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Lessons learnt from the epidemic of asthma

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Historical perspectives

In his 'Treatise on Asthma; Its Pathology and Treatment' published in 1860, Henry Hyde Salter, a London physician, described the disorder as '*paroxysmal dyspnoea of a peculiar character, generally periodic with intervals of healthy respiration between the attacks*'.¹ Although at that time the concept of allergy had not yet been introduced, Salter was aware of the hereditary nature of the disease, and its relation to 'idiosyncrasies' such as the emanations of horses, cats and other animals. Similarly, he appreciated the importance of emotional disturbances in asthma. However, it took a further 30 years before William Osler, in his first edition of *Principles and Practices of Medicine* (1892),² connected disordered airway function of asthma with a variety of pathological changes in the lung, including bronchial mucosal oedema, inflammation and the production of 'gelatinous' mucus. Osler also drew attention to the *bizarre and extraordinary* variety of circumstances which, at times induced a paroxysm, including exposure to allergens, emotions, diet and the common cold (Table 1). It is this hyper-responsiveness of the airways to a variety of direct and indirect stimuli that is characteristic of asthma and differentiates it from other lung diseases. Bronchial hyper-responsiveness (BHR) can be quantified in the laboratory using incremental doses of a stimulus such as methacholine and histamine (direct stimuli) or allergen, adenosine, cold dry air or exercise (indirect stimuli that cause airway narrowing through the release of mediators from airway cells). By measuring the

incremental fall in a measure of lung function such as the forced expiratory volume in 1 s (FEV₁), the concentration dose response curve in asthma is steeper and displaced to the left, allowing quantification in the form of a provocative concentration or dose of agonist that reduces lung function by 20% of baseline (PC₂₀). The lower the PC₂₀ value, the greater the level of BHR and, in general, the more severe the asthma.

While, as a key feature of asthma, physicians recognise the importance of BHR in causing the airways to contract too much and too easily, its underlying mechanism has remained a mystery.^{3,4} Of the many theories put forward to explain BHR, a combination of increased and dysfunctional airway smooth muscle⁴ and airway wall thickening⁵ has found wide acceptance. William Osler drew attention to a special type of airway inflammation that was a characteristic feature of asthma and the presence of octahedral crystals (Charcot-Leyden crystals) being present in the sputum. In 1860, Salter had already noticed what appeared to be binuclear cells present in asthmatic sputum, but their identification had to await the discovery by Paul Ehrlich of tetrabromofluorescein (eosin),⁶ which had 'a weak affinity for nuclear substances' but stained the granules of the binucleated cells present in asthmatic sputum and airways bright red and led to the naming of these cells—*eosinophils*. Experimenting with aniline dyes, Paul Ehrlich was also responsible for the discovery of mast cells and basophils, the other inflammatory cells implicated

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Table 1 Factors associated with asthma according to William Osler

Bronchial asthma
1. Spasm of the bronchial muscles 2. Swelling of the bronchial mucosal membrane 3. A special form of inflammation of the smaller bronchioles [<i>bronchiolitis exudative</i>: (Curschmann)] 4. Hay fever has many resemblances to asthma 5. The affection runs in families 6. The disease often begins in childhood and sometimes lasts into old age 7. Bizarre and extraordinary variety of circumstances which at times induce a paroxysm: - Climate and atmosphere, e.g. hay, dust, cat - Fright or violent emotion - Diet (overloading of the stomach) or certain foods - Cold infection 8. Sputum is distinctive: rounded gelatinous masses ('perles') and Curschmann spirals Octahedral crystals (Leyden)

in the inflammation of asthma. In 1922, Huber and Koessler further emphasized the prominence of eosinophils as the characteristic inflammatory cell in asthma,⁷ although they did notice that their numbers varied greatly from case to case, illustrating the heterogeneity of the inflammatory process.

Allergy and asthma

In their classical studies on *supersensitivity* by Prausnitz and Küstner in 1921,⁸ the identification of a reaginic antibody that could passively transfer the acute allergic response from one individual to another (later to be identified as the immunoglobulin IgE), provided a link between the inflammatory cell infiltrate of asthma and 'sensitization' to common environmental allergens. In 1873, Charles Blackley, a Manchester physician, in his book entitled *Experimental Researches on the Causes and Nature of Cattanhus Aestivus*,⁹ described a strong association between exposure to allergens and upper and lower airway disease. The precise mechanisms whereby allergens could evoke such a vigorous response had to await the discovery of histamine by Dale and Laidlaw in 1911.¹⁰ Half a century later, Riley and West¹¹ made the connection between histamine, tissue mast cells and the allergic response. Thus, the scenario of asthma being a process of IgE generation by B lymphocytes and plasma cells, mast cell activation, and eosinophil recruitment orchestrated by T lymphocytes, with release of a wide range of pharmacologically active mediators that contract airway smooth muscle and promote oedema formation, provided a plausible mechanism underlying asthma pathogenesis. Over the last half century, acceptance of these concepts has led to the introduction of therapies

that include inhaled β_2 -adrenoceptor agonists to relax airway smooth muscle, and topically active corticosteroids to suppress inflammation. However, while there have been progressive improvements in the efficacy, duration of action and therapeutic index of these drugs, unlike other complex diseases, there have been no new drugs that have been introduced that attack the underlying pathogenic processes.

Why is asthma increasing?

Despite having effective therapies for a large proportion of asthmatic patients, worldwide the last 30 years has witnessed a dramatic increase in disease prevalence, especially in the UK and other English-speaking countries, including New Zealand, Australia and North America.¹² Asthma prevalence is also increasing in countries such as Norway, where from 1972 to 1989 there has been a dramatic 3.5-fold increase, restricted to children and young adults.¹³ The epidemic of asthma has also spread to developing countries that have adopted a 'westernized' lifestyle. Careful studies investigating the association between indoor and outdoor allergen exposure and the rising trends in asthma have failed to demonstrate a causal link, even though allergy is a prime trigger for the asthmatic response. The recognition that the characteristic inflammation that occurs in asthmatic airways is the consequence of an altered T-lymphocyte response (designated Th2-like), in which there is enhanced production of a cluster of cytokines encoded on chromosome 5q32-34 (interleukins 3, 4, 5, 9, 13 and GM-CSF) involved in the generation of IgE and the recruitment and activation of mast cells, eosinophils and basophils, has provided a possible explanation

for the increasing worldwide trends in asthma and allergy.¹⁴ T-cell polarization towards a Th2-like phenotype requires a continuous presence of interleukin 4. The production of interleukin 12 and/or interleukin 18 by antigen-presenting cells such as dendritic cells, inhibits Th2 polarization through the induction of γ -interferon by Th1-like T cells. Increased production of IL12 and IL18 is under the control of the innate immune response, and is strongly influenced by Toll-like receptors (TLR), especially TLR4 and TLR9, whose ligands are bacterial-derived endotoxin (lipopolysaccharides) and CpG DNA oligonucleotides, respectively.¹⁵

By studying the 1958 British Birth Cohort, David Strachan noticed an inverse association between hay fever prevalence and the number of younger children in the family.¹⁶ This family size effect has been found in multiple studies, but most strongly expressed for measures of allergic sensitization, hay fever and eczema, although not consistently for asthma. One explanation advanced for this association is that infections and/or contact with the contents of micro-organisms acquired in the home in early childhood may lead to protection against the subsequent development of allergic disease (the *hygiene hypothesis*).¹⁷ This could help explain associations of hay fever with smaller households (particularly those with few older siblings), richer families, better quality housing and breast feeding. Strengthening the link between the changing environment and acquisition of allergy are recent studies showing a strong protective effect of being born and brought up on a livestock farm,^{18,19} but the level of protection again was strongest for allergic sensitization (atopy) rather than the development of asthma.²⁰ It is suggested that on livestock farms, high exposure to bacterial products such as lipopolysaccharide and CpG DNA oligonucleotides stimulates innate immune responses, via production of IL-12, IL-18 and γ -interferon, that inhibit Th2 and promote Th1 responses.²¹ While such a hypothesis is attractive, studies in mice in which Th1 response has been superimposed upon a Th2 response have not only failed to show reversal of the latter, but led to enhanced inflammation.²² A more plausible explanation for the key role that innate immunity plays in censoring the atopic phenotype in relation to exposure to the natural environment is the development of T-cell tolerance, involving the production of IL-10 and TGF- β by subsets of Th3 regulatory (T_R) T cells.²² In mice, the T_R cells that develop at these mucosal sites inhibit inflammatory responses mediated by both Th2 and Th1 mechanisms, which helps explain why Th1-associated diseases such as diabetes, rheumatoid arthritis and psoriasis

have also increased in parallel with allergic disease.²³ Therapies that may enhance the tolerance process by upregulating Th3 and T_R cells, including the administration of probiotics such as lactobacillus, oral allergen immunotherapy, and the administration of modified allergen immunotherapy using peptide allergens that differentiate protective immune from anaphylactic responses, or allergens given with CpG (a ligand for the TLR9) or conjugated with CpG motifs, offer exciting new therapeutic approaches for allergic disease.

Molecular targets of monoclonal antibody therapy

The development of a specific monoclonal IgG1 antibody targeted to the C ϵ 3 domain of human IgE has created a new therapeutic opportunity for intervening in allergic disease by depriving the mast cell of the IgE required for its activation (Figure 1).²⁴ If the high affinity receptor for IgE (FC ϵ R1) is not occupied by IgE, it undergoes rapid internalization, thereby enhancing the protective effect of anti-IgE therapy. Systemic administration of humanized anti-IgE (TNX-901) in patients with peanut allergy has a profound effect in protecting them against subsequent anaphylactic responses following peanut exposure.²⁵ In patients with allergic asthma, another anti-IgE monoclonal antibody (omalizumab) almost totally inhibited the early (mast-cell-dependent) and late (inflammatory-cell-dependent) responses to inhalation allergen challenge and the associated influx of eosinophils into the airways.²⁶ Clinical trials of anti-IgE therapy in over 1000 patients with asthma requiring regular inhaled corticosteroids

Humanized monoclonal anti-IgE antibody: Omalizumab

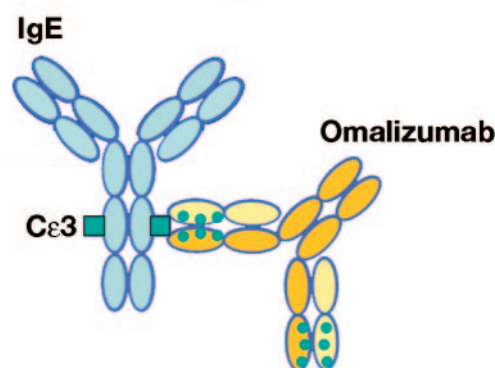


Figure 1. Epitope to C3 domain of the heavy chain of IgE to which the therapeutic humanized monoclonal antibody omalizumab is directed. The same region of IgE binds with high affinity to the α -chain of the IgE receptor.

(including children) have demonstrated efficacy, but this is not complete.²⁷ In patients with more severe allergic disease requiring high dose inhaled corticosteroids in addition to long acting β_2 -adrenoceptor agonists, anti-IgE therapy proved efficacious with useful improvements in indices of asthma, but between subjects the response was variable, and fell short of full resolution of the disease.²⁸

An alternative targeted approach has been to block eosinophil recruitment by blocking the pro-eosinophilic cytokine IL-5 (Figure 2), using anti-IL-5 monoclonal antibodies such as mepolizumab. Single-dose mepolizumab has an impressive effect in reducing both circulating and sputum eosinophils, but in contrast to studies in non-human primates, this was not paralleled by any inhibition of early- or late-phase allergen-induced bronchoconstriction.²⁹ In a large subsequent clinical trial involving over 300 asthmatic patients, mepolizumab administered on three occasions at 4-week intervals, resulted in >95% reduction in circulating eosinophils and 80% reduction in sputum eosinophils, but this was not accompanied by any evidence of clinical efficacy.³⁰ In a separate study, this treatment caused a 55% reduction in tissue eosinophils³¹ and clear loss of subepithelial staining of the *lamina reticularis* for tenascin C and other matrix molecules.³² This disappointing clinical result raises the possibility that asthma expressed clinically and as disordered airway function extends beyond IL-5 and possibly eosinophil-driven inflammation.

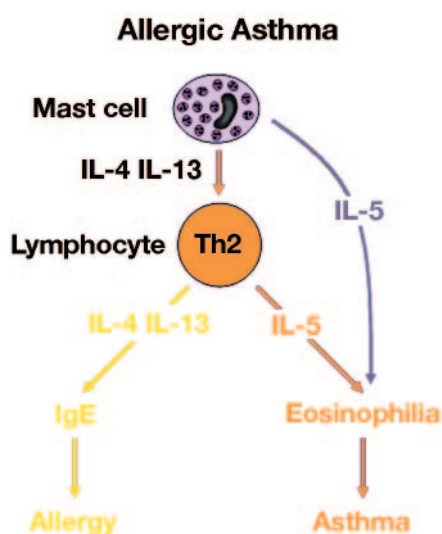


Figure 2. Simplified scheme to illustrate the source and role of IL-5 in eosinophil recruitment.

Asthma is more than an inflammatory disease: evidence for disordered repair

A proven way of reducing airway eosinophils, as well as other Th2-linked inflammatory processes that is highly efficacious, is the early and regular use of inhaled corticosteroids.^{33,34} However, two large 3-year longitudinal studies that investigated the effects of inhaled beclamethosone dipropionate (CAMP) or budesonide (START) have shown little or at best only a partial effect on the natural history of asthma, as measured by the post-bronchodilator FEV₁.^{35,36} One reason for this is that inflammation *per se* may not explain the full pathophysiology of this complex disease, especially the observed increase in smooth muscle³⁷ and thickening of the airway³⁸ that is characteristic of chronic asthma. Longitudinal paediatric studies such as the German Multi-Asthma Study (MAS)³⁹ have revealed a close association of allergen exposure in early infancy with subsequent childhood sensitization but *not* to the development of asthma. Analyses of the published epidemiological literature indicate that the population attributable risk of atopy or allergen exposure on asthma is <40%, and for dust mite exposure, is only 4%.^{40,41} This apparent low impact of mite exposure might explain the relatively poor clinical responses observed in asthma with extensive dust mite reduction strategies.⁴² Other factors that have been linked to the inception of asthma include passive smoking, diet (low antioxidant and high salt, saturated fat and protein), selected virus infections (e.g. RSV), prolonged breast feeding, exposure to certain drugs (e.g. paracetamol) and prematurity. Since many of these environmental agents interact with the airway epithelium, abnormal tissue injury and repair creates an appropriate microenvironment for persistent airway inflammation and remodelling in asthma to persist, resembling a chronic wound response.⁴³ (Figure 3). While allergy becomes an important triggering mechanism for attacks of asthma or aggravating established asthma, chronic asthma is a disorder where the microenvironment generated by the airway itself is able to sustain chronic inflammation that is difficult to reproduce in animal models of allergen sensitization and challenge.

Among the pathological abnormalities of asthma that Huber and Kessler⁷ drew attention to in their classical publication was hyaline thickening of the epithelial basement membrane, which they considered a diagnostic feature of the disease. Subsequently, transmission electron microscopy and immunostaining of matrix proteins have revealed

the presence of increased collagen types I, III and V fibronectin, tenascin and perlecan in the *lamina reticularis* underneath a normal-looking basement membrane.^{44,45} The cells responsible for this matrix

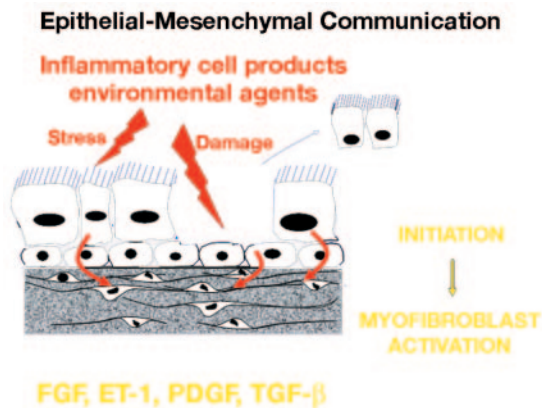


Figure 3. Interaction between the epithelium and underlying mesenchymal cells in asthma. Increased susceptibility of the epithelium to injury by environmental agents linked to an impaired repair response creates a chronic wound scenario to explain the chronic inflammatory and remodelling responses of asthma. Epithelial cell activation leads to the release of a range of growth factors that act on underlying mesenchymal cells.

are epithelial cells and myofibroblasts that lie beneath the epithelium.⁴⁶ (Figure 4). These structural changes, together with the evidence of epithelial injury and stress, have led us to suggest that a *primary* abnormality in persistent asthma is epithelial cell loss, not from cytotoxicity consequent upon the release of inflammatory products as commonly thought,⁴⁷ but due to premature programmed cell death (apoptosis).⁴⁸ This has been confirmed in endoscopic bronchial biopsies from patients with asthma by demonstrating increased 3' DNA nicking (Tunnel staining) in epithelial columnar cells and increased immunostaining for the p85 caspase-3 cleavage product of poly-ADP-ribose polymerase (PARP).⁴⁸ Apoptosis occurs in patches of columnar cells, in association with PARP-positive epithelial cell clumps recovered by bronchoalveolar lavage that resemble Creola bodies. Using bronchial brushing to obtain epithelial stem cells suitable for *in vitro* culture, confluent epithelial monolayers from asthmatic airways exhibited increased susceptibility to oxidant-induced apoptosis, even after the cells had been passaged two or three times.⁴⁸ This epithelial cell phenotype may have a genetic basis, or be the result of early environmental cell programming.

Comparison of normal and asthmatic bronchial epithelium

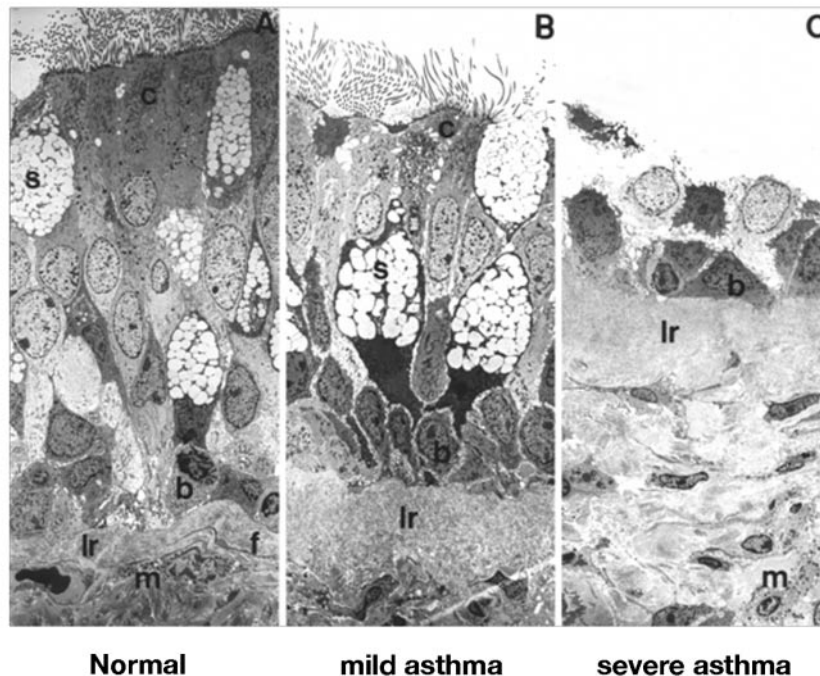


Figure 4. Transmission electron micrographs of the normal (left), mild asthmatic (middle) and severe asthmatic (right) airway epithelium. Note the epithelial disruption involving columnar cells in severe asthma and the deposition of collagen in the lamina reticularis, characteristic of this disease.

When the caspase pathways become activated in epithelial cells, there occurs rapid disruption of cytokeratin filaments and cell adhesion molecules involved in maintaining tight junctions, so that the cells lose their attachments and are lost into the airway lumen. This loss of columnar cells is paralleled by a marked up-regulation of epidermal growth factor receptor (CerbB1, Her1, EGFR), whose level of expression is proportional to disease severity, and is resistant to corticosteroid treatment.⁴⁹ Activation of EGFR by ligands such as EGF, transforming growth factor (TGF) α and amphiregulin results in basal cell proliferation to reconstitute the epithelium, in a process referred to as healing without scar formation, or by 'primary intention'. However, when the asthmatic epithelium is examined for evidence of a proliferative response using biomarkers such as proliferating cell nuclear antigen (PCNA) or Ki67, the level of immunostaining of these markers is no different from that observed in the epithelium of normal controls.^{50,51} Despite evidence of marked epithelial injury and stress in asthma, the epithelium fails to mount an appropriate repair response.

One reason for this is the inhibition of cell cycling. Airway epithelial cells are richly endowed with the protein P_{21}^{waf} , whose function in the cytoplasm is to increase epithelial cell survival, and in the nucleus, to arrest cell division at the G_1 phase.⁵¹ In mild asthma, immunostaining for P_{21}^{waf} shows that it is mostly restricted to the cytoplasm of columnar cells, where it functions to enhance cell survival when the cells are confronted with oxidant injury. However in more severe disease, a higher proportion of the P_{21}^{waf} is localized to the basal epithelial cells and, more specifically, to the nucleus, where it suppresses cell proliferation.⁵² Using confluent epithelial cell culture, *in vitro* inhibition of the EGFR tyrosine kinase followed by physical or chemical injury to the cells results in increased production of pro-fibrogenic cytokines including fibroblast growth factor-2 (FGF-2), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1) and endothelin 1 (ET-1), all of which exert proliferative effects on airway fibroblasts in co-culture experiments.⁵³ Under these conditions, active TGF- β is also released through the action of epithelial-derived MMP9 and cathepsins on the latent form of the growth factor. The active form of TGF- β further inhibits epithelial cell proliferation by promoting translocation of P_{21}^{waf} to the nucleus.

Active TGF- β also provides a differentiation signal to the underlying fibroblasts, by generating connective tissue growth factor (CTGF), which converts these cells into myofibroblasts.⁵⁴ Asthmatic

myofibroblasts contain bundles of α -actin and heavy chain myosin, indicative of their contractile phenotype, and also secrete large amounts of matrix proteins and proteoglycans. In addition, myofibroblasts generate large quantities of pro-inflammatory cytokines including eotaxin, GM-CSF, vascular endothelial growth factor (VEGF) and endothelin-1, indicative of their ability to maintain a chronic inflammatory response. While the precise relationship between myofibroblast transformation and the increase in underlying airway smooth muscle has yet to be established, in the bladder, outflow obstruction causes myofibroblasts to be converted into abnormal smooth muscle.⁵⁵ RNA micro-array analysis of airway myofibroblasts reveals a very high proportion of genes associated with the contractile properties of smooth muscle.⁵⁶ The observation that both asthmatic mesenchymal cells cultured from airway biopsies, and airway smooth muscle microdissected from resected lung specimens,⁵⁶ exhibit enhanced proliferative capacities (in the absence of exogenous growth factors) and secrete larger quantities of cytokines and growth factors, reinforces the case that these cells are able to support airway inflammation and remodelling.⁵⁷ The increase in mast cells, but not eosinophils or T lymphocytes, in asthmatic airway smooth muscle, has been shown to relate to BHR and variable airflow obstruction in asthma, and is not likely to result from secretion of mast cell growth factors (such as stem cell factor (SCF) and TGF- β) and chemotactic factors (such as RANTES and IP-10) being secreted by the abnormal smooth muscle and myofibroblasts.⁵⁸

Altered communication between the epithelium and underlying fibroblasts has similarities with the branching morphogenesis that occurs during lung development in the fetus, in which there is activation of the epithelial mesenchymal trophic unit (EMTU) involving intermittent production of both epithelial and fibroblast growth factors⁵⁹ (Figure 3). Although activation of the EMTU is implicated in providing the stimulus for asthma chronicity, this does not mean that Th-2-mediated inflammation is not important. Th-2 cytokines, especially IL4, IL9 and IL13, have powerful effects on the differentiating epithelium by altering its trajectory away from a ciliated stratified structure towards a mucus-secreting (goblet-cell-enriched) phenotype.⁶⁰ Although normal and asthmatic epithelial cells exhibit similar responses to IL4 and IL13 to enhancing the release of mediators such as IL8 and GM-CSF, asthmatic epithelial cells have an enhanced capacity for forming goblet cells via the generation of the EGFR ligand TGF α , which is a powerful stimulus for goblet cell differentiation.⁶¹

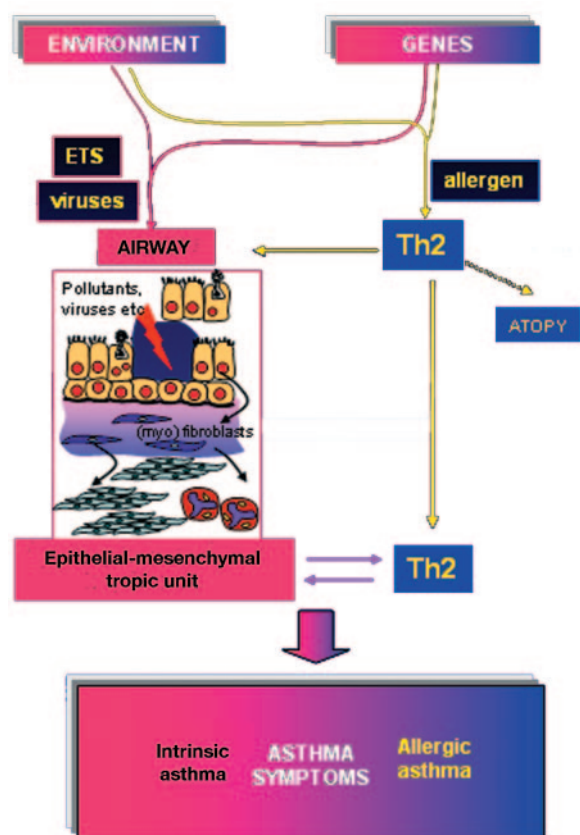


Figure 5. Interaction(s) between the inflammatory and EMTU components in the pathogenesis of chronic asthma.

Both IL-4 and IL13 are also able to increase the epithelial production of the profibrogenic growth factor TGF- β 2, which is insensitive to corticosteroids.⁶² An interplay between Th-2-mediated inflammation and an activated EMTU provides the necessary micro-environment for maintaining chronic airway inflammation as well as remodelling that together are linked to BHR (Figure 5).

By analogy with chronic mucosal inflammation at other anatomical sites, it has been thought that the increase in airway smooth muscle and other features of airway wall remodelling occurs as a direct consequence of long-standing chronic airway inflammation.⁶³ However, recent bronchial biopsy studies in asthmatic children have shown that 'remodelling' changes are present at the inception of asthma. In a bronchial biopsy study involving 4–14-year-old children, those with asthma had increased thickening of the subepithelial *lamina reticularis*, and increased expression of EGFR, but reduced evidence of proliferation in the presence of increased P₂₁^{waf} expression.^{64–66} These changes are identical to those observed in adult asthma. Importantly, these changes appeared independent of eosinophil infiltration,^{65,66} and suggest that for

Th2-type inflammation to be expressed in the lower airways, a parallel series of lung morphogenetic factors are required, in which activation of the EMTU is paramount.

The genetic basis of asthma: identification of a new asthma gene

As both Salter and Osler recognized, asthma and allergy run in families.^{1,2} However, twin and extensive pedigree studies show that BHR is inherited independently of atopy.⁶⁷ The underlying mechanism(s) responsible for BHR in asthma is regarded as 'the Holy Grail' of asthma, since they are fundamental to the paroxysms of airway obstruction that lead to the clinical expression of the disease.⁶⁸ Explanations for BHR include mucosal swelling, excessive airway smooth muscle (ASM) shortening, an increase in smooth muscle mass causing greater force generation, and an excessive velocity of contraction linked to altered cross-bridge cycling.

A significant advance in defining the genetic origin of BHR has come from genome-wide screens investigating linkage between asthma and its partial phenotypes with chromosomal regions. In one of these (Figure 6), involving 420 affected sib-pair families (including 360 from the south coast of England), strong linkage was found between asthma and microsatellite markers on chromosome 20p13. Subsequent physical mapping and DNA sequencing of the region of linkage followed by both case-control and family-based single nucleotide polymorphism (SNP) allelic association studies, led to the identification of a *disintegrin* and *metalloprotease* (ADAM) 33 as the gene responsible for the linkage signal.⁶⁹ Haplotypes comprising combination of SNPs in the exonic, intronic and 3'-non-coding regions of ADAM33 greatly strengthened the statistical association with asthma. The level of statistical significance for both the linkage and allelic association in relation to asthma was further strengthened when doctor-diagnosed asthma as the phenotype was conditioned by the presence of BHR, but was weakened by total serum IgE or specific IgE. This suggested that ADAM33 was more closely associated with structural and functional responses of asthmatic airways, rather than manifestations of atopy. In the mouse, a locus for BHR (*bhr1*) has been mapped to a region on chromosome 2 (74 cM) that is syntenic to chromosome 20p13 and very close to the location of the mouse orthologue of ADAM33 (73.9 cM).⁶⁹

Genetic Mapping of ADAM33

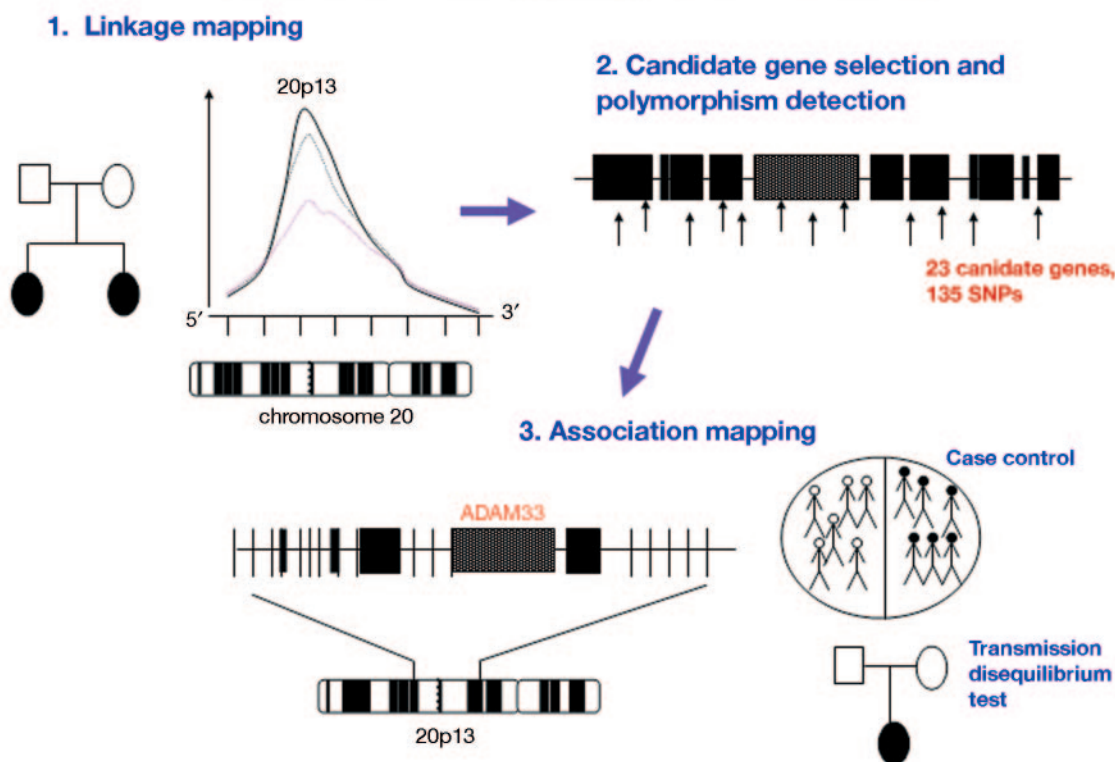


Figure 6. The positional cloning of the new candidate gene ADAM33. Sequentially, this included a genome-wide scan using microsatellite markers, fine mapping of the region of linkage to asthma, cloning and sequencing of contigs expressed in bacterial artificial chromosomes, identification of possible candidate genes, SNP typing of these potential candidates, case control, TDT and haplotype association studies and, finally, cell and tissue expression of mRNA.

ADAM33 is the most recently described member of the ADAM gene family of zinc-dependent metalloproteases that includes TNF α -converting enzyme (ADAM17, TACE). It is phylogenetically most closely related to human ADAM12 and ADAM19. However, unlike other members of the ADAM family, ADAM33 mRNA is selectively expressed in mesenchymal cells, supporting the view that its abnormal function maybe linked to the pathogenesis of BHR and airway wall remodelling.⁷⁰ While the functions of ADAM33 are still largely unknown, in common with other ADAM proteins it possesses eight domains, including a catalytic domain, a disintegrin domain, a cysteine-rich domain, an EGF-like domain, a transmembrane domain, and cytoplasmic domains. As shown by Asakura *et al.*,⁷¹ in the pathogenesis of hypertensive-induced cardiac hypertrophy, the catalytic site (in this case of ADAM12) has the capacity to generate heparin-binding EGF-like growth factor (HB-EGF), which was considered responsible for prolonging myocyte survival. A similar function of ADAM33, releasing soluble growth factors that promote

airway mesenchymal cell proliferation and survival, provides one mechanism whereby gain of function maybe associated with BHR.⁷² We have found that at least six alternatively spliced variants of ADAM33 exist, but the majority of transcripts expressed in either normal or asthmatic airway mesenchymal cells do not possess the catalytic subunit.⁷³ Thus, independent of its proteolytic enzymic function, other mechanisms linking ADAM33 to BHR include enhanced differentiation to a contractile phenotype, fusion of myocytes into a single muscle unit, and enhanced migration to build abnormal muscle bundles. The recent demonstration of ADAM33 mRNA expression in mouse lungs at the onset of branching morphogenesis⁷⁴ suggests that this gene maybe important in lung development, and that subtle polymorphic changes could predispose infant airways to asthma. Of the 58 single nucleotide polymorphisms so far identified in ADAM33, only seven lead to changes in amino acid sequence, the majority of which are clustered in the transmembrane and cytoplasmic domains.⁷⁵ The combinations of SNPs that are most closely

associated with BHR and asthma are also clustered at this end of the gene, including the intronic and the 3' untranslated region (the S, T and V domains). At this time, it is not known which parts of the ADAM33 molecule are responsible for its genetic association with asthma, although confirmation of association of relevant SNPs in *ADAM33* has been confirmed in both family and population-based studies in Caucasian, Hispanic and Black USA citizens.⁷⁵ Those influencing activation of the catalytic site of ADAM33, its intracellular transport, alternative splicing and mRNA stability appear to exert the strongest associations. Most recently, a number of these SNPs have been shown to predict the accelerated decline in baseline lung function over 20 years in chronic asthma⁷⁶ and to predict increased airways resistance in 3- and 5-year-old children born to allergic parents.⁷⁷ These observations add to the view that ADAM33 is in some way involved in the pathogenesis of airway wall re- (or pre-) modelling.

The involvement of tissue specific genes such as *ADAM33* in predisposing individuals to asthma provides an explanation for the independent heritability of BHR from atopy, the occurrence of asthma in individuals who are not atopic (e.g. intrinsic and some forms of occupational asthma), the morphological and functional features of airway wall remodelling and thickening, linked to an accelerated decline in lung function over time in those with more chronic and severe disease, and the relative failure of inhaled corticosteroids to influence the natural history of asthma, despite their ability to effectively suppress airway inflammation. Based on this, an alternative strategy to therapy would be to enhance the ability of the asthmatic epithelium to defend itself against the environment and repair without activation of underlying mesenchymal cells, i.e. to target the EMTU. Possibilities might include the use of inhaled surfactant, antioxidants or activators of epithelial tyrosine kinases that increase the resistance of the airway epithelium to injury.

Postscript

Ending on a historical note, it is worth noting that in a footnote to John Forbes's 1838 translation of Laenec's third edition⁷⁸ commenting on the effective treatment of asthma (my italics): 'Asthma... in every other case a more correct pathology in this disease will put us in the way of a more rational practice, instead of wasting our efforts in attempting to ward off paroxysms of a purely spasmodic nature by measures directed to the nervous system, our

attention will be directed to the real disease—the structural alteration and preternatural sensibility of the bronchial membrane'.

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