

Expression and Regulation of a Disintegrin and Metalloproteinase (ADAM) 8 in Experimental Asthma

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Asthma, a complex chronic inflammatory pulmonary disorder, is on the rise despite intense ongoing research. To elucidate novel pathways involved in asthma pathogenesis, we used transcript expression profiling in a murine model of asthma. Employing asthma models induced by different allergens (ovalbumin and *Aspergillus fumigatus*) we uncovered the involvement of ADAM8, a member of a disintegrin and metalloproteinase (ADAM) family. *In situ* hybridization of mouse lungs revealed strong ADAM8 induction in peribronchial and perivascular inflammatory cells as well as in bronchiolar epithelial cells following allergen challenge. Sequence analysis of lung ADAM8 cDNA identified a novel splice variant of ADAM8 that contained an additional exon in juxtaposition to the transmembrane domain. Allergen-induced ADAM8 mRNA accumulation in the lung was dose- and time-dependent. Transgenic or pharmacologic delivery of interleukin (IL)-4 or IL-13 to the lungs resulted in a marked increase of ADAM8 expression. Gene-targeted mice studies revealed that ovalbumin-induced ADAM8 was largely dependent upon signal transducer and activator of transcription (STAT) 6 and the IL-4 receptor α -chain. Thus, ADAM8 is an allergen-, IL-4-, and IL-13-induced gene in the experimental asthmatic lung. Taken together with the role of ADAM33 in asthma, these results suggest that allergic lung responses involve the interplay of diverse members of the ADAM family.

Despite intense ongoing asthma research, there is currently an epidemic of atopic disease in the western world and the incidence is on the rise (1). Experimentation in the asthma field has largely focused on analysis of the cellular and molecular events induced by allergen exposure in sensitized animals (primarily mice) and humans. These studies have identified elevated production of immunoglobulin (Ig)E, mucus hypersecretion, airway obstruction, complement proteins, eosinophilic inflammation, and enhanced bronchial reactivity to spasmogens in the asthmatic response (2). Clinical and experimental investigations have demonstrated a strong correlation between the presence of CD4⁺ T helper 2 lymphocytes (Th2 cells) and disease severity, suggesting an integral role for these cells in the pathophysiology of asthma (3, 4). Th2 cells are thought to induce asthma through the secretion of an array of cytokines (interleukin [IL]-4, -5, -6, -9, -10, -13) that activate inflammatory and residential effector pathways both directly and indirectly (5, 6). In particular, IL-4 and IL-13 are produced at elevated levels in the asthmatic lung and are thought to be central regulators of many of the hallmark features of

disease (6, 7). IL-4 promotes Th2 cell differentiation, IgE production, airway eosinophilia, morphologic changes to the respiratory epithelium, and airway hyperreactivity (8–10). IL-13 induces IgE production, mucus hypersecretion, eosinophil recruitment and survival, airway hyperreactivity, the expression of CD23, adhesion systems, and chemokines (11–15). IL-4 and IL-13 share similar signaling requirements in part, such as utilization of the IL-4 receptor (R) α -chain (16, 17) and the induction of janus kinase-1 and signal transducer and activator of transcription (STAT) 6 (18–20). Although these studies have provided the rationale for the development of multiple therapeutic agents that interfere with specific inflammatory pathways, the development of an asthma phenotype is likely to be related to the complex interplay of a large number of additional genes and their polymorphic variants (21, 22).

Aiming to elucidate novel pathways involved in the pathogenesis of allergic asthma, we took an empiric approach using DNA microarray analysis of whole-lung RNA (23). To identify allergen-induced genes in the lung that were not specific to a particular experimental regime, we induced experimental asthma using two distinct experimental protocols. In one model, mice were intraperitoneally sensitized with the allergen ovalbumin (OVA) and subsequently challenged with intranasal OVA or control saline on two occasions separated by 3 d. In another model, experimental asthma was induced by the repeated intranasal applications of *Aspergillus fumigatus* antigens. It is noteworthy that this model involves a unique mucosal sensitization route (24) and that *A. fumigatus* is a ubiquitous common aeroallergen. Importantly, both asthma models exhibit similar phenotypes, including Th2-associated eosinophilic inflammation, mucus overproduction, and airway hyperresponsiveness. Lung RNA from saline- and allergen-challenged mice (18 h after the last challenge) was subjected to microarray analysis using the Affymetrix chip U74Av2 (Affymetrix, Santa Clara, CA) that contains oligonucleotide probe sets representing 12,422 genetic elements, one of the largest collections of characterized mouse genes commercially available. This led to the identification of 291 genes that were commonly involved in disease pathogenesis, rather than unique to a particular allergen or mode of disease induction. These “asthma signature” genes provide a valuable opportunity to define new pathways involved in the pathogenesis of allergic airway inflammation. This manuscript focuses on one such gene that encodes for a disintegrin and metalloproteinase (ADAM) 8.

The ADAM family of type I transmembrane proteins consists of at least 35 members that contain characteristic structural motifs including a zinc-dependent metalloproteinase-, disintegrin-, cysteine-rich region, an EGF-like domain, and a cytoplasmic tail that contains a consensus SH3-binding sequence (25, 26). Complex structural organization of ADAM proteins allows them to be involved in multiple processes, such as shedding of plasma membrane molecules including cell adhesion molecules, cytokines (e.g., tumor necrosis factor [TNF]- α), chemokines, and growth factors (26, 27). For example, ADAM10 has been shown to convert CXCL16 from a membrane-bound scavenger receptor

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Abbreviations: a disintegrin and metalloproteinase, ADAM; bronchoalveolar lavage fluid, BALF; Clara cell 10, CC10; interleukin, IL; ovalbumin, OVA; receptor, R; signal transducer and activator of transcription, STAT; T helper 2, Th2; tumor necrosis factor, TNF.

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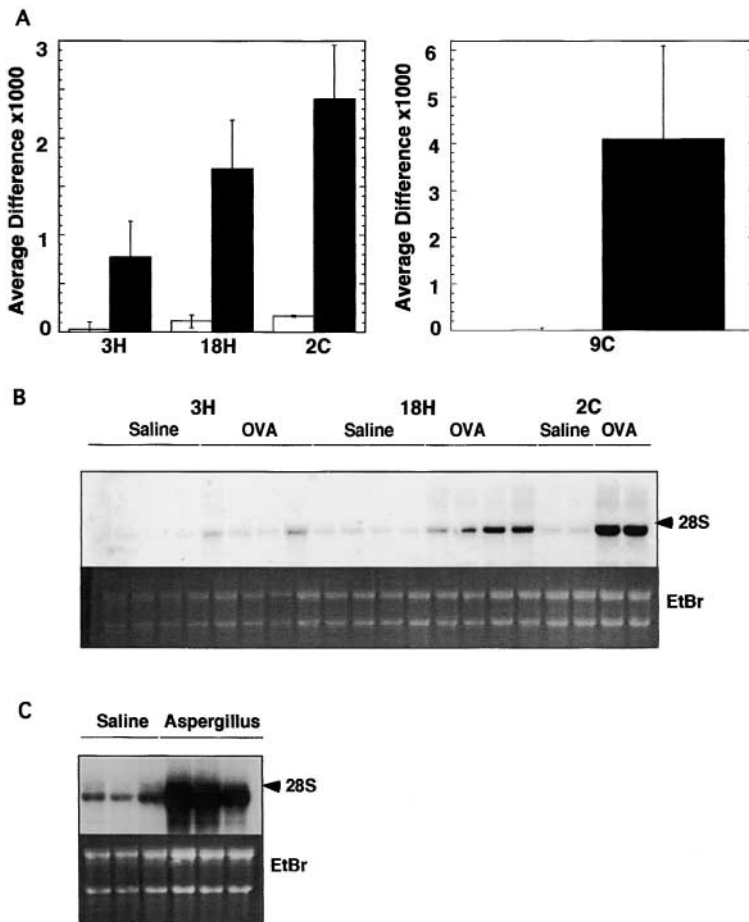


Figure 1. ADAM8 expression is induced in experimental asthma. In A, the expression of ADAM8 in the lungs of OVA- (left panel) or *Aspergillus*- (right panel) challenged mice as measured by microarray analysis is shown. The average difference for the hybridization signal after saline (white bar) and allergen (black bar) challenge at different time points is depicted. Time points are as follows: 3H: one challenge, 3 h; 18H: one challenge, 18 h; 2C: two challenges, 18 h; 9C: nine challenges, 18 h. Error bars represent the standard deviation. In B, Northern blot analysis of ADAM8 expression after OVA challenge is shown. Time points are as in A. In C, the expression of ADAM8 after intranasal challenges with *Aspergillus fumigatus* antigen or saline is shown. The ethidium bromide (EtBr)-stained gel is also shown in B and C. Each lane represents one mouse.

to a soluble chemoattractant (28). Additionally, ADAM proteins have been implicated in cell adhesion, signaling, proliferation, death, and inflammation. Notably, ADAM33 has recently been identified as a human asthma susceptibility gene based on a genome-wide genetic linkage study (29); recently single nucleotide polymorphisms in ADAM33 have also been associated with asthma (30). In this manuscript, we chose to focus on the ADAM8 gene because our gene chip analyses indicated that ADAM8 (but not numerous other ADAM family members) was specifically induced in the experimental murine asthmatic lung. Furthermore, we were interested in examining this molecule because of the diverse roles of ADAM family members in inflammatory processes. Furthermore, the low affinity IgE receptor CD23 has recently been shown to be a substrate for ADAM8 *in vitro*, further implicating ADAM8 in allergic responses (31). We report that ADAM8 is an allergen-, IL-4-, and IL-13-induced gene that is regulated by STAT6-dependent and -independent signaling pathways in experimental asthma.

Materials and Methods

Mice

Balb/c mice were obtained from the National Cancer Institute (Frederick, MD) and housed under specific pathogen-free conditions. Mice deficient in STAT6 or IL-4R α in the Balb/c background were obtained from Jackson Laboratories (Bar Harbor, ME). IL-13-deficient mice and mice deficient in both IL-4 and IL-13 were kindly provided by Dr. Andrew McKenzie (Medical Research Council Laboratory of Molecular Biology, Cambridge, UK) (32). Mice carrying the IL-4 Clara cell 10 (CC10) lung transgene (9) containing wild-type or deleted copies of the gene for STAT6 (33) have been previously reported (34). Mice carrying the tetracycline-inducible IL-13 transgene under the regulation

of the CC10 lung promoter have been previously described (35). In brief, a two-transgenic system was used to target the expression of IL-13 to the lung in an externally regulated fashion. The activator mice, containing the CC10-rtTA-hGH transgene, were kindly provided by Dr. Jeffrey Whitsett (Cincinnati Children's Hospital Medical Center) (36). Mice carrying the TetO-CMVp-IL-13-bGH transgene were bred with activator mice, and the resulting bitransgenic mice (CC10-rtTA-IL-13) were fed doxycycline-impregnated food at 4 wk of age for 2 wk.

Experimental Asthma Induction

Mice were sensitized by intraperitoneal injection with 100 μ g of OVA and 1 mg aluminum hydroxide in saline on Days 0 and 14. On Days 24 and 27, the mice were lightly anesthetized with inhaled isoflurane and challenged intranasally with 50 μ g OVA or saline. In other experiments, mice were challenged with nine doses of intranasal *A. fumigatus* antigen over the course of 3 wk (37). The allergen challenge was performed by applying 50 μ l to the nares using a micropipette with the mouse held in a supine position. After instillation, the mice were held upright until alert. Mice were killed 18 h after allergen challenge. IL-4 administration via intranasal delivery in lightly anesthetized (isoflurane) mice was performed as previously described (38). Briefly, recombinant murine IL-4 (2 μ g in 50 μ l 0.9% saline) was delivered in conjunction with monoclonal antibody (10 μ g) directed against IL-4 (reagents kindly provided by Dr. Fred Finkelman, University of Cincinnati); this allows for the half-life of IL-4 to be increased from several minutes to $\sim$ 24 h. Dosing was every other day for six doses. Recombinant murine IL-13 (4 μ g in 20 μ l 0.9% saline [a generous gift from Dr. Debra Donaldson, Wyeth Research, Cambridge MA]) was administered via intratracheal delivery in ketamine-anesthetized mice. Dosing was for five consecutive days.

The right upper lobe of the lungs was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin using standard histologic techniques. The remaining lungs were placed in Trizol (Gibco BRL, Carlsbad, CA) and homogenized for RNA isolation.

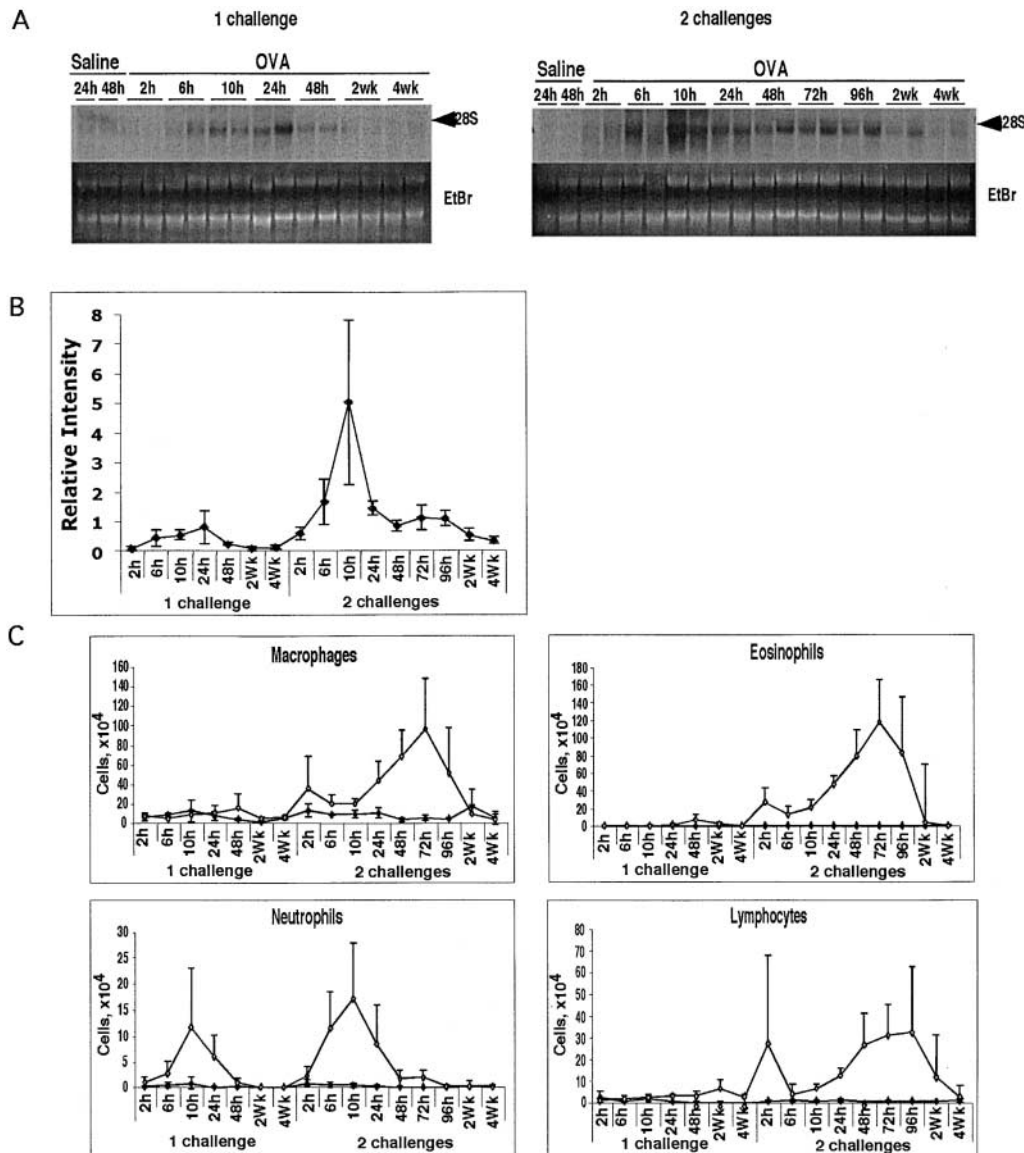


Figure 2. Kinetics of ADAM8 mRNA induction. In **A**, Northern blot analyses of lung RNA after one (left panel) or two (right panel) OVA challenges are shown. The ethidium bromide (EtBr)-stained gels are also shown. In **B**, quantification of ADAM8 mRNA is shown at different time points after one or two OVA challenges. The data are expressed as Relative Intensity, calculated as a ratio of ADAM8 signal intensity to 18S RNA signal intensity. Relative intensity for the ADAM8 signal in saline-challenged mice was 0.17 ± 0.19 (mean $\pm$ SD, $n = 4$). In **C**, the kinetics of cell accumulation in BALF after one or two OVA challenges are shown. The data are expressed as mean $\pm$ SD ($n = 4$ mice). In **B** and **C**, note that the x axis is not linear. The abbreviations of hours (h) and weeks (wk) are used.

Microarray Hybridization and Analysis

Microarray hybridization was performed by the Affymetrix Gene Chip Core facility at Cincinnati Children's Hospital Medical Center, as previously described (23). Briefly, RNA was converted to cDNA with Superscript choice for cDNA synthesis (Invitrogen, Carlsbad, CA) and subsequently converted to biotinylated cRNA with Enzo High Yield RNA Transcript labeling kit (Enzo Diagnostics, Farmingdale, NY). After hybridization to the murine U74Av2 GeneChip (Affymetrix), the gene chips were automatically washed and stained with streptavidin-phycoerythrin by using a fluidics system. The chips were scanned with a Hewlett Packard GeneArray Scanner (Palo Alto, CA). This analysis was performed with one mouse per chip ($n > 3$ for each allergen challenge condition and $n > 2$ for each saline challenge condition). From data image files, gene transcript levels were determined using algorithms in the Microarray Analysis Suite Version 4 software (Affymetrix). Differences between saline- and OVA-treated mice were also determined using the GeneSpring software (Silicon Genetics, Redwood City, CA). Data for each allergen challenge time point were normalized to the average of the saline-treated mice.

Northern Blot Analysis

Lung RNA (10–20 μ g) was electrophoresed in an agarose-formaldehyde gel, transferred to Gene Screen transfer membranes (NEN, Boston, MA) in $10\times$ SSC, and cross-linked by ultraviolet radiation. Probes were obtained

from clones from the IMAGE consortium (GenBank AA154162 for ADAM8, GenBank AA277194 for ADAM33), and labeled with ^{32} P using the Klenow reaction with random priming. Blots were hybridized under standard conditions. Following washes, membranes were exposed to film for 1–5 d. ImageQuant 1.1 software (Molecular Dynamics, Sunnyvale, CA) was used to quantify a hybridization signal intensity after densitometry. Relative intensity was calculated as the ratio of ADAM8 hybridization signal to 18S rRNA band detected by ethidium bromide staining.

In Situ Hybridization of Mouse Lung

In situ hybridization was performed as described (39). In brief, murine ADAM8 cDNA in plasmid pT7T3 (Incyte Genomics, St. Louis, MO) was linearized by *Eco*RI or *Not*II digestion, and antisense and sense RNA probes, respectively, were generated by T3 and T7 RNA polymerase (Riboprobe Gemini Core System II transcription kit; Promega, Madison, WI), respectively. The radiolabeled [α S35-UTP] probes were hybridized and washed under high-stringency conditions.

Results

DNA Microarray Analysis Identifies ADAM8 as an Allergen-Induced Gene in Experimental Asthma

Global quantitative microarray analysis revealed that ADAM8 mRNA expression was significantly increased in both the

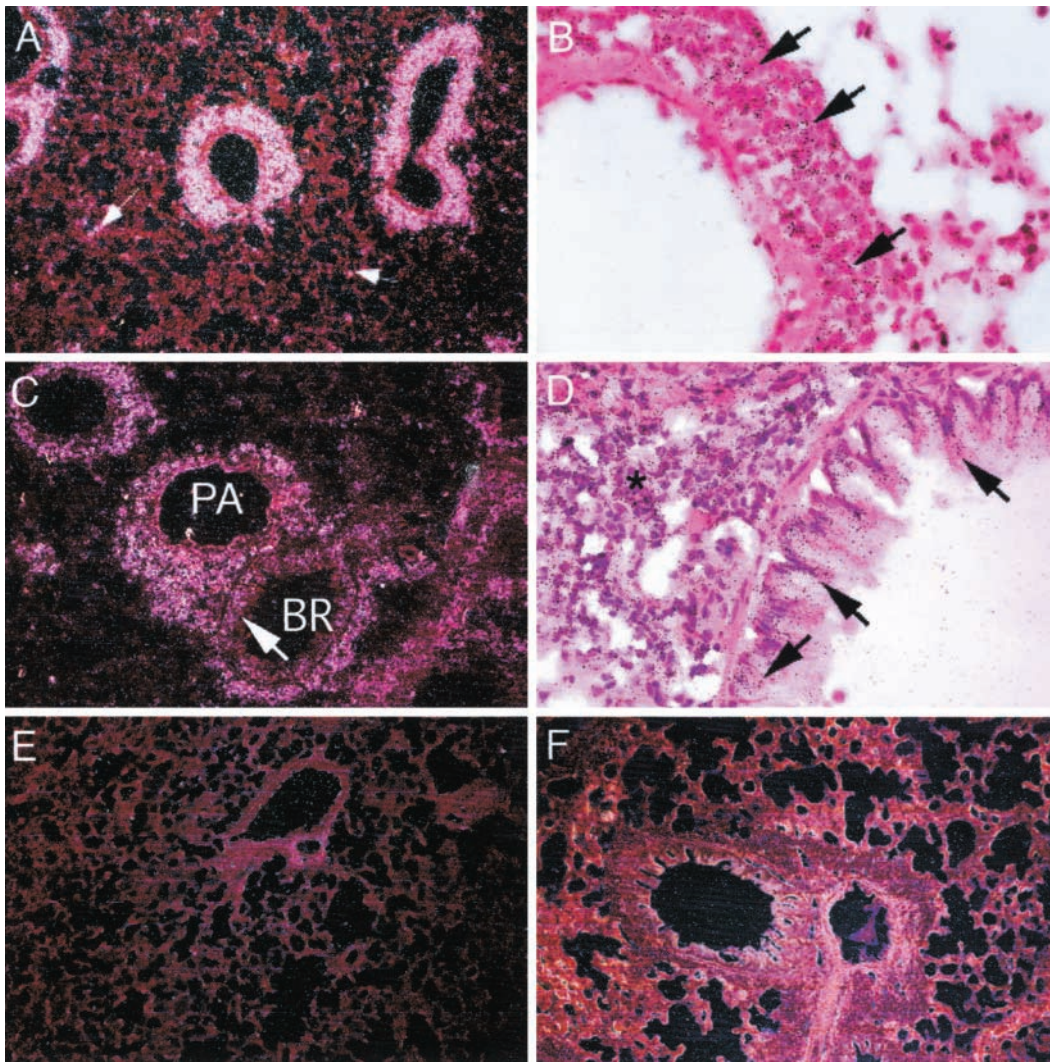


Figure 3. ADAM8 mRNA *in situ* hybridization. Panels A–D show lung sections from allergen-challenged mice (18 h after two OVA challenges) hybridized with the antisense riboprobe for ADAM8. The dark-field (A, C) and brightfield (B, D) images are shown. In A, there is a strong signal (bright white grains) in the perivascular region, and a nonuniform scattered expression in individual cells in the alveolar tissue (arrows). In B, ADAM8-positive cells are in the intense inflammatory infiltrate in the perivascular region and are composed mainly of eosinophils. In C, the inflammatory infiltrate surrounding a pulmonary arteriole (PA) and a respiratory bronchiole (BR) are ADAM8-positive. In addition, there is expression present in the bronchiole epithelial layer (arrow), and bright field analysis of the high-power magnification (D) reveals positive expression in the bronchiole lining cells (arrow) and inflammatory cells (asterisk), but no signal present in the underlying smooth muscle layer. As a control, the saline-challenged lung is hybridized with the ADAM8 antisense probe as shown in E. In F, an OVA-challenged lung is hybridized with the ADAM8 sense control probe. Magnification: A–E, $\times 100$; B and F, $\times 400$.

Aspergillus- and OVA-induced asthma models (Figure 1A). Interestingly, microarray analysis revealed very specific dysregulation of ADAM8 compared with other members of the ADAM family of proteins that were represented on a chip. In particular, ADAM-1a, -2, -4, -5, -7, -8, -9, -10, -11, -12, -15, -17, -19, -23, -28, decysin, and ADAMTS-1 were represented on the microarray chip; however, only ADAM-5, ADAM-9, ADAM-10, ADAM-15, and ADAM-19 had a detectable hybridization signal (data not shown). Notably, none of these members of the ADAM family were increased during induction of experimental asthma. Although ADAM33 sequences were not present on the microarray chip, we examined its expression by Northern blot analysis but did not detect its induction during experimental asthma in two independent experiments (data not shown).

Northern Blot Analysis Demonstrates ADAM8 Induction by Allergen

We subsequently determined by Northern blot analysis that ADAM8 was indeed induced by allergen challenge in both OVA- and Aspergillus-induced models (Figures 1B and 1C, respectively) compared with its low level of constitutive expression in the saline-challenged lung. OVA-induced ADAM8 expression was time- and dose-dependent during the progression of experimental asthma. Figure 1B demonstrates that ADAM8 mRNA

was induced after the first allergen challenge, and induced even higher after two OVA allergen challenges.

We were interested in examining the induction and decline of ADAM8 mRNA accumulation in the lung following the first and second OVA challenge. After the first OVA challenge, ADAM8 mRNA peaked between 6 and 24 h and declined to baseline by 48 h. After two allergen challenges, ADAM8 mRNA accumulated at a much higher level, peaking at 10 h and remaining elevated even after 2 wk compared with saline-challenged lungs (Figures 2A and 2B). We were next interested in correlating ADAM8 mRNA accumulation with differential leukocyte recruitment into the bronchoalveolar lavage fluid (BALF). After both OVA challenges, peak ADAM8 mRNA expression correlated with neutrophil levels. However, the sustained ADAM8 expression more strongly correlated with eosinophils and lymphocytes. It is important to note that while ADAM8 mRNA remained elevated at 2 and 4 wk after the second OVA challenge, BALF leukocytes had already returned to control levels by this time.

ADAM8 *In Situ* Hybridization

To begin to address the distribution ADAM8 mRNA-positive cells in the allergic lung, we performed *in situ* hybridization for ADAM8 mRNA. OVA/aluminum hydroxide-sensitized mice

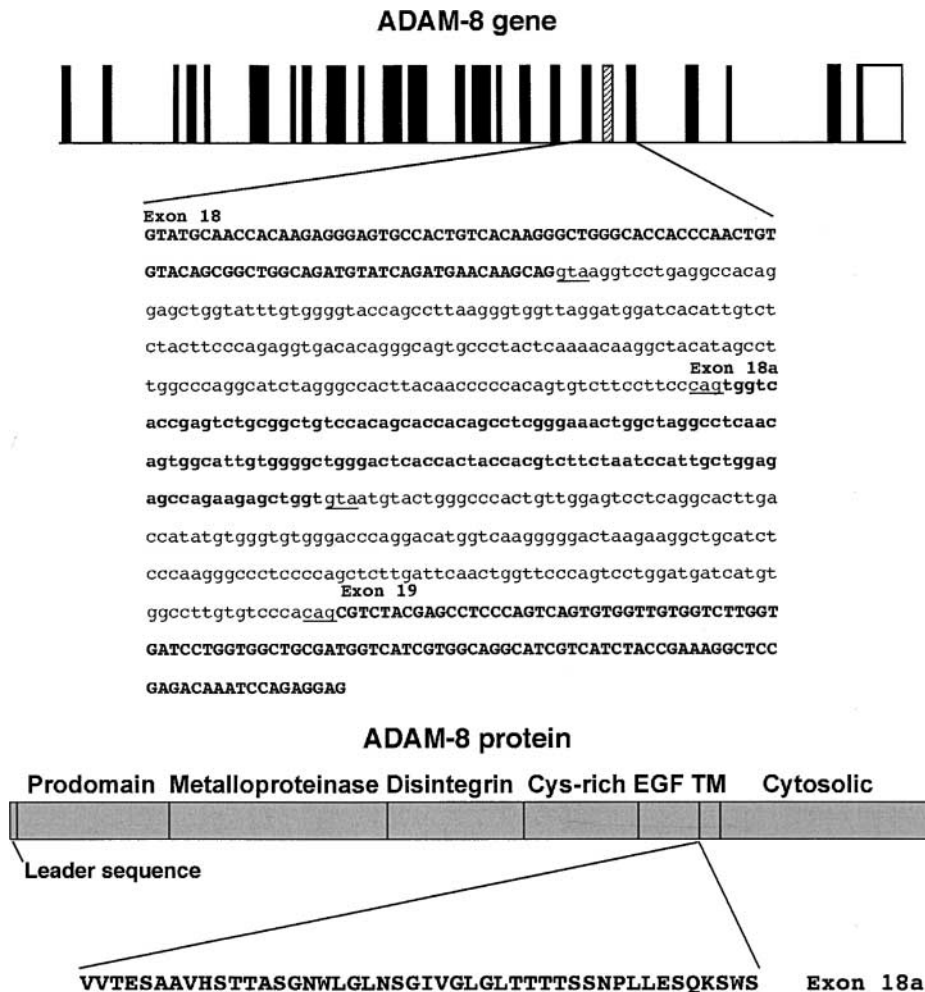


Figure 4. Schematic representation of ADAM8 alternative splice variant. The upper part depicts ADAM8 gene consisting of 23 exons (filled boxes represent translated regions, open box represents 3' untranslated sequence). The genomic sequence between exons 18 and 19 is shown. Upper case letters represent exon sequences, lower case letters represent intronic sequences. The alternative exon sequence within the intron is shown in bold lower case letters. The positions of the splice donor and acceptor sites are underlined. The bottom part depicts schematic representation of ADAM8 protein divided into its functional domains and the position and amino acid sequence of the new alternate exon. The exon 18a sequence has been deposited to GenBank under accession no. AY523626.

were challenged with intranasal OVA or saline, and *in situ* hybridization was performed on lung tissue obtained 18 h after the second allergen or saline challenge. Antisense staining of OVA-challenged lungs revealed specific ADAM8 mRNA expression in a variety of cells (Figures 3A–3D). The strongest staining for ADAM8 mRNA was seen in perivascular and peribronchial inflammatory cells, especially prominent in eosinophils in the interstitial tissue and blood vessels. A weaker expression was also seen in mononuclear cells that most likely represented macrophages. In addition, some of the severely inflamed respiratory bronchioles showed ADAM8 expression in the epithelial lining cells as shown in Figures 3C and 3D. There was no signal in the smooth muscle cells of the bronchial airways or arterioles, alveolar lining cells, and endothelial cells. As a control, no specific staining was observed when the OVA-challenged lungs were stained with the sense probe (Figure 3E). In addition, antisense and sense staining of the saline-challenged lung revealed no detectable staining (Figure 3F and data not shown).

ADAM8 mRNA Is Differentially Spliced in the Lung

Because ADAM8 has not been previously examined in the lung, we were interested in sequencing the gene product derived from OVA-challenged lung RNA. To accomplish this, we isolated lung RNA 18 h after a second dose of OVA, performed RT-PCR using primers that spanned the full open reading frame of ADAM8 cDNA, and then sequenced the subcloned PCR product. This analysis revealed that the majority of the ADAM8 sequences were identical to the published sequences (e.g., Gen-

Bank X13335), but we also isolated three clones (of 33 tested clones) of ADAM8 cDNA that contained an additional exon of 135 bp between exons 18 and 19. This alternate exon, designated exon 18a, was flanked by perfect exon acceptor and donor sequences (Figure 4). This exon produced an in-frame sequence that added 45 amino acids between the EGF-like and transmembrane domains of ADAM8 (Figure 4). Exon 18a was comprised mainly of uncharged and hydrophobic amino acids (56% and 36%, respectively) and was rich in serine (20%) and threonine (16%). A search of existing databases failed to find sequences similar to this exon.

Pharmacologic Delivery of IL-4 and IL-13 Induces Lung ADAM8

Because asthma is a Th2-associated process, we were interested in testing the hypothesis that pharmacologic delivery of IL-4 and IL-13 was sufficient for induction of ADAM8. To test this hypothesis, we administered IL-4 or IL-13 by repeated application to the respiratory tract of anesthetized mice. These protocols induce several features of experimental asthma including eosinophilic inflammation, chemokine induction, mucus production, and airway hyperreactivity (6, 40, 41). Administration of both Th2 cytokines induced marked levels of ADAM8 mRNA compared with saline-treated control mice (Figures 5A and 5B).

Chronic Overexpression of IL-4 or IL-13 Potently Induces Lung ADAM8 *In Vivo*

We were next interested in testing the hypothesis that chronic overexpression of IL-4 or IL-13, particularly in the lungs, was

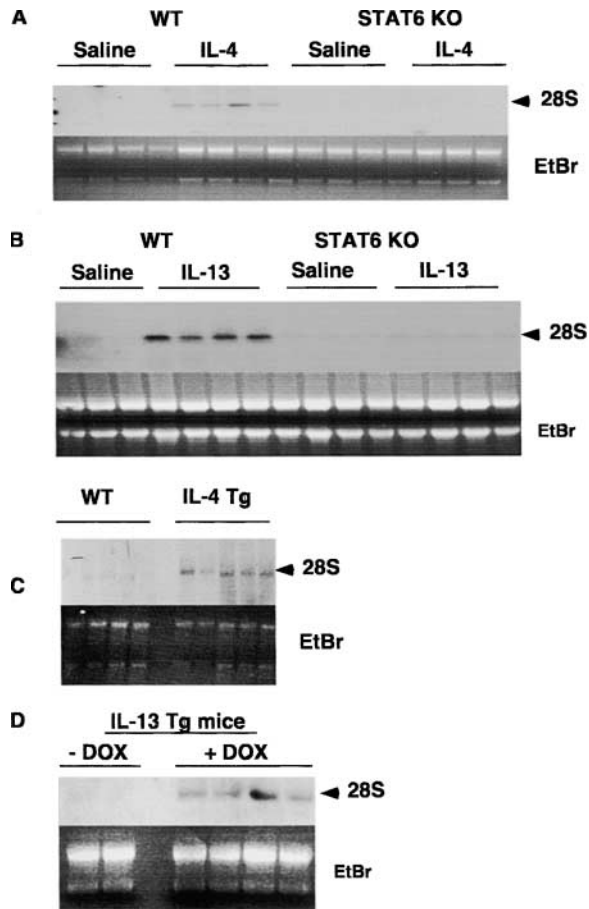


Figure 5. IL-4 and IL-13 induce ADAM8 expression in the lung. Northern blot analyses of ADAM8 expression in mouse lungs. In *A* and *B*, wild-type and STAT6-deficient mice were pharmacologically treated with saline, IL-4, or IL-13. In *C* and *D*, IL-4 or IL-13 lung transgenic mice were examined for ADAM8 mRNA expression. The IL-13-inducible transgenic mice were fed a control (–) or doxycycline-supplemented (+) diet for 2 wk. The ethidium bromide (EtBr)-stained gels are also shown. Each lane represents one mouse.

sufficient for induction of ADAM8. Mice that overexpress the IL-4 or the IL-13 transgene in pulmonary epithelium (under the control of the CC10 promoter) have several features of asthma including eosinophil-rich inflammatory cell infiltrates, mucus production, and changes in baseline airway tone (42). We hypothesized that ADAM8 mRNA would be induced by the IL-4 and IL-13 transgenes. Indeed, IL-4 lung transgenic mice had a marked increase in the level of ADAM8 mRNA compared with wild-type mice (Figure 5C). Similarly, mice with the IL-13 transgene under the control of the doxycycline-regulated promoter had elevated levels of ADAM8 mRNA after 2 wk of doxycycline administration, as shown in Figure 5D.

IL-4 and IL-13 Induction of Lung ADAM8 Are Dependent on STAT6

To test the role of STAT6 in the induction of ADAM8 *in vivo*, we delivered IL-4 to wild-type and STAT6-deficient mice. As shown in Figure 5A, IL-4-induced ADAM8 expression was largely STAT6-dependent. We also delivered IL-13 to STAT6-deficient mice and found that ADAM8 was largely dependent upon STAT6 (Figure 5B).

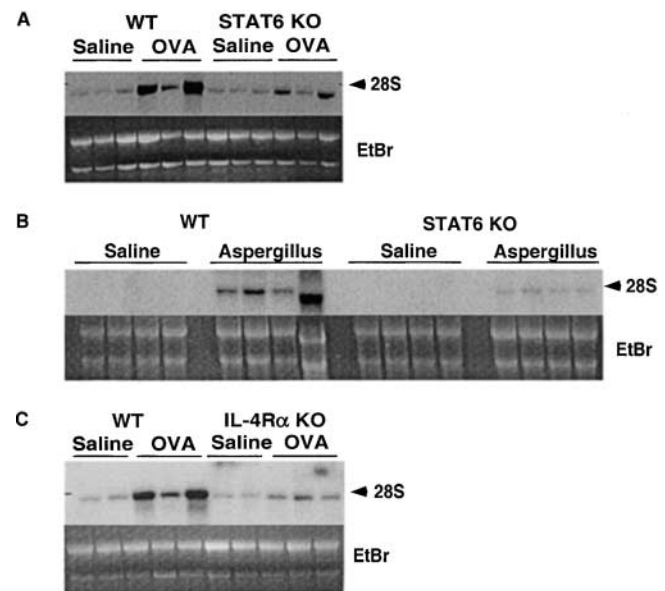


Figure 6. Regulation of allergen-induced ADAM8 expression by STAT6 and IL-4R α . Northern blot analyses of ADAM8 lung mRNA expression in saline- or OVA- (*A*, *C*) and Aspergillus- (*B*) challenged mice 18 h after the last allergen challenge. Wild-type (WT), STAT6-deficient (KO) (*A*, *B*), and IL-4R α KO (*C*) mice were challenged with saline, OVA, or *Aspergillus fumigatus* allergen as indicated in the figures. The ethidium bromide (EtBr)-stained gels are also shown. Each lane represents one mouse.

Allergen-Induced ADAM8 Expression Is Primarily STAT6-Dependent

We were next interested in determining if allergen-induced ADAM8 mRNA expression was STAT6-dependent. This would help determine if allergen-induced ADAM8 was predominantly downstream from IL-4/IL-13 signaling. Notably, mice deficient in STAT6 had markedly reduced expression of ADAM8 after two OVA challenges compared with OVA-challenged wild-type mice (Figure 6A). Similarly, *A. fumigatus*-induced ADAM8 was largely STAT6-dependent (Figure 6B).

Allergen-Induced ADAM8 Is Primarily Regulated by IL-4R α

We were interested in further examining the signaling mechanism involved in allergen-induced expression of ADAM8. Because activation of STAT6 signaling by IL-4 and IL-13 is usually mediated by the IL-4R α chain, we examined ADAM8 induction in OVA-challenged mice that lack functional IL-4R α . As shown in Figure 6C, ADAM8 induction was significantly attenuated in IL-4R α -deficient mice as compared with wild-type mice.

Discussion

To understand the complex mechanisms involved in the pathogenesis of allergic lung inflammation, we employed transcript expression profile analysis to define a set of “asthma signature” genes. We aimed to identify allergen-induced genes that had not been previously associated with asthma. We chose to focus on the ADAM8 gene because this molecule had properties potentially important in asthma pathogenesis and because other members of the ADAM family have been implicated in immune responses (26, 43, 44). For example, ADAM17 is involved in ectodomain shedding of the cell surface precursor form of TNF- α (27). Additionally, a recent human genome search revealed that ADAM33 was an asthma-associated gene (29). Interestingly, although the microarray chip contained sequences that represented over 17

members of the ADAM family, there was only one ADAM gene (ADAM8) that was significantly induced by allergen challenge. Notably, although ADAM8 had not been studied in a context of allergy or lung inflammation, a recent publication has identified CD23 as a specific substrate for the metalloproteinase associated with ADAM8 (31). In particular, ADAM8 is able to convert membrane-bound CD23 into a soluble form that has been shown to be involved in the regulation of IgE synthesis and the activation of macrophages to release a variety of proinflammatory mediators (45–47). Notably, soluble CD23 has been shown to be elevated in such diseases as asthma and rheumatoid arthritis (48), implicating a possible involvement of ADAM8. Additionally, CD23 involvement in airway hyperreactivity and inflammatory cell migration during allergic airway inflammation has been demonstrated in murine asthma models using CD23-deficient mice (49–51).

As an initial investigation, we directed our attention to the expression and regulation of ADAM8 in experimental asthma. Our results demonstrate several important findings concerning ADAM8. First, we identify ADAM8 as a member of the “asthma signature” genome, demonstrating that it is induced by diverse allergens and modes of disease induction. Related to this finding, we have identified a splice variant of the ADAM8 gene. It will be interesting to determine the functional significance of exon 18a and the specific cells that express this variant. Second, we demonstrate that ADAM8 induction is amplified by prior respiratory allergen exposure, as demonstrated by the significantly enhanced expression of ADAM8 following the second allergen challenge compared with the first allergen challenge. Third, we demonstrate that whereas ADAM8 induction initially parallels the cellular infiltrate into the BALF, they are dissociated by 96 h after allergen challenge (when ADAM8 expression remains sustained whereas leukocyte levels primarily return to baseline). Fourth, using *in situ* hybridization we demonstrate that ADAM8 is expressed by multiple cell types in the inflamed lung. In particular, eosinophils, not previously known to express ADAM8, are identified as a major source of ADAM8 mRNA. Induction of ADAM8, which is 65.6% identical to its human ortholog also known as CD156, does not occur solely because it is a marker of the cellular infiltrate because inflamed lung structural cells (e.g., bronchial epithelium) also express ADAM8. Bronchial epithelial cells as well as alveolar macrophages could be a cellular source of ADAM8 in lungs at late time points after inflammation is largely resolved (e.g., 2–4 wk after the second allergen challenge). Fifth, we demonstrate by two different approaches (pharmacologic and transgenic) that the Th2 cytokines IL-4 and IL-13 are strong inducers of ADAM8 in the lung. Previous studies have reported that the ADAM8 gene is upregulated in cell lines by several cytokines including interferon- γ , TNF- α , and lipopolysaccharide (52, 53). Our results are the first to associate ADAM8 with Th2 immune responses, thus broadening the potential involvement of this molecule in diverse inflammatory responses. Although we have identified ADAM8 to be part of the “asthma signature” genes, further study is needed to determine whether other lung diseases result in induction of ADAM8 expression. Finally, we demonstrate that mice deficient in STAT6 or the shared subunit of the IL-4 and IL-13 receptor (IL-4R α) have a large attenuation of allergen-induced ADAM8 mRNA accumulation. Interestingly, subsequent analysis of ADAM8 *cis*-regulatory element clusters using TraFaC software (54) has revealed the presence of two putative STAT6 binding sites located 4–5 kilobases upstream of the first ADAM8 exon (our unpublished data). These collective results place ADAM8 downstream from Th2 cytokine signaling, suggesting that its regulation by STAT6 may involve a direct mechanism, at least in part.

The tissue and cellular distribution of ADAM8 differs from that reported for ADAM33. ADAM33 has been detected in lung tissue, bronchial smooth muscle cells, and lung fibroblasts but not in leukocytes or bronchial epithelium (55). It is notable that we were not able to detect induction of murine ADAM33 mRNA in experimental asthma even though the human gene has been reported to be expressed in lung tissue (29, 55). This suggests intrinsic differences between murine and human ADAM33, which may be a result of the murine asthma model inadequately mimicking the human disease. Alternatively, human ADAM33 may exert its effect on asthma by a systemic rather than a lung-specific mechanism, or ADAM33 may be expressed in the lungs at levels below the detection of our assay. Additionally, recent publications have challenged the importance of ADAM33 in asthma pathogenesis (56). In any event, these collective results highlight distinct and important potential roles for both ADAM8 and ADAM33 in the pathogenesis of asthma.

In summary, we have demonstrated that ADAM8 is an allergen-induced gene expressed during the induction of experimental asthma by a mechanism largely downstream from IL-4, IL-13, and STAT6 signaling. It has been proposed that ADAM8 may play a role in cell migration due to its active metalloproteinase domain and also due to potential adhesion properties of its disintegrin domain. Indeed, transgenic mice expressing the ectodomain of ADAM8 under control of the α 1-antitrypsin promoter demonstrated defects in leukocyte infiltration following oxazolone-mediated contact hypersensitivity or casein-induced peritonitis (57). These results suggest that ADAM8 is likely to contribute to the pathogenesis of allergic lung inflammation at least in part by regulating leukocyte recruitment. In addition, the ability of ADAM8 to induce ectodomain shedding of CD23 may have important implications for the development of allergic airway inflammation. Collectively, these results provide evidence for the involvement of multiple ADAM family members in the orchestration of allergic lung inflammation. As such, it will be important to further dissect the specific involvement of each ADAM member with different aspects of allergic disease.

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