

Molecular mechanisms in allergy and clinical immunology

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Understanding the pathophysiology of severe asthma to generate new therapeutic opportunities

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Although asthma is defined in terms of reversibility of airflow obstruction, as the disease becomes more severe and chronic, it adopts different characteristics, including a degree of fixed airflow obstruction and corticosteroid refractoriness. Underlying these phenotypes is evidence of airway wall remodeling, which should be distinguished from the increase in smooth muscle linked to airways hyperresponsiveness. Aberrant epithelial-mesenchymal communication leads to a chronic wound scenario, which is characterized by activation of the epithelial-mesenchymal trophic unit, epithelial damage, the laying down of new matrix, and greater involvement of neutrophils in the inflammatory response. In allergic asthmatic patients who remain symptomatic despite high-dose corticosteroid therapy, blockade of IgE with omalizumab confers appreciable clinical benefit. Chronic severe asthma is also accompanied by a marked increase in TNF- α production that might contribute to corticosteroid refractoriness. Based on this, TNF blockade with the soluble fusion protein etanercept produces improvement in asthma symptoms, lung function, and quality of life paralleled by a marked reduction in airways hyperresponsiveness. Identification of novel susceptibility genes, such as a disintegrin and metalloprotease 33 (ADAM33), will provide further targets against which to direct novel therapies for asthma, especially at the more severe end of the disease spectrum. (*J Allergy Clin Immunol* 2006;117:496-506.)

Key words: Severe asthma, airway remodeling, tumor necrosis factor, etanercept, anti-IgE, omalizumab, a disintegrin and metalloprotease 33, airway responsiveness, new treatments

Abbreviations used

ADAM: A disintegrin and metalloprotease
APP: Amyloid precursor peptide
BAL: Bronchoalveolar lavage
BHR: Bronchial hyperresponsiveness
COPD: Chronic obstructive pulmonary disease
ECM: Extracellular matrix
EMTU: Epithelial-mesenchymal trophic unit
LABA: Long-acting β_2 -agonist
LTRA: Leukotriene receptor antagonist
SNP: Single nucleotide polymorphism
TIMP: Tissue inhibitor of metalloprotease

Asthma is a disorder of the conducting airways characterized by T_H2 -mediated inflammation and enhanced mediator release. In mild disease the inflammatory response, attendant bronchial hyperresponsiveness (BHR), and variable airflow obstruction are highly responsive to inhaled corticosteroids, positioning these drugs as first-line controller therapy for this disease. However, in persistent and severe asthma inhaled corticosteroids are only partially effective, and patients often require intermittent or continuous oral corticosteroids. This severe end of the disease spectrum accounts for approximately 10% of the asthmatic population.¹ It is these patients who have the highest morbidity and mortality and account for approximately 30% of the health cost of this disease through unscheduled doctor visits, hospital admissions, and corticosteroid side effects. Although a wide range of other treatments are used in these patients that include cytotoxic agents and nebulized bronchodilators, these are of variable benefit, with appreciable side effects.² Thus severe corticosteroid-refractory asthma represents a major unmet clinical need.³

Because of difficulty in accessing the airways in patients with severe asthma, it is only recently that additional insight has been gained into the underlying

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mechanisms that separate refractory asthma from that which is highly corticosteroid responsive. The first important point to note is the level of heterogeneity of severe disease.² On the one hand, there is extensive evidence of inflammation spreading to involve all the airways, including the most peripheral, with some alveolar involvement,⁴ whereas on the other hand, there are cases in which there is scant evidence of inflammation but an increase in smooth muscle, extensive remodeling of the airway epithelium and walls, or both² and also many intermediate phenotypes. As the disease adopts a more chronic phenotype, despite high-dose inhaled corticosteroids, the degree of bronchodilator reversibility becomes progressively impaired because of a combination of matrix deposition and reduced access of inhaled aerosols to airways obstructed by mucus and cell debris. When inflammation is present, its character also changes to involve neutrophils either alone or in addition to eosinophils.^{5,6} It had been widely thought that the neutrophilia was the result of chronic corticosteroid therapy. Although oral (but not inhaled) corticosteroids induce a circulating and sputum neutrophilia,⁷ presumably through their capacity to release neutrophil precursors from the bone marrow and their capacity to inhibit apoptosis, there is increasing evidence that in severe asthma the neutrophils are in an activated state and therefore likely to contribute to tissue damage.⁸

Longitudinal cohort studies have established that a proportion of severe asthma has its origins in childhood and tracks throughout life.^{9,10} A combination of computed tomographic imaging and bronchial biopsy studies have shown that severe asthma in childhood also exhibits features of airway remodeling,¹¹ comprising epithelial injury and matrix deposition,¹² as well as eosinophilic-neutrophilic inflammation.¹³ These findings, which occur early in the natural history of severe asthma, indicate that genetic and environmental factors might exert their effects early in life,¹⁴ although in wheezing babies evidence of remodeling is not present.¹⁵

When compared with mild disease, severe asthma has a number of associated features that include high prevalence of sinusitis, intolerance to nonsteroidal anti-inflammatory drugs, increased body mass index, and psychiatric disorders.^{2,5,16} Early-onset severe asthma has a high incidence of accompanying atopy with associated disorders, such as eczema, food allergy, and rhinitis,¹⁷ whereas late-onset asthma is characterized by less atopy⁶ but a greater association with occupational exposures¹⁸ and a clinical presentation that is frequently confused with that of chronic bronchitis with frequent infectious exacerbations.¹⁹

From a clinical perspective, lack of adherence to treatment is an important cause for increased asthma severity. There is now strong evidence that a high proportion of patients with severe disease can see improvement through attention to treatment adherence.²⁰ Asthma guidelines recommend a combination of an inhaled corticosteroid and a long-acting β_2 -agonist (LABA), usually administered twice daily.²¹ The clinical trial base for this approach is extensive, although for some patients, the replacement of a LABA with a leukotriene receptor antagonist (LTRA) or

the addition of an LTRA^{22,23} produces further benefit, especially in those patients shown to be intolerant to nonsteroidal anti-inflammatory drugs.²⁴ There is little doubt that the uptake of inhaled corticosteroids is the principal factor in the decreasing asthma mortality in countries in which this has been observed,²⁵ although recently some concerns over the widespread use of inhaled LABAs, as well as short-acting β_2 -agonists, have emerged.²⁶ As a rule, LABAs should not be used without an inhaled corticosteroid, and in children with severe asthma, in whom LABAs are reportedly less effective,²⁷ an LTRA is frequently effective.

Although there continues to be an ongoing debate about the principal mechanisms of corticosteroid-refractory asthma, several abnormalities have emerged.²⁸ These include the capacity of certain cytokines, such as TNF- α and IL-1 β , to activate p38 mitogen-activated protein kinase, thereby interfering with nuclear localization of glucocorticosteroid receptors,²⁹ reduced acetylation of a lysine residue in histone 4 leading to impaired activation of anti-inflammatory genes,³⁰ and cytokine induction of an alternatively spliced variant of the glucocorticosteroid receptor (β -variant) that is capable of binding to DNA but not to corticosteroids, thereby acting as a dominant-negative inhibitor.^{31,32} Of considerable interest is the recent finding that tobacco smoking markedly impairs corticosteroid responsiveness in asthma,³³ although responsiveness to other controller therapies, such as LTRAs, are said to remain intact.³⁴ The most likely mechanism that explains this is reduced histone deacetylase activity, as recently described in corticosteroid refractoriness in chronic obstructive pulmonary disease (COPD).³⁵

The recognition that current asthma therapy is failing to control the disease in a significant proportion of patients with severe asthma has created an opportunity for novel therapeutic approaches. Although a number of strategies targeting therapy at specific aspects of the inflammatory response, such as IL-4, IL-5, and IL-10, have not resulted in the predicted clinical benefits,³⁶ there is a reason to be optimistic about some new approaches both in controlling severe chronic asthma and in preventing exacerbations.

TARGETING IgE

IgE has long been known as a major therapeutic target in atopic disease after its discovery in 1967; however, only recently has this become possible in severe atopic asthma with the introduction of neutralizing IgG biologic agents targeted to that part of the IgE molecule that interacts with the high- and low-affinity IgE receptor. Once it was shown that anti-IgE therapy was highly effective at reducing free IgE in the circulation by forming small complexes,³⁷ the way was open to generate a therapeutic agent that could be used in severe allergic asthma. The result was omalizumab, an IgG1 mAb administered once or twice monthly as a subcutaneous injection. Extensive clinical trials have confirmed the efficacy of omalizumab in severe allergic asthma, both in increasing disease control and reducing

exacerbations.³⁸ Although therapy is limited to those patients with a total serum IgE level of between 30 and 700 IU/mL, its efficacy is high, with approximately two thirds of patients benefiting considerably.³⁹ Because treatment with omalizumab has been shown to have a marked effect on the allergic inflammatory response,^{40,41} it is difficult to explain why there is little or no response in approximately 30% of patients with severe allergic asthma.⁴² However, the physicians' overall clinical assessment after 16 weeks of therapy has been shown to be highly reliable in separating responders from nonresponders. Another unexplained feature of anti-IgE therapy is the apparent lack of effect on nonspecific BHR when measured by means of methacholine challenge, yet this therapy has a marked effect in reducing many aspects of airway inflammation.^{40,41} A recent study⁴³ has, however, shown that at both 4 and 12 weeks, omalizumab markedly reduces airway responsiveness to adenosine 5'-monophosphate, which in large part causes bronchoconstriction through activation of the adenosine A_{2B} receptor on mast cells (therefore an indirect challenge), but not to methacholine (a direct challenge).⁴⁴ There have been no studies of omalizumab that have examined its effect on methacholine responsiveness in patients with severe asthma.

One of the advantages of a systemic therapy such as omalizumab is the ability to improve allergic inflammation at sites other than the lung, including rhinitis, sinusitis, conjunctivitis, and food allergy,⁴⁵ which probably contributes to the marked increase in quality-of-life measures observed with this therapy in patients with severe asthma.⁴⁶ The longest period of anti-IgE treatment is 4 years. Because this therapy is directed to one of the principle pathophysiologic pathways of asthma, it will be of great interest to determine whether, over time, omalizumab is able to influence the natural history of asthma.

ANTI-TNF THERAPY IN SEVERE ASTHMA

Severe asthma exhibits an altered inflammatory cell profile manifest both in secretions and in tissue biopsy specimens and involving neutrophils or a mixture of neutrophils and eosinophils.^{5,47} TNF- α is a major therapeutic target in a range of chronic inflammatory disorders in which neutrophils are involved. These include rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, Crohn's disease, psoriasis, glomerulonephritis, sarcoidosis, and Behçet's disease, all of which are characterized by T_H1-type immune response associated with excess generation of TNF- α . Although asthma is predominantly considered an eosinophilic disorder involving T_H2 cytokines,⁴ as the disease becomes more severe and chronic, it adopts T_H1-type characteristics, with greater involvement of neutrophils⁵ and evidence of increased tissue destruction and remodeling.⁴⁸ In 1995, we first suggested that TNF- α was a potential mediator of severe asthma on the basis of an immunohistochemical assessment of patients who had died from asthma during the 1970s New Zealand epidemic.⁴⁹ Genetic association studies in

asthma have also shown association between TNF- α gene polymorphism, BHR, and asthma.^{50,51} Inhalation of TNF- α in rodents,⁵² guinea pigs,⁵³ and healthy⁵⁴ or asthmatic⁵⁵ human subjects leads to BHR accompanied by airway neutrophilia. In murine models of asthma, either a deficiency in TNF- α receptors,⁵⁶ or administration⁵⁷ or induction of a TNF- α autoantibody⁵⁸ resulted in marked attenuation of antigen-induced airway inflammation and BHR. Many different cell types produce TNF- α , but those especially relevant to asthma include T lymphocytes, monocytes, macrophages, mast cells, eosinophils, and epithelial cells.⁵⁹ In a recent study⁶⁰ we have shown that patients with severe corticosteroid-dependent asthma have higher concentrations of TNF- α in their bronchoalveolar lavage (BAL) fluid, whereas there was no difference in levels between patients with mild asthma and healthy control subjects. The increased TNF- α level is a feature of more persistent and corticosteroid-refractory asthma rather than of asthma per se and extends previous reports in which increased TNF- α -immunoreactive cells and a 30-fold greater TNF- α gene expression in bronchial biopsy specimens from patients with severe asthma compared with those with symptomatic nonsteroid-treated asthma.⁶¹ Immunostaining localized TNF- α predominantly to mast cells, as we have previously reported in biopsy specimens from patients with mild asthma.⁶²

In a similar population of patients with severe asthma, Berry et al⁶¹ have shown that when compared with healthy subjects or those with well-controlled asthma, their circulating mononuclear cells express increased cell-surface levels of membrane-associated TNF- α , TNF- α -converting enzyme (a disintegrin and metalloprotease 17 [ADAM17]), and the TNF receptors p55 and p75. Because anti-TNF- α strategies modify inflammatory diseases that persist despite corticosteroid therapy,⁶³ we have assessed anti-TNF- α in severe asthma. In an open-label study, we used the p75 fusion protein etanercept, which interacts with both TNF- α and TNF- β to prevent cytokine binding to the p55 and p75 cell-surface receptors. Patients with severe asthma treated with high-dose inhaled and oral corticosteroids received etanercept, 25 mg subcutaneously twice weekly for 12 weeks. This treatment resulted in a marked improvement in asthma symptoms, lung function, and BHR.⁶⁰ However, in contrast to omalizumab, the most striking feature of anti-TNF therapy was the improvement in BHR (measured as PC₂₀ methacholine) which, at the end of treatment, improved by 2.5 doubling dilutions beyond the change already achieved with corticosteroids (Fig 1). Recognizing that this study was uncontrolled, Berry et al⁶¹ have recently reported the beneficial effect of etanercept in a small 10-week cross-over trial involving 10 patients with refractory asthma, achieving a 3.5 doubling dose increase in PC₂₀, a remarkable 0.85-point increase in asthma quality-of-life score, and an improvement in postbronchodilator FEV₁ value. Of particular interest in this study was their identification of peripheral blood mononuclear cell (PBMC) TNF- α membrane expression as a biomarker of the treatment response. In another 2-week study of etanercept, but this time in

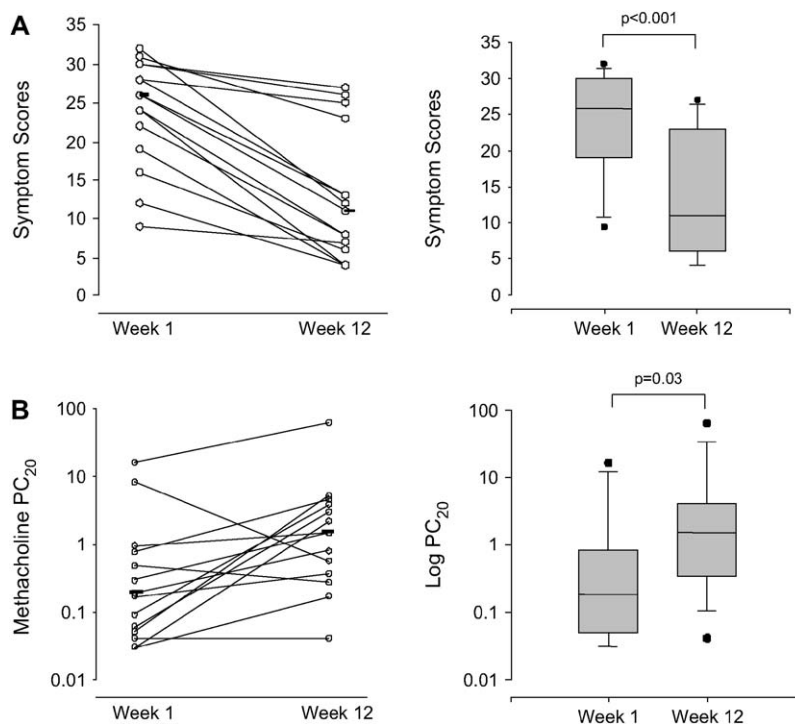


FIG 1. Effect of etanercept administered for 12 weeks on symptoms (A) and methacholine hyperresponsiveness measured as PC₂₀ (B) in patients with severe corticosteroid-refractory asthma. Reproduced with permission from BMJ Publishing Group from Howarth et al.⁶⁰

patients with mild allergic asthma, there was a marked increase in p75 levels in BAL fluid consistent with penetration of the p75 fusion protein into the airways.⁶⁴ Of further interest in this study was a lack of effect of etanercept either on airway inflammation assessed by means of BAL or on methacholine responsiveness, further strengthening the view that TNF- α is preferentially involved as a mediator of severe disease. Large clinical trials in patients with severe asthma using both etanercept and blocking mAbs, against TNF- α , are now in progress.

AIRWAY WALL REMODELING: ROLE OF SUSCEPTIBILITY GENES

BHR is a fundamental abnormality in asthma that increases in proportion to disease severity and is functionally antagonized by β_2 -adrenoceptor agonists. Despite extensive research on the topic, the mechanisms underlying BHR are still not known for certain, but an increase in airway smooth muscle involving both hyperplasia⁶⁵ and hypertrophy⁶⁶ and alterations to its physicochemical properties are considered important.⁶⁷ These events seem to be especially relevant in more severe disease, when BHR becomes refractory to corticosteroids and the airways no longer respond to the smooth muscle relaxant effect of deep inspiration.⁶⁸ Two recent studies in which airway smooth muscle was examined in bronchial biopsy specimens in patients with severe and mild-to-moderate asthma has confirmed the increase in severe disease and a reduced

distance between the epithelial and smooth muscle layer.^{65,69} BHR is to be differentiated from the progressive decrease in lung function that occurs in chronic asthma over time. The term *remodeling* has been used to describe the deposition of new matrix in the airway walls⁷⁰ and is associated with proliferation and activation of myofibroblasts.⁷¹ In severe asthma epithelial injury and impaired repair lead to the release of profibrogenic growth factors (including members of the epidermal growth factor, fibroblast growth factor, and TGF- β families) that act on primitive mesenchymal cells to convert them into myofibroblasts. These stromal cells exhibit greatly increased capacity to synthesize new extracellular matrix (ECM) and generate potent mitogens and differentiation factors for smooth muscle cells.^{72,73} Because aberrant communication between the epithelium and underlying mesenchyme reproduces some of the events involved in fetal lung development,¹⁸ the term *epithelial-mesenchymal trophic unit* (EMTU) has been applied to describe this component of asthma,⁷³ as also described by Plopper and colleagues when investigating the effects of inhaled environmental factors in airway morphogenesis in nonhuman primates.^{74,75} While the EMTU interacts with cells and mediators associated with T_H2-type inflammation, which are corticosteroid sensitive,⁷⁶ as the EMTU assumes a more active role in driving remodeling in severe and chronic disease, ensuing airflow obstruction becomes increasingly refractory to corticosteroids⁷⁷ and dissociated from some of the environmental factors important in milder disease, such as environmental allergen exposure.

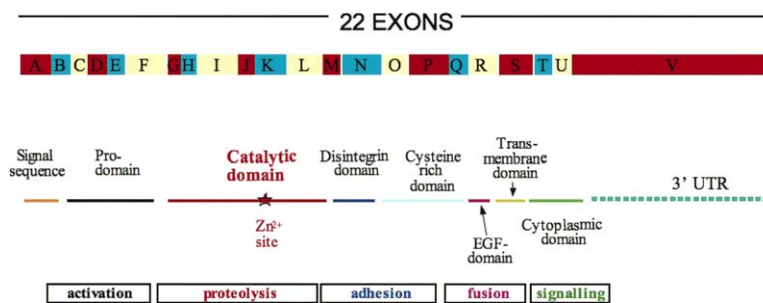


FIG 2. Domain structure of ADAM33 in relation to function. Each exon of the ADAM33 gene is identified by letters A through V (top). EGF, epidermal growth factor; UTR, untranslated region. Reprinted by permission from Macmillan Publishers Ltd: Van Eerdewegh et al.⁹¹

Using a newly developed bronchoscopic ultrasonographic probe, we have recently shown that BHR is inversely related to airway wall thickness in patients with asthma.^{78,79} A similar conclusion has been drawn when wall thickness was measured with computed tomography.⁸⁰ These observations appear to run counter to the mathematic models that predict a positive association between BHR and airway wall thickness. Recognizing that the airway epithelium in patients with asthma is more susceptible to injury,⁸¹ including repeated bronchoconstriction,^{82,83} inhaled environmental insults, and mechanical stress associated with BHR, these are likely to be important stimuli for activating the EMTU in chronic asthma. Through the release of epithelial- and smooth muscle-derived fibrogenic growth factors, increased ECM deposition would be expected to increase stiffness of the airways, thereby reducing the narrowing caused by repeated smooth muscle contraction. However, over time, these events could lead to progressive fixed airflow obstruction, a feature characteristic of chronic asthma.⁸⁴ In support of this, there is altered matrix production throughout the bronchial wall in asthma,⁸⁵ and *in vitro* studies have shown that ECM components differentially affect smooth muscle differentiation and contractility.⁸⁶ Thus, in some patients persistent asthma might progress to disease in which regulatory processes within the ECM counteract smooth muscle contractility and lead to permanent impairment in lung function, whereas in others these regulatory pathways are less prominent, and BHR, diurnal variation in lung function, and reversibility are more characteristic.

SUSCEPTIBILITY GENES IN SEVERE ASTHMA

The observation that severe asthma in early childhood tracks into adulthood^{9,10} suggests that there are important susceptibility genes for disease severity and chronicity in addition to those that predict asthma itself. Several genes have been associated with severe disease, including the Gly 16 allele of the β_2 -adrenoceptor gene,⁸⁷ the C-509 T allele of TGF- β ,⁸⁸ the Ile 158 Ile allele of tissue inhibitor of metalloprotease (TIMP) 1,⁸⁹ and IL-4 and IL-4RA.⁹⁰ ADAM33 is the first novel gene that appears to be closely associated with both BHR⁹¹ and accelerated decrease in

baseline lung function.⁹² ADAM33 comprises 22 exons, which encode a full-length molecule of 812 amino acids comprising 7 domains, pro-, catalytic, disintegrin, cysteine-rich, epidermal growth factor, transmembrane and cytoplasmic, serving the functions of activation, proteolysis, adhesion, fusion, and signalling (Fig 2).^{91,93} ADAM33 is highly polymorphic, containing in excess of 100 single nucleotide polymorphisms (SNPs). To clarify the role of ADAM33 as a gene that contributes to asthma risk in the general population, Blakey et al⁹⁴ have undertaken a meta-analysis of currently published association studies of ADAM33 and asthma supplemented by additional populations from the United Kingdom and Iceland. Combined analysis involving 1299 patients and 1665 control subjects in case-control studies and 4561 individuals in family studies, they showed that the F+1 and ST+7 variants were significantly associated with asthma. According to the authors, additional risk imparted by these variations could account for 50,000 excess cases of asthma in the United Kingdom alone.

Expression of ADAM33 mRNA is restricted to mesenchymal cells, which includes airway fibroblasts, myofibroblasts, and smooth muscle (Fig 3).^{91,95,96} In airway fibroblasts we have identified full-length ADAM33 and 6 alternatively spliced variants lacking the catalytic domain (Fig 4).⁹⁷ Indeed, less than 2% of the mRNA transcripts of ADAM33 in airway fibroblasts contained the enzymic active-site sequence. Using an antibody directed to the cytoplasmic domain of the protein, we have also found that the majority of ADAM33 exists as alternatively spliced isoforms⁹⁷ that localize to myofibroblasts and smooth muscle cells. Again, full-length ADAM33 containing the catalytic domain was present at very low levels. This might suggest that the various alternative splice variants serve as dominant negative regulators of the full-length molecule.

The catalytic domain has recently been crystallized and shown to contain a unique loop close to the active site that is likely to influence substrate specificity (Fig 5).⁹⁸ Screening of peptide sequences has identified stem cell factor, amyloid precursor peptide (APP), the insulin B chain, and TNF-related activation-induced cytokine as substrates for cleavage, but kinetic analysis indicate that these are not likely to be the natural substrates.⁹³ Indeed,

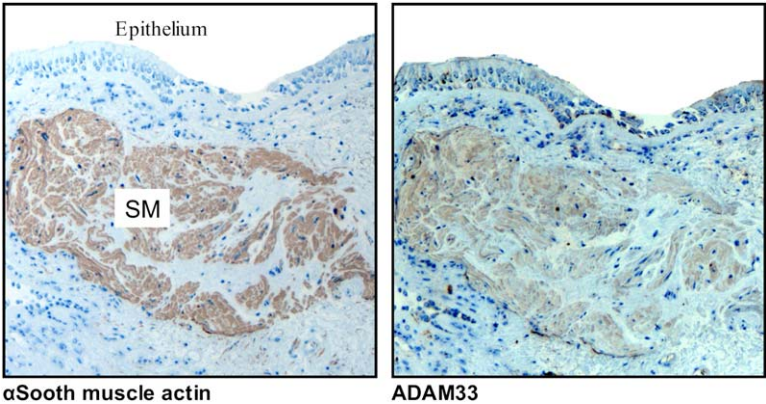


FIG 3. Localization of ADAM33 protein to airways smooth muscle (SM) in a bronchial biopsy specimen from a patient with severe asthma. Reprinted with permission from Haitchi et al.¹¹⁶ Official Journal of the American Thoracic Society. © American Thoracic Society.

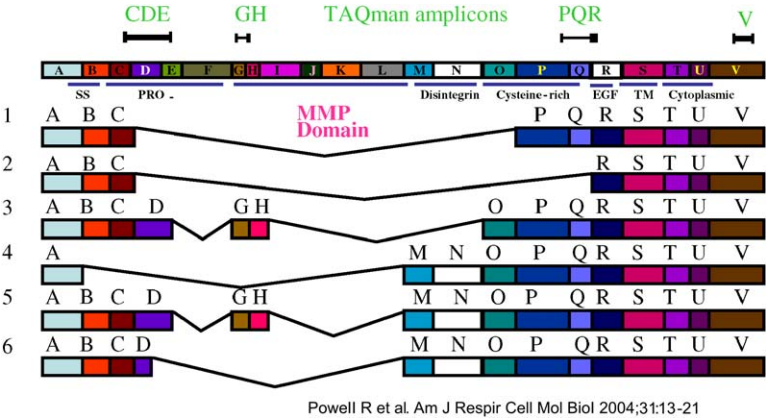


FIG 4. Alternatively spliced variants of ADAM33 in human fibroblasts identified by means of PCR. EGF, Epidermal growth factor; TM, transmembrane. Reprinted with permission from Powell et al.⁹⁷ Official Journal of the American Thoracic Society. © American Thoracic Society.

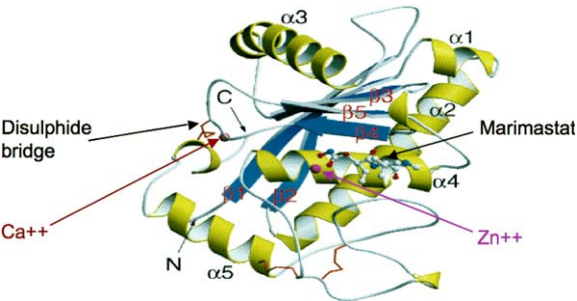


FIG 5. Three-dimensional structure of ADAM33 determined by means of crystallography in complex with the inhibitor Marimastat (shown as the stick and ball construct). The substrate binding site contains unique features that are specific for ADAM33, suggesting that it has a limited substrate specificity. C, C-terminal end; N, N-terminal end. Reprinted with permission from Orth et al.⁹⁸

on the basis of a novel peptide generated from APP and used in kinetic studies, Zou et al⁹⁹ have shown a 100-fold increase in the efficiency of cleavage when compared

with that of native APP. Also, different from other ADAMs, ADAM33 activity was effectively inhibited by TIMP-3 and TIMP-4 but only weakly by TIMP-1.⁹⁹

One important function of the ADAM proteins is their ability to interact selectively with other molecules through their disintegrin domain. This unusual property of proteolytic enzymes has led to ADAMs being regarded as “beloved liberators” (ie, using their adhesion property to seek their substrate).¹⁰⁰ Using recombinant ADAM disintegrin domains, Huang et al¹⁰¹ have recently shown that although ADAM12 inhibited cell migration mediated by $\alpha_4\beta_1$ integrin, but not by $\alpha_5\beta_1$ integrin, and ADAM17 displayed the reciprocal effect, ADAM33 shares with ADAM19 the capacity to inhibit cell migration mediated by both β_1 integrins.

AIRWAY PHENOTYPES ASSOCIATED WITH ADAM33

Although asthma is defined on the basis of airflow limitation that is reversible with β_2 -agonists, as the

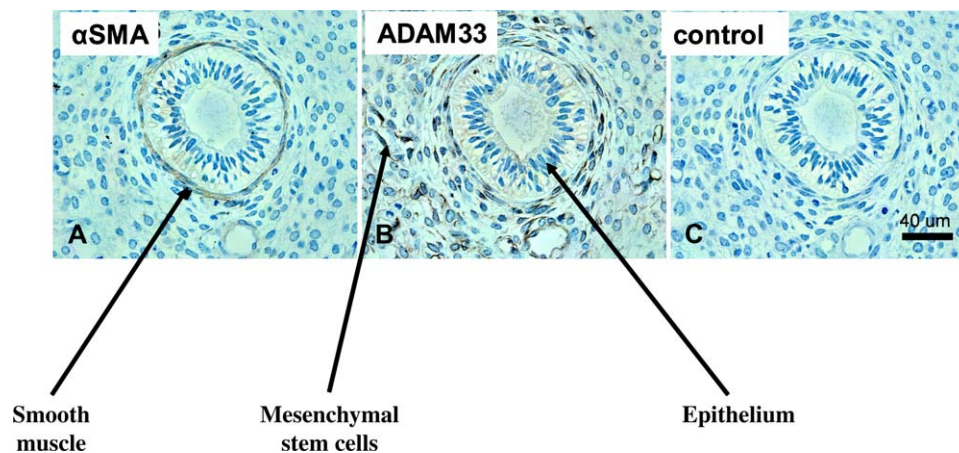


FIG 6. Immunolocalization of ADAM33 in 8- to 12-week human fetal lung tissue. ADAM33 immunoreactivity is mostly found around bronchial tubes in primitive mesenchymal cells but also in airways smooth muscle. α SMA, α Smooth muscle actin. Reprinted with permission from Haitchi et al.¹¹⁶ Official Journal of the American Thoracic Society. © American Thoracic Society.

disease adopts a more chronic phenotype, there occurs a progressive decrease in baseline lung function that is either nonreversible or difficult to reverse. In a cohort of Dutch patients with chronic asthma followed for 23 years, inhaled corticosteroids reduced the rate of decrease in baseline FEV₁ values in male subjects but not female subjects.¹⁰² Because severe persistent asthma is more prevalent in female subjects,⁶ this sex difference in treatment response with inhaled corticosteroids implied different mechanisms. It has been assumed that the underlying process responsible for the accelerated decrease in baseline lung function in nonsmoking asthmatic subjects is airway wall remodeling. There is evidence that in patients with chronic disease the airways are less distensible¹⁰³ and that this correlates with the thickness of the subepithelial reticular basement membrane.¹⁰⁴ Using a combination of endobronchial and transbronchial biopsy specimens, Bergeron et al¹⁰⁵ have shown that collagen deposition and α -smooth muscle actin immunoreactivity were markedly increased in the peripheral, as well as the central, airways in patients with chronic asthma but that this was only partially responsive to corticosteroids, presumably by modifying inflammation.¹⁰⁶ Van Rensen et al¹⁰⁷ have investigated a range of inflammatory markers in airway biopsy specimens that might predict an accelerated decrease in lung function in patients with asthma, but only the number of CD8+ lymphocytes achieved significance.

Although many studies have investigated the genetics of asthma susceptibility, longitudinal studies on the natural history of asthma or asthma outcomes are scarce.¹⁰⁸ ADAM33 is a good candidate because of its localization to mesenchymal cells.^{91,109} In the original UK population association studies revealed that 7 SNPs and 16 haplotypes in the ADAM33 gene were significantly associated with asthma and more specifically with BHR.⁹¹ Jongepier et al⁹² have investigated 9 ADAM33 SNPs in 200 Dutch patients with chronic severe asthma for whom they have longitudinal FEV₁ data spanning 20

years. Using a linear fixed-effect model taking account of other variables, the SNPs S2 and Q-1 significantly predicted a more rapid annual decrease in postbronchodilator FEV₁ values. The genetic association of ADAM33 with indices of disease chronicity in asthma increased the possibility that it might be involved in other chronic lung disorders, such as COPD, as a remodeling gene. To investigate this further, van Diemen et al¹¹⁰ genotyped ADAM33 in 1390 subjects from a general population in the Netherlands for which they have longitudinal lung function measurements over 25 years. By investigating 8 SNPs of ADAM33 and the same statistical approach as they had applied to chronic asthma in the general population, the S-2, Q-1, and S-1 variants, when compared with the wild type, predicted and accelerated the decrease in FEV₁ values of 4.9, 9.4, and 3.6 mL/y. They also reported a significantly higher prevalence of the F+1, S-1, S-2, and T-2 SNPs in subjects with COPD. On the basis of these findings, genetic variation in ADAM33 could exert more than one effect on the airways, either by influencing BHR through an effect on smooth muscle differentiation or contractility and on fixed airway obstruction and progressive decrease in lung function through ECM deposition by airway (myo)fibroblasts and smooth muscle cells.

Outcomes in adult asthma are, to a large extent, determined in childhood. Both genetic and environmental factors can influence baseline lung function and BHR independently of allergic sensitization. Longitudinal birth cohort studies have established that chronic severe asthma in children is also accompanied by a progressive loss of postbronchodilator baseline lung function over time.¹¹¹⁻¹¹³ Two recent large intervention studies with inhaled corticosteroids in children with asthma (Childhood Asthma Management Program and Inhaled Steroid Treatment as Regular Therapy in Early Asthma Study) revealed good short-term benefit but little or no long-term effect on either BHR or reduction in postbronchodilator baseline lung function as a reflection of remodeling.^{114,115} As observed

in adult airway tissue, fibroblasts, and smooth muscle, multiple alternatively spliced variants of ADAM33 are also present both at the mRNA and protein levels in 6- to 8-week human fetal lungs. In cultured human fetal lung tissue, the application of immunohistochemistry and laser confocal microscopy revealed that ADAM33 was localized to both developing airway smooth muscle and to primitive mesenchymal progenitor cells that surrounded the developing airways (Fig 6).¹¹⁶ The possibility exists that variation in ADAM33 could influence baseline lung function and predispose to the onset of asthma. In a study involving 302 three-year-old and 504 five-year-old children born of asthmatic and allergic parents (the Manchester Allergy and Asthma Study), 10 asthma-related SNPs in *ADAM33* have been shown to predict reduced lung function when measured as specific airway resistance, with the strongest association at both ages being to the F+1 SNP, which is close to the catalytic domain.¹¹⁷ At age 5 years, highly significant association was also seen between asthma-related *ADAM33* SNPs and reduced baseline FEV₁ values and the presence of wheeze in the first year of life. The F1, ST+5, V-3, and V5 SNPs associated significantly with the postbronchodilator FEV₁ value, but there was no association between any SNP and allergic sensitization.

Thus what began as a search for asthma susceptibility genes led to the identification of *ADAM33*. Subsequent studies suggest that not only is ADAM33 involved in asthma but also in COPD, it is a factor determining infant lung function and possibly is a gene involved in branching morphogenesis of the developing lung. It is still not clear which components of ADAM33 or its expression are responsible for its link to these lung diseases, but the recent identification of its epigenetic regulation by methylation of CpG islands¹¹⁸ suggests it is tightly regulated. Most recently, using an antibody directed to the catalytic domain of ADAM33, Lee et al¹¹⁹ have reported the presence of a 55-kd soluble form of ADAM33 in the BAL fluid of asthmatic, but not healthy, subjects, as well as confirming its presence in smooth muscle and epithelial basement membrane lamina reticularis. The levels of soluble ADAM33 were greater in patients with moderate-to-severe asthma compared with levels in those with mild asthma or healthy control subjects, with protein levels correlated inversely with FEV₁ values. If this finding is confirmed, then there will be further interest in ADAM33 as a new biomarker of asthma severity.

CONCLUSIONS AND FUTURE DIRECTIONS

Although current asthma therapy generally treats the disease as an homogeneous entity with combinations of inhaled corticosteroids, LABAs, short-acting β_2 -adrenoceptor agonists, and LTRAs, increasingly different phenotypes are being recognized against which specific therapies are being directed, such as anti-IgE and anti-TNF biologic agents. With greater understanding of molecular mechanisms and the identification of further novel

susceptibility genes in asthma, we are likely to witness the development of new diagnostic tests and treatments that are targeted to individual disease phenotypes. If such trends continue, then it is possible that treatments will be discovered that modify the natural history of asthma and reverse the changes associated with airway remodeling, rather than simply suppressing inflammation and dilating the airways.

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