

Proteome Analysis of Chronically Inflamed Lungs in a Mouse Chronic Asthma Model

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Key Words

Asthma · Bronchoalveolar lavage fluid · Airway remodeling · Ovalbumin

Abstract

Background: Asthma is a chronic airway inflammatory disease characterized by airway wall remodeling. The mechanisms underlying airway remodeling in asthma are not fully understood. There is an urgent need to investigate global protein profiling of chronically inflamed lungs to identify novel pathogenic molecules and biomarkers for chronic asthma. In this study, we described the first differentially expressed proteome of lung tissue and bronchoalveolar lavage fluid from a mouse chronic asthma model. **Methods:** BALB/c mice sensitized to ovalbumin were challenged with ovalbumin aerosol 3 times per week for 8 weeks. The lung tissue and lavage fluid proteins were resolved by 2-dimensional gel electrophoresis, and differentially expressed proteins were identified by matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry. **Results:** Airway goblet cell hyperplasia, smooth muscle hyperplasia, subepithelial fibrosis, airway hyperresponsiveness, pulmonary inflammatory cell infiltration and elevated serum ovalbumin-specific IgE level were observed in our chronic asthma model. We have identified at least 100 protein spots that were differentially expressed in chronically

inflamed lungs, and the identity of 66 protein spots was confirmed. **Conclusions:** Many of these proteins, including cytoskeleton-related proteins, Ca²⁺-binding proteins and antioxidant proteins, may be related to the development of airway remodeling, and they should be evaluated further as potential therapeutic targets and biomarkers for chronic asthma.

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Introduction

Allergic asthma is a chronic airway disorder characterized by airway inflammation, airway remodeling and airway hyperresponsiveness. Airway remodeling refers to structural changes in the airways as a result of repeated cycles of airway injury and repair, causing an abnormal deposition of extracellular matrices, goblet cell hyperplasia, subepithelial fibrosis and airway smooth muscle hyperplasia and hypertrophy [1]. The process of airway remodeling appears to be poorly reversible by current treatments with anti-inflammatory agents and bronchodilators. Varying degrees of airway remodeling can be observed in most asthmatic patients even when they are asymptomatic [2, 3]. Protein changes that underline airway remodeling in asthma are not fully understood. To capture the full spectrum of protein alterations during

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remodeling in asthma, a global protein profiling study of the chronically inflamed lungs is urgently needed.

Proteomics is a powerful tool for large-scale protein studies of complex diseases such as asthma [4, 5]. Indeed, there are a number of recent reports focusing on the proteome of either lung tissues or bronchoalveolar lavage (BAL) fluid obtained from animal models of acute asthma [6–11]. While those reports revealed valuable data about allergic airway inflammation, acute asthma models lack airway remodeling features commonly observed in human asthma [12, 13]. The purpose of the present study was to investigate differential protein profiling in chronically inflamed lungs isolated from mice with chronic asthma based on protocols reported by several laboratories [13–17]. Chronic asthmatic mice demonstrated airway remodeling features like airway goblet cell hyperplasia, smooth muscle hyperplasia and subepithelial fibrosis. Proteins from lung tissues and BAL fluid were resolved by 2-dimensional gel electrophoresis (2-DE) and identified by peptide mass fingerprinting using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) tandem mass spectrometry (MS/MS).

Materials and Methods

Mouse Chronic Asthma Model

Animal experiments were performed according to the Institutional Guidelines for Animal Care and Use Committee of the National University of Singapore. Male BALB/c mice ($n = 10$), 6–8 weeks of age (Interfauna, East Yorkshire, UK), were sensitized by intraperitoneal injections of 20 μ g ovalbumin (OVA; grade V, Sigma, St. Louis, Mo., USA) together with 4 mg $\text{Al}(\text{OH})_3$ in 0.1 ml PBS on days 0 and 14. From day 18, animals were challenged with low-dose 0.5% OVA aerosol, as compared with 1% OVA aerosol in a mouse acute asthma model [7], for 30 min/day 3 times a week for 8 weeks at the same frequency and duration as in previous reports [14–17]. Control mice ($n = 10$) were sensitized with OVA but challenged with sterile PBS. In separate groups of mice, airway hyperresponsiveness to inhaled methacholine and serum IgE levels was monitored at 2-, 4-, 6- and 8-week intervals, as described previously [18]. Twenty-four hours after the last OVA challenge, mice were sacrificed by cervical dislocation. Tracheotomy was performed and a cannula was inserted into the trachea. Ice-cold PBS (0.5 ml $\times$ 2) was instilled into the lungs and BAL fluid was collected. Lung tissues were immediately frozen in liquid nitrogen. BAL fluid and lung tissues were stored at -80°C .

Lung Histology

Lung tissues were fixed in 10% formalin. Paraffin-embedded sections (5 μ m) were stained with hematoxylin and eosin to evaluate general morphology and cell infiltration. Mucus-secreting goblet cells were visualized with periodic acid-Schiff stain. Masson trichrome stain was used for the assessment of subepithelial

fibrosis [19]. Fibronectin deposition was detected using immunohistochemistry, as previously described [20]. All slides were examined in a random and blinded fashion.

Measurement of Airway Hyperresponsiveness and Serum OVA-Specific IgE Level

In order to monitor changes in airway responsiveness throughout the 8-week development of chronic lung inflammation in the same animals, we used the unrestrained noninvasive plethysmography approach to measure Penh values. Mouse airway responsiveness to increasing concentrations of methacholine (5–80 mg/ml, Sigma) was measured at 2-, 4-, 6- and 8-week time intervals using a single-chamber whole-body plethysmograph (Buxco, Sharon, Conn., USA), as previously described [18]. The highest Penh value obtained during each methacholine challenge was expressed as percentage of basal Penh value in response to PBS challenge. Recently, the use of Penh for airway responsiveness has generated controversies, and it is not a substitute for invasive mechanical indexes such as lung resistance [21]. Nevertheless, it has been shown that Penh values are highly correlated to lung resistance observed in BALB/c mice, the same strain of mice used in the present study. Blood was collected from tail vein, and the serum OVA-specific IgE level was determined by ELISA, as previously described [18].

Protein Sample Preparation

Frozen lung tissues were homogenized in a lysis buffer containing 8 M urea, 2 M thiourea, 4% CHAPS, 0.8% pharmalyte, 65 mM DTT, protease inhibitor cocktail, 5 μ g/ml DNase I and 20 μ g/ml RNase A at 4°C . Lysates were spun at 14,000 rpm for 15 min, and the supernatants were collected for proteomics study. BAL fluid samples were precipitated with TCA (10% final concentration). After centrifugation, the pellet was resuspended in ice-cold acetone and centrifuged again. The pellet was air dried and solubilized in the same lysis buffer, as described above. Protein concentration was measured using a modified Bradford assay.

2-DE and Image Analysis

One hundred micrograms of lung tissue and BAL fluid protein lysates were resolved by 2-DE. Gel staining with silver nitrate and protein spot detection, quantitation and matching were performed, as previously described [7]. Matched protein spots with statistically significant changes in optical density between the control (saline aerosol) and the asthmatic group (OVA aerosol) were selected for protein identification.

Protein Identification

Protein spots were excised from the stained gels and digested in porcine sequencing grade modified trypsin (Promega, Madison, Wisc., USA). Peptide mass fingerprint maps of tryptic peptides were generated by MALDI-TOF tandem MS/MS using a 4700 Proteomics Analyzer with a TOF-TOFTM optics workstation (Applied Biosystems, Foster City, Calif., USA). Peptide masses were searched against the NCBIInr database with the Mascot software (<http://www.matrixscience.com>) and the MS-Fit program (<http://prospector.ucsf.edu>). Proteins with a minimum of 4 matching peptides and the sequence coverage exceeding 20% with the matched proteins, and with a probability score of more than 75, were considered positive.

Statistical Analysis

Changes in optical intensities of all matched protein spots between groups ($n = 9$) were subjected to two-tail Student's t test using SPSS software (Chicago, Ill., USA). Data are expressed as means $\pm$ SD. The critical level for significance was set at $p < 0.05$.

Results

Characterization of a Mouse Chronic Asthma Model

Repeated OVA aerosol challenge of OVA-sensitized mice for 8 weeks induced airway morphological changes as demonstrated by increased inflammatory cell infiltration into the lamina propria and epithelial layer, thickening of peribronchial airway smooth muscle layer, goblet cell hyperplasia and increased subepithelial collagen and fibronectin deposition in the airways (fig. 1). Airway hyperresponsiveness in mice repeatedly challenged with OVA aerosol increased dramatically throughout the 8-week study period as compared with PBS aerosol challenge (fig. 2a). Nevertheless, the magnitude of airway hyperresponsiveness in asthmatic mice declined gradually over the 8-week period, which is consistent with the declining trend of airway hyperresponsiveness in some documented mouse chronic asthma models [15]. Similarly, the serum OVA-specific IgE level was markedly elevated in OVA-challenged mice at week 2 and then dropped steadily over the 8-week period upon repeated allergen challenge (fig. 2b). Nevertheless, the serum OVA-specific IgE level was still significantly higher than that in control mice, a finding consistent with a report by Sakai et al. [17].

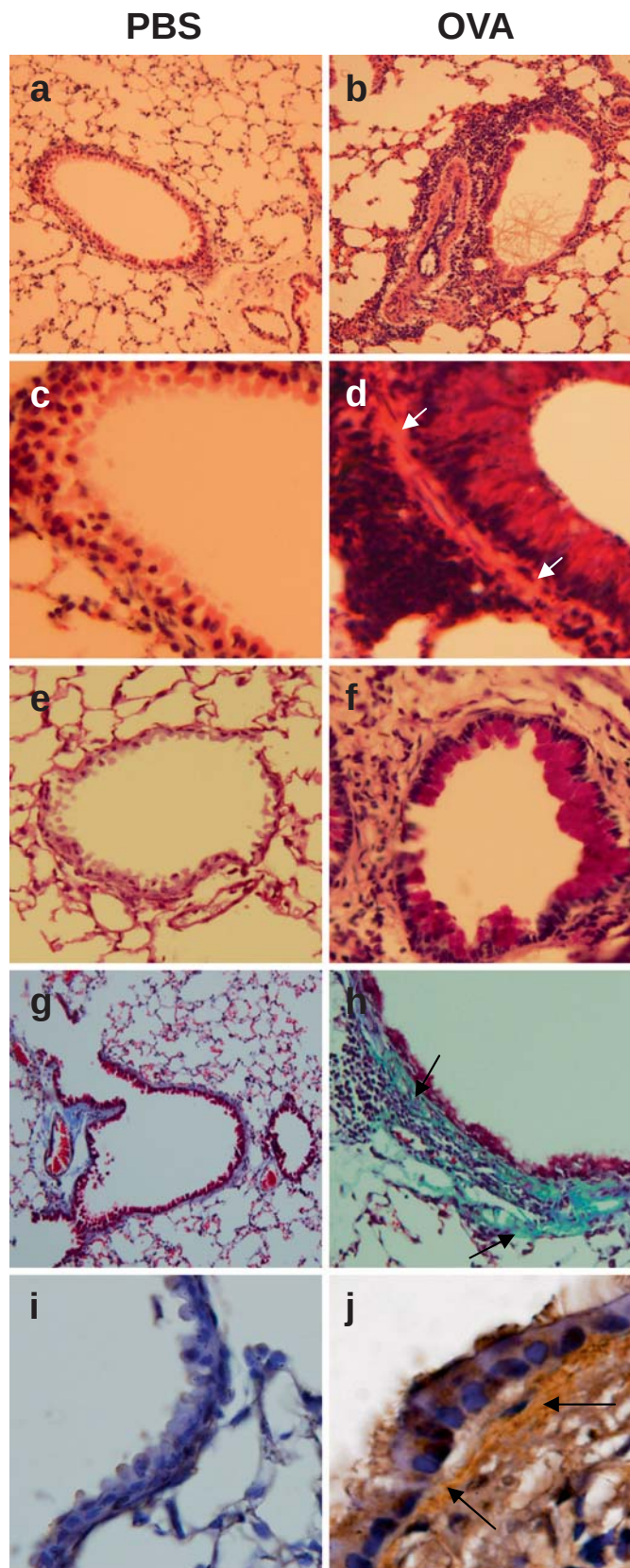


Fig. 1. Histological examination of lung remodeling in a mouse chronic asthma model. Lungs were collected 24 h after the last challenge. Representative photomicrographs using hematoxylin and eosin staining for general airway inflammation (**a, b**) and smooth muscle layer thickening (**c, d**), periodic acid-Schiff staining for goblet cell hyperplasia (**e, f**), Masson trichrome staining for subepithelial collagen deposition (**g, h**) and immunohistochemistry for fibronectin expression (**i, j**) ($n = 5-8$ for each group). $\times 40$ objective (**a-h**), $\times 100$ objective (**i, j**). White arrows and black arrows indicate structural changes.

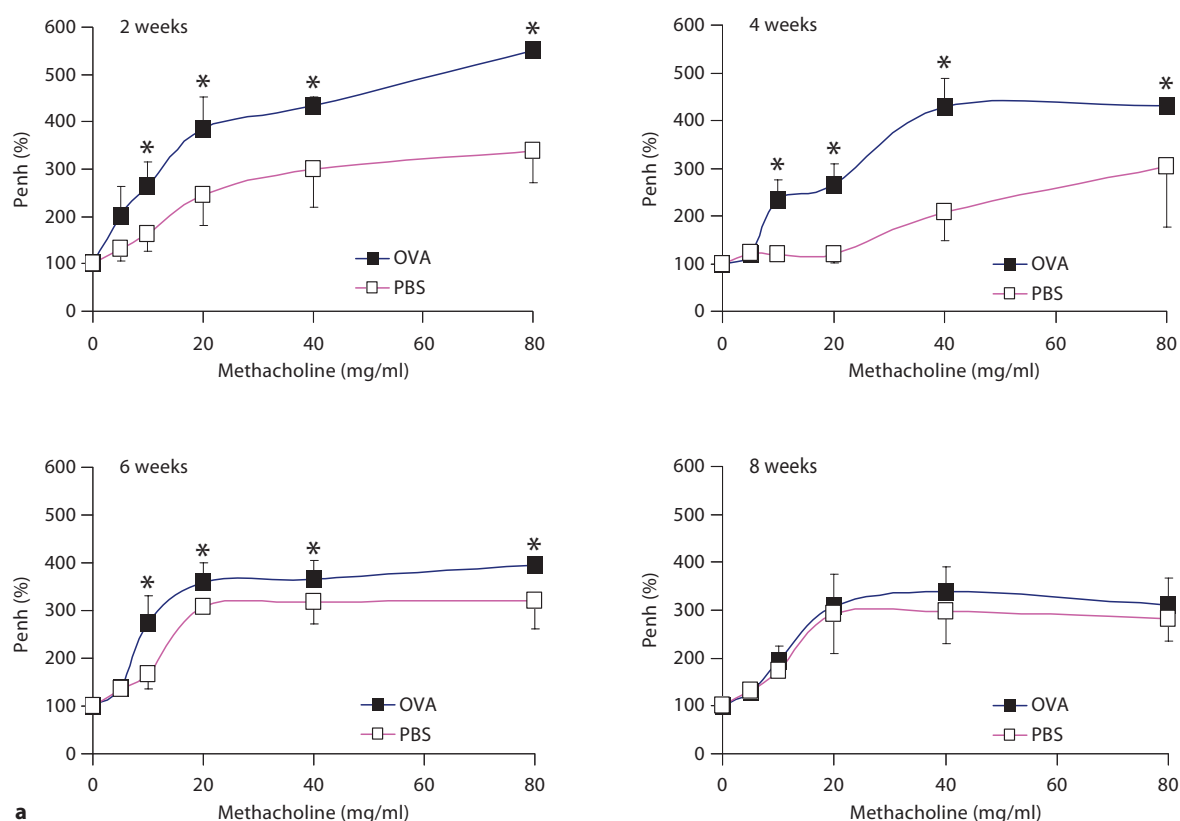
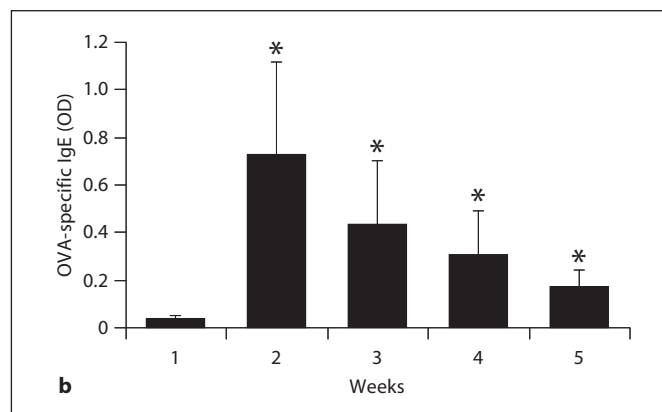


Fig. 2. Functional and biochemical examinations of a mouse chronic asthma model. **a** Airway responsiveness was measured after mice had been challenged with aerosolized PBS or OVA for 2, 4, 6 and 8 weeks using a single-chamber whole-body plethysmograph in which mice were exposed to increasing doses of inhaled methacholine ($n = 5$ for each group). **b** Serum level of OVA-specific IgE was measured using ELISA ($n = 15$ for each group) after mice had been challenged for 2, 4, 6 and 8 weeks. Data are expressed as means $\pm$ SD. * $p < 0.05$, compared with PBS control.



Proteomics of Chronically Inflamed Lungs

Lung tissue and BAL fluid samples were obtained from mice 24 h after the last OVA aerosol challenge. Protein samples from lung tissues were resolved in both pH 4–7 and pH 6–11 gels, whereas BAL fluid protein samples were only resolved in pH 4–7 gels due to limited protein amounts in BAL fluid. Following image analysis, about

1,000 protein spots were consistently detected in pH 4–7 gel for lung tissue, 500 protein spots in pH 6–11 gel for lung tissue and 700 protein spots in pH 4–7 gel for BAL fluid. A total of 100 differentially expressed protein spots were detected in chronically inflamed lungs with statistically significant changes in optical density and were subjected to peptide mass fingerprinting by MALDI-

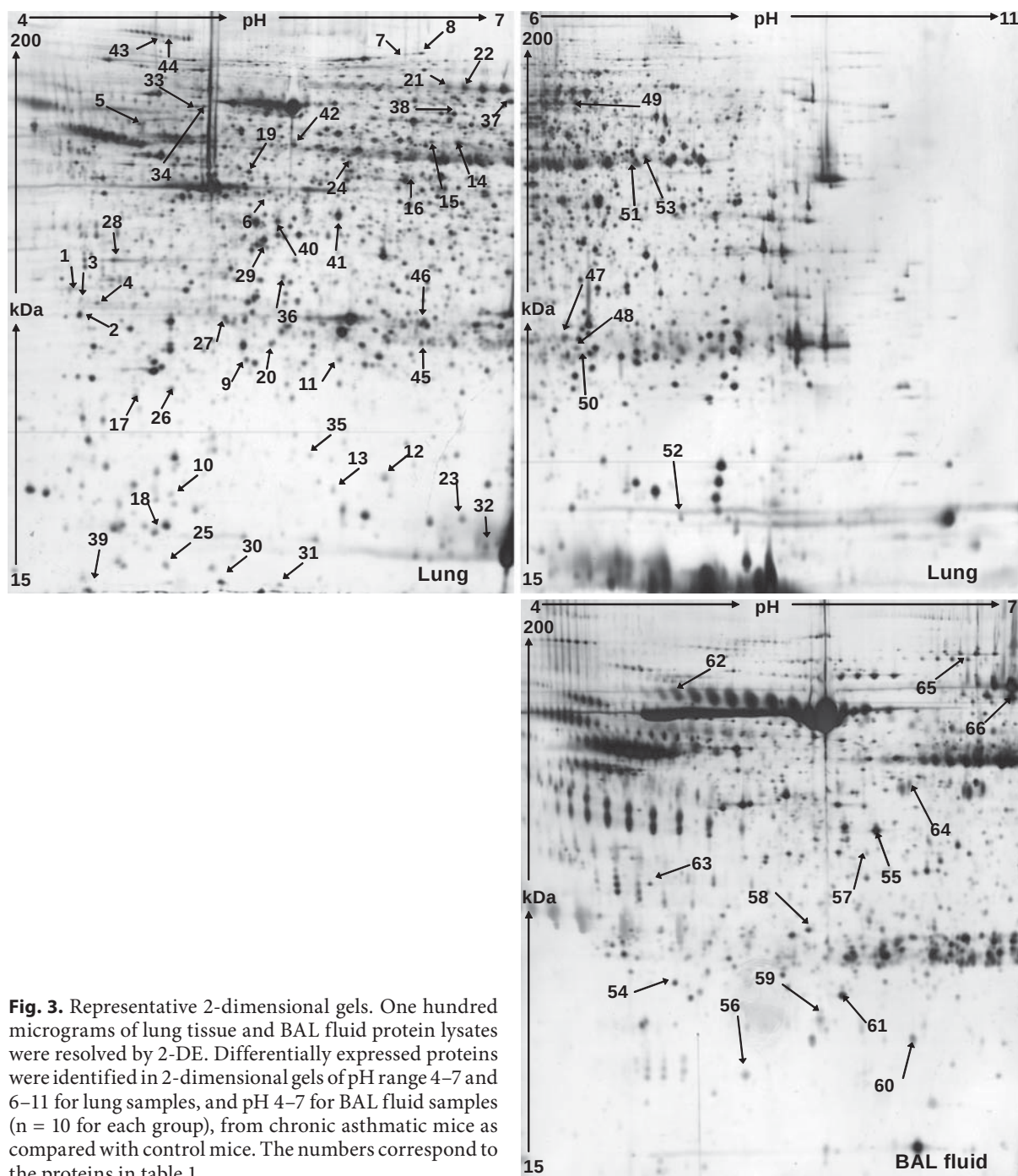


Fig. 3. Representative 2-dimensional gels. One hundred micrograms of lung tissue and BAL fluid protein lysates were resolved by 2-DE. Differentially expressed proteins were identified in 2-dimensional gels of pH range 4–7 and 6–11 for lung samples, and pH 4–7 for BAL fluid samples ($n = 10$ for each group), from chronic asthmatic mice as compared with control mice. The numbers correspond to the proteins in table 1.

TOF tandem MS/MS. Sixty-six spots were identified by the MS/MS, corresponding to 41 increases and 25 decreases in protein spot optical density (table 1). Forty-six proteins (spots 1–46) were identified in pH 4–7 gel for lung tissue, 7 proteins (spots 47–53) in pH 6–11 gel for

lung tissue and 13 proteins (spots 54–66) in pH 4–7 gel for BAL fluid. The location of the identified protein spots is shown in representative gels (fig. 3). Several proteins existed in multiple spots (e.g., Ym1 and lungkine) with slightly different molecular weights or different isoelec-

Table 1. Differentially expressed proteins in chronically inflamed lungs from mouse chronic asthma model

Proteins	Spot No.	Accession No.	Score ¹	Matched peptides, n	Coverage ² %	Fold change
<i>Cytoskeleton and related proteins</i>						
Lymphocyte cytosolic protein 1	33	gi 31543113	858	49	57	2.0
Lymphocyte cytosolic protein 1	34	gi 31543113	967	48	45	2.2
ARP 2/3 complex, subunit 5	35	gi 37805157	84	10	52	2.5
Capping protein (actin filament), muscle Z-line, β	36	gi 6753262	159	13	31	2.0
Moesin	37	gi 28703650	168	24	23	5.2
Actin-interacting protein 1	38	gi 6755995	234	25	43	-2.1
Cytokeratin 7	42	gi 14861854	197	19	30	2.1
<i>Immune response</i>						
Fizz1	39	gi 54611371	84	5	35	10.0
Ym2	40	gi 22123907	394	23	42	360
Ym2	41	gi 22123907	665	24	45	31.3
Ig κ chain variable region	45	gi 554065	79	3	33	3.7
Ig light chain variable region	46	gi 799373	80	6	36	2.3
Ig κ chain V-III region	47	gi 37196720	151	6	58	2.1
Ig κ chain V-III region	48	gi 37196720	80	4	32	2.5
Ig κ light chain variable region	50	gi 620021	90	6	50	2.5
Ig γ chain	51	gi 440121	85	9	22	16.2
Immunoglobulin heavy chain 4 protein (IgG1)	53	gi 23958196	120	8	20	7.7
Acidic mammalian chitinase	54	gi 37999744	295	14	49	2.4
Ym2	55	gi 22123907	158	18	46	71.5
Surfactant protein D	64	gi 6524992	215	14	37	2.1
Lungkine	59	gi 5031129	128	11	36	2.6
Lungkine	60	gi 5031129	130	12	34	2.3
Properdin factor B	65	gi 3986764	435	20	45	-3.8
<i>Cellular metabolism</i>						
Protein phosphatase 2, regulatory subunit A, isoform α	5	gi 8394027	221	31	40	-2.0
Serpinb6a protein	6	gi 34784412	216	15	29	2.3
Pyruvate carboxylase	7	gi 6679237	126	19	20	-2.2
Pyruvate carboxylase	8	gi 6679237	155	17	21	-2.3
Adenylate kinase 1	9	gi 10946936	257	15	51	2.0
Phosphohistidine phosphatase	10	gi 58037409	80	9	50	2.0
Glutathione peroxidase 3 precursor	11	gi 1170039	194	13	36	2.0
Superoxide dismutase 1	12	gi 45597447	469	12	44	-2.8
Ubiquitin-conjugating enzyme E2N	13	gi 18017605	80	5	33	-3.9
Carboxylesterase 3	14	gi 175124887	467	35	35	-2.0
Carboxylesterase 3	15	gi 175124887	128	25	30	-2.3
Proteasome 26S non-ATPase regulatory subunit 11	16	gi 33585718	215	24	40	-2.0
Proteasome subunit β type 9 precursor (LMP-2)	17	gi 17380240	305	17	43	2.1
Apolipoprotein A-I	20	gi 6753096	167	13	31	2.8
Ribosomal protein S12	23	gi 63741520	224	9	42	-2.0
Vesicle amine transport protein 1	24	gi 55715891	203	19	42	-2.6
Myotrophin	25	gi 6679961	196	6	32	-3.1
Ubiquitin-conjugating enzyme E2D 3	52	gi 13384718	75	4	36	-2.1
<i>Other acute phase protein</i>						
S100 calcium-binding protein A8	31	gi 7305453	133	8	49	-4.5
S100 calcium-binding protein A9	32	gi 6677837	242	14	41	-4.8
S100 calcium-binding protein A11	30	gi 21886811	140	8	57	-3.1
Transthyretin (prealbumin)	56	gi 56541070	263	10	39	-2.6
Pregnancy zone protein	57	gi 85861184	490	21	43	-3.3
Serum amyloid P component	58	gi 38174334	414	15	44	-2.9

Table 1 (continued)

Proteins	Spot No.	Accession No.	Score ¹	Matched peptides, n	Coverage ² %	Fold change
<i>Extracellular matrix</i>						
Procollagen, type VI, α 1	43	gi 6753484	178	15	23	2.0
Procollagen, type VI, α 1	44	gi 6753484	123	12	23	2.2
<i>Signal transduction</i>						
14-3-3 protein ϵ	1	gi 31981925	394	29	66	-2.1
14-3-3 protein ζ/δ	2	gi 76611326	77	6	25	-2.0
14-3-3 protein γ	3	gi 3065929	149	8	20	-2.0
14-3-3 protein β	4	gi 3065925	142	13	32	-2.3
<i>Transport</i>						
Cytochrome c oxidase, subunit Va	18	gi 6680986	219	8	28	-2.2
Ubiquinol-cytochrome c reductase core protein 1	19	gi 46593021	1,020	32	46	2.1
Transferrin	21	gi 20330802	147	23	24	4.4
Transferrin	22	gi 20330802	219	28	33	2.4
Ferritin light chain 1	61	gi 6753914	734	21	50	2.7
Hemopexin	62	gi 23956086	218	32	39	2.1
Gob-5 protein	49	gi 7513665	661	25	33	3.3
Gob-5 protein	66	gi 7513665	115	14	28	6.4
<i>Other calcium-binding proteins</i>						
Calpain, small subunit 1	27	gi 6753256	624	14	35	2.0
Sorcin, Sri protein	26	gi 13385076	385	15	40	2.0
Annexin A3	29	gi 7304887	745	24	52	2.1
Annexin A5	28	gi 1098603	513	33	71	2.3
Annexin A5	63	gi 1098603	430	23	42	2.5

¹ The score is $-10 \times \log(P)$, where P is the probability that the observed match is a random event. A protein score >75 is considered significant ($p < 0.05$).

² Coverage refers to peptide sequence homology of at least 20% to the matched protein.

tric points, which may be a result of post-translational modifications such as glycosylation and phosphorylation [22].

Discussion

Our results provide the first proteome map of chronically inflamed lungs in mice. The mouse chronic asthma model was confirmed by the development of airway remodeling such as thickening of peribronchial airway smooth muscle layer, goblet cell hyperplasia and increased subepithelial collagen and fibronectin deposition in the airways. Airway remodeling is a common feature of human asthma, and we hypothesized that proteome analysis of chronically inflamed mouse lungs may identify novel protein molecules that can be linked to chronic asthma. In this study, 100 protein spots have been

found to be differentially expressed in chronically inflamed mouse lungs. The identity of 66 protein spots was confirmed using MS/MS, and their potential biological connections with airway remodeling development are discussed.

A dramatic elevation in the protein level of members of the chitinase and Fizz family proteins including Ym2 (spots 40, 41 and 55), acidic mammalian chitinase (AMCase, spot No. 54) and found in inflammatory zone 1 (Fizz1, spot No. 39) was observed in mouse chronic lung inflammation. These proteins can be highly upregulated in alveolar macrophages and airway epithelium when stimulated with Th2 cytokines like IL-4 and IL-13 [23–25]. AMCase has recently been shown to be proinflammatory in a mouse acute asthma model via its chitinolytic activity [23]. Ym2, while lacking the catalytic domain, is structurally similar to AMCase and possesses a carbohydrate-binding site capable of recognizing glycos-

Table 2. Comparison of differentially expressed proteins identified by proteomics of lungs from chronic asthma versus acute asthma models

Protein	Acute asthma ¹	Chronic asthma
Acidic mammalian chitinase	↑	↑
Actin-interacting protein 1	n.c.	↓
Annexin A3	↓	↑
Annexin A5	n.c.	↑
Apolipoprotein A1	n.c.	↑
APR2/3 complex, subunit 5	n.c.	↑
Properdin factor B	n.c.	↓
Capping protein, muscle Z-line, β	n.c.	↑
Cytokeratin 7	n.c.	↑
Ferritin light chain 1	↑	↑
Fizz1	n.c.	↑
Gob-5	↑	↑
Hemopexin	↑	↑
Heptoglobin	↑ or ↓	n.c.
Ig light chain variable region	↑	↑
Ig γ chain	n.c.	↑
Ig heavy chain 4 (IgG1)	n.c.	↑
Lungkine	↑	↑
Lymphocyte cytosolic protein 1	n.c.	↑
Moesin	n.c.	↑
Myotrophin	n.c.	↓
Procollagen type VI α1	n.c.	↑
Pregnancy zone protein	n.c.	↓
Protein phosphatase 2A	n.c.	↓
S100 calcium-binding protein A8	↑	↓
S100 calcium-binding protein A9	n.c.	↓
S100 calcium-binding protein A11	n.c.	↓
Sorcin	n.c.	↑
Superoxide dismutase 1	n.c.	↓
Surfactant protein A	↓	n.c.
Surfactant protein B	↓	n.c.
Surfactant protein D	↑	↑
Transferrin	↑	↑
Transthyretin	↓ or ↑	↓
Ubiquitin-conjugating enzyme E2D	n.c.	↓
Ubiquitin-conjugating enzyme E2N	n.c.	↓
Vesicle amine transport protein 1	↑	↓
Ym1 (chitinase 3-like 3)	↑	n.c.
Ym2 (chitinase 3-like 4)	↑	↑
14-3-3 proteins	n.c.	↓

¹ Data obtained from the literature [6–11]. n.c. = No change.

aminoglycans on cell surface and heparin/heparan sulfate in the extracellular matrix. Since the Ym2 level was the most highly elevated among the 3 chitinase and Fizz family members, Ym2 appears to play a crucial role in effector immune cell recruitment and airway wall remod-

eling [23, 24]. In contrast to the proteomes of mouse acute asthma models previously reported showing upregulation of 3 chitinases, namely Ym1, Ym2 and AMCase [7, 8, 10], we were unable to observe any increase in Ym1 level in the mouse model of chronic asthma (table 2). Fizz1, also known as resistin-like molecule α or hypoxia-induced mitogenic factor, was found to be upregulated in B cells, macrophages and dendritic cells in the draining lymph nodes from a mouse pulmonary nematode infection model, indicating a potential role of Fizz1 in immunomodulation of airway inflammation [25]. Fizz1 may also contribute to airway remodeling by virtue of its pulmonary proliferative, angiogenic and antiapoptotic properties observed in a mouse hypoxia model [26] and its stimulatory effect on myofibroblast differentiation by inducing α-smooth muscle actin and type I collagen production in a rat lung fibrosis model [27].

Actin cytoskeleton is critical for cell morphology, locomotion and adhesion. Among the elevated actin-binding proteins identified in the chronically inflamed mouse lungs, moesin (spot No. 37) was the most elevated. It belongs to the ERM family of 3 highly homologous proteins: ezrin, radixin and moesin [28]. ERM proteins serve as linkers of plasma membrane proteins to actin cytoskeleton and induce F-actin reorganization essential for cell adhesion, migration and phagocytosis. Moesin is the predominant ERM isoform expressed in leukocytes and endothelium, indicative of its critical role in immune cell trafficking and inflammation. In addition, moesin has been shown to mediate tumor necrosis factor-α-induced pulmonary microvascular endothelial paracellular gap formation leading to plasma exudation and edema [29]. The rest of the identified actin-related proteins including lymphocyte cytosolic protein 1 (also known as L-plastin, spots 33 and 34), actin-related protein 2/3 complex (spot No. 35) and capZ (spot No. 36) were significantly increased by about 2-fold in lung lysate samples. They are essential for cell migration and adhesion probably by promoting and stabilizing actin polymerization [30–32]. In contrast, 2 regulators of cytoskeleton formation, actin-interacting protein 1 (spot No. 38) and protein phosphatase 2 (spot No. 5), were decreased in a mouse chronic asthma model. They bind and activate cofilin, which in turn binds to G- and F-actin and induces actin severing and depolymerization [31, 33]. Therefore, reduction in actin-interacting protein 1 and protein phosphatase 2 may further enhance actin filament formation and stabilization. The present study showed a new insight into the roles of cytoskeleton-related proteins in chronically inflamed lungs.

Another cytoskeleton protein that was increased in mouse chronic asthma is cytokeratin 7 (spot No. 42). The expression of cytokeratin 7 is restricted to cells with epithelial origin. Cytokeratin 7 can be a marker for the discriminating diagnosis of lung cancer [34]. The increase in cytokeratin 7 in chronically inflamed lungs may be indicative of the hyperplasia of mucus glands as demonstrated in our histological findings. Another protein elevated in chronically inflamed lungs that has been linked to mucus secretion is gob-5 (spots 49 and 66). Gob-5 has also been found to be upregulated in a mouse acute asthma model reported by our laboratory [7]. Gob-5 (alias mCLCA3), a member of the calcium-activated chloride channel family, has been shown to play a critical role in active export of chloride ions that leads to mucin secretion from airway epithelial cells such as goblet cells [35]. However, recent studies showed that gob-5 is a secreted nonintegral membrane protein, and therefore, is not an ion channel [36], and that gob-5 is not essential for mucus overproduction in a mouse asthma model [37]. The discrepancies in the role of gob-5 in asthma from these reports remain to be resolved. Nevertheless, it is now believed that gob-5 may play a role as a coiniciator or potentiator in regulating chloride channel activity for mucus production [36, 37].

The role of oxidative stress in the development of asthma has been well documented [38]. Superoxide dismutase (SOD) plays a critical role in protecting the lungs against oxidative damage. SOD exists in 3 isoforms: the cellular form (SOD1), the mitochondrial form (SOD2) and the extracellular form (SOD3). Studies revealed that decreased SOD1 activity in asthmatic airways resulted in airway epithelial apoptosis and hyperresponsiveness, whereas overexpression of SOD1 inhibited allergen-induced airway inflammation in a mouse asthma model [39, 40]. We have observed a major drop in SOD1 level in chronically inflamed lungs (spot No. 12), indicating an impaired antioxidative mechanism in chronic asthma.

Ca²⁺-binding proteins like S100A8 (calgranulin A) and S100A9 (calgranulin B) have been linked to leukocyte differentiation, inflammation and wound healing [41]. Elevated levels of S100A8, S100A9 and S100A11 have been observed in rheumatoid arthritis and cancers [41, 42]. In addition, BAL fluid levels of S100A8 and S100A9 were increased upon acute allergen challenge in asthmatics [43]. In a striking contrast, levels of S100A8, S100A9 and S100A11 (spots 30–32) were markedly reduced in chronically inflamed lungs. In chronic asthma, pulmonary transforming growth factor- β level is high and this cytokine plays a critical role in airway remodeling. A re-

cent report revealed that transforming growth factor- β significantly suppressed S100A8 expression in fibroblasts in vitro [44], which may explain the reduction in S100 proteins in our mouse chronic asthma model. Furthermore, Th2 cytokines like IL-4 and IL-13 have also been shown to suppress S100 protein expression [45]. Besides, S100A8 has been shown to possess an antioxidant function in inflammation [46]. The decrease in airway S100 protein levels implies further weakening of oxidative defense in chronic asthma.

The level of 4 isoforms (β , γ , ϵ , ζ/δ) of 14-3-3 proteins (spots 1–4) was reduced in our mouse chronic asthma model. 14-3-3 proteins can interact with a vast array of different proteins and play a role in diverse functions like cell cycle regulation, signaling transduction and apoptosis [47]. A recent study revealed that inactivation of 14-3-3 proteins exacerbated cardiac hypertrophy and fibrosis in diabetic mice [48]. The role of 14-3-3 in asthma pathogenesis is unknown. We speculate that reduction in 14-3-3 isoforms in chronically inflamed lungs may be linked to lung remodeling development such as smooth muscle cell and goblet cell hypertrophy and subepithelial fibrosis.

In conclusion, this is the first report of proteome analysis of chronic lung inflammation and remodeling in a mouse chronic asthma model. We have identified at least 100 differentially expressed protein spots in chronically inflamed lungs, and many of these proteins may play a role in the development of chronic asthma. Apart from some proteins like AMCase, Ym2 and gob-5 that were also detected in mouse acute asthma models, most identified proteins such as cytoskeleton-related proteins, Ca²⁺-binding proteins and other signaling molecules were only observed in the mouse chronic asthma model, which may be important for airway morphological changes (table 2). The present study provides new insight into the better understanding, and future studies, of the mechanisms and therapeutics of airway remodeling in asthma.

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