

Original article

Increased expression of *ADAM33* and *ADAM8* with disease progression in asthma

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Background: *ADAM33*, a disintegrin and metalloproteinase 33 gene, has been identified as a risk factor for asthma and bronchial hyperresponsiveness and has been postulated as a gene for airway remodeling. *ADAM8* is strongly induced by allergens and T_H2 cytokines in the lung in experimental asthma.

Objectives: To assess the importance of these genes in asthma pathogenesis and to investigate whether expression relates to disease severity or deterioration in lung function, we measured the mRNA and protein expression of both genes in bronchial biopsies of subjects with asthma and control subjects.

Methods: RNA was extracted from frozen endobronchial biopsies of mild, moderate, and severe adults with asthma and controls. Subjects with moderate and severe asthma were taking corticosteroids. The mRNA transcript of both genes was measured by real time RT-PCR using specific primers. Protein expression was examined by immunohistochemistry on paraffin sections.

Results: *ADAM33* mRNA expression was significantly higher in both moderate and severe asthma compared with mild asthma ($P < .05$) and controls. Immunostaining for *ADAM33* was increased in the epithelium, submucosal cells, and smooth muscle in severe asthma compared with mild disease and controls. *ADAM8* mRNA expression was significantly increased in all asthma groups compared with controls. Increased inflammatory cells stained positive for *ADAM8* in both moderate ($P < .05$) and severe asthma ($P < .005$) compared with mild disease.

Conclusions: These results demonstrate increased expression of both ADAM genes as asthma severity increases.

Clinical implications: These genes may contribute to the remodeling process that occurs with asthma progression and

may have implications for future treatment in severe disease. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: ADAM8, ADAM33, asthma severity, bronchial biopsies, immunohistochemistry, mRNA

Asthma is a chronic respiratory disease of the airways characterized by predominantly T_H2-mediated inflammation, bronchial hyperresponsiveness, and airflow obstruction that is initially completely reversible but that may become fixed and irreversible over time as airway remodeling develops. It is a complex disorder with variable clinical and physiologic presentations in which genetics and the environment interact to modify both the susceptibility to and the severity of the disease. In recent years, 2 novel genes, both members of the ADAM family of proteins, have emerged as asthma-associated genes. The *ADAM33* gene was first identified using positional cloning as a gene significantly associated with bronchial hyperresponsiveness and asthma.¹ More recently, specific induction of *ADAM8* was reported in a murine model of allergic asthma by microarray analysis of whole-lung RNA.²

The ADAM family of type 1 transmembrane proteins is a subgroup of the zinc-dependent metalloproteinase superfamily^{3,4} comprising over 30 members that are structurally very complex. ADAM proteins are membrane-anchored metalloproteinases composed of multiple domains that facilitate their participation in many cellular processes. Structurally, they comprise an N-terminal secretion signal sequence followed by pro-metalloprotease, disintegrin-like, cysteine-rich, epidermal growth factor (EGF)-like, transmembrane and cytoplasmic domains.⁵ They are activated by autocatalytic removal of the prodomain. Cell adhesion, proliferation, differentiation, signaling and apoptosis, as well as the inflammatory response are but a few of the processes in which ADAM proteins are thought to play a role.⁶

ADAM33 has been identified as an asthma susceptibility gene in ethnically diverse populations.^{1,7-9} It is highly polymorphic containing more than 70 single nucleotide polymorphisms (SNPs), some of which are particularly associated with bronchial hyperresponsiveness, a hallmark of asthma.^{1,9,10} However, the exact nature of the association is unclear. Given the high gene expression in bronchial smooth muscle and fibroblasts,^{11,12} a functional

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Supported by the Richard and Edith Strauss Foundation Canada, GlaxoSmithKline, and a Research Institute Fellowship from McGill University Health Centre (to S.C.F.).

Disclosure of potential conflict of interest: J. Bourbeau has received grant support from AstraZeneca, Abbott, Altana, Boehringer Ingelheim, GlaxoSmithKline, BMS, and Pfizer and is on the speakers' bureau for Boehringer Ingelheim, GlaxoSmithKline, Abbott, and Pfizer. The rest of the authors have declared that they have no conflict of interest.

Received for publication July 13, 2006; revised December 21, 2006; accepted for publication December 28, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.12.665

Abbreviations used

ADAM: A disintegrin and metalloproteinase
 ASMC: Airway smooth muscle cells
 BALF: Bronchoalveolar lavage fluid
 BHR: Bronchial hyperresponsiveness
 FVC: Forced vital capacity
 NHBE: Normal human bronchial epithelial cells
 SNP: Single nucleotide polymorphism

role for *ADAM33* in the pathophysiology of airway remodeling in asthma has been suggested.^{13,14} The recent association of *ADAM33* SNPs with a more rapid decline in FEV₁ in asthma over a 20-year period in a Dutch population¹⁵ suggests a role for the gene in disease progression. However, the relevance of the gene expression to physiologic changes remains more difficult to establish. The recent observation by Lee et al¹⁶ of an inverse correlation between FEV₁ (% predicted) and *ADAM33* protein levels in bronchoalveolar lavage fluid (BALF) of Korean subjects with asthma has provided the first evidence of such a link.

ADAM33 was initially described as having 2 isoforms, α and β .¹⁷ The β -isoform lacks 26 amino acids (exon Q) between the cysteine-rich and EGF-like domains in comparison with the native gene. This lack of maturation coupled with localization to the endoplasmic reticulum suggests that the β -isoform exerts a dominant negative effect on protease activity.¹⁸

The less well-studied *ADAM8* (also known as CD156) is another member of the ADAM family that has lately been implicated as playing a role in allergic lung inflammation. Initially identified in macrophage cell lines¹⁹⁻²¹ and subsequently in neurons and oligodendrocytes,²² it has more recently been identified as an "asthma signature gene" based on microarray analysis.² King et al² showed strong induction of *ADAM8* by allergens and T_H2 cytokines in experimental murine asthma, with high expression in eosinophils and lymphocytes in BALF. It is postulated to have a functional role in the recruitment of leukocytes. To date, no human data are available on *ADAM8* expression in asthma.

The aim of the current study was to examine the mRNA and protein expression of both *ADAM33* and the lesser known *ADAM8* in bronchial biopsy specimens from groups of subjects with well-defined severities of asthma to see whether the expression of either gene relates to disease severity. Specifically, in relation to *ADAM33*, we hypothesized that higher expression occurs at the mRNA level of the gene in subjects with asthma compared with controls and that it is more highly expressed in the more severely diseased subgroups with asthma.

MATERIALS AND METHODS

Subject recruitment

Subjects with well-defined moderate and severe asthma were recruited from the Montreal Chest Institute and Sacre

Coeur Hospital based on the criteria of the American Thoracic Society workshop for Refractory Asthma.²³ Subjects with mild asthma²⁴ and control subjects were recruited from the Montreal Chest Institute alone. The protocols for the study were reviewed and approved by the Ethics Committees of both hospitals, and informed consent was obtained from all participating subjects.

In order to be suitable for enrollment, subjects with severe asthma had to fulfill at least 1 of 2 major criteria: (1) treatment with daily oral steroids for more than 50% of the previous 12 months; or (2) treatment with high-dose inhaled steroid (≥ 1000 μ g fluticasone or equivalent per day) and at least 1 other add-on therapy (long-acting β_2 -agonist, leukotriene receptor antagonist, theophylline) continuously over the previous 12 months. In addition, 2 or more minor criteria were required: (1) daily use of short-acting β -agonist; (2) persistent airflow obstruction as documented by prebronchodilator FEV₁ less than 70% and FEV₁/FVC ratio less than 80% predicted; (3) at least 1 urgent care visit in the previous 12 months; (4) ≥ 3 steroid bursts in the previous 12 months; (5) prompt deterioration with less than 25% dose reduction of steroids; or (6) a near-fatal asthma event in the last 3 years.

Subjects with moderate asthma were required to fulfill all of the following criteria: (1) well-controlled asthma on at least 200 μ g/day of fluticasone or equivalent, but not exceeding 1000 μ g/day, with or without the use of a long-acting β -agonist; (2) 2 or less steroid bursts in the previous 12 months and none in the past 3 months and the total number of days on oral steroids could not exceed 30 days in the previous 12 months; (3) FEV₁ greater than 70% predicted and greater than 90% of personal best from the previous 2 years; and (4) no more than 1 nonscheduled urgent care or office visit in the previous 12 months.

Compliance with medication was assessed using the MDILog (Medtrac Technologies; Lakewood, Colo), an electronic device that was attached to each inhaler thereby permitting accurate monitoring of inhaler use. We included in the moderate and severe groups only those subjects who took at least the prescribed 4 inhalations of fluticasone and salmeterol (Advair 250/25 μ g) per day on at least 70% of days in a month run-in period and were thus sufficiently compliant to be considered as having refractory asthma despite adequate treatment.

Subjects with mild asthma had FEV₁ readings greater than or equal to 70% postbronchodilator and required infrequent use of inhaled short-acting β_2 -agonist medication alone. It was necessary for each subject with asthma to display either a 12% or greater improvement in FEV₁, with an absolute increase of at least 200 mL from baseline, after administration of a β -adrenergic agonist or a PC₂₀ of less than or equal to 8 mg/mL of methacholine.

Control subjects were healthy nonsmokers without history of respiratory disease or symptoms suggestive of asthma. They had normal spirometry and were well at time of recruitment.

Exclusion criteria included chronic obstructive pulmonary disease, the presence of any other known pulmonary disease, HIV, metastatic cancer, congestive heart failure,

or any major comorbid disease that might affect asthma disease activity. It was necessary for all subjects with asthma to be free from respiratory tract infection for at least 1 month before enrollment in the study. All subjects had pulmonary function tests and skin prick allergen tests performed before bronchoscopy.

Immunohistochemistry

Bronchoscopy was performed under light sedation. Six endoscopic bronchial biopsies were obtained from the right lung at various segmental and subsegmental carinae using a biopsy forceps with fenestrated cup and a 2.8-mm channel (Olympus Biopsy Forceps 35C; Olympus Medical Systems Corp, Tokyo, Japan). Tissues were fixed in formaldehyde and embedded in paraffin before cutting. Staining was performed using a peroxidase-based method as previously described.²⁵ Briefly, sections of 5 μ m in thickness were de-paraffinated in xylene, dehydrated in ethanol, and washed in PBS. After permeabilization with Triton (0.2% in PBS), tissues were immersed in a solution of hydrogen peroxide in PBS (5% in PBS). The sections were then washed 3 times for 5 minutes in PBS before incubating with Universal Blocking Solution (DakoCytomation, Mississauga, Ontario, Canada) for 20–30 minutes.

Tissue sections were immunostained with polyclonal antibodies for *ADAM33*, (RP3; TriplePoint Biologics Inc., Forest Grove, Ore) raised against the cytoplasmic domain of the protein, at a concentration of 0.5 μ g/mL and *ADAM8* (R&D Systems, Minneapolis, Minn) at a concentration of 10 μ g/mL. The secondary antibodies, raised in swine and rabbit, respectively (DakoCytomation), were applied at concentrations of 1:100 and 1:500 respectively. In order to confirm specificity of the *ADAM33* antibody, sections were also stained with a ten-fold mass excess of the immunizing peptide for *ADAM33* (P3, TriplePoint Biologics Inc.) after preadsorption with the antibody for 1 hour at 4°C. Goat IgG served as the isotype control for *ADAM8*. The reactions were developed using StreptABComplex/HRP and DAB substrate according to manufacturer's instructions (DakoCytomation). Slides were examined and images were acquired by a BX51 Olympus microscope attached to a CoolSNAP-Pro color digital camera (Cارسen Group, Markham, Ontario, Canada) using the Image Pro-plus 4.0 system (Media Cybernetics, Silver Spring, Md). Scoring of epithelial staining (which refers to the percentage of the epithelium that stained positively) was performed blinded. The scoring system was as follows: 0 = no staining, 1 = 0% to 12.5%, 2 = 12.5% to 25%, 3 = 25% to 37.5%, 4 = 37.5% to 50%, 5 = 50% to 62.5%, 6 = 62.5% to 75%, 7 = 75% to 87.5%, and 8 = 87.5% to 100%. Submucosal inflammatory cell staining for each antibody was reported as the number of positively stained inflammatory cells per field.

RNA extraction and cDNA preparation from bronchial biopsies

Total RNA was isolated from frozen bronchial biopsies using RNeasy micro kit extraction columns. cDNA was generated in a 30- μ L reaction using 0.5- μ g total RNA

as template, oligo(dT)12–18 primer, and Superscript II Reverse Transcriptase, in the presence of RNA guard. *ADAM33* and *ADAM8* mRNA were measured by real-time reverse transcriptase polymerase chain reaction (RT-PCR) (LightCycler; Roche Diagnostics, Laval, Quebec, Canada) using specific primers.

Cell culture and stimulation of primary epithelial cells with T_H2 cytokines

Epithelial cells were isolated from endobronchial biopsies of mild steroid-naïve subjects with asthma and normal controls, and cells were characterized by immunofluorescence as previously described.²⁶ Cells were stimulated with IL-4 and IL-13 (1 ng/mL, 10 ng/mL, and 50 ng/mL) for 4 hours and 24 hours. RNA was extracted using the RNeasy Mini Kit according to the manufacturer's protocol. A total of 0.5- μ g RNA was reverse transcribed, and RT-PCR for *ADAM33* mRNA transcript was carried out using the Light Cycler.

Stimulation of ASMC and NHBE cells with dexamethasone

Human ASMC and normal human bronchial epithelial cells (NHBEs) were obtained from Clonetics and grown in their optimal media (SmGM-2 and BEGM, respectively) at 37°C in a humidified incubator with 5% CO₂ as recommended by the supplier. Confluent cells were plated at passages 5 and 6. Cells were stimulated with dexamethasone (Sigma D1756; Sigma Chemical Company, St Louis, Mo) at concentrations of 0.1, 1.0, and 10 μ mol/L for 4, 24, and 48 hours. RNA was extracted as described above, and 0.5 μ g of total RNA was reverse transcribed. *ADAM33* and *ADAM8* mRNA expression was assessed by real-time RT-PCR using specific primers.

Preparation of standards for real-time RT-PCR

Quantification of the housekeeping gene, ribosomal protein S9 (*S9*), was achieved by constructing standard curves using serial dilutions of purified cDNA. The primers were designed to span at least 1 intron. Primers for *ADAM33* were designed so that the sense primer traversed the splice junction between exons P and Q and the antisense primer started in exon R. All PCR amplicons were purified using the QIAquick PCR purification kit and cloned in pGEM-T (pGEM-T Vector System; Promega, Madison, Wisc). Plasmids were sequenced to confirm identity and integrity. Large-scale preparations of each construct were purified using the HiSpeed Plasmid Maxi Kit. A 10-fold dilution series ranging from 10¹⁰ to 10¹ of each purified pGEM-T plasmid was then prepared in Tris-HCl pH 8.0.

Primer sequences were as follows: *S9*-sense 5'-TGCTGACGCTTGATGAGAAG-3'; *S9*-antisense 5'-CGCAGAGAGAAGTCCGATGTG-3'; *ADAM 33* α -isoform-sense 5'-GACCTAGAATGGTGTGCCA GA-3'; *ADAM 33* α -isoform-antisense 5'-AGCCTGGC TTGTACAG AAG-3'; *ADAM8*-sense 5'-GCAACCAC AAGCAGGA GTG-3'; and *ADAM8*-antisense 5'-CAGA ACCACCAC CACGAAG-3'.

TABLE I. Subject demographics

Groups*	Severe (n = 15)	Moderate (n = 14)	Mild (n = 8)	Controls (n = 7)
Sex (M:F)	8:7	8:6	4:4	5:2
Age (y)	49 (25-62)	47 (23-62)	43 (28-55)	29 (20-44)
Disease duration (y)	15.5 (1-50)	31 (1-54.4)	18 (10-26)	N/A
FEV ₁ (L)	2.2 (0.3)	3.0 (0.3)	3.2 (0.4)	3.6 (0.6)
FEV ₁ % predicted	67.7 (7.2)	86.8 (3.2)	94.4 (7.1)	104.7 (8.0)
FVC (L)	3.4 (0.4)	3.9 (0.3)	4.4 (0.5)	4.4 (0.6)
FVC% predicted	85.4 (6.7)	95.8 (3.5)	107.4 (7.1)	107.2 (6.2)
FEV ₁ /FVC (%)	64.9 (5.0)	73.3 (1.6)	75.0 (1.9)	81.9 (3.4)
Atopy (M:F)	7:7	8:6	3:4	0

M, Male; F, female.

*Age and disease duration are shown as median (range). All other data are expressed as means $\pm$ SEM.

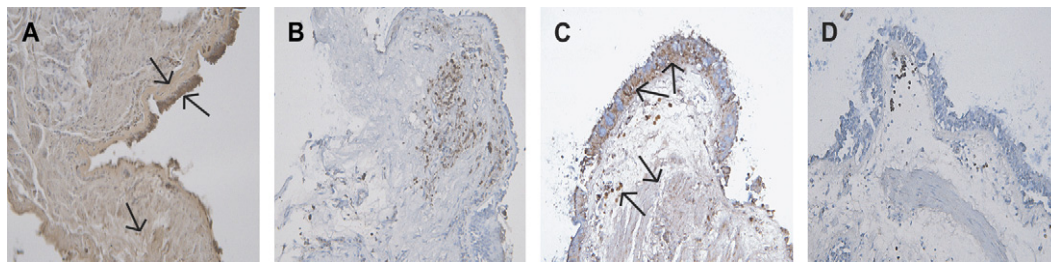


FIG 1. Representative examples of immunohistochemistry of bronchial biopsies showing staining for *ADAM33* in a subject with severe asthma (**A**) compared with a normal control subject (**B**). Arrows point to the positive staining in the epithelium, subepithelium, smooth muscle, and submucosal cells. **C**, Positive staining in a patient with moderate asthma that is abolished by preadsorption of the antibody with immunizing peptide (**D**). Magnification $\times 200$.

Real-time RT-PCR

Relative quantifications of *S9*, *ADAM33*, and *ADAM8* were performed using the LightCycler. PCR reactions were performed in a 20- μ L volume containing 7 μ L water, 1 μ L cDNA, 0.3 μ mol/L of each primer, and 10 μ L of 2X QuantiTect SYBR Green PCR Master Mix. Specificity of the amplified products was assessed by means of melting curve analysis and gel electrophoresis. Ratios of the number of copies of each gene to the number of copies of the housekeeping gene were calculated.

STATISTICS

All values are reported as means $\pm$ SEM unless otherwise stated. Single (1-way) ANOVA was used to analyze differences between the groups followed by Tukey HSD post-hoc multiple comparison tests. A difference of *P* less than or equal to .05 was taken to be statistically significant.

RESULTS

Subjects recruited

A total of 37 patients with asthma and 7 control subjects underwent bronchoscopy. Patient characteristics are shown in Table I. The mean FEV₁% predicted postalbuterol was 63.8 ± 5.8 in the severe group, which indicates significant airflow obstruction despite compliance with

high-dose medication. The median age of onset of asthma was 39.5 years (range, 1.0-56.0) in the group with severe disease, much later than that of the moderate (16 years; range, 0.6-54) and mild (29.5 years; range, 5-22) groups with asthma, which indicates a more rapidly progressive process in severe asthma. All but 2 subjects with asthma were atopic as demonstrated by elevated IgE levels and positive skin prick tests to common allergens. All subjects were nonsmokers.

Immunohistochemical staining of *ADAM33* in bronchial biopsies

Staining for *ADAM33* was identified in the epithelium, subepithelium, submucosal inflammatory cells, and smooth muscle of subjects with asthma (Fig 1). Smooth muscle was identified in all biopsy specimens and stained positively for *ADAM33* protein in 100% of severe and moderate cases, one third of mild biopsies, and no control biopsies. The epithelium stained positively for *ADAM33* protein in 85% of subjects with asthma. The epithelium of control biopsies stained only weakly positive. PreadSORption of the antibody with the immunizing peptide led to almost complete blockade of staining in all positive cells. The scores for epithelial staining of *ADAM33* are shown in Fig 2, A. The mean epithelial score for *ADAM33* expression in the severe group was $5.1 (\pm 0.5)$, significantly higher (*P* < .001) than that observed in the mild (1.8 ± 0.4) and control (1.0 ± 0.0) groups. No significant difference was found between severe and moderate

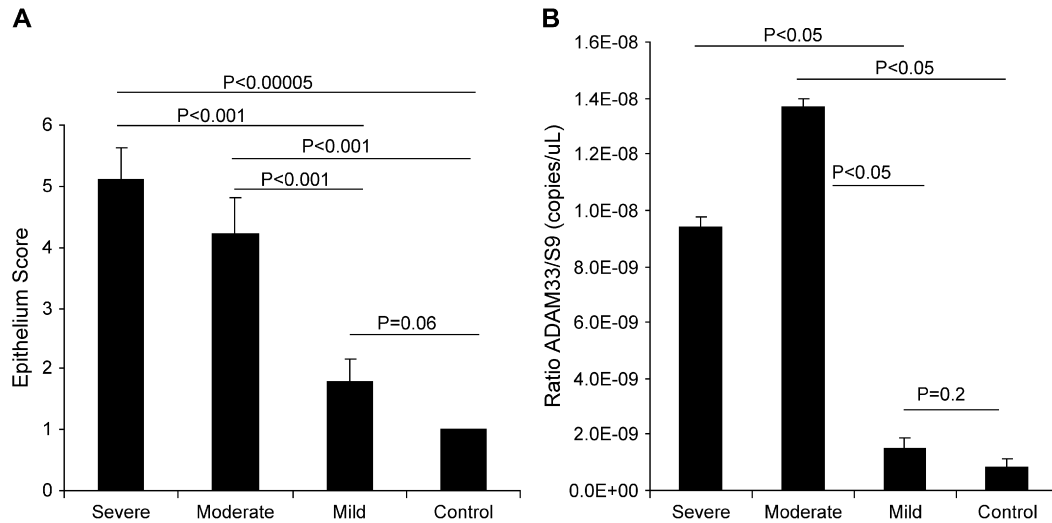


FIG 2. Epithelial scores of *ADAM33* immunostaining (A) and mRNA transcript (B) for *ADAM33α* in bronchial biopsy samples of subjects with mild, moderate, and severe asthma compared with controls.

asthma ($P = .14$). The mean epithelial score for the moderate group was $4.2 (\pm 0.6)$, which is again significantly higher than that of the mild ($P < .001$) and control groups (1.0 ± 0.0 , $P < .0001$). The number of inflammatory cells per field that stained positively for *ADAM33* did not differ statistically between the groups.

mRNA expression of *ADAM33* in bronchial biopsies

mRNA transcript for *ADAM33* α -isoform was detected in all bronchial biopsy samples examined. Expression was highest in the group of subjects with moderate asthma and lowest in the control group. No statistical difference was found at the mRNA level between severe and moderate asthma ($P = .4$). However, the ratio of *ADAM33* expression to housekeeping gene was significantly higher in severe asthma compared with mild asthma ($P < .05$) and controls ($P < .005$) (Fig 2, B). The same was true when mRNA expression of the gene was compared between subjects with moderate asthma and those with mild disease and control subjects ($P < .05$). In relation to physiologic parameters, we did not detect a significant correlation between the mRNA transcript levels of *ADAM33* and FEV₁% predicted ($r = -0.2$).

Protein and mRNA expression of *ADAM8* in human bronchial biopsies

Biopsies from the same cohort of subjects were examined for expression of mRNA transcript of *ADAM8* by real-time RT-PCR and protein expression by immunohistochemistry. Staining for *ADAM8* was observed in submucosal inflammatory cells and the epithelium of biopsies with asthma. No expression was observed in airway smooth muscle (Fig 3). Minimal staining was observed in control biopsies. The mean number of positively stained inflammatory cells per field in the severe group was 21 ± 2.32 , significantly higher than that observed

in mild asthma (6.25 ± 2.32 ; $P < .005$) and control subjects (6.2 ± 1.93 , $P < .0005$) (Fig 4, A). Significantly higher numbers of inflammatory cells stained positively per field in the moderate group compared with the mild and control groups ($P < .005$). No differences were observed between moderate and severe disease ($P = .9$) or between mild asthma and controls ($P = 1.0$). mRNA expression of *ADAM8* in human bronchial biopsies is shown in Fig 4, B. The ratio of *ADAM8* mRNA to the housekeeping gene was highest in the severe group and lowest in the control group. In fact, there was negligible expression of the gene in some control samples despite consistent expression of the housekeeping gene. mRNA transcript of *ADAM8* was significantly higher in biopsies of all 3 groups of subjects with asthma compared with controls. Despite a trend toward a difference between severe and mild asthma ($P = .06$), the differences in mRNA expression of the gene between the various groups with asthma examined did not reach statistical significance.

Effect of T_H2 cytokines on *ADAM33* expression in bronchial epithelial cells

To confirm our observation that *ADAM33* is expressed by epithelial cells, we examined primary bronchial epithelial cells from both control subjects and donors with asthma for mRNA expression of the same spliced variant of *ADAM33* and compared the level of expression with that observed in primary ASMCs (Fig 5, A). Although the expression of mRNA transcript of the gene was higher in epithelial cells from subjects with asthma compared with normal bronchial epithelial cells, the expression was several fold lower than that detected in ASMCs at baseline. mRNA transcript of the gene was induced in normal bronchial epithelial cells stimulated with IL-4. Maximal induction of *ADAM33* mRNA occurred at 24 hours after stimulation of the cells with 10 ng/mL

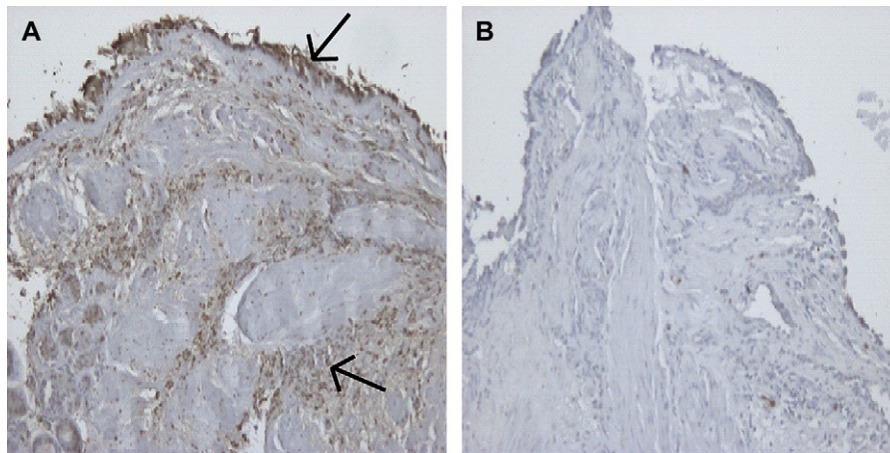


FIG 3. Representative examples of immunostaining for *ADAM8* in a subject with severe asthma (**A**) and a normal control subject (**B**). Arrows point to the positive staining in the epithelium and submucosal inflammatory cells. Note the lack of smooth muscle staining. Magnification $\times 200$.

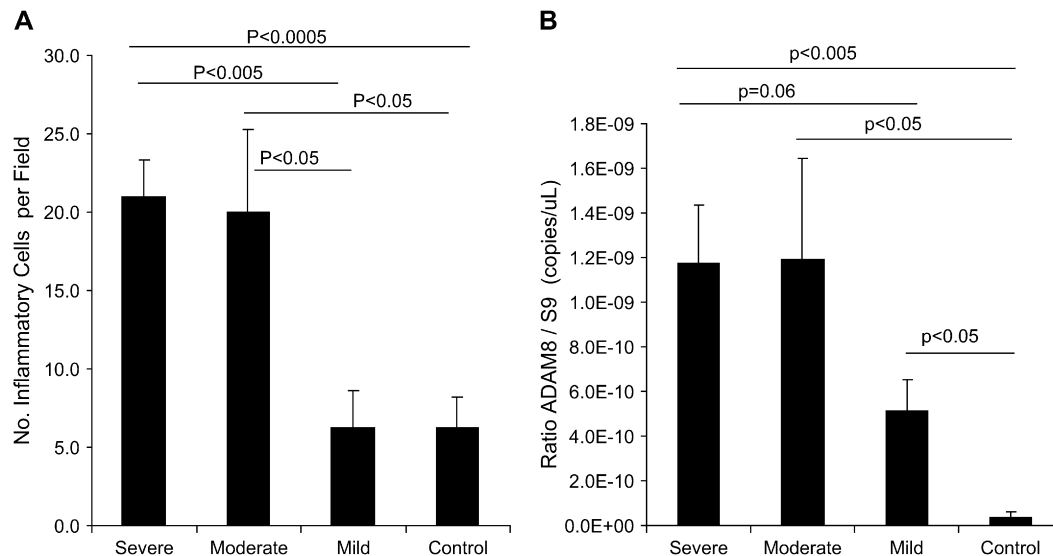


FIG 4. Inflammatory cell score for *ADAM8* immunostaining (**A**) and mRNA transcript for *ADAM8* relative to housekeeping gene (**B**) in bronchial biopsies of subjects with asthma compared with normal controls.

IL-4 (Fig 5, B). No induction of the gene occurred after stimulation of the cells with IL-13.

Effect of dexamethasone on *ADAM33* and *ADAM8* expression in NHBE cells and ASMC

In vitro stimulation of primary NHBE and ASM cells with increasing doses of dexamethasone did not have any effect on the mRNA expression of either *ADAM* gene at any of the time-points examined.

DISCUSSION

The prevalence of clinically important asthma in many countries is around 5% in adults and 10% in children, but definitions based on hyperresponsiveness or wheeze in

the past 12 months have produced rates of up to 30% in children. The prevalence and chronic nature of this disease represents a significant economic burden.³

Bronchial hyperresponsiveness (BHR) is the marker that distinguishes asthma from other diseases of the airways. Alterations in smooth muscle shortening and contractility along with increased smooth muscle mass relative to disease severity are thought to be the most likely explanations for BHR and the excessive airway narrowing that is observed in established disease.^{27,28} These structural changes in airway cells that occur with disease progression come under the term “remodeling,” a process describing the tissue repair that occurs in the airways in response to acute injury or inflammation.²⁹ Despite the long-held concept that airway remodeling is the result of long-standing inflammation, it has been shown recently

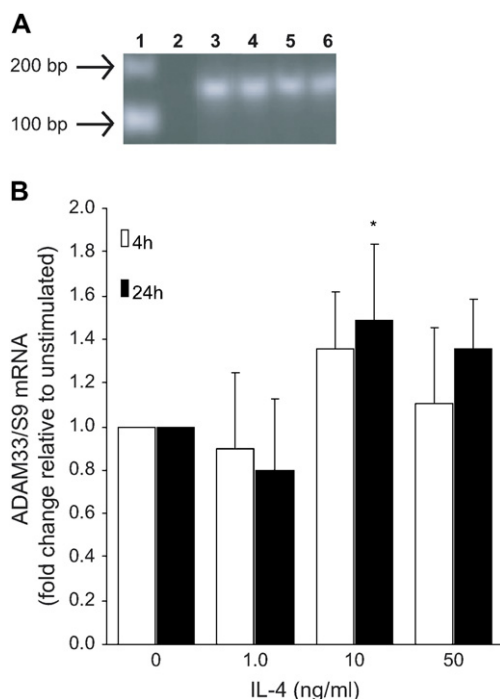


FIG 5. Agarose gel showing mRNA detection of *ADAM33 α* isoform in primary bronchial epithelial cells (**A**). PCR product of *ADAM33 α* was 159 bp. Lane 1 = ladder; lane 2 = negative control; lane 3 = primary unstimulated epithelial cells from subjects with asthma at 24 hours; lane 4 = NHBE unstimulated at 24 hours; lanes 5 and 6 = NHBE cells stimulated with 10 ng/mL and 50 ng/mL, respectively, at 24 hours. **B**, The effect of IL-4 on *ADAM33 α* mRNA expression in NHBE cells. Cells were treated with 1.0 ng/mL, 10 ng/mL, and 50 ng/mL IL-4. RNA was extracted at 4 and 24 hours and reverse transcribed. cDNA was assessed by real-time RT-PCR. Data shown are means $\pm$ SEM of 3 independent experiments, * $P < .05$.

that features of remodeling, particularly thickening of the lamina reticularis, are present in children with asthma.^{30,31}

The identification of *ADAM33* as a susceptibility gene for asthma and more importantly the association of polymorphisms of the gene with BHR and asthma have paved the way for a myriad of studies endeavoring to clarify the exact functional role of the genetic polymorphisms in the pathogenesis of asthma. It is not clear whether a gain or loss of gene function underpins the development of BHR and asthma.³² However, the high gene expression in cells of mesenchymal origin suggests an association with the remodeling process. The very recent associations of SNPs in *ADAM33* with childhood asthma in a Japanese population³³ and the detection of several protein isoforms in cultured human embryonic lung tissue and surrounding mesenchyme³⁴ suggest that variation in the *ADAM33* gene may predispose to the onset of asthma and bronchial hyperresponsiveness in childhood.

ADAM33 has at least 2 isoforms that are expressed in different lung cell types as a result of exon skipping. In fact, Powell et al¹² have demonstrated the existence of multiple novel isoforms of the gene in human airway fibroblasts. Several important SNPs have been mapped to

introns within *ADAM33*, which suggests that they may regulate the splicing of *ADAM33* transcripts.

We found a statistically significant increase in mRNA expression of *ADAM33* between mild asthma and both the moderate and the severe subgroups, which lends support to the idea that expression of the gene may be regulated at the mRNA level. All subjects with moderate and severe asthma were taking high-dose inhaled fluticasone, and some of the subjects with severe disease were also taking oral corticosteroid medication, whereas subjects with mild asthma were steroid naive. This result indicates the relative unresponsiveness of *ADAM33* to steroid intervention, which supports the relevance of this molecule to disease severity.

Haitchi et al³⁴ previously reported no difference in mRNA expression between bronchial biopsies from subjects with asthma and control biopsies. The discrepancy in results is most likely explained by the lower number of subjects examined in that study and the fact that the group with asthma comprised a mixed group of disease severities with fairly well-preserved lung function. We, in fact, also did not detect a statistically significant difference between mild asthma and controls in terms of mRNA expression of the tested spliced variant of *ADAM33*. With regard to physiologic parameters, whereas Lee et al¹⁶ have previously reported a significant negative correlation between protein expression of *ADAM33* in BAL samples and FEV₁% predicted in asthma, we found no significant correlation between mRNA expression of the gene and FEV₁% predicted.

ADAM33 is reported in the literature to be selectively expressed by mesenchymal cells.^{1,11} However, 2 recent papers examining bronchial biopsies of subjects with asthma and normal subjects have reported staining in the epithelium, but neither group has specifically attributed this staining to *ADAM33* expression. Haitchi et al³⁴ report weak immunoreactivity in bronchial epithelial cells but attribute this to nonspecific cross-reactivity with epithelial cytokeratins. Lee et al¹⁶ describe positive epithelial staining for *ADAM33* protein in 30% of bronchial biopsies studied from subjects with asthma and in a much smaller percentage of normal biopsies. In our hands, we found positive epithelial staining in greater than 80% of biopsies from subjects with asthma with weak staining in normal epithelium. We found a statistically significant increase in the epithelial staining as asthma severity progressed from mild to severe. We subsequently confirmed mRNA expression of *ADAM33* *in vitro* in primary bronchial epithelial cells with higher expression observed in epithelial cells from donors with asthma compared to cells from normal controls. Stimulation of NHBE cells with IL-4 led to induction of *ADAM33* mRNA at 24 hours with maximal effect observed at a concentration of 10 ng/mL. Interestingly, IL-13 had no significant effect on mRNA expression of the gene.

Our results indicate an upregulation of *ADAM33* in activated bronchial epithelium in asthma. An element of deposition of secreted protein produced by smooth muscle and fibroblasts on the epithelium may also account for the heavy epithelial staining observed in some of the

more severely diseased subjects. Although mRNA gene expression was higher in biopsies from subjects with moderate and severe asthma, it is not possible to say exactly what cells are contributing to this increased expression. It is likely to be a combination of airway smooth muscle cells, epithelial cells, and fibroblasts.

Human *ADAM8* is located on chromosome 10q26.3 and is highly expressed in granulocytes and monocytes.²¹ The murine homolog has also been observed in activated glial cells and neurons and is thought to play a role in neurodegenerative diseases. Our interest in *ADAM8* in relation to bronchial asthma stems from the recent discovery that CD23, a low-affinity IgE receptor, is a substrate for *ADAM8* *in vitro*, which implies a role for this gene in allergy and inflammation.³⁵ Added to this, King et al² subsequently showed *ADAM8* to be upregulated by allergens and Th2 cytokines in a mouse model of experimental asthma, which further implicated the gene in allergic responses. To our knowledge, we describe the first human study examining the expression of *ADAM8* in bronchial asthma. Here, we report increased mRNA expression in all groups of subjects with asthma examined compared with controls. We also show higher expression of the protein in submucosal inflammatory cells of subjects with asthma compared with controls and lack of expression by airway smooth muscle cells.

We found no evidence of induction of either *ADAM33* or *ADAM8* in bronchial epithelial cells or ASMCs after stimulation with dexamethasone. We also could not detect any significant expression of either ADAM gene at the protein level by immunohistochemistry in a group of 8 subjects with chronic obstructive pulmonary disease who were taking long-acting β -agonists and high-dose inhaled fluticasone (data not shown). We therefore conclude that the increased expression of both of these ADAM genes that we observed with increased severity of asthma was caused by the disease itself and is specific to asthma and cannot be attributed to corticosteroid medication.

In conclusion, it seems that most likely interplay occurs among several members of the ADAM family in asthma pathogenesis. We have demonstrated an increase in both mRNA and protein expression of *ADAM33* with increasing disease severity in asthma, which further implicates this gene in airway remodeling. *ADAM33* is associated with bronchial hyperresponsiveness and is a susceptibility gene for more rapidly progressive disease.¹⁵ How exactly polymorphisms of this gene alter airway function and cause hyperreactivity needs to be elucidated. We have also shown that *ADAM8* mRNA is increased in asthma compared with control subjects and that protein expression is predominantly in submucosal inflammatory cells and the lung epithelium. *ADAM8* may play a role in leukocyte recruitment and mobilization and may contribute to ectodomain shedding of CD23, thereby giving it potential as a target for therapeutic intervention.

We are grateful to Cathy Fugère, Leo Cicora, and Elsa Schotman for their assistance in the collection and processing of samples.

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