Disease-Specific Gene Expression Profiling in Multiple Models of Lung Disease

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***Rationale*: Microarray technology is widely employed for studying the molecular mechanisms underlying complex diseases. However, analyses of individual diseases or models of diseases frequently yield extensive lists of differentially expressed genes with uncertain relationships to disease pathogenesis.**
***Objectives*: To compare gene expression changes in a heterogeneous set of lung disease models in order to identify common gene expression changes seen in diverse forms of lung pathology, as well as relatively small subsets of genes likely to be involved in specific pathophysiological processes.**
***Methods*: We profiled lung gene expression in 12 mouse models of infection, allergy, and lung injury. A linear model was used to estimate transcript expression changes for each model, and hierarchical clustering was used to compare expression patterns between models. Selected expression changes were verified by quantitative polymerase chain reaction.**
***Measurements and Main Results*: A total of 24 transcripts, including many involved in inflammation and immune activation, were differentially expressed in a substantial majority (9 or more) of the models. Expression patterns distinguished three groups of models: (*1*) bacterial infection (n = 5), with changes in 89 transcripts, including many related to nuclear factor-κB signaling, cytokines, chemokines, and their receptors; (*2*) bleomycin-induced diseases (n = 2), with changes in 53 transcripts, including many related to matrix remodeling and Wnt signaling; and (*3*) T helper cell type 2 (allergic) inflammation (n = 5), with changes in 26 transcripts, including many encoding epithelial secreted molecules, ion channels, and transporters.**
***Conclusions*: This multimodel dataset highlights novel genes likely involved in various pathophysiological processes and will be a valuable resource for the investigation of molecular mechanisms underlying lung disease pathogenesis.**

Keywords: gene expression; infection; asthma; fibrosis

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Gene expression profiling by microarray technology has become a widely used strategy for investigating the molecular mechanisms underlying many complex human diseases.

What This Study Adds to the Field

Gene expression profiling of a large set of diverse mouse lung disease models can be used to identify relatively small sets of genes that are associated with specific types of lung disease.

Gene expression profiling by microarray technology has become a widely used strategy for investigating the molecular mechanisms underlying many complex human diseases. Lung diseases that have been studied using microarrays include idiopathic pulmonary fibrosis (1, 2), hypersensitivity pneumonitis (2), nonspecific interstitial pneumonia (2), chronic obstructive pulmonary disease (3–5), primary pulmonary hypertension (6), asthma (7, 8), and others. These studies have resulted in many novel observations, and are a promising step toward development of individually targeted therapies (9). However, microarray studies of human disease have important limitations, including the heterogeneity of human populations and diseases, the inability to manipulate and examine specific pathways in humans, and the limited ability to safely acquire samples from affected tissues.

To overcome some limitations of human studies, many investigators have used microarrays to study animal models. Arrays have been used to study many models, including influenza infection (10), acute lung injury (11–13), bronchopulmonary dysplasia (14), idiopathic pulmonary fibrosis (15), bleomycin-induced lung injury (16), and asthma (17–19). These studies have their own limitations, as animal models typically exhibit some features of a disease but not others, and sometimes even features that are not components of the human disease being modeled. The search for relevant gene expression changes is further challenged by the identification of very large and unwieldy datasets of differentially expressed genes associated with these animal studies, which result, in part, from the use of genetically homogeneous populations and standardized approaches for inducing disease. These studies typically involve a single animal model, or, occasionally, a small number of models of the same disease. The use of genetically homogeneous populations

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and standardized approaches for inducing disease and collecting tissue for analysis facilitates the identification of large numbers of differentially expressed genes. In some cases, these approaches have identified individual genes (20) or small sets of genes (19) of special interest. More often, these studies have generated long lists of common gene expression changes (21, 22), making it difficult to determine which gene expression changes are important for key pathologic features of the disease being modeled, and also how various models relate to one another. In the present study, we hypothesized that relationships between lung gene expression and molecular mechanisms underlying pulmonary disease pathogenesis could be elucidated by comparing gene expression changes seen in a wide variety of models of lung pathology. To this end, we used a novel microarray-based approach using a heterogeneous set of models of various lung diseases, including bacterial infection, fibrosis, and allergic inflammation. By using a consistent approach to study each of these models, and by including a wide variety of models rather than one or two models of a single lung disease, we identified small sets of signature gene expression profiles associated with various forms of lung disease. Many of the genes identified here have not previously been associated with these lung diseases. Some of the results of these studies have been previously reported in the form of an abstract (23).

METHODS

Mouse Models of Lung Disease

Animal experiments were performed at the University of California, San Francisco, Vanderbilt University, and the University of Texas, Houston, and were approved by the institutional animal care and use committees at these institutions. Table 1 provides a summary of the 12 mouse models of lung disease that were used to assemble the dataset, with references to the published protocol descriptions and phenotypes of each. Microarray data from 2 of the 12 models (IL-13 and ovalbumin allergy in BALB/c mice) have been previously reported as part of a study focusing on the role of IL-13 in allergic airway disease (19), and were reanalyzed for inclusion in the large dataset reported here. Data from the other 10 of these 12 models have not been reported previously. In general, models were analyzed at a single time point, as specified in Table 1. These time points were chosen based upon previous studies and represent time points commonly used by investigators employing the models for analysis of pathophysiology. The one exception was that we studied mice at both 7 and 21 days after bleomycin injury, as earlier time points have been considered as models of acute lung injury, whereas later time points are used as models of the pulmonary fibrosis model. For each model, experimental groups were studied, along with control groups that were matched for genetic background, age, and sex.

Microarray Studies

Lung RNA from individual experimental or control mice was used to generate fluorophore-labeled cDNA, which was hybridized to 70-mer oligonucleotide microarrays, as previously described (19). Additional information about the array experiments is provided in the online supplement, and a complete description of the arrays, the experimental protocols, and the raw array data are available from the Gene Expression Omnibus website (http://www.ncbi.nlm.nih.gov/geo; accession number GSE4231). A total of 86 arrays (47 experimental samples and 39 control samples) passed quality control and were included in the main dataset.

Microarray Data Analysis

A linear model was used to estimate relative transcript expression (fold difference) for each experimental group compared with the corresponding control group, as described in the online supplement. Genes with missing values were excluded, and hierarchical clustering performed, as previously described (24).

PubMed Literature Database Mining

The PubMatrix literature mining tool (25) (http://pubmatrix.grc.nia.nih.gov/) was used to systematically determine whether genes identified in our microarray data analysis had been previously associated with lung diseases of interest. Details are provided in the online supplement.

Real-Time Polymerase Chain Reaction

Expression changes of selected genes were verified by quantitative real-time polymerase chain reaction (PCR), using either TaqMan probes or SYBR-Green for detection, as previously described (26). Gene expression differences were calculated from the mean values for experimental and control groups using the ΔΔCt (delta delta cycle threshold) method and three to four housekeeping genes. Additional details about PCR methods are provided in the online supplement.

RESULTS

Gene Transcript Expression Changes in 12 Different Lung Disease Models

We obtained lung RNA from groups of mice representing 12 lung disease models and used DNA microarrays to analyze expression in these models. These models feature a wide range of disease-inducing stimuli, including infectious agents, toxins, allergens, and transgene overexpression that have varied effects on the lung (Table 1). RNA samples from individual mice were analyzed on separate DNA microarrays using a standardized protocol performed in a single laboratory. By using a standardized approach to sample processing, array hybridization, and data analysis, we were able to make extensive comparisons within the dataset. A final data set was assembled using results from 86 microarrays. The complete data set is publicly available from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo; accession number GSE4231). Analyses of each of the 12 models in the dataset revealed numerous transcript expression changes in each model (Figure 1). The average number of statistically significant transcript expression changes within each of the 12 models (experimental group vs. the appropriate control group) was 663 (4% of the 16,463 transcripts represented on the arrays). On average, 460 transcripts had increased expression (including 289 that were increased more than twofold), and 203 transcripts had decreased expression (68 by more than twofold). The largest number of expression changes was seen in *Mycoplasma pulmonis*–infected lungs, which had 1,573 differentially expressed transcripts, including 812 changes that were twofold or greater. The smallest number of expression changes was observed in *Nippostrongylus brasiliensis*–infected mice (275 transcripts significantly different from control, including 157 changes that were twofold or greater).

Transcript Expression Changes Observed in Most or All Models

There was a small set of transcripts that had statistically significant changes in most of the 12 different models; 23 transcripts were increased in at least 9 of the 12 models examined (Table 2). Although the character of the inflammatory response seen in these 12 models is varied, many of the same transcripts associated with inflammation and immune response activation were induced in most of the models. These include chemokine ligands and receptors and proteins involved in leukocyte recruitment, homing, and phagocytosis, antigen recognition and processing, and in the acute inflammatory-phase response. Several of the genes among this set make proteins that mediate or are mediated by initial host immune responses, including early IFN-γ production (*Ccl2*, *Ccr5*, *Cxcl9*, *Itgb2*, *Fcgr3*, *Ctss*), oxidative burst (*Ncf1*), calcium mobilization (*Ccl9, Lilrb4*), and chemotaxis

TABLE 1. MOUSE MODELS OF LUNG DISEASE

Model	Description	Phenotype	Reference(s)
Aspergillus extract	C57BL/6 mice were sensitized via intranasal administration of *Aspergillus fumigatus* antigen mixture (n = 4) or saline alone (n = 3) 5 times at 4-d intervals and killed 4 h after the final challenge.	Th2 cytokine production, eosinophilic inflammation, airway hyperreactivity, goblet cell hyperplasia, elevated serum IgE, airway fibrosis	52
Bleomycin-induced acute lung injury	A single dose of bleomycin (1.1 U/kg body weight in 60 μl saline; n = 5) or saline alone (n = 4) was administered intratracheally to C57BL/6 mice. Mice were killed at 7 d. For bleomycin; n = 5 for both 7 and 21 d; for controls; n = 4 for 7 d and n = 2 for 21 d.	Inflammation (neutrophils, lymphocytes, macrophages), pulmonary edema	29
Bleomycin-induced pulmonary fibrosis	A single dose of bleomycin (1.1 U/kg body weight in 60 μl saline; n = 5) or saline alone (n = 2) was administered intratracheally to C57BL/6 mice. Mice were killed at 21 d.	Pulmonary fibrosis	28
IL-13 overexpression	Transgenic mice expressing IL-13 under the control of a Clara cell–specific promoter (n = 5) were studied at 6–8 wk of age. IL-13–overexpressing mice that lacked STAT6, a signaling molecule that is required for IL-13–induced pathology, were used as control animals (n = 4).	Eosinophilic inflammation, airway hyperreactivity, goblet cell hyperplasia, fibrosis, emphysema	19, 49
LPS aerosolized	*E. coli* LPS (7 ml of 1.0 mg/ml solution of LPS; n = 2) was delivered to C57BL/6 mice as a continuous aerosol (driving flow rate of 8 L/min) generated by a small-volume nebulizer over 30 min. Mice were killed 4 h after exposure. Controls were the LPS i.p. control mice, as described above.	Neutrophilic inflammation in lungs	(53)
LPS i.p.	*E. coli* LPS (5 μg/g BW; n = 3) or saline alone (n = 3) was administered to C57BL/6 mice via a single i.p. injection. Mice were killed 4 h after injection.	Systemic inflammation	54
M. pulmonis	C3H mice were inoculated intranasally with *M. pulmonis* (strain CT7; 10^5 cfu; n = 5) or PBS alone (n = 4). Mice were killed after 7 d.	Chronic inflammation, pulmonary edema, marked airway remodeling	55
M. tuberculosis	C57BL/6 mice were exposed to aerosols (5 ml suspension) containing *M. tuberculosis* (H37RV; 470 cfu; n = 3) or sterile DI water alone (n = 3) for 40 min and analyzed 21 d later.	Macrophage inflammation	56
N. brasiliensis	BALB/c mice were injected with 500 third-stage *N. brasiliensis* larvae (n = 3) or saline alone (n = 3) at the base of the tail and maintained on water supplemented with antibiotics (2 g/L/neomycin sulfate, 100 mg/L chloramphenicol) for 5 d before being killed.	Th2 cytokine production, eosinophil and basophil influx, goblet cell hyperplasia, airway fibrosis	57, 58
OVA allergy (C57BL/6 and BALB/c)	Mice were sensitized with OVA (50 μg in 10 mg alum, i.p.) or with alum alone (controls) on days 0, 7, and 14 and challenged with OVA (1 mg in 50 μl saline) or saline alone (controls) on days 21, 22, and 23. Mice were analyzed on Day 24. For C57BL/6 mice; n = 3 for OVA and 4 for controls; for BALB/c mice; n = 5 for OVA and controls.	Th2 cytokine production, eosinophilic inflammation, airway hyperreactivity, goblet cell hyperplasia, elevated serum IgE	19, 49
P. aeruginosa	*P. aeruginosa* (PA103: 5×10^7 cfu in 50 μl; n = 3) or lactated Ringer's solution alone (n = 2) was instilled intratracheally into BALB/c mice via the oropharynx. Mice were killed 2 h after treatment.	Severe alveolar epithelial cell injury, inflammation, edema formation	59

Definition of abbreviations: i.p. = intraperitoneal; OVA = ovalbumin; PBS = phosphate-buffered saline.

(*Ccl2*). Their up-regulation across most models suggests that these genes may play a role in the more common features of disease pathogenesis, and not the more distinct features associated with individual pathogens. Only one transcript, flavin, containing monooxygenase 3 (*Fmo3*), a xenobiotic-metabolizing enzyme that is normally expressed in the terminal bronchiolar epithelium of the postnatal lung (27), was decreased in all of the models. Thus, our results suggest that decreases in *Fmo3* expression occur with many lung pathologies, which might lead to altered metabolism of xenobiotics, including drugs and nicotine in several lung diseases. Systematic analysis of the PubMed literature revealed that 19 of the 24 transcripts listed in Table 2 have been previously associated with lung disease(s), but 5 (21%) had no previously reported association with any of the types of lung disease represented by our models (*Evi2b*, *Ms4a6d*, *500001M20Rik*, *2610307O08Rik*, *S100a4*).

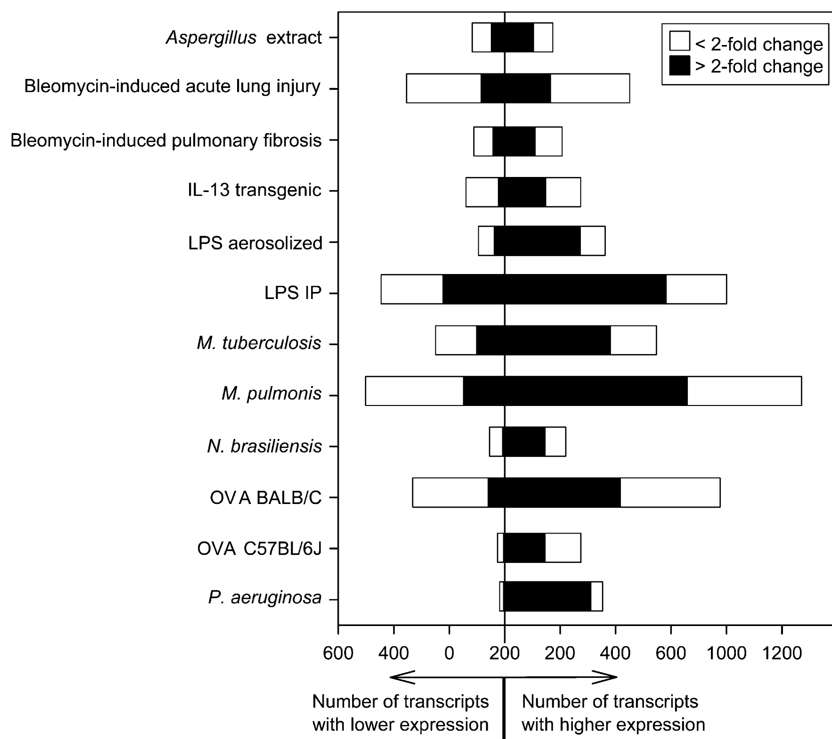

Figure 1. Transcript expression changes in 12 different lung disease models. Lung RNA samples from experimental and control mice representing all of the 12 models listed in Table 1 were analyzed using microarrays. A linear model was fit using results from all samples to estimate relative transcript expression (fold-difference) and determine statistical significance for each experimental group compared with its corresponding control group. The graph represents the number of significantly differentially expressed transcripts (adjusted $P < 0.05$) for each model. IP = intraperitoneal; OVA = ovalbumin.

Relationships between the 12 Lung Disease Models

The models we studied were heterogeneous, and could be divided into groups in a number of ways. For example, groups could be defined based upon the nature of the stimulus (infectious organism, allergen, or other) or the duration of the stimulus (acute, subacute, or chronic). Rather than using a predetermined set of groupings based upon selected characteristics of the models, we instead chose to form groups based on similarities in gene expression patterns. Hierarchical clustering was used to examine the relationships between transcript expression changes in the 12 models (Figure 2). Clustering analysis of the 50 most highly differentially expressed transcripts from each model revealed 3 major clusters of expression patterns. A cluster of five models had expression patterns that were similar to one another, but quite distinct from the other seven models. The five models in that cluster were all models of bacterial infection, including three induced by infection with live bacteria (*M. pulmonis*, *Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa*) and two triggered by administration of the bacterial toxin, LPS, either by aerosol inhalation or by intraperitoneal injection. The remaining seven models could be further subdivided into two clusters of two and five models each. The cluster with two models included models that represent different phases (7 and 21 d) of the response to intratracheal administration of the toxic chemotherapeutic agent, bleomycin. These are often used as models of acute lung injury and pulmonary fibrosis, respectively (28, 29). The final cluster of five models included all three of the allergic models studied (ovalbumin challenge in two different mouse strains and *Aspergillus* extract challenge), along with mice infected with the nematode parasite *N. brasiliensis* and mice with transgenic overexpression of the cytokine, IL-13. Increased production and activity of T helper type 2 (Th2) cytokines, including IL-13, are known to be central features of each of these five models. Although the clustering shown in Figure 2 resulted from analysis of the 50 most highly differentially expressed genes from each of the models, similar clusters were seen when fewer transcripts (25 transcripts) or more transcripts (100 or 200 transcripts) were used for clustering (data not shown), indicating that the relationships between models were reasonably robust. These clustering results suggest that there are patterns of transcript expression changes that are characteristic of the response to bacterial infection, bleomycin-induced lung injury and repair, and Th2 inflammation, and suggested that the data set could be used to identify those characteristic expression changes.

Transcript Expression Changes in Bacterial Infection Models

We used information from all 12 models to identify transcript expression changes that were seen in most or all bacterial infection models, but not in other models. A total of 90 gene transcripts were significantly increased in at least four of the five bacterial infection models, but not in any of the other seven models (Table 3 and Table E1 in the online supplement). A substantial fraction of these (27 transcripts) have a functional relationship with the nuclear factor (NF)-κB pathway, which plays a central role in innate immunity and can be triggered both by LPS (via Toll-like receptors [TLRs]) and by host cytokines, including tumor necrosis factor (TNF) and IL-1. Transcripts that were selectively increased in bacterial infection models encoded IL-1α, IL-1β, TNF, an IL-1 receptor (IL-1R2), and the receptor, CD14, which is important for LPS recognition by TLR4. Transcripts encoding MyD88, which is critical for proximal TLR signaling, and several proteins that are related to the TNF receptor–associated factors were also increased selectively in these models, as were transcripts encoding NF-κB2 and Rel, the IκB family member, Nfkbiz, and two NF-κB/IκB regulators (Bcl10 and Map3k8). Several of the transcripts that were selectively increased in bacterial infection models are regulated by NF-κB in various systems (*see* Table 3). Transcripts related to other cytokines, especially IFNs, were also well represented in the set. Many of the genes that were selectively induced in

TABLE 2. GENES THAT WERE DIFFERENTIALLY EXPRESSED IN AT LEAST 9 OF THE 12 MODELS

Description	Symbol	*Aspergillus* Extract	Bleomycin-induced Acute Lung Injury (*7 d*)	Bleomycin-induced Fibrosis (*21 d*)	IL-13, Transgenic	LPS, Aerosolized	LPS, i.p.	*M. pulmonis*	*M. tuberculosis*	*N. brasiliensis*	OVA BALB/C	OVA C57BL/6J	*P. aeruginosa*
Chemokines/chemotaxis													
CC chemokine ligand 2	Ccl2	1.5	4.8*	3.6*	3.7*	7.4*	14.7*	15.0*	10.8*	2.5	5.5*	4.4*	13.4*
C-C chemokine ligand 8	Ccl8	16.9*	3.1*	4.8*	2.7*	1.0	0.9	13.4*	8.6*	17.8*	25.0*	10.3*	0.8
C-C chemokine ligand 9	Ccl9	4.6*	2.3*	3.0*	4.9*	3.1*	1.8	2.4*	1.5	3.3*	3.6*	2.9*	1.5
C-C chemokine receptor 5	Ccr5	1.8	3.2*	3.2*	2.5*	2.9*	5.4*	14.7*	2.3*	1.9	6.3*	4.2*	1.4
CXC chemokine ligand 9	Cxcl9	1.0	2.0*	2.5*	1.1	2.4*	21.0*	17.0*	65.3*	2.7*	6.8*	4.4*	2.4
Leukocyte glycoproteins													
Glycoprotein 49A	Gp49a	1.9	3.2*	4.1*	4.8*	4.3*	9.3*	10.3*	3.3*	2.5	4.3*	1.7	4.7*
Lymphocyte antigen 86	Ly86	2.2*	2.7*	2.7*	2.3*	0.9	3.0*	3.2*	3.2*	2.6*	5.0*	3.6*	1.0
Leukocyte homing													
Integrin β2	Itgb2	2.9*	2.1*	2.0*	3.1*	1.2	1.6	3.1*	3.7*	2.4*	2.3*	2.2*	1.7
Phagocytosis													
C-type lectin domain protein, family 7 member a	Clec7a	3.4*	2.4*	3.4*	4.9*	1.6	0.8	2.8*	5.5*	2.4*	5.2*	3.9*	3.0*
Fc receptor, IgG, low affinity III	Fcgr3	2.2*	2.4*	3.6*	2.1*	1.8	3.1*	4.9*	3.9*	2.5*	2.2*	2.2*	1.9
Neutrophil cytosolic factor 1	Ncf1	1.6*	1.5*	1.6*	1.1	1.5	1.9*	2.8*	2.2*	1.3	2.1*	1.6*	2.3*
Leukocyte Ig-like receptor, subfamily B, member 3	Lilrb3	1.8*	1.7*	2.6*	1.1	2.3*	3.8*	4.8*	3.7*	1.8	2.2*	2.0*	2.3*
Monooxygenases/electron transport													
Thromboxane A synthase 1, platelet	Tbxas1	1.8*	1.6*	1.9*	2.3*	1.0	1.1	2.1*	1.8*	2.4*	4.5*	2.1*	0.9
Flavin containing monooxygenase 3	Fmo3	−2.8*	−2.3*	−1.8	−4.3*	−4.0*	−1.8	−3.8*	−3.6*	−2.3*	−2.4*	−1.9*	−1.4
Antigen recognition/processing													
Cathepsin S	Ctss	3.4*	3.8*	4.2*	3.2*	1.0	1.4	4.0*	8.9*	2.8*	4.0*	3.2*	1.2
Leukocyte Ig-like receptor, subfamily B, member 4	Lilrb4	2.6*	3.1*	3.8*	4.4*	5.9*	10.0*	11.7*	5.2*	2.7*	3.1*	3.0*	5.8*
Acute-phase response													
Serine (or cysteine) peptidase inhibitor, clade A, member 3N	Serpina3n	1.7	3.4*	2.3	2.7*	4.2*	6.5*	6.0*	2.0	5.6*	3.0*	3.2*	4.8*
Serum amyloid A3	Saa3	2.6	4.6*	6.6*	9.6*	46.7*	31.9*	55.8*	13.8*	3.8	8.7*	14.0*	10.6*
Others													
Ecotropic viral integration site 2b	Evi2b	1.8*	1.5*	1.7*	1.3	1.0	1.0	2.0*	1.9*	1.8*	1.9*	1.9*	2.4*
Membrane-spanning 4 domains, subfamily A, member 6D†	Ms4a6d	2.5*	2.6*	1.5	2.6*	1.5	5.9*	6.1*	3.5*	2.7*	2.4*	2.4*	1.5
Solute carrier family 26, member 4†	Slc26a4	15.8*	3.2*	3.3	15.6*	14.1*	4.5*	4.0*	4.1*	13.8*	8.7*	4.8*	3.5
RIKEN cDNA 1500001M20 gene†	1500001M20Rik	1.7	3.7*	2.5*	2.3*	4.6*	6.5*	6.9*	2.6	4.9*	2.5*	2.7*	4.2*
RIKEN cDNA 2610307O08 gene†	2610307O08Rik	1.2	2.1*	2.0*	1.8*	1.8	2.8*	3.5*	2.9*	1.9*	2.2*	1.9*	2.2
S100 Ca-binding protein A4	S100a4	2.0*	2.4*	3.7*	2.2*	1.5	1.5	2.8*	2.6*	2.4*	2.9*	3.4*	1.2

Definition of abbreviations: i.p. = intraperitoneal; OVA = ovalbumin; PBS = phosphate-buffered saline; *P. aeruginosa* = *Pseudomonas aeruginosa*; Th = T helper cell.
Values represent fold change compared with controls.
* Significantly different from controls by *t* test.
† This gene was not found to be associated with asthma, allergy, bacterial infection, bleomycin, or pulmonary fibrosis in the PubMed database.

bacterial infection models have known roles in leukocyte recruitment and activation in various systems. These include chemoattractants, other secreted molecules, cell adhesion molecules, and other surface receptors. For example, the transcript for the chemokine, Cxcl9 (monokine induced by IFN-γ), which is a chemoattractant for Th1 lymphocytes, was increased 17- and 65-fold in lungs infected by *M. pulmonis* and *M. tuberculosis*. Only two transcripts were decreased in at least four bacterial infection models, but not in any of the other models (Table E1). These are HOXA5, a transcription factor that suppresses angiogenesis (30), and D0H4S114, which encodes P311, a protein that may regulate alveolar regeneration (31). We found that 69 of the 89 transcripts (78%) were associated with bacterial infection in the published literature. However, our systematic review of PubMed found no previous reports of associations between the remaining 20 transcripts (indicated by a "†" symbol in Table 3 and "‡" in Table E1) and bacterial infection, LPS, or pneumonia.

Within the set of five bacterial infection models, the two models with the most highly correlated expression pattern were the aerosolized and intraperitoneal LPS administration models (Figure 2). Comparison of expression of all transcripts represented on the arrays showed a high degree of correlation, but there were some transcripts that were highly increased in one of

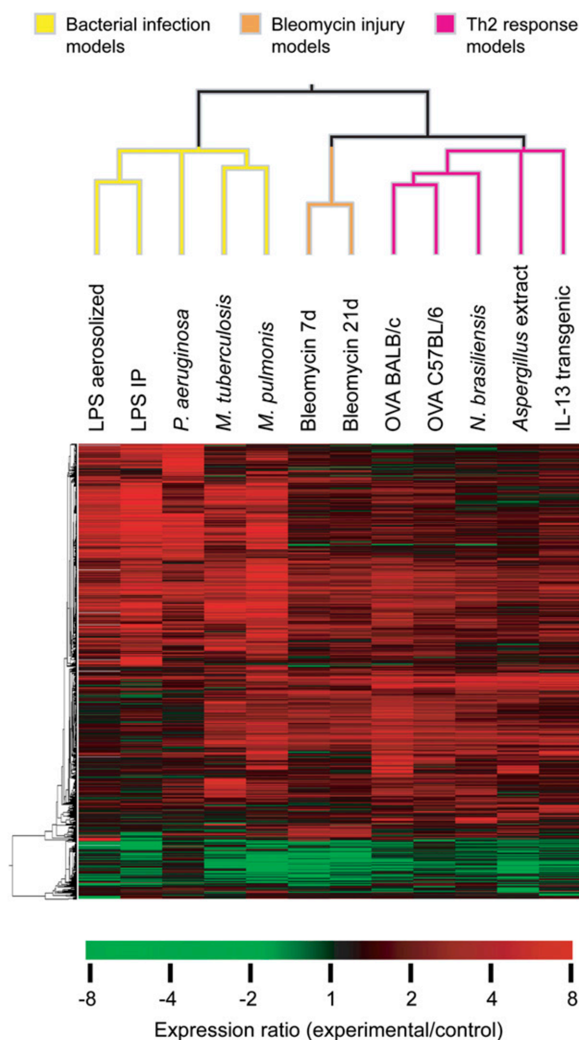

Figure 2. Transcript expression relationships among 12 lung disease models. Hierarchical clustering was performed using the most highly differentially expressed transcripts for each model. For each model, all transcripts with statistically significant expression changes (adjusted $P < 0.05$) were identified. The 50 differentially expressed transcripts with the largest fold change (either increases or decreases) were selected from each model, resulting in 510 total unique transcripts, and these were used for clustering. IP = intraperitoneal; OVA = ovalbumin.

these models but not the other (Figure 3). Transcripts with statistically significant increases in the intraperitoneal LPS model, but not the aerosolized LPS model, included many likely to play a role in acute inflammatory responses, such as neutrophilic granule protein (23.9-fold for intraperitoneal vs. 1.2-fold for aerosolized), cathelicidin antimicrobial peptide (15.4 vs. 1.3-fold), myeloperoxidase (8.4- vs. −1.2-fold), inducible NO synthase (Nos2, 5.7- vs. 1.0-fold), E-selectin (7.9- vs. 1.9-fold), IL-6 (8.7- vs. 2.6-fold), and the chemokines Ccl3 (13.5- vs. 3.8-fold), Cxcl7 (4.4- vs. 1.2-fold), and Cxcl11 (9.0- vs. 3.3-fold). In contrast, transcripts with significant increases in the aerosolized LPS model, but not the intraperitoneal model, included several likely to be involved in the lung epithelial response to LPS, including keratin 4 (18.9-fold for aerosolized vs. −2.4-fold for intraperitoneal), keratin 13 (16.7- vs. −2.7-fold), small proline rich-like 1 (7.0- vs. −3.0-fold), and aquaporin 3 (2.1- vs. −1.2-fold).

Transcript Expression Changes in Bleomycin-induced Lung Disease

We used a similar approach to identify transcript expression changes that were characteristic of the bleomycin-induced acute lung injury (7 d after exposure) and bleomycin-induced pulmonary fibrosis (21 d after exposure) (32). Only 12 transcripts were significantly increased in these 2 models (7 and 21 d after bleomycin exposure), but were not significantly increased in any of the other 10 models. Because both allergic airway disease and bacterial infections can induce fibrosis, we reasoned that this approach was too restrictive. By including transcripts that were increased in both bleomycin models and in just 1 of the other 10 models, we identified 20 additional transcripts. The 32 transcripts that were increased in both bleomycin models and in at least 1 of the other 10 models are listed in Table 4. Although the transcript expression changes were all statistically significant, fold change values were mostly modest (~1.5- to 3-fold). In fact, the list includes only 3 transcripts (elastin, pleckstrin homology-like domain family A member 3, and procollagen, type V, α2) that were among the 50 most highly up-regulated transcripts at 7 d after bleomycin, and 3 transcripts (elastin, laminin-α1, and latent transforming growth factor [TGF]-β binding protein) that were among the 50 most highly up-regulated transcripts at 21 days after bleomycin exposure. Nonetheless, previous studies make it clear that a substantial number of the 32 transcripts that were selectively increased after bleomycin exposure have functions relevant to fibrosis. These include seven transcripts that encode extracellular matrix proteins (*Lamc2*, *Lama1*, *Col5a2*, *Col18a1*, *Eln*, *Emilin1*, *Fbn1*), some of which have already been found to be up-regulated in pulmonary fibrosis (33, 34). Three additional transcripts are also involved in extracellular matrix formation and remodeling: *Loxl2*, a member of the lysyl oxidase family of matrix cross-linking enzymes that is already known to be dramatically increased after bleomycin exposure (1); *Mmp2*, a matrix metalloproteinase implicated in lung matrix remodeling (35); and stratifin, an inducer of the matrix metalloproteinases, Mmp1 and Mmp3. The profibrogenic cytokine TGF-β is believed to be the most important cytokine linked to the induction of pulmonary fibrosis (28). Our analysis identified one TGF-β superfamily member, growth differentiation factor 15, two TGF-β binding proteins (integrin-αv and latent TGF binding protein 2), and follistatin, an antagonist of the injury-repairing TGF-β superfamily member, activin, which blocks pulmonary fibrosis when administered to bleomycin-treated rats (36). Finally, we found that two transcripts encoding proteins in the Wnt (wingless-type MMTV integration site family) pathway (Wnt7b and Wnt1-inducible signaling pathway protein 1) were selectively up-regulated in the bleomycin models. The Wnt pathway, which regulates cell growth, differentiation, polarity, and adhesion, is key for numerous developmental processes, and has increasingly been implicated in the pathogenesis of diseases, including pulmonary fibrosis (37). The roles of other transcripts that were selectively increased in bleomycin lung injury, such as *Klhdc8a*, *Nnat*, and *Psrc1*, remain to be explored. We found PubMed reports of associations between pulmonary fibrosis or bleomycin and only eight (25%) of the transcripts that were selectively increased in the bleomycin models. The remaining 24 transcripts (indicated by a "†" symbol in Table 4) therefore represent novel candidates that may play roles in lung fibrosis.

Using a similar approach to search for transcripts with decreased expression, we identified 21 transcripts that were significantly decreased in both bleomycin models and in 1 or none of the other 10 models (Table E2). The large majority (19/21, or 90%) of these transcripts were not associated with pulmonary fibrosis or bleomycin in our systematic analysis of PubMed.

TABLE 3. GENE EXPRESSION CHANGES CHARACTERISTIC OF BACTERIAL INFECTION MODELS

Description	Symbol	LPS, Aerosolized	LPS, i.p.	*P. aeruginosa*	*M. tuberculosis*	*M. pulmonis*
NF-κB pathway–related genes						
Ligands						
IL-1α	Il1a	2.5*	1.9	12.3*	2.5*	11.5*
IL-1β	Il1b	7.2*	15.3*	18.1*	3.5*	20.7*
TNF	Tnf	5.3*	5.8*	36.9*	7.7*	29.7*
Receptors						
CD14	Cd14	10.4*	8.2*	5.6*	2.9*	12.7*
IL-1 receptor type II	Il1r2	5.7*	5.8*	6.1*	1.6	15.7*
Signaling from receptors to NF-κB						
Myeloid differentiation primary response gene 88	Myd88	2.9*	3.4*	2.4*	1.7	3.0*
Sphingosine kinase 1†	Sphk1	2.9*	4.1*	3.0*	1.1	2.1*
TRAF family member–associated NF-κB activator	Tank	1.8*	2.6*	1.5	1.7*	1.7*
TRAF type zinc finger domain containing 1	Trafd1	2.2*	3.2*	1.4	2.5*	3.1*
Baculoviral IAP repeat-containing 2	Birc2	1.8*	2.8*	2.1*	1.1	1.6*
Baculoviral IAP repeat-containing 3	Birc3	2.3*	2.0*	3.9*	1.1	2.0*
NF-κB/Rel/IkB family members						
NF of κ light polypeptide gene enhancer in B-cells 2, p49/p100	Nfkb2	4.0*	4.3*	3.4*	1.6	2.8*
NF of κ light polypeptide gene enhancer in B-cell inhibitor, zeta	Nfkbiz	3.9*	8.7*	17.6*	2.5*	6.5*
Avian reticuloendotheliosis viral (v-rel) oncogene related B	Relb	3.8*	3.5*	3.5*	1.7	2.4*
Regulators of NF-κB/IκB						
B-cell leukemia/lymphoma 10	Bcl10	1.8	2.1*	2.3*	1.8*	1.9*
Mitogen-activated protein kinase kinase kinase 8	Map3k8	2.2*	4.5*	3.0*	1.4	3.7*
Transglutaminase 2, C polypeptide	Tgm2	2.4*	2.4*	1.3	2.2*	2.5*
Genes induced by the NF-κB pathway						
Growth arrest and DNA damage–inducible 45β	Gadd45b	2.6*	2.6*	4.5*	1.0	3.3*
MARCKS-like 1	Marcksl1	4.0*	10.2*	3.2*	1.4	4.5*
Pentaxin-related gene	Ptx3	4.1*	6.2*	4.2*	1.4	3.8*
Prostaglandin-endoperoxide synthase 2	Ptgs2	1.9	4.6*	14.3*	2.5*	3.8*
Radical S-adenosyl methionine domain containing 2	Rsad2	6.4*	30.1*	1.9	2.9*	5.9*
Superoxide dismutase 2, mitochondrial	Sod2	3.3*	4.6*	2.6*	2.0*	3.5*
Thymidylate kinase family LPS-inducible member	Tyki	5.6*	19.6*	1.3	3.7*	3.9*
TNF-α–induced protein 2	Tnfaip2	8.3*	5.5*	5.4*	3.7*	4.4*
TNF-α–induced protein 3	Tnfaip3	3.3*	9.3*	14.6*	2.1*	7.4*
IFN-related genes						
IFN-α and -β receptor 2	Ifnar2	1.6	2.4*	2.0*	2.1*	1.9*
IFN-dependent positive-acting transcription factor 3g	Isgf3g	2.2*	2.9*	1.5	2.0*	2.1*
IFN-γ–induced GTPase	Igtp	2.8*	4.6*	1.7	2.6*	2.9*
IFN regulatory factor 1	Irf1	2.9*	4.9*	4.9*	3.6*	4.2*
IFN-induced protein 35†	Ifi35	1.9*	3.1*	1.2	2.0*	1.8*
IFN-induced protein with tetratricopeptide repeats 3	Ifit3	3.2*	6.1*	1.4	2.1*	2.7*
IL-18 receptor accessory protein	Il18rap	1.9*	2.3*	3.7*	1.6	3.5*
Ubiquitin-specific protease 18	Usp18	3.4*	9.9*	1.2	2.5*	3.4*
Other cytokines and receptors						
Colony-stimulating factor 1 (macrophage)	Csf1	3.0*	3.3*	4.4*	2.2*	1.5
Colony-stimulating factor 3 (granulocyte)	Csf3	3.8*	8.6*	7.5*	1.6	4.2*
NF, IL-3, regulated	Nfil3	2.5*	4.5*	4.4*	1.2	2.0*
Pre–B-cell colony–enhancing factor 1	Pbef1	2.1*	4.1*	1.8	2.3*	3.3*
Leukocyte adhesion, migration, and activation						
CD80 antigen	Cd80	2.3*	2.5*	2.7*	1.2	1.7*
Chemokine (C-C motif) ligand 20	Ccl20	4.8*	2.6*	17.1*	1.7	7.8*
C-type lectin domain family 4, member e	Clec4e	1.5	2.1*	4.0*	1.6*	9.3*
Killer cell lectin-like receptor, subfamily A, member 2	Klra2	1.0	2.2*	1.9*	3.9*	3.2*
Lectin, galactose binding, soluble 9	Lgals9	2.1*	5.2*	1.1	3.5*	2.2*
Matrix metallopeptidase 8	Mmp8	4.0*	11.3*	7.1*	1.5	18.0*
Paired Ig-like type 2 receptor α	Pilra	2.2*	2.0*	2.5*	2.6*	3.6*
S100 calcium binding protein A8 (calgranulin A)	S100a8	8.2*	5.7*	5.1*	1.7	18.7*
S100 calcium binding protein A9 (calgranulin B)	S100a9	15.8*	42.0*	8.9*	2.3	19.3*
Selectin, lymphocyte	Sell	3.0*	9.0*	3.9*	1.2	4.2*
Sialic acid-binding Ig-like lectin E†	Siglece	1.9*	2.1*	2.1*	1.0	2.5*
Stress response signaling-related genes						
Thioredoxin reductase 1†	Txnrd1	2.6*	2.5*	1.7*	1.0	1.7*
Jnk signaling						
Basic leucine zipper transcription factor, ATF-like	Batf	2.4*	4.8*	2.4*	1.8	6.3*
Dual specificity phosphatase 16	Dusp16	1.9*	3.5*	2.4*	−1.3	2.2*
Zinc finger protein 36	Zfp36	2.8*	3.2*	10.8*	1.1	3.5*
p38 MAPK signaling						
MAPK-activated protein kinase 2	Mapkapk2	1.5	2.3*	4.1*	2.6*	2.3*
p53 Signaling						
Promyelocytic leukemia	Pml	2.0*	5.4*	1.5	2.2*	1.8*

Definition of abbreviations: ATF = activating transcription factor; IAP = inhibitor of apoptosis protein; IκB = inhibitor of NF-κB; i.p. = intraperitoneal; MAPK = mitogen-activated protein kinase; MARCKS = myristoylated protein kinase C substrate; NF = nuclear factor; TNF = tumor necrosis factor; TRAF = TNF receptor–associated factor.

Data include selected genes that were significantly increased in at least four of the five bacterial infection models, but not in any of the other seven models. Values represent fold change compared with controls.

* Significantly different from controls by *t* test.

† This gene was not found to be associated with bacterial infection in the PubMed database.

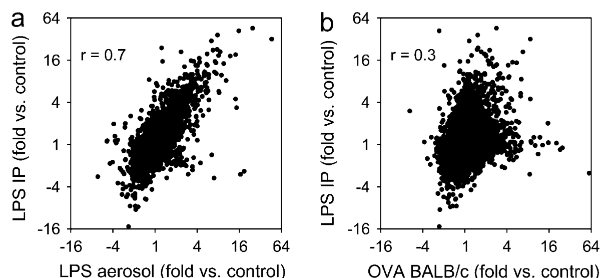

Figure 3. Similar pattern of gene expression changes in the intraperitoneal and aerosolized LPS models. Changes in gene expression in the intraperitoneal and aerosolized LPS models were highly correlated, although there were some genes that behaved differently in these two models (*a*). By comparison, expression changes in two models from different groups (intraperitoneal [IP] LPS and ovalbumin [OVA] challenge) were not highly correlated (*b*). Fold- difference measurements for all 16,463 transcripts represented on the arrays are shown in each plot, as are correlation coefficients (*r*).

The two bleomycin-induced models had surprisingly similar overall patterns of gene expression even though they analyzed gene expression at much different time points after bleomycin administration (7 d for the acute lung injury model and 21 d for the fibrosis model). Studies of additional lung injury models will be required to determine which of these expression changes are selective for bleomycin-induced injury and which are seen in other models of acute lung injury and/or pulmonary fibrosis.

Transcript Expression Changes in Th2 Inflammation Models

The third group included five models with Th2 inflammation. Within this group, the two ovalbumin allergy models (in BALB/c and C57BL/6 mice) were the most closely related (Figure 2). However, it is remarkable that other models provoked by very different stimuli—infection with the parasite *N. brasiliensis*, overexpression of a single Th2 cytokine (IL-13), or a more complex antigen mixture from a fungus (*Aspergillus*)—also had similar effects on gene expression. We identified 26 transcripts that were characteristic of the Th2 inflammation models (Table 5). These include seven transcripts encoding secreted proteins produced by epithelial cells, including the mucin glycoproteins Muc5ac and Muc5b. All but one of these seven transcripts were up-regulated in Th2 models and also in *M. pneumoniae* infection, which all are characterized by prominent mucus overproduction. Five molecules involved in ion transport were also increased in Th2 models. Some of the 26 transcripts have previously been implicated in the pathogenesis of allergic airway disease and asthma or in additional forms of Th2 inflammation, including acidic chitinase (Chia) (38–41), the secreted mucins (19, 42, 43), the calcium-activated chloride channel, Clca3 (44), 12,15-lipoxygenase (Alox15) (7, 45), and arginase (Arg1) (46–48). Most of the transcripts in Table 5 that encode proteins that are secreted by epithelial cells or are involved in ion transport have previously shown to be induced in response to the direct effects of IL-13 on epithelial cells (19), which is believed to be central to the pathogenesis of allergen-induced mucus production (19, 49). There were no transcripts that were decreased in at least four of the five Th2 models and in one or none of the other models. Half (13/26) of the transcripts identified in this analysis were not found to be associated with allergy or asthma in the PubMed database (Table 5).

Validation of Array-based Transcript Expression Measurements

To determine whether transcript expression changes measured using microarrays could be verified using a different method, we used quantitative real-time PCR to measure the expression of selected transcripts. We had sufficient material available to study three of the bacterial infection models, each of the two bleomycin models, and three of the Th2 models. In the 83 cases in which the arrays showed at least 1.5-fold higher expression of a transcript in a disease model, 75 of the PCR reactions (90%) also showed at least 1.5-fold higher expression (Table E3). Six of the eight PCR reactions that failed to show at least 1.5-fold higher expression were assays for Zgpat or Atp2a1 (each primer pair was used for the three allergic models), indicating a clear discrepancy between array and PCR results for these two transcripts. Array and PCR estimates of the magnitude of the fold difference often differed. The most dramatic differences were usually seen in cases where PCR measurements showed very large differences and arrays showed smaller differences. PCR typically has greater sensitivity and dynamic range than arrays (50, 51), and we used a conservative array analysis method (no background subtraction) that tends to underestimate fold-difference. We conclude that array findings of differential expression were very frequently confirmed by quantitative PCR, although the magnitude of the change was often underestimated by the arrays.

Conclusions

The results of the microarray analyses presented here demonstrate abundant transcript expression changes in the context of lung disease. The dataset of transcript expression profiles generated here is publicly available, and will provide a useful resource for investigators studying pulmonary disease pathogenesis. By using multiple models of varying lung disease, we identified transcript expression changes that were common to many forms of lung disease and transcripts whose expression was changed only among specific lung disease models. We described a series of characteristic transcript expression changes for the various categories of lung disease, including bacterial infection, bleomycin-induced lung disease, and Th2 (allergic) inflammation.

We chose to study a wide variety of experimental systems rather than studying a single system, an approach that has some different strengths and weaknesses than other array-based approaches. One important limitation of our approach is that we did not study most model systems at multiple time points, as it would not have been practical for us to do so with so many models. Instead, we generally chose a single time point when there is known to be established pathology, based on previous work by investigators who developed the models and use them to investigate mechanism. Therefore our approach will not detect transient gene expression changes that occur before the time point that we analyzed and may contribute to the development of pathology. A second limitation is that different types of mice were used in different models. Because models included age-, sex-, and strain-matched control animals, we were able to identify gene expression changes due to disease as opposed to those which were simply due to baseline differences between strains. Nonetheless, it is likely that genetic and environmental differences between the cohorts of mice used for the various models complicate the process of trying to identify common features between models. A third limitation is that our set of models included only two "lung injury" models, and in both cases the injury was initiated by bleomycin administration. Additional studies will be required to determine which of the bleomycin lung injury–associated gene expression changes seen in our studies are also found in other lung injury models. Like studies of lung gene expression in just one model, our approach is also limited because arrays are not sufficiently sensitive to

TABLE 4. GENE EXPRESSION INCREASES CHARACTERISTIC OF BLEOMYCIN-INDUCED LUNG DISEASE

Description	Symbol	Bleomycin-induced Acute Lung Injury (*7 d*)	Bleomycin-induced Fibrosis (*21 d*)	Other Model with Increased Expression
Extracellular matrix				
Elastin	Eln	4.8*	4.1*	*Aspergillus* extract 3.0*
Elastin microfibril interfacer 1†	Emilin1	1.7*	1.7*	LPS aerosolized 2.1*
Fibrillin 1	Fbn1	2.3*	1.9*	*M. pulmonis* 1.6*
Laminin-α1	Lama1	1.8*	3.1*	—
Laminin-γ2	Lamc2	2.0*	2.0*	—
Procollagen, type V, α2†	Col5a2	2.9*	2.2*	*M. pulmonis* 2.7*
Procollagen, type XVIII, α1	Col18a1	1.5*	1.7*	—
Regulation of extracellular matrix				
Lysyl oxidase–like 2†	Loxl2	1.9*	2.0*	—
Matrix metallopeptidase 2	Mmp2	2.3*	2.3*	*Aspergillus* extract 2.1*
Stratifin†	Sfn	2.6*	2.3*	*M. pulmonis* 2.4*
TGF-β				
Growth differentiation factor 15	Gdf15	2.4*	2.0*	*P. aeruginosa* 2.4*
Integrin-αV	Itgav	1.5*	1.7*	LPS aerosolized 1.9*
Latent TGF-β binding protein 2†	Ltbp2	1.8*	3.1*	*Aspergillus* extract 2.2*
Regulation of TGF-β				
Follistatin	Fst	2.4*	2.3*	—
Wnt signaling				
Wnt1-inducible signaling pathway protein 1†	Wisp1	1.9*	1.9*	—
Wingless-related MMTV integration site 7B†	Wnt7b	2.1*	1.6*	—
Development/morphogenesis/cell cyle/differentiation				
Carboxypeptidase X1 (M14 family)†	Cpxm1	1.9*	2.1*	OVA BALB/c 2.5*
Cyclin G1	Ccng1	2.7*	2.4*	—
Integral membrane protein 2A†	Itm2a	2.2*	2.5*	*Aspergillus* extract 1.9*
Keratin 18†	Krt18	1.8*	2.1*	*M. pulmonis* 2.1*
MAP/microtubule affinity–regulating kinase 1†	Mark1	1.4*	1.9*	—
Pleckstrin homology-like domain, family A, member 3†	Phlda3	3.4*	2.2*	—
Transcription factor AP-2, α†	Tcfap2a	1.4*	1.7*	—
Other targeting/signaling				
Aldo-keto reductase family 1, member B8†	Akr1b8	1.7*	2.4*	LPS aerosolized 2.2*
ATPase inhibitory factor 1†	Atpif1	1.6*	1.8*	OVA BALB/c 1.5*
Chondrolectin†	Chodl	1.8*	2.0*	IL-13 overexpression 1.5*
Lactamase, β†	Lactb	1.9*	1.5*	—
S100 Ca-binding protein A14†	S100a14	2.6*	2.1*	*M. pulmonis* 1.8*
S100 Ca-binding protein A6 (calcyclin)	S100a6	1.7*	2.0*	*M. pulmonis* 1.9*
Others				
Kelch domain containing 8A†	Klhdc8a	2.3*	2.4*	*M. pulmonis* 1.6*
Neuronatin†	Nnat	2.3*	1.9*	*Aspergillus* extract 1.9*
Proline/serine-rich coiled-coil 1†	Psrc1	2.4*	2.4*	*Aspergillus* extract 1.9*

Definition of abbreviations: AP = activator protein; MAP = microtubule-associated protein; MMTV = mouse mammary tumor virus; OVA = ovalbumin; TGF = transforming growth factor; Wnt = wingless-type MMTV integration site family.

Data include selected genes that were significantly increased in both bleomycin models and only 1 or none of the other 10 models. Values represent fold change compared with controls.

* Significantly different from controls by *t* test.

† Genes not found to be associated with bleomycin or pulmonary fibrosis in the PubMed database.

detect some gene expression changes, and because changes in gene expression that affect relatively small populations of cells within the lung may not be detectable.

The major advantage of the multimodel approach that we used is that it provides a way to generate small lists of genes associated with related groups of disease models rather than a much longer list of genes that are differentially expressed in a single model and may or may not have an association with specific pathological features of that model. It is likely that the relatively short lists generated using our approach are highly enriched for genes that are involved in pathogenesis, based on data from previous studies involving other experimental approaches. Other genes in these lists have not previously linked to lung disease pathogenesis. Based on their consistent and relatively specific associations with specific forms of lung disease, many of these signature genes are good candidates for further study. Our systematic review of the PubMed database of published literature indicated that many of these genes have not previously been associated with the diseases that were represented in the multiple model dataset. Although our analysis approach focused largely on genes that were differentially expressed across groups of models, this kind of multiple model dataset could also be used to identify gene expression changes that are unique for specific disease models. For example, we were able to identify gene expression changes that were different between the intraperitoneal and aerosolized LPS models and give insights into how the route of exposure affects the response to this bacterial component. Some approaches to analyzing array data have relied on selection of the most highly differentially expressed transcripts for further hypothesis generation and testing, but those approaches do not necessarily lead to the identification of disease-specific gene expression changes that are likely to be good targets for therapeutic intervention. In conclusion, the present study shows that the combined analysis of multiple disease models is a powerful method for identifying transcript expression changes that are characteristic of specific pathophysiological processes, even when the degree of differential expression is relatively modest.

TABLE 5. GENE EXPRESSION CHANGES CHARACTERISTIC OF TYPE 2 T HELPER CELL MODELS

Description	Symbol	OVA BALB/C	OVA C57BL/6J	*N. brasiliensis*	*Aspergillus*	IL-13 Transgenic	Other Model with Increased Expression
Secreted molecules							
Anterior gradient 2 (*Xenopus laevis*)†	Agr2	3.9*	6.1*	2.2*	2.5*	4.4*	*M. pulmonis* 3.1*
Chitinase, acidic	Chia	2.6*	1.7	7.6*	6.2*	8.5*	—
Mucin 5, subtypes A and C, tracheobronchial/ gastric	Muc5ac	13.0*	10.9*	5.4*	3.8*	4.2*	*M. pulmonis* 3.2*
Mucin 5, subtype B, tracheobronchial	Muc5b	3.3*	3.0*	3.1*	2.5*	4.6*	*M. pulmonis* 11.1*
Regenerating islet–derived 3γ†	Reg3g	5.5*	3.3*	1.7	2.7*	3.7*	*M. pulmonis* 14.5*
Small proline-rich protein 2A	Sprr2a	8.5*	2.9	10.1*	6.8*	5.8*	*M. pulmonis* 5.4*
Trefoil factor 2 (spasmolytic protein 1)	Tff2	4.3*	3.7*	2.6*	1.7	5.0*	*M. pulmonis* 6.4*
Channels/transporters							
ATPase, Na^+/K^+ transporting, α3 polypeptide†	Atp1a3	2.7*	1.6	2.9*	3.5*	2.2*	*P. aeruginosa* 2.9*
ATPase, Ca^{++} transporting, cardiac muscle, fast twitch 1†	Atp2a1	11.2*	2	3.6*	6.0*	6.7*	—
Chloride channel, calcium activated 3	Clca3	59.9*	54.2*	54.5*	62.9*	34.8*	—
FXYD domain-containing ion transport regulator 4†	Fxyd4	2.7*	2.5	8.7*	12.5*	4.1*	—
Solute carrier family 5 (sodium/glucose cotransporter), member 1†	Slc5a1	1.6*	1.2	2.5*	2.1*	1.9*	LPS i.p. 1.7*
Other genes							
RIKEN cDNA 9130211I03 gene†	9130211I03Rik	2.5*	2.1*	1.8*	1.8*	1	—
Arachidonate 15-lipoxygenase	Alox15	4.6*	2.3*	6.4*	3.8*	1	—
Arginase 1, liver	Arg1	9.0*	5.3*	7.2*	8.9*	4.6*	*M. pulmonis* 9.4*
Eosinophil-associated, RNase A family, member 11	Ear11	20.5*	5.3*	10.2*	6.7*	11.3*	—
Guanylate cyclase activator 1a (retina)†	Guca1a	10.2*	7.0*	1.3	3.5*	2.4*	*M. pulmonis* 3.9*
Heme-binding protein 2†	Hebp2	1.8*	1.3	1.8*	1.7*	1.7*	LPS aerosolized 2.2*
Integrin-αX	Itgax	2.2*	2.5*	3.3*	2.6*	3.7*	Bleomycin 21 d 3.4*
Megakaryocyte-associated tyrosine kinase†	Matk	2.8*	2.9*	2.5*	2.8*	1.6	—
Meteorin, glial cell differentiation regulator-like†	Metrnl	2.5*	1.8*	1.7	2.1*	1.9*	*M. pulmonis* 3.0*
Macrophage galactose N-acetyl-galactosamine specific lectin 1	Mgl1	4.6*	2.2*	2.2*	1.3	2.1*	Bleomycin 7 d 1.6*
NAD synthetase 1†	Nadsyn1	15.5*	5.0*	6.5*	10.3*	9.4*	Bleomycin 7 d 4.3*
Programmed cell death 1 ligand 2	Pdcd1lg2	2.8*	2.5*	3.6*	2.0*	1.6	*M. pulmonis* 2.2*
Scinderin	Scin	1.5*	2.2*	1.8*	1.9*	2.4*	—
Zinc finger, CCCH-type with G patch domain†	Zgpat	2.1*	2.7*	4.1*	2.8*	3.6*	—

Definition of abbreviations: CCCH = cysteine-cysteine-cysteine-histidine; OVA = ovalbumin.
Genes differentially expressed in at least four of five Th2 models and one or none of the other models.
* Significantly different from controls (*t* test).
† Genes not found to be associated with asthma or allergy in the PubMed database.

Conflict of Interest Statement: C.C.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. J.Y.H.Y. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. X.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.K.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.R.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. P.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.M.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. T.S.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. V.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. W.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.V. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.J.E. received $2,500 in 2007 for serving on an advisory board for Johnson & Johnson.

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