54**, 2807–16.
- Wenzel, S.E. (2006) Asthma: defining of the persistent adult phenotypes. *Lancet* **368**, 804–13.
- Wenzel, S.E., Schwartz, L.B., Langmack, E.L. *et al.* (1999) Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* **160**, 1001–8.
- Wenzel, S.E., Balzar, S., Cundall, M. & Chu, H.W. (2003) Subepithelial basement membrane immunoreactivity for matrix metalloproteinase 9: association with asthma severity, neutrophilic inflammation, and wound repair. *J Allergy Clin Immunol* **111**, 1345–52.
- Wicks, J., Haitchi, H.M., Holgate, S.T., Davies, D.E. & Powell, R.M. (2006) Enhanced upregulation of smooth muscle related transcripts by TGF beta2 in asthmatic (myo) fibroblasts. *Thorax* **61**, 313–19.
- Williams, T.J. & Jose, P.J. (2000) Role of eotaxin and related CC chemokines in allergy and asthma. *Chem Immunol* **78**, 166–77.
- Wilson, J.W. & Robertson, C.F. (2002) Angiogenesis in paediatric airway disease. *Paediatr Respir Rev* **3**, 219–29.
- Wilson, S.J., Shute, J.K., Holgate, S.T., Howarth, P.H. & Bradding, P. (2000) Localization of interleukin (IL)-4 but not IL-5 to human mast cell secretory granules by immunoelectron microscopy. *Clin Exp Allergy* **30**, 493–500.
- Woodman, L., Sutcliffe, A., Kaur, D. *et al.* (2006) Chemokine concentrations and mast cell chemotactic activity in BAL fluid in patients with eosinophilic bronchitis and asthma, and in normal control subjects. *Chest* **130**, 371–8.
- Xystrakis, E., Kusumakar, S., Boswell, S. *et al.* (2006) Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest* **116**, 146–55.
- Yang, M., Wu, T., Cheng, L., Wang, F., Wei, Q. & Tanguay, R.M. (2005) Plasma antibodies against heat shock protein 70 correlate with the incidence and severity of asthma in a Chinese population. *Respir Res* **6**, 18.
- Ye, Y.M., Nahm, D.H., Kim, C.W. *et al.* (2006a) Cytokeratin autoantibodies: useful serologic markers for toluene diisocyanate-induced asthma. *Yonsei Med J* **47**, 773–81.
- Ye, Y.M., Nahm, D.H., Kim, S.H. *et al.* (2006b) Circulating autoantibodies in patients with aspirin-intolerant asthma: an epiphenomenon related to airway inflammation. *J Korean Med Sci* **21**, 412–17.
- Ying, S., Humbert, M., Barkans, J. *et al.* (1997) Expression of IL-4 and IL-5 mRNA and protein product by CD4⁺ and CD8⁺ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* **158**, 3539–44.
- Ying, S., Meng, Q., Zeibecoglou, K. *et al.* (1999) Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* **163**, 6321–9.
- Ying, S., Humbert, M., Meng, Q. *et al.* (2001) Local expression of epsilon germline gene transcripts and RNA for the epsilon heavy chain of IgE in the bronchial mucosa in atopic and nonatopic asthma. *J Allergy Clin Immunol* **107**, 686–92.
- Ying, S., O'Connor, B., Ratoff, J. *et al.* (2005) Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* **174**, 8183–90.
- Ying, S., Meng, Q., Corrigan, C.J. & Lee, T.H. (2006) Lack of filaggrin expression in the human bronchial mucosa. *J Allergy Clin Immunol* **118**, 1386–8.