

Dissecting asthma using focused transgenic modeling and functional genomics

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Background: Asthma functional genomics studies are challenging because it is difficult to relate gene expression changes to specific disease mechanisms or pathophysiologic features. Use of simplified model systems might help to address this problem. One such model is the IL-13/Epi (IL-13-over-expressing transgenic mice with STAT6 expression limited to epithelial cells) focused transgenic mouse, which isolates the effects of a single mediator, IL-13, on a single cell type, the airway epithelial cell. These mice develop airway hyperreactivity and mucus overproduction but not airway inflammation.

Objective: To identify how effects of IL-13 on airway epithelial cells contribute to gene expression changes in murine asthma models and determine whether similar changes are seen in people with asthma.

Methods: We analyzed gene expression in ovalbumin allergic mice, IL-13-overexpressing mice, and IL-13/Epi mice with microarrays. We analyzed the expression of human orthologues of genes identified in the mouse studies in airway epithelial cells from subjects with asthma and control subjects.

Results: In comparison with the other 2 models, IL-13/Epi mice had a remarkably small subset of gene expression changes.

Human orthologues of some genes identified as increased in the mouse models were more highly expressed in airway epithelial cells from subjects with asthma than in controls. These included calcium-activated chloride channel 1, 15-lipoxygenase, trefoil factor 2, and intelectin.

Conclusion: The combination of focused transgenic models, DNA microarray analyses, and translational studies provides a powerful approach for analyzing the contributions of specific mediators and cell types and for focusing attention on a limited number of genes associated with specific pathophysiologic

aspects of asthma. (*J Allergy Clin Immunol* 2005;116:305-11.)

Key words: Asthma, IL-13, calcium-activated chloride channel, trefoil factor, 15-lipoxygenase, intelectin

Asthma results from a complex interplay between genetic and environmental factors.¹ Microarray studies of lung tissue from subjects with asthma and from animals with experimental allergic asthma typically reveal hundreds of genes that are differentially expressed in comparison with normal lung.²⁻⁴ However, it has been difficult to relate gene expression changes to specific mediators, cell types, or pathophysiologic features.

Models that focus on one particular mediator or cell type or on specific pathophysiologic features might help overcome this obstacle. We developed a transgenic mouse model to analyze effects of a single cytokine, IL-13, on a single cell type, the nonciliated airway epithelial cell.⁵ IL-13 expression is increased in the airways of subjects with asthma,^{6,7} and IL-13 is necessary and sufficient for the development of experimental asthma.⁸⁻¹⁰ IL-13 activates the signaling molecule signal transducer and activator of transcription factor 6 (STAT6) in many cell types.¹¹ Overexpression of IL-13 in the airways of mice with normal STAT6 expression (tg-IL-13 mice) causes mucus overproduction, airway hyperreactivity, inflammation, fibrosis, and emphysema.¹⁰ To isolate the effects of IL-13 on airway epithelial cells, we produced mice that overexpress IL-13 in the airway and express STAT6 only in nonciliated airway epithelial cells. These IL-13/Epi mice developed mucus overproduction and airway hyperreactivity but not inflammation, fibrosis, or emphysema.⁵

Here we apply a genomics-based approach to identify gene expression changes in the IL-13/Epi focused model, the tg-IL-13 model, and a conventional allergic asthma model. Inclusion of the focused model allowed us to pinpoint a remarkably small number of gene expression changes that were consistently associated with airway hyperreactivity and mucus production. Furthermore, we used these results to guide translational studies that show the relevance of some of these gene expression changes in people with asthma. These experiments demonstrate that focused transgenic models combined with microarrays can lead to an improved understanding of the pathogenesis of complex diseases such as asthma.

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Abbreviations used

GAPD: Glycerdehyde-3-phosphate dehydrogenase
 IL-13/Epi: IL-13-overexpressing transgenic mice with
 STAT6 expression limited to epithelial cells
 tg-IL-13: IL-13-overexpressing transgenic mice
 STAT6: Signal transducer and activator of transcription
 factor 6
 UCSF: University of California San Francisco

METHODS**Mice**

The University of California San Francisco (UCSF) Committee on Animal Research approved the use of mice for these experiments. Care and use of animals complied with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals by Awardee Institutions (#3400-01). Three groups of transgenic mice were used in these experiments: (1) CC10-IL-13⁺, *Stat6*^{+/-} mice (tg-IL-13 mice); (2) CC10-IL-13⁺ *Stat6*^{-/-} mice; and (3) CC10-IL-13⁺, *Stat6*^{-/-}, CC10-hStat6⁺ mice (IL-13/Epi mice). CC10 refers to the Clara cell specific promoter used to express IL-13 and human STAT6 (hSTAT6).¹² The development and characterization of these transgenic mice have been previously described.⁵ Mice used here were backcrossed 5 times onto the Balb/c genetic background. tg-IL-13 mice have an intact *Stat6* gene and IL-13-driven activation of STAT6 in a wide range of cells in the lung, resulting in airway inflammation, mucus overproduction, airway hyperreactivity, subepithelial fibrosis, and emphysema. IL-13⁺ *Stat6*^{-/-} mice (used as negative controls) lack STAT6 and did not develop any detectable IL-13-induced lung pathology. IL-13/Epi mice also lack mouse STAT6 but express human STAT6 selectively in airway epithelial cells. hSTAT6 is functional in mice, and epithelial-restricted activation of Stat6 by IL-13 induced airway hyperreactivity and mucus production without airway inflammation, subepithelial fibrosis, or emphysema. Wild-type Balb/c mice 6 to 8 weeks old were used for the ovalbumin challenge model. There were 5 mice in each experimental and control group.

Antigen sensitization and challenge

Mice were sensitized by intraperitoneal administration of 50 µg grade V ovalbumin mixed with adjuvant (10 mg aluminum potassium sulfate) 3 times at weekly intervals. Control mice received adjuvant alone. Beginning 1 week after the last injection, mice were challenged 3 times by intranasal administration of ovalbumin (1 mg in 50 µL PBS) at daily intervals. Control mice were challenged with PBS alone. Tissues were harvested for isolation of RNA 24 hours after the last challenge.

Isolation and labeling of RNA from mice

Whole-lung RNAs were purified by using Trizol (Invitrogen, Carlsbad, Calif). Integrity of all RNA samples used in this study was confirmed with a model 2100 bioanalyzer (Agilent Technologies, Inc, Palo Alto, Calif). Cy3-labeled and Cy5-labeled lung cDNAs were prepared as described.¹³ To obtain samples enriched for airway epithelial cell RNA, the superior portion of the trachea was cannulated, and the trachea and proximal major bronchi were excised from the thorax and slowly perfused with 0.35 mL lysis buffer (RNeasy kit; Qiagen Inc, Valencia, Calif). RNA was isolated from perfusate according to the manufacturer's instructions. Because the amount of RNA obtained from tracheal perfusates was only ~1 µg, a T7 RNA polymerase-based method¹³ was used to prepare Cy3-labeled and Cy5-labeled amplified cRNAs for array hybridizations.

Microarray analysis

Lung gene expression was analyzed by hybridizing Cy5-labeled cDNA from mouse lungs (5 mice per group, each hybridized separately) along with Cy3-labeled reference lung cDNA pooled from wild-type mice. Tracheal perfusate samples were analyzed similarly, except that amplified cRNA from each mouse was compared with an amplified cRNA reference pool made by using tracheal perfusate samples from wild-type mice. DNA microarrays used in these experiments were produced by using the Operon Biotechnologies (Huntsville, Ala) Mouse Genome Oligo 2.0 set of 70-mer oligonucleotides, supplemented by some additional 70-mers. A MIAME-compliant description of the array experiments and the raw array data are available from Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>, accession number GSE1438).

We used an approach that allowed us to estimate differential gene expression between the various groups we studied on the basis of linear models, as previously described.^{14,15} To determine whether there were significant differences in gene expression between groups, we calculated the odds ratio (probability of being differentially expressed/probability of not being differentially expressed). When the log₂ of the odds ratios (known as the B-statistic) was greater than 0, we classified the gene as differentially expressed.^{16,17} Hierarchical clustering, a method for grouping together genes with similar expression patterns, was performed by using Acuity 4.0 software (Axon Instruments, Union City, Calif). In addition, genes were classified on the basis of expression patterns to determine whether they were increased relative to controls in 1, 2, or all 3 of the experimental groups (ovalbumin, tg-IL-13, and IL-13/Epi) as follows. Seven pseudogene vectors were created to represent genes that were increased only in 1 of the 3 models ([0,0,1]; [0,1,0]; and [1,0,0]), genes increased equally in 2 models ([0,1,1]; [1,0,1]; [1,1,0]), and genes increased equally in all 3 models ([1,1,1]). Each differentially expressed gene was assigned a vector in 3-dimensional space according to the median log fold-change gene expression values determined for that gene in the 3 experimental groups. All vectors were scaled to unit length, and each gene was matched to the closest pseudogene, as determined by Euclidean distance.

Human subjects

These studies were approved by the UCSF Committee on Human Research and conducted in compliance with the Declaration of Helsinki principles. Written informed consent was obtained from all subjects. All subjects were adult nonsmokers (<10 pack-year total smoking history with last cigarette >1 year before the study). Medical histories were obtained, physical examinations were performed, symptom questionnaires were collected, and spirometry was performed as described.¹⁸ Airway reactivity was measured by determining the PC₂₀.¹⁹ The 28 healthy control subjects had no history of lung disease and were not hyperreactive (PC₂₀ > 16 mg/mL). All 30 subjects with asthma had a previous physician diagnosis of asthma, were hyperreactive (PC₂₀ < 8 mg/mL), and used only short-acting inhaled β-adrenergic-agonist medications for therapy. Individuals with an asthma exacerbation or respiratory infection within the previous 6 weeks or significant medical problems other than asthma and those using inhaled or systemic corticosteroids or leukotriene antagonists were excluded.

Bronchial epithelial brushings

Bronchoscopy was performed, and bronchial brushings were obtained randomly from right or left lower lobe bronchial segments by using 4 disposable cytology brushes. The brushes were gently vortexed in sterile saline. Cells from all brushes were pooled, yielding a single sample for each subject. An aliquot was removed for cytocentrifugation, stained with Diff-Quik (Baxter, McGraw Park,

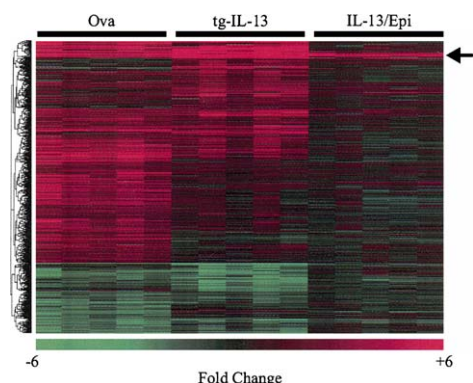


FIG 1. Lung gene expression changes in 3 mouse asthma models. Differentially expressed genes were arranged by hierarchical clustering. Each column represents data from 1 of 5 individual mice in each group. Colors represent fold-change compared with the appropriate controls. The arrow indicates a small group of genes that were increased in all 3 models. *Ova*, Ovalbumin.

III), and examined by light microscopy. On average, the bronchial brushings contained 97% epithelial cells. Total RNA was extracted by using the RNeasy kit. Thirty-four of the 58 human epithelial cell RNA samples analyzed for this study were analyzed separately for another study (Woodruff et al, unpublished data).

Cultured human bronchial epithelial cells

Air-liquid interface cultures were established by using published protocols.²⁰ Primary normal human bronchial epithelial cells (lot 3F1191; Cambrex Bio Science, Baltimore, Md) were seeded onto 12-mm-diameter Corning Transwells (Corning, NY) containing 0.4- μ m pores (4-5 replicates per condition). Cells were submerged in bronchial epithelial growth media (Clonetics) until confluent (3 days), and then the apical media was removed and the basolateral media was changed to differentiation media (1:1 of Dulbecco modified Eagle media to bronchial epithelial growth media) for 8 days. After the differentiation period, IL-13 (0-10 ng/mL) was maintained in the basolateral media over the period of the next 4 days. At the end of the IL-13 exposure, total RNA was isolated by using the RNeasy kit.

Analysis of gene expression by real-time PCR

Primers and a probe for mouse genes (see Table E1 in the Journal's Online Repository at www.mosby.com/jaci) and human genes (see Table E2 in the Online Repository at www.mosby.com/jaci) were designed by using Primer Express software (Perkin Elmer, Boston, Mass). First-strand cDNA synthesis and PCR were performed by using ABI Prizm 7700 or 7900 Sequence Detection Systems (Applied Biosystems, Foster City, Calif). For mouse lung and cultured human airway epithelial cell studies, cycle thresholds for each gene were normalized to those of glyceraldehyde-3-phosphate dehydrogenase (GAPD). For human bronchial brushing samples, a 2-step PCR approach²¹ was used, and transcript copy numbers were normalized on the basis of the geometric mean expression values of 3 house-keeping genes (GAPD, elongation factor 1 α 1, and cyclophilin A) as described.²² To identify genes that were differentially expressed between subjects with asthma and normal subjects, we performed a Wilcoxon rank-sum test and calculated the corresponding adjusted *P* value by using the Westfall and Young²³ maxT algorithm implemented in Bioconductor's *multtest* package (Bioconductor; open source software for bioinformatics, www.bioconductor.org).²⁴

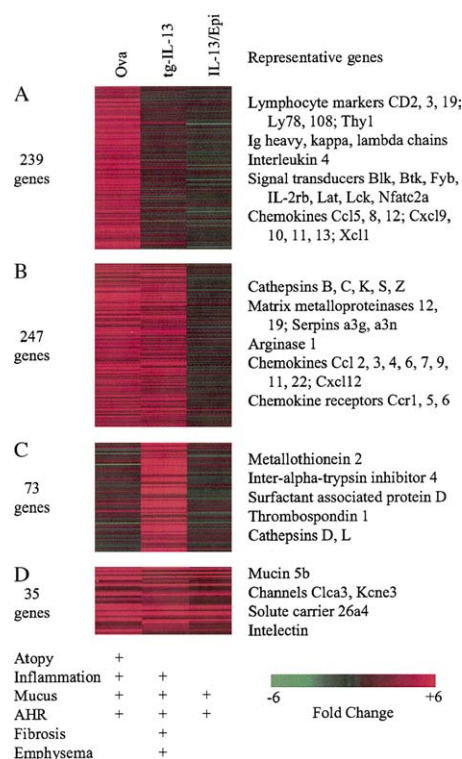


FIG 2. Gene expression patterns. Grouping revealed genes with increased expression in (A) the ovalbumin (*Ova*) model only, (B) the *Ova* and tg-IL-13 models, (C) the tg-IL-13 model only, and (D) all 3 models. The number of genes (left) and representative genes (right) is shown for each group. Phenotypic attributes of each model are shown at the bottom. *AHR*, Airway hyperreactivity.

RESULTS

Microarray analysis of gene expression in mouse asthma models

We used DNA microarrays to analyze gene expression in 3 murine models of asthma. The first model was composed of mice that were sensitized and challenged with ovalbumin (ovalbumin model). The second model was composed of mice with transgenic overexpression of IL-13 in the lung (tg-IL-13 model); these mice had an intact gene for STAT6, a key signaling molecule required for IL-13 activity. The third model was composed of IL-13 transgenic mice with STAT6 expression limited to non-ciliated airway epithelial cells (IL-13/Epi model). In whole-lung samples, 805 gene transcripts were differentially expressed (increased or decreased) in at least 1 model (Fig 1). Of these, 509 genes were increased or decreased by ≥ 2 -fold in at least 1 model. There were 583 gene expression changes in the ovalbumin model and 351 changes in the tg-IL-13 model. In contrast, only 18 changes were seen in the IL-13/Epi focused transgenic model. Comparison of the focused transgenic model to the other models allowed for the identification of a small subset of gene expression changes that are attributable to a specific mechanism and also are relevant to pathogenesis in complex systems.

TABLE I. Genes induced by allergen and by direct effects of IL-13 on airway epithelial cells*

Symbol	Description	Fold-change by arrays			Fold-change by PCR		
		Ova	tg-IL-13	IL-13/Epi	Ova	tg-IL-13	IL-13/Epi
Clca3	Chloride channel calcium activated 3	78.3	59.8	28.2	820.3	1530.7	1448.2
Slc26a4	Solute carrier family 26, member 4	9.9	29.8	20.0	18.7	40.1	10.3
Retnla	Resistin-like α	4.3	23.8	23.7	ND†	ND	ND
Itln	Intelectin	7.6	10.8	19.2	9.5	73.8	146.2
Nadsyn	NAD synthetase 1	17.2	12.5	6.4	0.7	0.5	0.8
Atp2a1	Ca ⁺⁺ transporting ATPase	15.3	8.0	3.9	0.4	0.1	0.9
AMCase	Acidic mammalian chitinase	2.5	12.3	8.6	3.7	7.4	4.4
Muc5ac	Mucin 5, subtypes A and C	12.1	6.8	3.8	33.5	23.4	7.8
Agr2	Anterior gradient 2	3.8	7.7	4.3	11.1	21.6	10.2
Tff1	Trefoil factor 1	3.3	6.4	5.7	52.6	592.2	224.7
Reg3g	Regenerating islet-derived 3 γ	6.4	4.1	3.2	ND	ND	ND
Pigr	Polymeric immunoglobulin receptor	2.5	8.4	2.3	1.5	12.3	2.2
Muc5b	Mucin 5, subtype B	4.0	5.1	2.8	3.6	7.4	13.2
D630002J15Rik	RIKEN cDNA D630002J15 gene	2.8	4.3	2.0	ND	ND	ND
Scin	Scinderin	2.8	2.9	3.0	21.9	21.8	34.5
Alox15	15-lipoxygenase	3.9	1.9	2.0	5.2	1.6	2.8
H2-Q7	Histocompatibility 2, Q region locus 7	2.4	2.1	2.1	ND	ND	ND
1110001D15Rik	RIKEN cDNA 1110001D15 gene	2.1	2.1	2.4	ND	ND	ND
Tff2	Trefoil factor 2	4.5	4.5	1.8	3.5	8.6	4.9

*Genes with a 2.0-fold or greater increase in expression in both the ovalbumin (Ova) allergic model and the IL-13/Epi model are included. Array fold-change values represent the larger median fold change from lung or tracheal perfusate compared with the appropriate control group.

†ND indicates that we did not use PCR to determine fold change for these mouse genes, which do not have obvious human orthologues.

TABLE II. Characteristics of human subjects*

	Control subjects	Subjects with asthma	P
Number	28	30	—
Age	36 $\pm$ 8	38 $\pm$ 13	NS
Sex	16 F/12 M	18 F/12 M	NS
PC ₂₀ (mg/mL)	60.8 $\pm$ 1.4	0.9 $\pm$ 1.3	<.0001
FEV ₁ /forced vital capacity (%)	80.6 $\pm$ 1.4	71.1 $\pm$ 1.4	<.0001
FEV ₁ (% predicted)	106.5 $\pm$ 13.2	85.2 $\pm$ 13.9	<.0001

*Values represent means $\pm$ SDs.

To aid in interpretation, we grouped transcripts according to the magnitude of change in expression across the 3 models. Transcripts were grouped by determining whether the expression changes were seen in 1, 2, or all 3 of the models (see Table E3 in the Online Repository at www.mosby.com/jaci). There were 17 transcripts with at least 2-fold increases in the IL-13/Epi model but only 3 transcripts with at least 2-fold decreases; therefore, we focused our subsequent analyses on increased genes. A total of 239 transcripts were increased in the ovalbumin model but not induced (or induced to a much lesser extent) in the tg-IL-13 or IL-13/Epi models (Fig 2, A). These changes are likely largely attributable to allergen-induced lymphocyte activation and to production of mediators other than IL-13. There were 247 genes increased similarly in the ovalbumin and tg-IL-13 models but not in the IL-13/Epi model (Fig 2, B). These allergen-induced genes are apparently increased because of IL-13 effects on cells other than nonciliated airway epithelial cells, and the large

number of gene transcripts is consistent with the idea that effects of IL-13 on these cells are important in allergic inflammatory responses.^{8,9} A total of 73 transcripts were increased in the tg-IL-13 model but not increased (or increased to a much lesser extent) in the other 2 models (Fig 2, C). These represent genes that are induced by prolonged high-level overexpression of IL-13 but not by acute allergen challenge and may be involved in the pathogenesis of subepithelial fibrosis and emphysema, pathologic features present in the tg-IL-13 model but not the other 2 models.

We were especially interested in identifying genes that were increased in all 3 models. Analysis of whole-lung samples revealed 35 genes that had similar fold increases in all models (Fig 2, D). Because some epithelial gene expression changes might have been undetectable in whole lung samples, we also analyzed gene expression in tracheal perfusate samples enriched for airway epithelial cell RNA. In these samples, we detected 276 genes with altered expression (increased or decreased) in at least 1 model (see Table E4 in the Online Repository at www.mosby.com/jaci). Of these, 107 genes were changed by $\geq$ 2-fold. There were 43 genes changed in the ovalbumin model and 239 in the tg-IL-13 model. There were 76 genes changed in the IL-13/Epi model, but only 22 of these were changed by 2-fold or more. Some genes had similar expression changes in both analyses, but many other changes were identified only in lung samples or only in tracheal samples. In part, this reflects the fact that the tracheal sample was enriched for large airway epithelial cells, whereas the whole-lung sample contained smaller airway epithelial cells as well as many other cell types. In

TABLE III. Gene expression in airway epithelial cells from control subjects and subjects with asthma

Symbol	Description	Fold Difference*	Median gene copy number		Adjusted <i>P</i>
			Control	Asthmatic	
Clca1†	Chloride channel calcium activated 1	216.5	0.06×10^4	8.21×10^4	.0001
Itln	Intelectin	3.4	1.73×10^5	6.12×10^5	.0002
Alox15	15-lipoxygenase	1.3	2.28×10^6	3.27×10^6	.06
Tff2	Trefoil factor 2	2.7	0.55×10^4	1.00×10^4	.06
Muc5ac	Mucin 5, subtypes A and C	1.5	1.67×10^7	2.07×10^7	NS
Tff1	Trefoil factor 1	1.4	1.47×10^5	2.17×10^5	NS
Agr2	Anterior gradient 2	1.1	0.90×10^7	1.04×10^7	NS
Slc26a4	Solute carrier family 26, member 4	0.8	3.72×10^4	3.35×10^4	NS
Scin	Scinderin	1.0	4.70×10^5	4.99×10^5	NS
Muc5b	Mucin 5, subtype B	−5.0	1.84×10^6	0.42×10^6	.0001
AMCase	Acidic mammalian chitinase	—	ND‡	ND	—

*Fold difference between subjects with asthma and control subjects, adjusted for age and sex.

†Human Clca1 is orthologous to mouse Clca3.

‡ND indicates that this transcript was not detected in most samples from both groups.

addition, we expect that there were differences in the sensitivity of the lung and tracheal sample analyses because we did not need to use RNA amplification for lung samples but did need to amplify tracheal samples. By combining microarray results from whole-lung and tracheal perfusate samples, we identified 18 genes that were increased at least 2-fold by allergen and by direct effects of IL-13 on airway epithelial cells (Table I). We also included trefoil factor 2 because previous PCR analysis⁵ suggested that the microarrays underestimated changes in expression of this gene.

PCR validation of gene expression changes in mouse models

In preparation for translational studies, we generated a validated list of genes with human orthologues that were increased in both ovalbumin and IL-13/Epi mice. Five transcripts listed in Table I did not have obvious human orthologues. Eleven of the remaining 14 transcripts were increased by at least 2-fold in both ovalbumin allergic and IL-13/Epi mice by PCR (Table I). Our mouse modeling approach therefore resulted in the selection of 11 genes that could be studied in people with asthma.

Airway epithelial gene expression in human subjects with and without asthma

We used real-time PCR to analyze gene expression in airway epithelial cells from 30 subjects with mild to moderate asthma and 28 controls (Table II). There were substantial and highly significant increases in the expression of the calcium activated chloride channel Clca1 (orthologue of murine Clca3) and intelectin in subjects with asthma (Table III and Fig 3, A). There were smaller increases in expression of 15-lipoxygenase and trefoil factor 2 in subjects with asthma. One transcript, mucin 5b, was less abundant in subjects with asthma, and other transcripts examined were not significantly different. We were unable to consistently detect acidic mammalian chitinase transcripts in either group by using 2 different sets of PCR primers and probes.

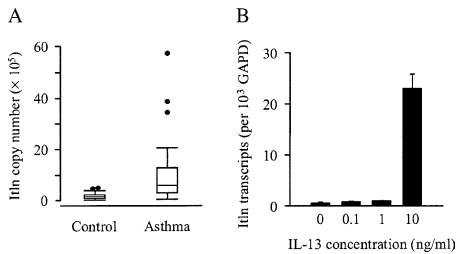


FIG 3. Intelectin (*Itln*) expression in human airway epithelial cells. **A**, Intelectin gene expression by airway epithelial cells from control subjects and subjects with asthma. Medians and interquartile ranges are shown. **B**, IL-13 treatment of cultured human airway epithelial cells induced increased expression of intelectin transcripts.

IL-13 induces intelectin expression in cultured human airway epithelial cells

One of the transcripts substantially increased in airway epithelial cells from subjects with asthma was intelectin, a pattern recognition molecule that has not been previously implicated in asthma. IL-13 treatment of cultured primary human bronchial epithelial cells led to a large increase in intelectin transcripts (Fig 3, B), demonstrating that IL-13 directly increases intelectin expression and that other cell types are not required.

DISCUSSION

We combined focused transgenic modeling, functional genomics, and translational studies in human subjects to help understand important aspects of the complex pathogenesis of asthma. As expected, microarray analysis of an allergic model of asthma revealed hundreds of gene expression changes. Analysis of the tg-IL-13 model, where IL-13 acts on many different cell types to produce extensive lung pathology, also showed hundreds of changes, many of which were similar to those seen in the allergic model. By using the data from these 2 models,

it would have been extremely challenging to determine how particular gene expression changes relate to specific disease mechanisms or to select a reasonable number of candidate genes for further study. By analyzing a third model (IL-13/Epi) that focused on the effects of a single mediator, IL-13, on a single cell type, the airway epithelial cell, we were able to generate a dramatically shorter list of differentially expressed genes. These expression changes were associated with airway hyperreactivity and mucus overproduction, which are features of all 3 models, but not with inflammation, fibrosis, or emphysema, which are absent in the IL-13/Epi model.

Our approach was designed to reduce the complexity inherent in microarray studies of disease pathogenesis. Other microarray studies have reduced complexity by analyzing homogeneous populations of cultured cells or cells isolated from tissues by laser capture microscopy or flow-cytometric sorting. Although those approaches can be useful, an important advantage of the focused transgenic model approach that we describe here is that it isolates the effects that a single mediator exerts on a single cell type *in vivo*.

We used translational studies to determine whether the gene expression changes first identified by using mouse models were also seen in human disease. Four of the orthologues identified in the models (the chloride channel *Clca1*, intelectin, 15-lipoxygenase, and trefoil factor 2) were increased in airway epithelial cells from people with asthma. In another study involving a subset of the human subjects used for this study, we found that expression of *Clca1* transcripts and protein was increased in airway epithelial cells from subjects with asthma (Woodruff et al, unpublished data). Previous reports suggest that induction of the *mClca3/hClca1* chloride channel in airway epithelial cells is important for mucus production.²⁵⁻²⁷ 15-Lipoxygenase produces 15*S*-hydroxyicosatetraenoic acid, which has been reported to trigger mucus secretion in dogs,²⁸ promote contraction of human airway smooth muscle,²⁹ and potentiate increases in allergen-induced early asthmatic responses in human beings.³⁰ Trefoil factor 2 was shown to be increased in an allergic mouse model of asthma,³¹ and we now show that trefoil factor 2 is increased in airway epithelial cells from people with asthma. Trefoil factor 2 promotes migration of cultured bronchial epithelial cells³² and contributes to repair of injured intestinal epithelium.³³ Intelectin is a recently described pattern recognition molecule not previously implicated in asthma. There are 2 related intelectins, *Itln1* and *Itln2*. The probes and primers that we used were designed to recognize *Itln1*, but they might not distinguish between these 2 very closely related sequences. The molecular patterns recognized by intelectin include furanosides such as galactofuranose.³⁴ Galactofuranosyl residues are present in bacterial and fungal cell walls and in protozoan parasites but not in mammalian cells. The possible contributions of intelectin to asthma pathogenesis require further exploration, but intelectin in the airway might alter the response of subjects with asthma to infection or colonization with bacterial or fungal pathogens.

We did not find significant increases in expression of the other human homologues that we examined. To some extent, this likely reflects difficulties inherent in detecting gene expression changes in limited samples (epithelial brushings) taken from human subjects with stable mild to moderate disease, as opposed to whole-lung analysis of mice with homogeneous genetic backgrounds housed in a controlled environment and subjected to a potent disease-inducing stimulus. One transcript, *Muc5b*, was significantly increased in each of the mouse models, but expression of both the transcript and the protein is decreased in people with asthma (Woodruff et al, unpublished data). Differences in *Muc5b* expression might relate to the fact that human *Muc5b* is expressed predominantly in submucosal glands.³⁵ These glands are abundant in human airways but are absent in the mouse, with the exception of a single gland in the trachea. This is an example of the limits of mouse modeling. Our PCR assay was unable consistently to detect the transcript for another orthologue, *AMCase*, but a recent report demonstrated that airway epithelial production of *AMCase* is also increased in subjects with asthma.³⁶ Including translational studies helped to clarify the relevance of the animal models to human disease and allowed us to draw novel inferences about the activity of a specific mechanism in human disease.

We conclude that the combination of focused transgenic models, DNA microarray analyses, and translational studies provides a powerful approach for analyzing the contributions of specific mediators and cell types and for focusing attention on a limited number of genes associated with specific pathophysiologic aspects of complex diseases like asthma.

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