

CHEST[®]

Official publication of the American College of Chest Physicians



Proteomic Analysis of Peripheral T-Lymphocytes in Patients With Asthma

Hye Cheol Jeong, Sang Yeub Lee, Eun Joo Lee, Ki Hwan Jung, Eun Hae Kang, Sung Yong Lee, Je Hyeong Kim, Eun Kyung Park, Sang Hoon Lee, Chang Sub Uhm, Yunjung Cho, Chol Shin, Jae Jeong Shim, Han Kyeom Kim, Kwang Ho In, Kyung Ho Kang and Se Hwa Yoo

Chest 2007;132;489-496; Prepublished online June 5, 2007;
DOI 10.1378/chest.06-2980

The online version of this article, along with updated information and services can be found online on the World Wide Web at:
<http://chestjournal.org/cgi/content/abstract/132/2/489>

CHEST is the official journal of the American College of Chest Physicians. It has been published monthly since 1935. Copyright 2007 by the American College of Chest Physicians, 3300 Dundee Road, Northbrook IL 60062. All rights reserved. No part of this article or PDF may be reproduced or distributed without the prior written permission of the copyright holder
(<http://www.chestjournal.org/misc/reprints.shtml>). ISSN: 0012-3692.

A M E R I C A N C O L L E G E O F
 C H E S T
P H Y S I C I A N S[®]

Proteomic Analysis of Peripheral T-Lymphocytes in Patients With Asthma*

Hye Cheol Jeong, MD, PhD; Sang Yeub Lee, MD, PhD; Eun Joo Lee, MD; Ki Hwan Jung, MD; Eun Hae Kang, MD; Sung Yong Lee, MD, PhD; Je Hyeong Kim, MD, PhD; Eun Kyung Park, PhD; Sang Hoon Lee; Chang Sub Uhm, MD, PhD; Yunjung Cho, MD, PhD; Chol Shin, MD, PhD, FCCP; Jae Jeong Shim, MD, PhD; Han Kyeom Kim, MD, PhD; Kwang Ho In, MD, PhD; Kyung Ho Kang, MD, PhD, FCCP; and Se Hwa Yoo, MD, PhD

Background: Asthma is chronic airway inflammation that occurs together with reversible airway obstruction. T-lymphocytes play an important role in the pathogenesis of asthma. Proteomic technology has rapidly developed in the postgenomic era, and it is now widely accepted as a complementary technology to genetic profiling. We investigated the changes of proteins in T-lymphocytes of asthma patients by using standard proteome technology: two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and a database search.

Methods: The proteins of CD3+ T-lymphocytes were isolated from whole blood of six steroid-naive asthmatic patients and of six healthy volunteers. 2D-PAGE was performed and the silver-stained protein spots were comparatively analyzed between the asthma and control groups using an image analyzer. Some differentially expressed spots were identified by MALDI-TOF-MS and database search. The messenger RNA expressions of some identified proteins were examined by real-time polymerase chain reaction (RT-PCR).

Results: Thirteen protein spots in the T-lymphocytes of the asthmatic patients were increased and 12 spots were decreased compared to those of the normal subjects. Among the identified proteins, the increased expression of the messenger RNA of phosphodiesterase 4C and thioredoxin-2 and the decreased expression of the messenger RNA of glutathione S-transferase M3 were confirmed by RT-PCR in the asthmatic patients.

Conclusions: Proteomic examination of the peripheral T-lymphocytes revealed some differentially expressed proteins in the asthmatic patients. The possibility of using the differentially expressed proteins as important biomarkers and therapeutic targets in asthma patients warrants further studies. (CHEST 2007; 132:489–496)

Key words: asthma; proteomics; T-lymphocytes

Abbreviations: 2D-PAGE = two-dimensional polyacrylamide gel electrophoresis; EGFR = epidermal growth factor receptor; FDR = false discovery rate; GST = glutathione S transferase; GST-M3 = glutathione S transferase-M3; HSP-70 = heat shock protein 70 kD; MALDI-TOF-MS = matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PDE = phosphodiesterase; PDE4C = human phosphodiesterase 4C; PTP = protein tyrosine phosphatase; RT-PCR = real-time polymerase chain reaction; TPR = tetrapeptide repeat

Asthma is a complex genetic disorder that displays various phenotypes, and this is attributed to the interactions among many genes and environmental factors.¹ The major pathophysiologic findings of asthma are airway inflammation, goblet-cell hyperplasia with mucus hypersecretion, and hyperresponsiveness to various nonspecific stimuli.² T-lymphocytes

and especially the T-helper type 2 lymphocytes are known to play a central role in the inflammatory process of bronchial asthma.³

To understand the pathophysiologic mechanisms of disease, it is important to know which genes, gene transcripts, proteins, and metabolites are specifically expressed in the disease. Nair et al⁴ stressed the

importance of the protein synthesis from the expression because the proteins produced by genes are ultimately responsible for most biological functions. Especially, because proteins undergo many post-translational modifications that affect their structures and functions, their ultimate phenotypes often differ from the genetic information. Proteomics is a rapidly growing field after the completion of the human genome project.⁵ Proteomics is a complex process involving the purification and identification of individual protein from all the proteins expressed in cells or tissues. Protein separation by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and protein identification by mass spectrometry are standard approaches in proteomics. 2D-PAGE is a powerful research technique that separates proteins based on their charge and molecular weight. Simultaneous examination of hundreds of polypeptides in a single sample has become possible by performing 2D-PAGE. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a widely used technique for identifying proteins by measuring the time of flight after generating the ions of the proteins with a laser.⁵ Several proteome profiles of bronchial asthmatic lungs have been reported; however, most of them were from animal studies.^{6–9} The purpose of this study is to explore the differentially expressed proteins between the T-lymphocytes of normal human and those of the asthmatic patients with using 2D-PAGE and MALDI-TOF-MS.

MATERIALS AND METHODS

Study Subjects

Six patients with asthma were enrolled (three men and three women; mean age, 28 years). Asthma was diagnosed according to the criteria of the American Thoracic Society.¹⁰ All asthmatic patients were nonsmokers, and two of the asthmatic patients had atopy. None of the patients with asthma had complications from other lung disease, and they did not have a history suggesting systemic viral infections, tumors, or autoimmune diseases. Systemic steroid was not used in asthmatic patients during the previous 3 months.

Six healthy volunteers (two men and four women; mean age, 26 years) were enrolled from graduate students at the College of Medicine, Korea University, and served as control subjects. All control subjects were nonsmokers and had normal findings on chest radiography, normal airway reactivity, and normal pulmonary function test results; and they had no current respiratory symptoms, were nonatopic, and had no respiratory infections within the previous month. None were receiving any medications. Informed consents were obtained from each subject for their participation in the study, and the approval of the protocol was obtained by the Clinical Research Ethics Committee of Korea University Medical Center.

Processing of Samples

Twenty milliliters of blood were taken from each person and mixed with 20 mL of normal saline solution and 4 mL of density gradient medium (Ficoll-Paque, S.G. 1.077; Amersham Pharmacia Biotech; Upsala, Sweden). After centrifugation (1,200 revolutions per minute for 12 min without break), the mononucleated cell layer was separated and the CD3+ T-lymphocytes were independently purified from each blood sample by the methods of a T-cell-negative isolation procedure with using monoclonal antibody (Dyna; Dynal Biotech; Lake Success, NY). Twelve samples of T-lymphocytes from 12 persons were ready for the following processes.

2D-PAGE

CD3+ T-lymphocytes of each person were lysed with buffer (containing 7 mol/L urea/2 mol/L thiourea, 4% 3-[(3-cholamidopropyl)dimethylamino]-1-propanesulfonate, 40 mmol/L Tris hydrochloride, 100 mmol/L dithiothreitol, and 1 × protease inhibitor cocktail) [Roche Diagnostic Corporation; Indianapolis, IN]. The extracted protein concentration was determined by a modified Bradford protein assay (Bio-Rad; Richmond, CA).¹¹

Isoelectric focusing was performed on 17-cm immobilized pH gradient strips (Bio-Rad), with a pI 3–10 linear gradient, on a isoelectric focusing cell (IPGphor; Bio-Rad). After the proteins were mixed with rehydration solution (8 mmol/L urea, 2% 3-[(3-cholamidopropyl)dimethylamino]-1-propane sulfonate, 50 mmol/L dithiothreitol, 0.5% immobilized pH gradient buffer pH 3–10, and a trace of bromophenol blue), this was followed by focusing for 1 h at 200 V, 1 h at 500 V, 1 h at 1,000 V, 1 h at 8,000 V subsequently at 8,000 to 64,000 V/h. The pH gradient strip (Immobiline Drystrip; Amersham Pharmacia Biotech) after isoelectric focusing was equilibrated with buffer (6 mmol/L urea, 50 mmol/L Tris, 30% glycerol, 2% sodium dodecyl sulfate, 1% dithiothreitol, and a trace of bromophenol blue) for 20 min, and then applied onto a 10% polyacrylamide gel (10 to 12 cm). The second-dimension separation was performed sequentially (Ettan DALTsix; Amersham Pharmacia Biotech). The separated spots were visualized by the silver-based staining technique.¹² Six

*From the Division of Respiratory and Critical Care Medicine (Dr. Jeong), Department of Internal Medicine, College of Medicine, Pochon CHA University, Seongnam; Division of Respiratory and Critical Care Medicine (Drs. Sang Yeub Lee, E. J. Lee, Kang, In, and Yoo), Department of Internal Medicine, Anam Hospital, Korea University, Seoul; Division of Respiratory and Critical Care Medicine (Drs. Sung Yong Lee, Shim, and Kang), Department of Internal Medicine, Guro Hospital, Korea University, Seoul; Department of Internal Medicine (Drs. Jung, J. H. Kim, and Shin), Ansan Hospital, Korea University, Ansan; Department of Anatomy (Drs. Park, Uhm, and Mr. S. H. Lee), College of Medicine, Korea University, Seoul; Department of Laboratory Medicine (Dr. Cho), College of Medicine, Korea University, Seoul; and Department of Pathology (Dr. H. K. Kim), College of Medicine, Korea University, Seoul, Korea. The authors have no conflicts of interest exist to disclose. Manuscript received December 19, 2006; revision accepted April 26, 2007.

Reproduction of this article is prohibited without written permission from the American College of Chest Physicians (www.chestjournal.org/misc/reprints.shtml).

Correspondence to: Sang Yeub Lee, MD, PhD, Division of Respiratory and Critical Care Medicine, Department of Internal Medicine, College of Medicine, Korea University, 126-1, 5ga Anam Dong, Seongbuk gu, Seoul, 136-705, South Korea; e-mail: pulsy@korea.ac.kr

DOI: 10.1378/chest.06-2980

control samples and six asthma samples were analyzed individually in the 2D-PAGE technique.

The silver-stained two-dimensional gels were scanned on an image scanner (Powerlook 1100; UMAX; Taipai, Taiwan). Analyses of spot-intensity calibration, spot detection, background abstraction, and matching of approximately 12 2D-PAGEs were performed using software (ProteomWeaver; Definiens; Munich, Germany). All selected spots were present in all gels. The differentially expressed spots were analyzed with using Mann-Whitney *U* test (SPSS, version 10.0.05; SPSS; Chicago, IL). Correction for multiple comparisons was done according to the false discovery rate (FDR) method described by Benjamini et al¹³ using the FDRalgo software for Microsoft Windows made available to the public at www.math.tau.ac.il/~ybenja/ with a significance value of 0.05; *p* values larger than the FDR α value that was calculated using this software were considered statistically nonsignificant.

Identification of Protein Spots

Spots of interest were analyzed (Voyager 4307 MALDI-TOF-MS; Applied Biosystems; Foster City, CA). The proteins were identified with peptide mass fingerprinting data using a search program (MS-FIT; available at: <http://prospector.ucsf.edu/ucsfhtml3.4/msfit.html>).

Real-time Polymerase Chain Reaction

We performed real-time polymerase chain reaction (RT-PCR) for validation study about some identified proteins with using a Miniprep method (Qiagen; Valencia, CA). Primers were designed from the sequence of human phosphodiesterase 4C (PDE4C), glutathione S transferase-M3 (GST-M3), and thioredoxin-2 complementary DNAs that were published in Gene Bank. The nucleotide sequences of primers were as following (PDE4C, 5'AGAGTGGTACCAGAGCAAGA3'; GST-M3, 5'TCTATGGT-TCTCGGGTACTG3'; thioredoxin-2, 5'AGCTCTGTTCCAC-TTTTCT3').

RESULTS

2D-PAGE

More than 300 spots were identified in the 2D-PAGE gels from the T-lymphocytes of the normal and asthmatic patients. The general distribution pattern of the spots in the silver-stained gels was similar in both groups (Fig 1, *top left, A*, and *top right, B*). The spots in the area of pI 4 to 7, and molecular weight of 20 to 100 kd were analyzed by an image analysis program (ProteomWeaver). Protein spots of the normal and asthma groups were compared, and 25 proteins showed different intensity, suggesting the differential expression. Among them, the intensities of 13 spots were significantly increased and the intensities of 12 spots were decreased in the asthma group compared to the control group. The 25 selected spots of the 2D-PAGE of an asthmatic, nonsmoking, 27-year-old man are shown in Figure 1, *bottom, C*.

Identification of the Differentially Expressed Proteins by MALDI-TOF-MS

After destaining, extraction, and lysis with trypsin, the individual spots were identified by MALDI-TOF-MS and this was followed by a database search (MS-FIT). On the list of 20 candidate proteins for each spot, the final protein was determined by comprehensively considering the corresponding experimental isoelectric point, the molecular masses, the number of matched peptides, and the sequence coverage. Thirteen up-regulated and 12 down-regulated proteins in the asthmatic group were identified (Table 1). Testing for multiple comparisons by FDR analysis resulted in an FDR threshold (α) value of 0.0396468.

PDE4C, heat shock protein 70 kd (HSP-70), protein tyrosine phosphatase (PTP), β -arrestin-1b, thioredoxin-like 2, LLR protein, glutathione reductase, major histocompatibility complex class I antigen, epidermal growth factor receptor (EGFR), pyrroline 5-carboxylate reductase isoform, and FLJ25770 protein were increased in the T-lymphocytes of asthmatic patients. Dynein, cytoplasmic light intermediate polypeptide 2, vimentin, zinc finger protein 76, tubulin β_2 , T-cell receptor β -chain, cyclin-dependent kinase 6, pyridoxal kinase, phenylalanine hydroxylase, GST-M3, and tetratricopeptide repeat (TPR)-containing protein were decreased in the T-lymphocytes of the asthmatic patients.

RT-PCR

We performed RT-PCR using PDE4C-, thioredoxin-2-, and GST-M3-specific primers. The results confirmed that the messenger RNAs of PDE4C and thioredoxin-2 were expressed at very low levels in the blood of normal subjects, and they were present at considerably higher levels in the blood of asthmatic patients. The messenger RNA level of GST-M3 in the asthmatic patients was obviously less than that in normal subjects (Fig 2). These results substantiate the specific up-regulation of PDE4C and thioredoxin-2 and the down-regulation of GST-M3 in the blood of asthmatic patients compared with those of the normal subjects.

DISCUSSION

This is the first proteomic approach using the human T-lymphocytes of blood in asthmatic patients. Although the proteomic data of the T-lymphocytes of normal human has been reported, no study on T-lymphocytes in asthmatic patients has been reported.¹⁴

It is known that the asthmatic process that triggers the immune system can lead to excessive release of

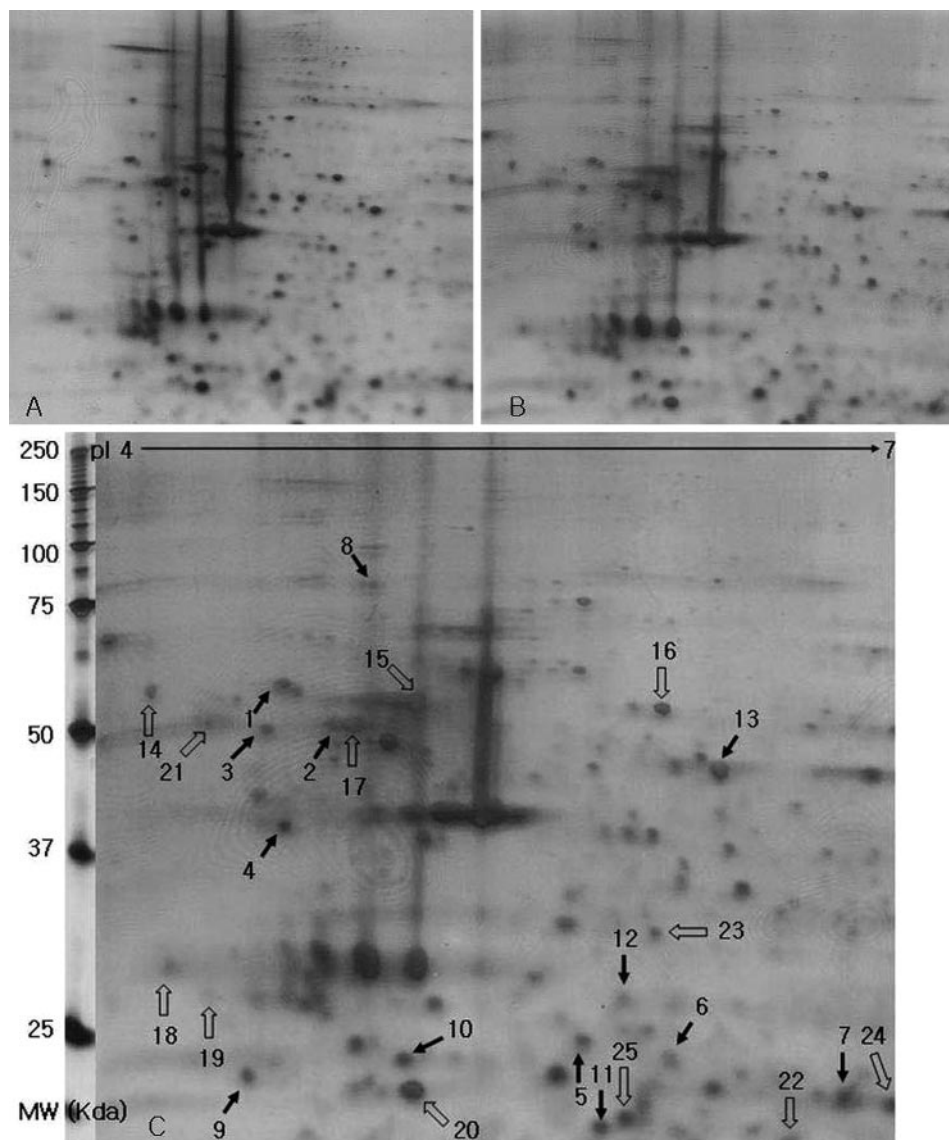


FIGURE 1. Representative 2D-PAGE gels of the peripheral T-lymphocytes of normal patients (*top left, A*) and asthmatic patients (*top right, B*). Each image shows a similar expression pattern of spots between *top left, A*, and *top right, B*. Twenty-five selected and identified spots are shown by the numbered arrows in a 2D-PAGE gel (*bottom, C*) of an asthmatic, nonsmoking, 27-year-old man (solid arrows indicate increased spots of proteins; empty arrows indicate decreased spots of proteins).

various cytokines and inflammatory mediators, which are produced by T-cells, infiltrated mononuclear cells, eosinophils, and local mast cells into the lung. Among the inflammatory cells, T-lymphocytes play major roles in the pathogenesis of bronchial asthma.^{3,15} So, we performed proteomic analysis of the peripheral T-lymphocytes of asthmatic patients.

In this study, we identified several proteins that were previously unknown or known to be associated with the pathogenesis of asthma. The function of phosphodiesterase (PDE) is to hydrolyze cyclic adenosine monophosphate and/or cyclic guanosine monophosphate. Crocker et al¹⁶ demonstrated that the PDE activity in

CD4+ T cells from asthmatic patients was elevated over that in the cells from nonatopic controls. *In vivo* and *in vitro* studies¹⁷ have established that selective PDE₄ inhibitors suppress the activity of many proinflammatory and immune cells. Additionally, it was reported that PDE₄ inhibition decreased the MUC5AC expression induced by EGFR in cultured human airway epithelial cells and in human isolated bronchus.¹⁸ Our study showed the result that the proteomic expressions of EGFR and PDE₄ were slightly increased in T-lymphocytes of asthmatic patients compared to healthy persons.

Oxidative stress occurs in many allergic and im-

Table 1—Identification of the Proteins With Altered Expression Levels in the Human T-Lymphocytes of Asthma Patients Relative to Normal Persons*

No.	Protein Name	Expression	MS-FIT Database Access No.	Cov, %	T Mass, kd/pI	O Mass, kd/pI	Fold Difference	p Value
1	HSP-70	↑	33112639	16	74/6.3	62/4.6	2.1	0.008
2	PDE4C	↑	19684163	7	67/4.7	56/4.8	1.3	0.015
3	PTP	↑	44890587	19	58/6.3	55/4.5	2.6	0.013
4	β-Arrestin-1b	↑	3514099	18	46/5.8	43/4.6	2.3	0.028
5	Thioredoxin-like 2	↑	11066205	19	36/4.8	30/5.8	1.4	0.001
6	Pyroline 5-carboxylate reductase isoform	↑	4960118	24	33/5.7	28/6.0	1.4	0.046
7	Unnamed protein product	↑	35745	15	48/8.2	25/6.6	1.5	0.031
8	Unnamed protein product	↑	7020470	45	26/6.0	75/5.0	1.3	0.022
9	LLR protein	↑	37181346	20	28/6.4	28/4.5	1.3	0.018
10	Glutathione reductase	↑	31829	47	25/6.1	29/5.2	1.6	0.014
11	FLJ25770 protein	↑	23271807	11	25/5.4	20/5.7	2.0	0.009
12	Major histocompatibility class I antigen	↑	27729531	46	31/5.6	33/5.9	1.5	0.023
13	EGFR	↑	51234111	11	70/6.8	50/6.5	1.2	0.006
14	Dynein, cytoplasmic, light intermediate polypeptide 2	↓	19684163	21	54/5.9	59/4.2	5.1	0.010
15	Vimentin	↓	44890587	33	53/5.1	60/5.2	2.4	0.014
16	Zinc finger protein 76	↓	30583471	15	61/5.7	58/6.2	1.5	0.046
17	Tubulin β-2	↓	20809886	22	49/4.8	56/4.9	2.8	0.019
18	T-cell receptor β-chain	↓	3089433	27	34/6.2	32/4.2	2.8	0.038
19	Pyridoxal kinase	↓	32186842	35	26/6.1	29/4.3	3.0	0.018
20	Pyridoxal kinase	↓	32186842	29	26/6.1	27/5.3	1.3	0.012
21	Phenylalanine hydroxylase	↓	20070771	13	51/6.3	56/4.4	1.4	0.041
22	Unnamed protein product	↓	7023347	29	16/6.9	17/6.5	2.5	0.013
23	Cyclin-dependent kinase 6	↓	51094901	23	36/6.0	35/5.9	1.5	0.022
24	GST-M3	↓	14250650	35	26/5.4	24/6.8	2.2	0.001
25	Tetratricopeptide repeat-containing protein	↓	20378333	35	20/5.0	18/5.8	1.5	0.010

*Cov = coverage; T Mass = theoretic mass; O Mass = observed mass; pI = isoelectric point.

munologic disorders. Asthma is also associated with reduced antioxidant defenses,^{19,20} and glutathione is one of the most important antioxidants. Glutathione reductase is a cofactor for generating reduced glutathione. In our study, glutathione reductase was increased and GST-M3 was decreased in the asthmatic patients. Glutathione S transferases (GSTs) are components of the phase II xenobiotic defense pathway that utilizes the lipid and DNA products of oxidative stress.²¹ Several reports^{22,23} have shown that the reduced antioxidant capacity of GST is related to the increased risk of allergic asthma. Polymorphism at the GST genetic locus is associated with the expression of bronchial hyperresponsiveness and atopic phenotypes in asthma.^{24–26}

Thioredoxin was slightly increased in the asthmatic group in this study. Thioredoxin is known to possess antioxidant activity that regulates redox-sensitive molecules such as nuclear factor-κB and glucocorticoid receptors.^{27,28} Yamada et al²⁹ first reported that the serum levels of thioredoxin were positively correlated with the severity of asthma.

Our study showed the increase of the HSP-70 in the asthma group. Heat shock protein protects the cells and tissues from the deleterious effects of

numerous mediators, reactive oxygen species, or tumor necrosis factor-α. Several studies^{30–32} have suggested that heat shock protein is correlated with the severity of asthma exacerbation.

In our study, the decreased expression of TPR-containing protein was observed in the asthma patients. The TPR domain consists of a 34-amino-acid motif, and it has many cellular functions such as mitosis, transcription, protein transport, and development.³³ A previous report³⁴ showed that some proteins with the TPR domain negatively regulated adenosine triphosphatase and HSP-70, but any detailed association with asthma has not yet been elucidated.

Several cytoskeletal proteins were decreased in the T-lymphocytes of asthma patients. Dynein is a microtubule-dependent motor protein. Vimentin attaches to the nucleus, endoplasmic reticulum, and mitochondria.³⁵ Tubulin β₂, a subunit of microtubules, integrates ciliary motion with the cellular functions via the cytoskeleton.³⁶ These cytoskeletal changes may illustrate the functional changes in the T-lymphocytes of asthma patient.

Some proteins related to the cell cycle were also observed in our study. PTPs work antagonistically with protein tyrosine kinases to regulate signal transduction

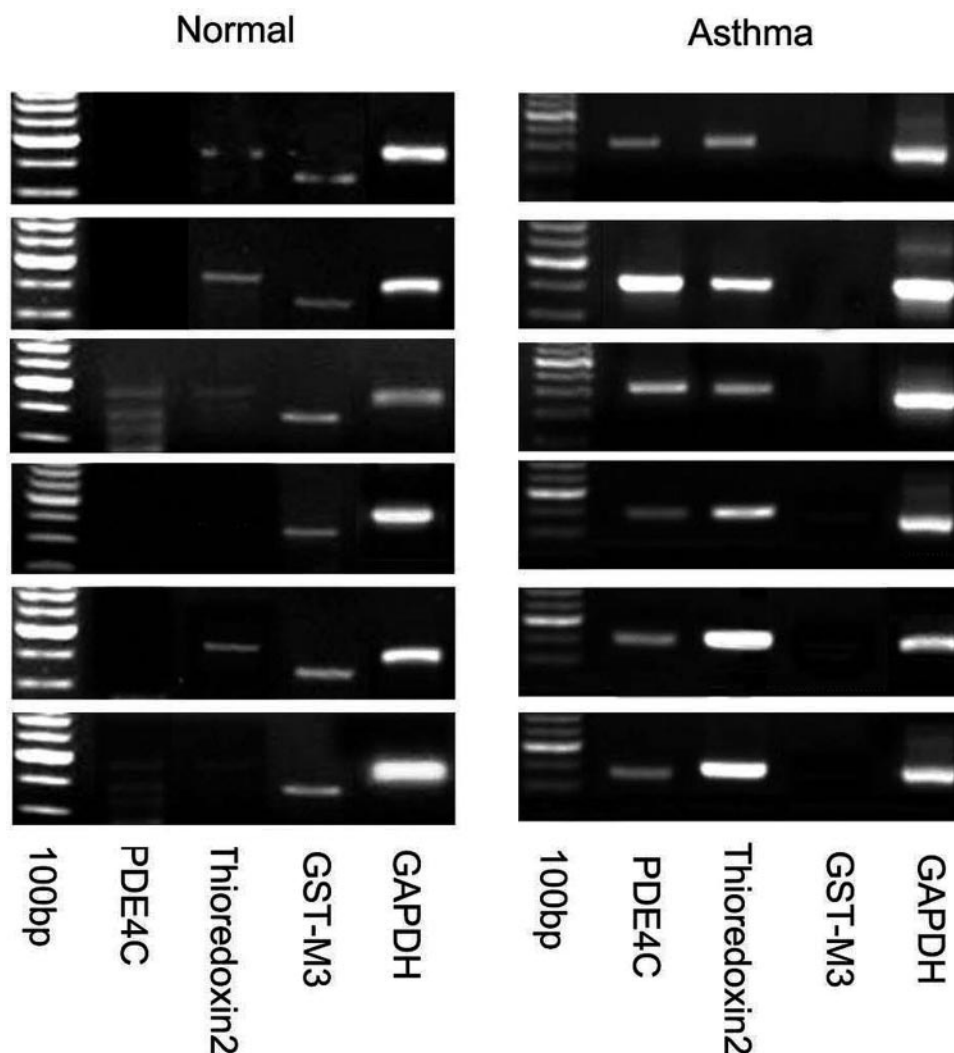


FIGURE 2. RT-PCR analysis of the messenger RNA expressions of the differentially expressed PDE4C protein, thioredoxin-2, and GST-M3 protein. RT-PCR of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control for RNA variation. 100bp = 100 base pair.

in a cell. Protein tyrosine kinases phosphorylate the tyrosine residues on a substrate protein, and PTPs remove these phosphates from substrate tyrosines (dephosphorylation). Kamata et al³⁷ reported that Src homology 2 domain-containing tyrosine phosphatase regulates the development of allergic airway inflammation. β -Arrestins are cytosolic proteins that mediate homologous desensitization of the G protein-coupled receptors by binding to agonist-occupied receptors and by uncoupling them from heterotrimeric G proteins. It was suggested in an animal study³⁸ that β -arrestin 2 was associated with T-cell migration and the development of asthma. Several previous studies^{39–41} have shown that T-cells expressing different β receptors may play different roles in regulating the airway inflammation in asthma. Cyclin-dependent kinase is also involved in the regulation of transcription and processing of messenger

RNA.⁴² Increased cyclin-dependent kinase inhibitors were discovered in the bronchial epithelium of asthmatic patients.^{43,44} The above-mentioned proteins show that an abnormal repair process may contribute to airway inflammation and remodeling during damage to the epithelium. Changes of several proteins (*ie*, pyridoxal kinase, phenylalanine hydroxylase, zinc finger protein, pyrroline-5-carboxylate reductase) were also observed in this study, and the relation of these proteins with the asthma is not yet clear.

Several studies^{45–47} have shown polymorphism of the human leukocyte antigen I genes in the asthma population. However, one study⁴⁸ showed that no definitive human leukocyte antigen association could be established with atopic or nonatopic asthma.

Our study may have some limitations. First, it is known that depending on the different physiologic

conditions, such as age, gender, fasting and feeding, changes in diet, physical activity, medications, pregnancy and the disease status, 2D-PAGE can show different panels.⁴ So, we selected nonsmokers and relatively young-aged persons. Second, we discovered changes of the proteins associated with the cell cycle, inflammation, or oxidative stress. Whether the changes of such proteins are pathognomonic markers of asthma or of a reactive phenomenon is questionable. Finally, posttranslational modifications might lead to a shift of the protein spot in the electrophoresis, which could have an influence on both spot identification and spot intensity.

Despite of the above limitations, our study is a meaningful first study on the proteomic changes of T-lymphocytes of asthmatic patients. It would be interesting and informative to observe the changes of the proteome profiles after steroid treatment. Studies on other inflammatory cells in asthma patients would also be necessary to understand the overall picture of proteome behavior in asthma patients. We suggest that some of the proteins, whose expressions are differentially regulated in the T-lymphocytes of asthmatic patients, may serve as important biomarkers and therapeutic targets of asthma.

In conclusion, proteomic analysis of the peripheral T-lymphocytes of asthma patients revealed some differentially expressed proteins compared with those of normal subjects. The possibility of using the differentially expressed proteins as important biomarkers and therapeutic targets in asthma patients warrants further studies.

REFERENCES

- Busse WW, Lemanske RF Jr. Asthma. *N Engl J Med* 2001; 344:350–362
- Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991; 87:893–910
- Kon OM, Kay AB. T cells and chronic asthma. *Int Arch Allergy Immunol* 1999; 118:133–135
- Nair KS, Jaleel A, Asmann YW, et al. Proteomic research: potential opportunities for clinical and physiological investigators. *Am J Physiol Endocrinol Metab* 2004; 286:863–874
- Zhu H, Bilgin M, Snyder M. Proteomics. *Annu Rev Biochem* 2003; 72:783–812
- Houtman R, Krijgsveld J, Kool M, et al. Lung proteome alterations in a mouse model for nonallergic asthma. *Proteomics* 2003; 3:2008–2018
- Roh GS, Shin Y, Seo SW, et al. Proteome analysis of differential protein expression in allergen-induced asthmatic mice lung after dexamethasone treatment. *Proteomics* 2004; 4:3318–3327
- Fehniger TE, Sato-Folatre JG, Malmstrom J, et al. Exploring the context of the lung proteome within the airway mucosa following allergen challenge. *J Proteome Res* 2004; 3:307–320
- Fajardo I, Svensson L, Bucht A, et al. Increased levels of hypoxia-sensitive proteins in allergic airway inflammation. *Am J Respir Crit Care Med* 2004; 170:477–483
- American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 136:225–244
- Stoscheck CM. Quantitation of protein. *Methods Enzymol* 1990; 182:50–69
- O'Connell KL, Stults JT. Identification of mouse liver proteins on two-dimensional electrophoresis gels by matrix-assisted laser desorption/ionization mass spectrometry of *in situ* enzymatic digests. *Electrophoresis* 1997; 18:349–359
- Benjamini Y, Drai D, Elmer G, et al. Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* 2001; 125:279–284
- Nyman TA, Rosengren A, Syrakki S, et al. A proteome database of human primary T helper cells. *Electrophoresis* 2001; 22:4375–4382
- Yssel H, Groux H. Characterization of T cell subpopulations involved in the pathogenesis of asthma and allergic diseases. *Int Arch Allergy Immunol* 2000; 121:10–18
- Crocker IC, Church MK, Ohia SE, et al. Beclomethasone decreases elevations in phosphodiesterase activity in human T lymphocytes. *Int Arch Allergy Immunol* 2000; 121:151–160
- Lipworth BJ. Phosphodiesterase-4 inhibitors for asthma and chronic obstructive pulmonary disease. *Lancet* 2005; 365:167–175
- Mata M, Sarria B, Buenestado A, et al. Phosphodiesterase 4 inhibition decreases MUC5AC expression induced by epidermal growth factor in human airway epithelial cells. *Thorax* 2005; 60:144–152
- Bowler RP, Crapo JD. Oxidative stress in allergic respiratory diseases. *J Allergy Clin Immunol* 2002; 110:349–356
- Bucchieri F, Puddicombe SM, Lordan JL, et al. Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. *Am J Respir Cell Mol Biol* 2002; 27:179–185
- Rushmore TH, Pickett CB. Glutathione S-transferases, structure, regulation, and therapeutic implications. *J Biol Chem* 1993; 268:11475–11478
- Kabesch M, Hoeffler C, Carr D, et al. Glutathione S transferase deficiency and passive smoking increase childhood asthma. *Thorax* 2004; 59:569–573
- Greene LS. Asthma and oxidant stress: nutritional, environmental, and genetic risk factors. *J Am Coll Nutr* 1995; 14:317–324
- Ivaschenko TE, Sideleva OG, Baranov VS. Glutathione-S-transferase micro and theta gene polymorphisms as new risk factors of atopic bronchial asthma. *J Mol Med* 2002; 80:39–43
- Sheehan D, Meade G, Foley VM, et al. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. *Biochem J* 2001; 360:1–16
- Tamer L, Calikoglu M, Ates NA, et al. Glutathione-S-transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for asthma. *Respirology* 2004; 9:493–498
- Laurent TC, Moore EC, Reichard P. Enzymatic synthesis of deoxyribonucleotides. *J Biol Chem* 1964; 239:3436–3444
- Nakamura H, Nakamura K, Yodoi J. Redox regulation of cellular activation. *Annu Rev Immunol* 1997; 15:351–369
- Yamada Y, Nakamura H, Adachi T, et al. Elevated serum levels of thioredoxin in patients with acute exacerbation of asthma. *Immunol Lett* 2003; 86:199–205
- Aron Y, Busson M, Polla BS, et al. Analysis of hsp70 gene polymorphism in allergic asthma. *Allergy* 1999; 54:165–170
- Tong W, Luo W. Heat shock proteins' mRNA expression in asthma. *Respirology* 2000; 5:227–230
- Bertorelli G, Bocchino V, Zhuo X, et al. Heat shock protein 70 upregulated to HLA-DR expression in bronchial asthma: effects of inhaled glucocorticoids. *Clin Exp Allergy* 1998; 28:551–560

- 33 Lamb JR, Tugendreich S, Hieter P. Tetratrico peptide repeat interactions: to TPR or not to TPR? *Trends Biochem Sci* 1995; 20:257–259
- 34 Ballinger CA, Connell P, Wu Y, et al. Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Mol Cell Biol* 1999; 19:4535–4545
- 35 Katsumoto T, Mitsushima A, Kurimura T. *Biol Cell* 1990; 68:139–146
- 36 Wheatley DN, Wang AM, Strugnell GE. Expression of primary cilia in mammalian cells. *Cell Biol Int* 1996; 20:73–81
- 37 Kamata T, Yamashita M, Kimura M, et al. Src homology 2 domain-containing tyrosine phosphatase SHP-1 controls the development of allergic airway inflammation. *J Clin Invest* 2003; 111:109–119
- 38 Walker JK, Fong AM, Lawson BL, et al. β -Arrestin-2 regulates the development of allergic asthma. *J Clin Invest* 2003; 112:566–574
- 39 Bakakos P, Pickard C, Smith JL, et al. TCR usage and cytokine expression in peripheral blood and BAL T cells. *Clin Exp Immunol* 2002; 128:295–301
- 40 Wahlstrom J, Gigliotti D, Roquet A, et al. T cell receptor V β expression in patients with allergic asthma before and after repeated low-dose allergen inhalation. *Clin Immunol* 2001; 100:31–39
- 41 Raulf-Heimsoth M, Merget R, Rihs HP, et al. T-cell receptor repertoire expression in workers with occupational asthma due to platinum salt. *Eur Respir J* 2000; 16:871–878
- 42 Loyer P, Trembley JH, Katona R, et al. Role of CDK/cyclin complexes in transcription and RNA splicing. *Cellular signaling* 2005; 17:1033–1051
- 43 Puddicombe SM, Torres-Lozano C, Richter A, et al. Increased expression of p21^{waf} cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. *Am J Respir Cell Mol Biol* 2003; 28:61–68
- 44 Fedorov IA, Wilson SJ, Davies DE, et al. Epithelial stress and structural remodelling in childhood asthma. *Thorax* 2005; 60:389–394
- 45 Apostolakis J, Toumbis M, Konstantopoulos K, et al. HLA antigens and asthma in Greeks. *Respir Med* 1996; 90:201–204
- 46 Wang WX, Yang SZ, Chui XW, et al. Association of HLA-Bw61 with asthma in the Chinese. *Tissue Antigens* 1988; 32:215–217
- 47 Hsieh KH, Shieh CC, Shieh RP, et al. Association of HLA-DQw2 with Chinese childhood asthma. *Tissue Antigens* 1991; 38:181–182
- 48 Torio A, Sánchez-Guerrero I, Muro M, et al. Analysis of the phenotypic distribution of HLA class I and class II in atopic and non-atopic asthma patients. *Eur J Immunogenet* 2000; 27:91–95

Proteomic Analysis of Peripheral T-Lymphocytes in Patients With Asthma

Hye Cheol Jeong, Sang Yeub Lee, Eun Joo Lee, Ki Hwan Jung, Eun Hae Kang, Sung Yong Lee, Je Hyeong Kim, Eun Kyung Park, Sang Hoon Lee, Chang Sub Uhm, Yujung Cho, Chol Shin, Jae Jeong Shim, Han Kyeom

Kim, Kwang Ho In, Kyung Ho Kang and Se Hwa Yoo

Chest 2007;132;489-496; Prepublished online June 5, 2007;

DOI 10.1378/chest.06-2980

This information is current as of October 28, 2007

Updated Information & Services	Updated information and services, including high-resolution figures, can be found at: http://chestjournal.org/cgi/content/full/132/2/489
References	This article cites 48 articles, 14 of which you can access for free at: http://chestjournal.org/cgi/content/full/132/2/489#BIBL
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://chestjournal.org/misc/reprints.shtml
Reprints	Information about ordering reprints can be found online: http://chestjournal.org/misc/reprints.shtml
Email alerting service	Receive free email alerts when new articles cite this article sign up in the box at the top right corner of the online article.
Images in PowerPoint format	Figures that appear in CHEST articles can be downloaded for teaching purposes in PowerPoint slide format. See any online article figure for directions.

