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Glutathione S - transferases in asthma: GSTP and GSTA may protect airway from oxidative stress in asthmatics

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손 성 욱

Abstract

Glutathione S-transferases in asthma: GSTP and GSTA may protect airway from oxidative stress in asthmatics

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Asthma is characterized by persistent airway inflammation. Reactive oxygen species (ROS) are known aggravating factors for airway inflammation in asthma. Glutathione S-transferases (GSTs) detoxify ROS and toxic compounds in environmental exposures such as tobacco smoke, and air pollution via glutathione-dependent

mechanisms. However little is known about the regulation of GST and expression of GST subtypes in asthma. The aim of this study was to evaluate how GSTs are regulated in asthma and to know their role in oxidative stress. We observed total GST activity and expression of GST subtypes in murine asthma models and GST expressions in induced sputum cells of asthmatics. Total GST activity was increased in BAL fluids of OVA - treated murine asthma model. GSTP and GSTA are highly expressed in peribronchiolar mononuclear inflammatory cells and epithelial cells in OVA - treated mice. However they are mainly expressed in epithelial cells in PBS - treated mice. GSTM are expressed in epithelial cells in both OVA and PBS - treated groups. GSTP1 mRNA expression was increased in the lung of OVA - treated mice compared with PBS - treated mice. GSTA1, GSTM1, and GSTT1 mRNA expressions were not different between both groups. However the protein levels of GSTs were low and not different in the lung between OVA - treated mice and PBS - treated mice. GSTA1 mRNA expression was increased in induced sputum cells of asthmatics compared with healthy controls. GSTA1, GSTM1, and GSTT1 mRNA expressions were not different between asthmatics and healthy controls. In asthmatics, GSTP1 and GSTA1 mRNA expressions were higher in induced sputum cells of asthmatics with $PC_{20} \leq 4$ mg/ml than those

with PC_{20} >4 mg/ml. GSTM1 and GSTT1 mRNA expressions were not different between two groups. These findings suggest that GSTs are upregulated in the airways of asthmatics in response to increased oxidative stress. GSTP and GSTA are thought to play an important role in protecting the airways of asthmatics compared with GSTM and GSTT.

Key words: asthma, Glutathione S - transferase, oxidative stress, reactive oxygen species

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INTRODUCTION

Asthma is a complex disorder characterized by airway hyperresponsiveness, obstructive change in pulmonary function, and persistent airway inflammation.[1] Asthmatic airways are exposed to oxidative stress induced not only by airway inflammatory cells, which includes alveolar macrophages, eosinophils, neutrophils, lymphocytes, and epithelial cells lining the airways, but also from environmental exposure to air pollution and cigarette smoke.[2] These inflammatory cells and environmental exposure have the capacity to generate reactive oxygen species (ROS).[3-6] ROS can reproduce the pathophysiologic features of asthma of airway smooth muscle contraction, increased airway reactivity and secretions, increased vascular permeability, and increased synthesis of chemoattractants.[4]

The antioxidant defenses within the airway are endogenous antioxidants, which are either enzymatic or nonenzymatic.[2] The enzymatic antioxidants include glutathione S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, and thioredoxin. The nonenzymatic antioxidants include low-molecular-weight compounds, such as glutathione (GSH), ascorbate, urate, bilirubin, α-tocopherol, and lipoic acid.

Concentrations of these antioxidants vary depending on both subcellular and anatomic location. The antioxidant defenses within the airway reside in the fluids lining the lungs, which are rich in a range of enzymatic and low-molecular-weight non-enzymatic antioxidants.[7]

GSTs comprise a family of phase II enzymes that catalyze the conjugation of reduced glutathione (GSH) through a sulfhydryl group to electrophilic sites on a wide variety of substrates found in air pollution, cigarette smoke, and mold.[8-10] The products of GST catalysis are more water soluble, promoting ROS detoxification and thereby protecting tissues from oxidative damage.[11,12] In humans, GSTs are divided into 8 families: alpha, kappa, mu, omega, pi, sigma, theta, and zeta.[12,13] Most GSTs exist as soluble dimeric proteins with subunit molecular weights of approximately 25 kDa in the cytosol except GST kappa, which is mitochondrial GST. Identity of subunits is based principally on amino acid sequence homology.[12] In general, members of the same class share more than 40% sequence identity but less than about 25% sequence identity with GSTs in other classes.[12,13] Several studies showed that GST polymorphisms are associated with some inflammatory diseases, including asthma. *GSTP1* Val105 allele has been reported to have significantly lower

GST enzyme activity.[14] The exposure to diesel exhaust particles, environmental tobacco smoke, ozone and mold each conferred an increased risk for wheezing in children who were carriers of the Val105 allele.[15-17]

Despite compelling genetic evidence supporting a strong role for GST in asthma, very little is known about the regulation of GST in asthma. Many of the cytosolic/soluble GST are expressed in liver and the function of GST has been mainly investigated in liver tissue.[17] The information about tissue-specific expressions of GST is relatively scant and the roles of GSTs against ROS in the airway of asthma are poorly understood.

The aim of this study was to investigate how GST expression and total GST activity are regulated in the airways of asthmatics and to determine the effect of GST on redox homeostasis in murine models of asthma.

Methods

OVA murine models

Wild-type BALB/c and C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, Me, USA). Six-week-old WT mice were sensitized with 75 μg of ovalbumin (OVA) and alum on days 0 and 7intraperitoneally, and challenged 4 times intranasally with 50 μg of OVA on days 14, 15, 21, and 22. Pulmonary function testing was assessed in conscious, unrestrained mice by using noninvasive whole-body plethysmography (Allmedicus, Seoul, Korea) on day 23, as previously described.[18] Bronchoalveolar lavage (BAL) fluid was harvested on day 23. Lung tissues were harvested on days 23, 24, and 2 weeks after the last challenge.

Total GST activity in murine BAL fluids

Total GST activity was assessed by using the GST assay kit (abcam, Cambridge, MA, USA) as described in manufacturer's protocol. Briefly, two microliters of BAL fluids were prepared in a total 50 μl with GST sample buffer, including a negative control with 50 μl of GST sample buffer only and a positive control (10 μl of GST positive control diluted 1:50) and 40 μl of GST assay buffer. Five microliters of glutathione was added to each well containing

the sample or control. For each well, a total 50 μl substrate reagents containing GST assay buffer 45 μl and GST substrate (CDNB) 5 μl were mixed. The absorbance was read once every minute at 380 nm using a plate reader to obtain at least 5 time points.

Western analysis

Lung lysates were prepared and Western analysis was undertaken with anti-GSTP antibodies, anti-GSTA antibodies, and anti-GSTM antibodies (abcam, Cambridge, MA, USA) as previously described.[19]

GST immunohistochemistry

For histologic analysis, murine lung were fixed in phosphate-buffered formalin, dehydrated in alcohol and embedded in paraffin. Slide-mounted paraffin sections were deparaffinized and rehydrated, and antigen retrieval was performed with high-pH target retrieval (DAKO, Glostrup, Denmark). After hydrogen peroxide inactivation and serum blocking, slides were incubated at 4°C for 18 hours with a 1:500 dilution of anti-GSTP antibodies (Enzo life Sciences, Ann Arbor, MI, USA), anti-GSTM antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-GSTA

antibodies (abcam, Cambridge, MA, USA). Sections were washed and incubated with biotinylated secondary antibody. Slides were developed with a peroxidase-labeled avidin detection system (Vector Laboratories, Burlingame, CA, USA).

Subjects

Sixty two patients attending the outpatient allergy clinic of Seoul National University Hospital were enrolled. Asthmatic patients (n=31) with mild or moderate persistent symptoms received inhaled corticosteroid and/or intermittent inhaled bronchodilator. Asthma was defined according to the criteria suggested by the American Thoracic Society (ATS).[20] The methacholine bronchial provocation test was performed as previously described.[21] They had a provocative concentration of methacholine causing a 20% fall in forced expiratory volume in one second (PC_{20}) with a dose of less than 16 mg/ml. They were not taking systemic steroids, were stable at the time of the study, and had not experienced a respiratory infection or asthma exacerbation during the month preceding this study. Control subjects (n=31) had normal spirometry and airway responsiveness (PC_{20}>16 mg/mL), no history of respiratory diseases, no current or past symptom suggesting asthma, and no respiratory infection during the month

before enrollment. All patients were non-smokers. All subjects were evaluated at baseline. Characteristics of subjects and symptoms were documented using their responses to a detailed structured questionnaire.

The study was approved by the Institutional Review Board of Seoul National University Hospital and all participants provided informed consent.

Sputum induction, processing, and RNA extraction

Sputum induction, processing, and RNA extraction were done as previously described.[22] Briefly, after pretreatment with 400 μg inhaled salbutamol, sputum was induced using an inhaled hypertonic saline aerosol at concentrations of 4.5% for 5, 10, 15, and 20 minutes. Sputum was added with 0.01M solution of dithiothreitol (DTE). The tube was vortex mixed, shaken for 20min in rocker at room temperature, filtered through 52mm nylon gauze to remove debris and mucus, and then centrifuged at 450 X g for 10 min. The cell pellet obtained was resuspended in phosphate-buffered saline (PBS) to a volume equal to the original sputum plus DTE volume. Total cell counting was carried out in a haemocytometer and cell concentrations were then adjusted to 1.0 X 10^6 cells/ml. Cytospins were prepared by adding 60 μl of this cell suspension to Shandon II

cytocentrifuge cups (Shandon Southern Instruments, Sewickley, PA, USA) and spun for 5 min at 500 rpm. Slides were stained with Diff Quik solution (Sysmex Co., Kobe, Japan) for the overall differential cell counting of leukocytes, bronchial epithelial cells and squamous cells. To determine cell differentiation, 300 nucleated cells per slide were counted and expressed as a percentage of intact round nucleated cells, excluding squamous epithelial cells. Sputum samples that contained >20% squamous epithelial cells were not analyzed.

Cells were isolated by brief centrifugation and then stored in 1 ml Trizol (Gibco, Carlsbad, CA, USA) at −80 ° C until required for further processing. RNA extraction was performed using phenol chloroform extraction and ethanol precipitation by following the manufacturer ' s instructions (Gibco, Carlsbad, CA, USA). The RNA contents of solutions were quantified using optical density (OD) at 260nm measured on a Nano - drop spectrophotometer (ND - 1000 Technologies, Wilmington, DE, USA). RNA obtained was stored at −80 ° C until required for further analysis.

Quantitative real time PCR

Total RNA (2 μ g) was used to synthesize first strand of cDNA from oligo - dT - primed RNA by reverse transcription (RT) using

reverse transcriptase (Promega, Madison, WI, USA). GSTs were amplified using an ABI 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA, USA) and a SYBR green master mix (Applied Biosystems, Carlsbad, CA, USA). Primer sequences for human and murine GSTs are described in table 1. Amplifications resulted in products of 200–600 bp, as determined by electrophoresis in 2% Tris-Acetate-EDTA (TAE) agarose gel containing bromide ethidium. GST concentrations were measured using Bio1D image analysis software (Vilber-Lourmat, Marne-La-Vallee, France). All results are normalized versus β-actin to compensate for differences in cDNA amounts.

Statistical analysis

A 2-tailed *t* test was used for comparing human GST mRNA levels. Mann-Whitney U-test was used for comparing murine GST mRNA levels and total GST activity. Results were considered significant when *P*-values were <0.05. Data were analyzed using PRISM software (Graphpad Software, Inc, La Jolla, CA, USA)

RESULTS

Total GST activity is increased in BAL fluid in OVA - challenged mice.

We examined total GST activity in C57BL/6 and BALB/c murine asthma model. In C57BL/6 mice, total GST activity in BAL fluid was significantly increased at 24 and 48 hours after the last OVA challenge ($P<0.05$, Fig 1A). Increased total GST activity returned to control levels at 2 weeks after last challenge. In BALB/c mice, total GST activity in BAL fluid tended to increase without statistical significance after the last OVA challenge ($P>0.05$, Fig 1B). Total GST activity returned to control levels at 2 weeks after last challenge.

Localization of GST in the murine lung

GST immunohistochemistry was performed on murine lungs treated with OVA and PBS to determine the localization of GST expression in the lung of BALB/c murine asthma model. Lung histologic findings showed that peribronchiolar infiltrations by mononuclear inflammatory cells were markedly increased in the OVA - treated mice compared with the PBS - treated mice (Fig 2A - C). GSTP and GSTA were expressed predominantly in mononuclear inflammatory cells and epithelial cells in the OVA - treated mice (Fig

2A, 2B). Macrophages in the airway were also stained. However GSTM was mainly localized in epithelial cells in OVA-treated mice (Fig 2C). In PBS-treated mice, GSTP, GSTA, and GSTM were mainly expressed in epithelial cells (Fig 2A-C).

GSTP1 mRNA expression is increased in the lung of OVA-treated mice.

We examined GST mRNA and protein expressions in the lung of C57BL/6 and BALB/c murine asthma model. GST mRNA expressions were evaluated at 24 hours after last challenge. GSTP1 mRNA expressions were increased in the lung after the last OVA challenge ($P<0.05$, Fig 3A). GSTA1, GSTM1 and GSTT1 mRNA expressions were not statistically different between OVA and PBS-treated mice ($P>0.05$, Fig 3B-3D). The expressions of GST proteins were evaluated 3 times (24, 48 hours and 2 weeks) after final challenge. GST proteins expressions were low and not different between OVA and PBS-treated mice (Fig 4). GST expressions were different according to time kinetics. GSTP was detected until 2 weeks after final challenges. GSTM was detected until 48 hours after final challenges. GSTA was detected until 24 hours after final challenges. GSTT expressions were not detected at 24 hours after final challenge.

GSTA1 mRNA expressions are increased in induced sputum cells of asthmatics.

Our data suggested the increase in GST expressions after OVA challenge in the murine models of asthma. To evaluate whether GST was similarly upregulated in airway cells in asthmatics, we quantified GST mRNA expressions in induced sputum cells, as well as control. There were no significant differences in age or sex between 2 groups. Similar to the observed upregulation of GST expression in the murine models of asthma, GSTA1 mRNA expression was increased in asthmatics compared with that seen in non-asthmatic controls (Fig 5B). GSTP1, GSTM1, and GSTT1 mRNA expressions were not statistically different between two groups (Fig 5A, 5C, 5D).

GSTP1 and GSTA1 mRNA expressions are increased in induced sputum cells of asthmatics with higher AHR than those with lower AHR.

Our data suggested GST expressions are increased in the airways of asthmatics. To evaluate whether GST was increased in airway cells of asthmatics according to airway hyperresponsiveness, we measured GST mRNA expressions in induced sputum cells of

asthmatics. There were no significant differences in age or sex between 2 groups. GSTP1 and GSTA1 mRNA expression were increased in asthmatics with $PC_{20} \leq 4$ mg/ml compared with those with $PC_{20} > 4$ mg/ml (Fig 6A and 6B). GSTM1 and GSTT1 mRNA expressions were not statistically different between two groups (Fig 6C and 6D).

Table 1. Primer sequences for human and murine GSTs

human	GSTP1	CCC TAC ACC GTG GTC TAT TTC C
		CAG GAG GCT TTG AGT GAG C
	GSTM1	TCT GCC CTA CTT GAT TGA TGG G
		TCC ACA CGA ATC TTC TCC TCT
	GSTA1	CTG CCC GTA TGT CCA CCT G
		AGC TCC TCG ACG TAG TAG AGA
	GSTT1	TGC CGC GCT GTT TAC ATC TT
		CGA AGG GAA TGT CGT TCT TCT T
murine	GSTP1	ATG CCA CCA TAC ACC ATT GTC
		GGG AGC TGC CCA TAC AGA C
	GSTM1	ATA CTG GGA TAC TGG AAC GTC C
		AGT CAG GGT TGT AAC AGA GCA T
	GSTA1	TAT TAT GTC CCC CAG ACC AAA GA
		CCT GTT GCC CAC AAG GTA GTC
	GSTT1	CCG TCG CGC CAT TTA TAT CTT
		CCC TCT TCA TGG GGT TCA CC

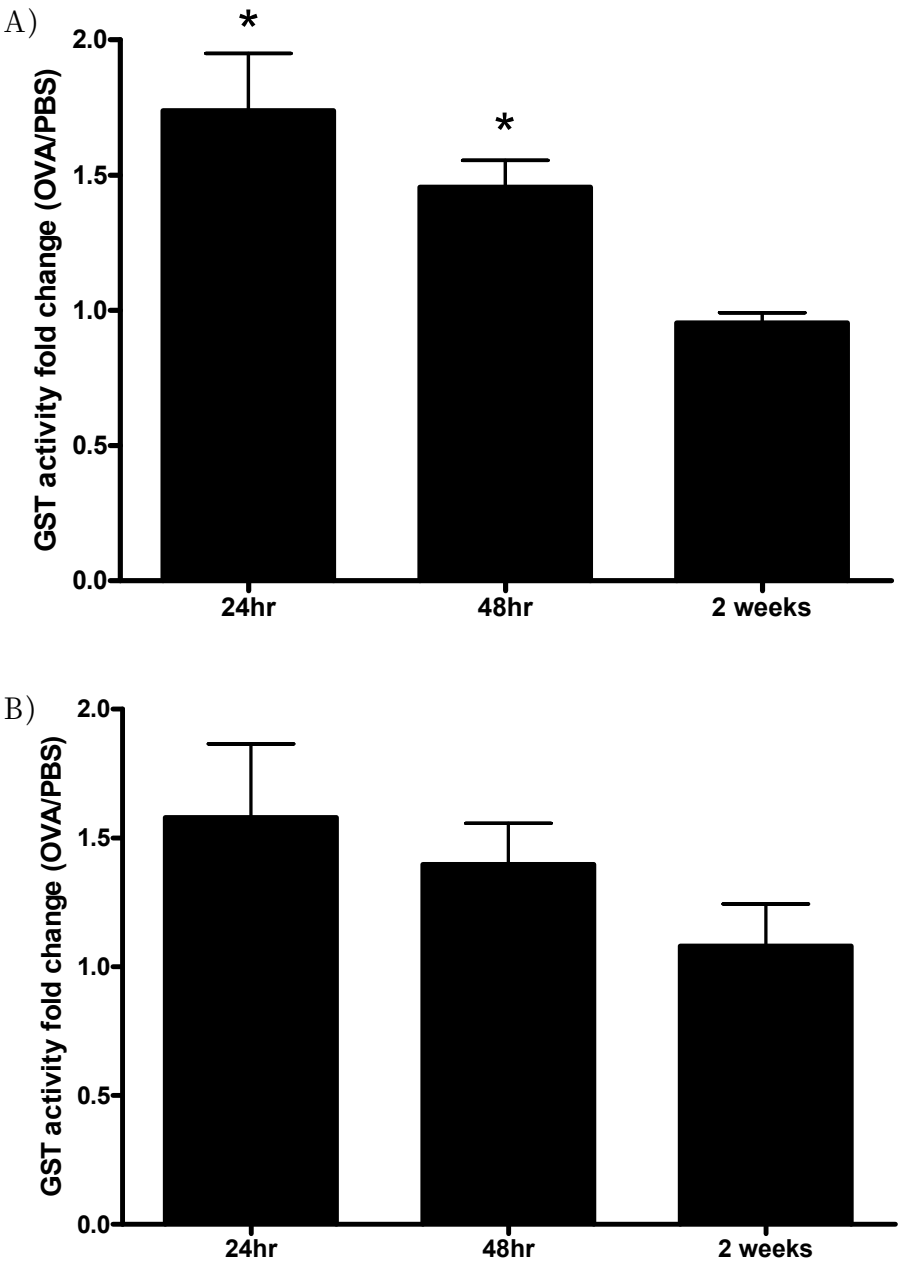
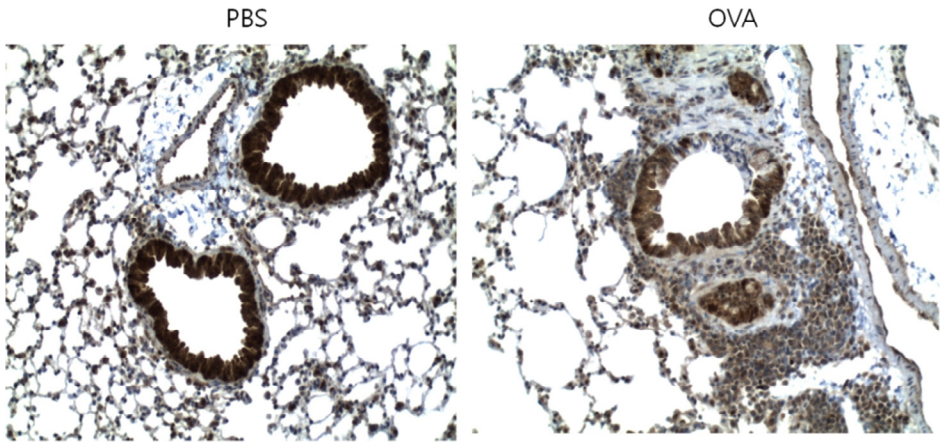


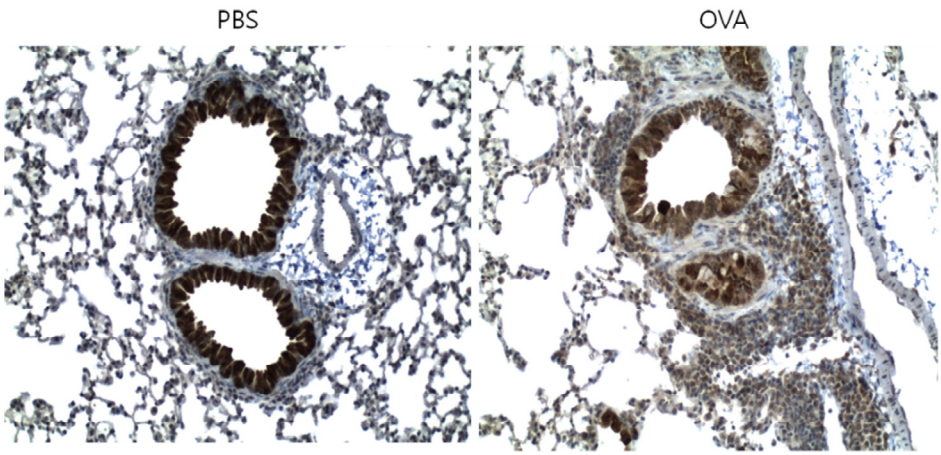
Figure 1. Total GST activity in BAL fluids of murine asthma models. C57BL/6 and BALB/c mice were sensitized intraperitoneally (i.p.) twice and challenged intranasally 4 times. Total GST activities were measured at 24, 48, and 2 weeks after final challenge in two groups. Data are represented as fold changes in total GST activity of OVA -

treated mice relative to PBS-treated mice. A) In C57BL/6 mice, total GST activity was higher at 24 and 48 hours after final challenge in OVA-treated mice than PBS-treated mice and returned to control levels after 2 weeks. B) In BALB/c mice, total GST activity tended to increase after final challenge in OVA-treated mice relative to PBS-treated mice without statistical significance. * $P<0.05$.

A) GSTP



B) GSTA



C) GSTM

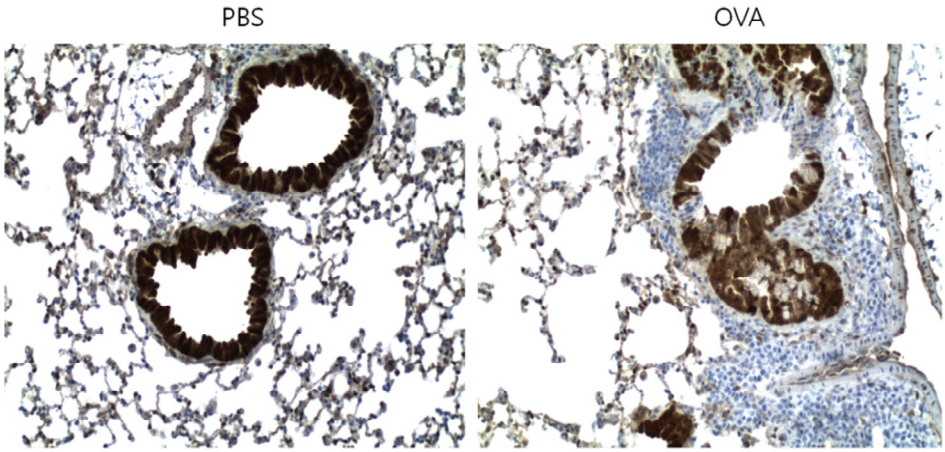


Figure 2. Localization of GSTs in the murine lung. BALB/c mice were treated with OVA and PBS, and analyzed for GSTP, GSTA, and GSTM expressions in the lung by means of immunohistochemistry. A and B) Wild-type mice treated with OVA displayed positive GSTP and GSTA expression predominantly in mononuclear inflammatory cells as well as epithelial cells. However GSTP and GSTA expression was mainly displayed in epithelial cells in PBS-treated mice. C) GSTM expression was displayed predominantly in epithelial cells in OVA and PBS-treated mice.

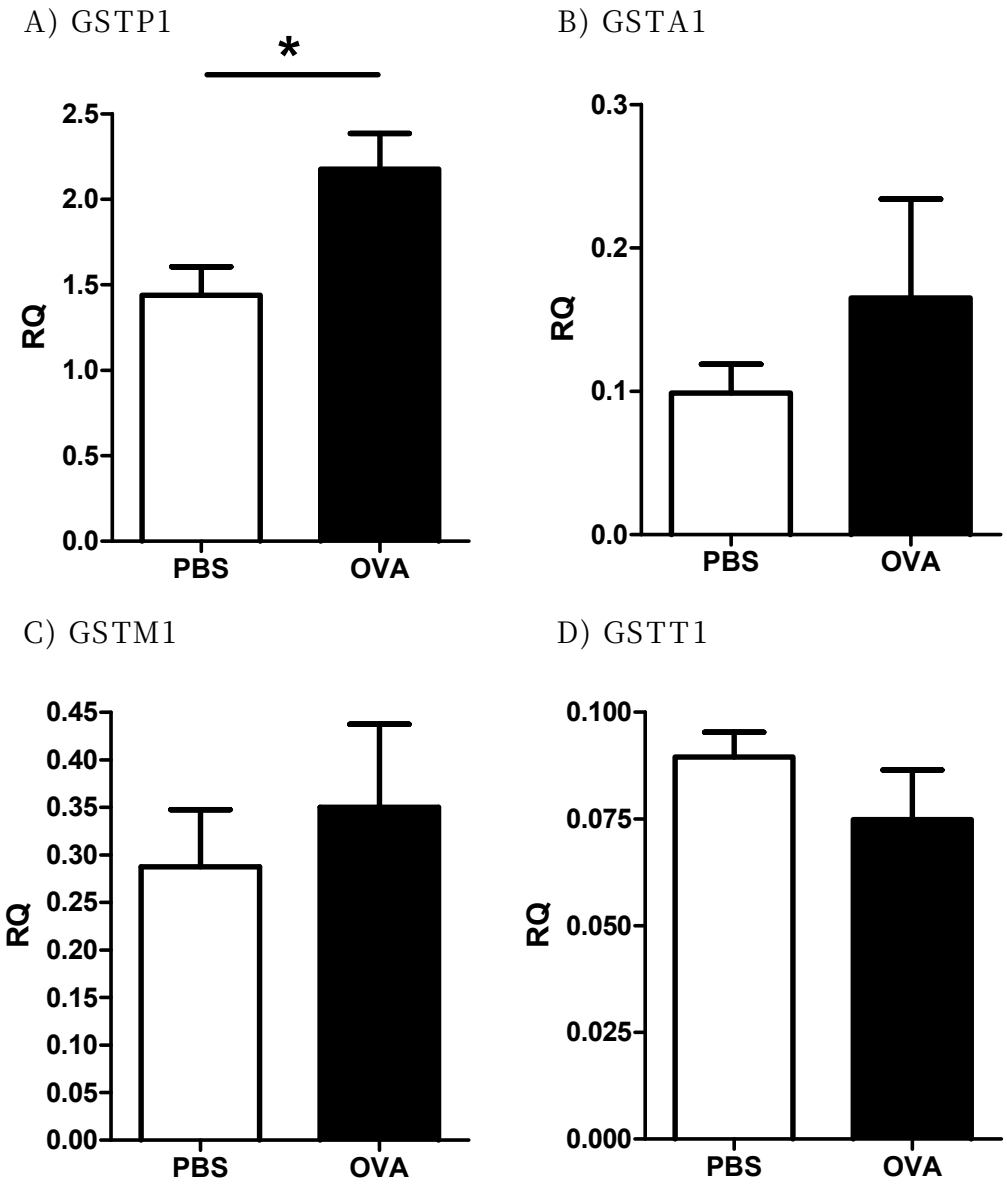


Figure 3. GSTs mRNA expressions in the murine lung. BALB/C mice were sensitized intraperitoneally twice and challenged intranasally 4 times with OVA. GSTP1, GSTA1, GSTM1, and GSTT1 mRNA expression were determined by means of real-time PCR. A) GSTP1 expression was higher in the lung of OVA-treated mice than PBS-treated mice. B-D) GSTA1, GSTM1 and GSTT1

expressions were not statistically different in the lung between OVA - treated mice and PBS - treated mice. * $P<0.05$.

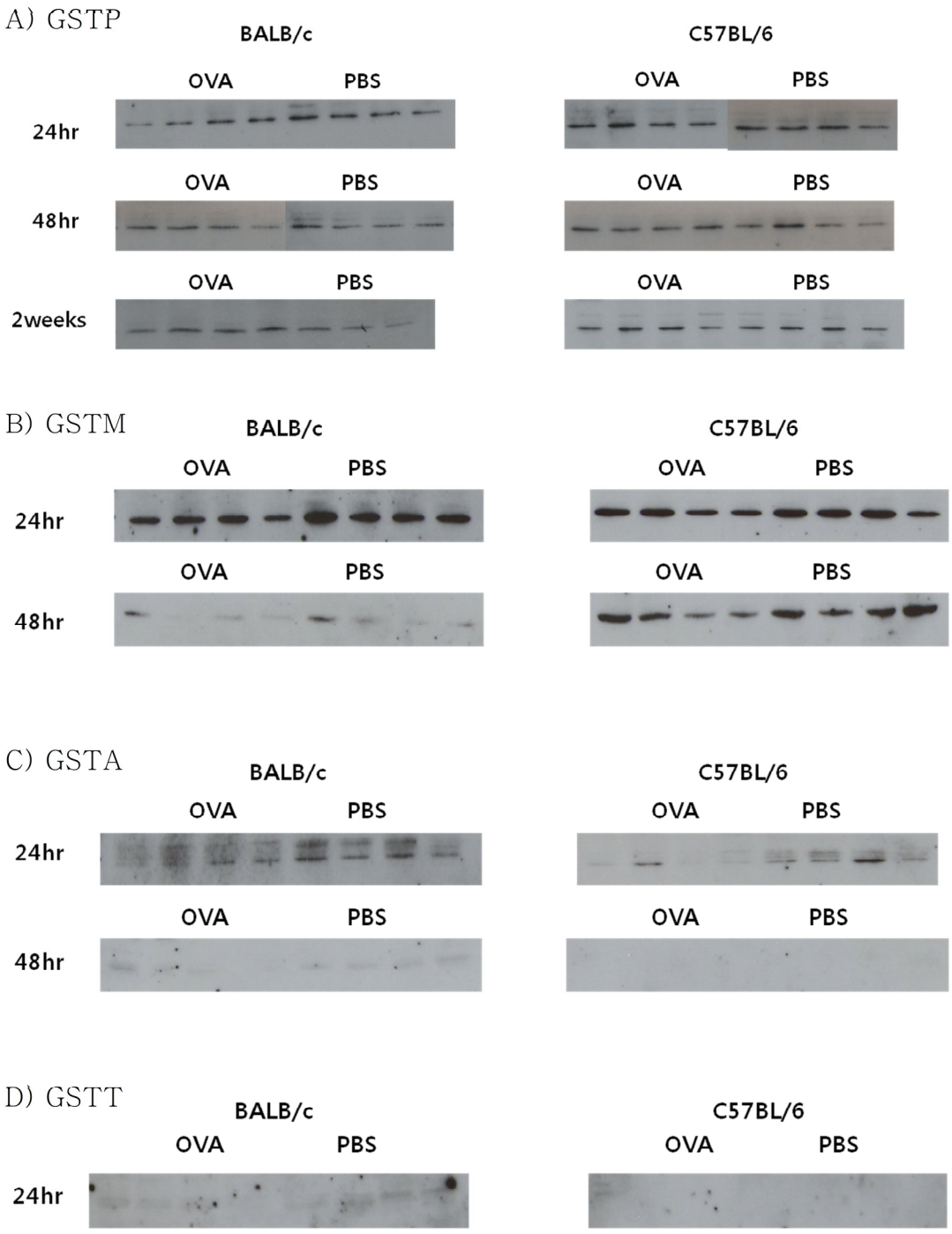


Figure 4. Western blotting for GSTs in the murine lung. A-D) Western analysis for GSTP, GSTM, GSTA, and GSTT revealed no significant difference between OVA-treated mice and PBS-treated

mice in the lungs of BALB/c and C57BL/6 murine asthma model. A) GSTP was detected until 2 weeks after final challenge. B) GSTM was detected until 48 hours after final challenge. C) GSTA was detected until 24 hours after final challenge. D) GSTT expressions were not detected 24 hours after final challenge.

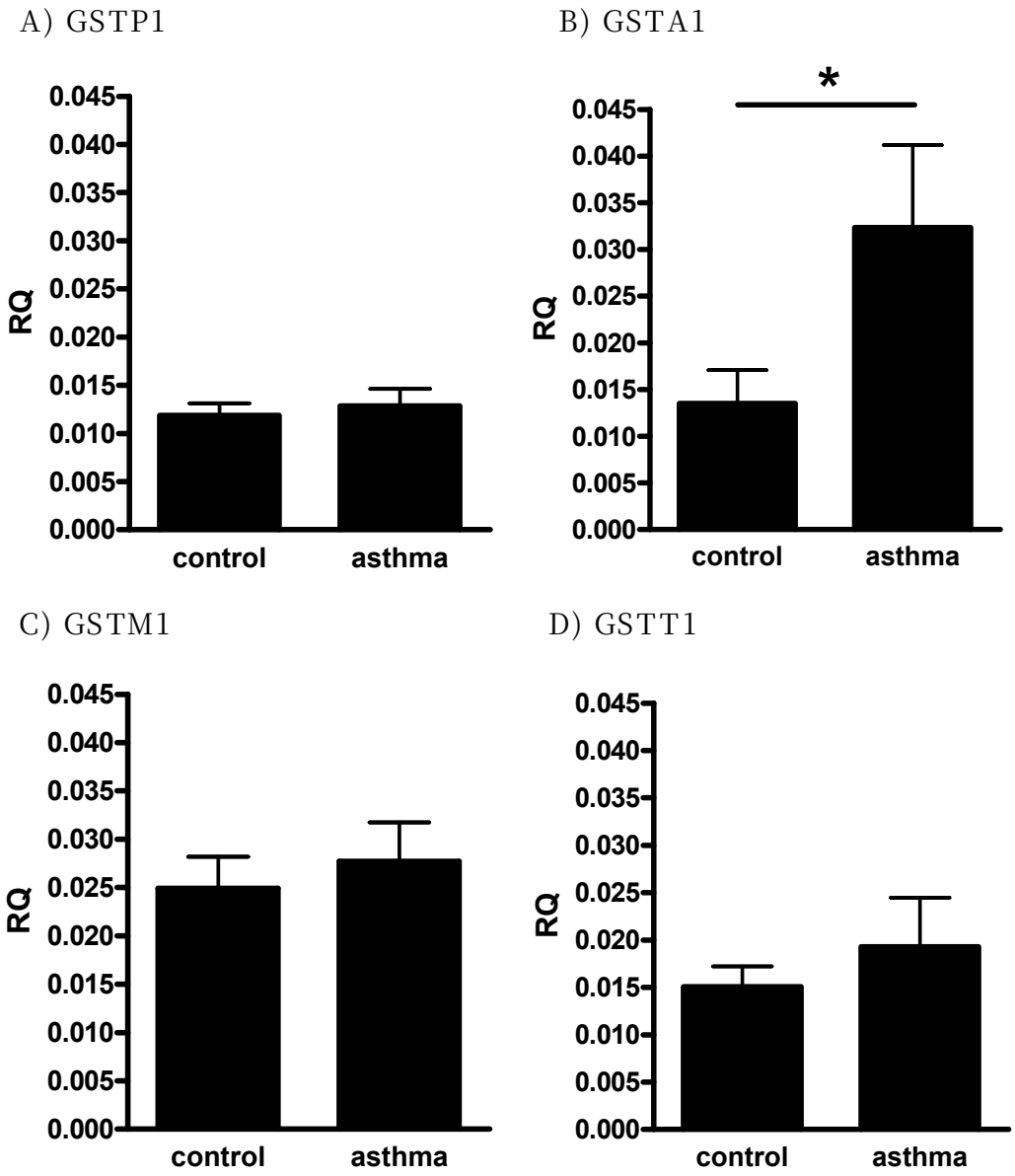


Figure 5. GSTs mRNA expressions in induced sputum cells. GSTP1, GSTA1, GSTM1 and GSTT1 mRNA expressions were evaluated in induced sputum cells by means of real-time PCR. B) GSTA1 expression was significantly increased in induced sputum cells from asthmatics compared with control subjects. A,C,D) GSTP1, GSTM1, and GSTT1 expressions were not statistically different in induced

sputum cells between asthmatics and control subjects. * $P<0.05$.

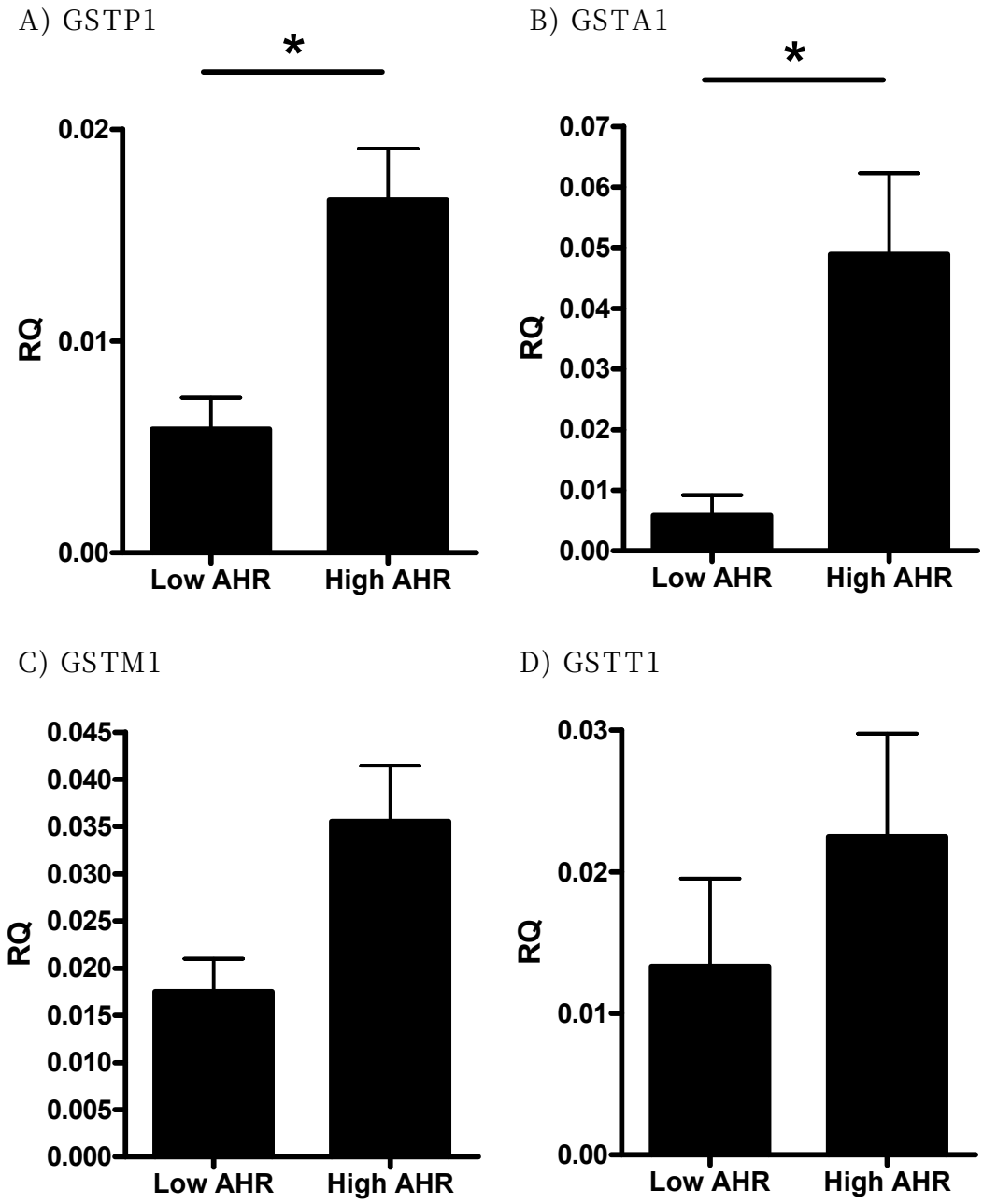


Figure 6. GSTs mRNA expressions in induced sputum cells from asthmatics according to airway hyperresponsiveness. GSTP1, GSTA1, GSTM1 and GSTT1 mRNA expressions were evaluated in induced sputum cells of asthmatics by means of real-time PCR.

High AHR groups included asthmatics with PC_{20} less than 4mg/ml and low AHR groups included asthmatics with PC_{20} >4 mg/ml. A and B) GSTP1 and GSTA1 expression was significantly increased in induced sputum cells from asthmatics with $PC_{20} \leq 4$ mg/ml compared with those with PC_{20} >4 mg/ml. C and D) GSTM1 and GSTT1 expressions tended to increase in induced sputum cells from asthmatics with $PC_{20} \leq 4$ mg/ml compared with those with PC_{20} >4 mg/ml without statistical significance. * $P<0.05$.

DISCUSSION

The prevalence of asthma has increased worldwide over the past decades. Asthma has become an epidemic phenomenon.[23] The increasing prevalence of asthma is important in developed countries, more than 15% in United Kingdom, New Zealand and Australia.[24] This disabling and life - threatening disease affects nearly 15 million adults and 5 million children in the United States.[25] Several factors have been proposed to account for the increasing prevalence of asthma. The genetic changes in populations would be too slow to account for such a rapid change in prevalence.[2] Changing environmental exposures may affect asthma prevalence.[2] Among environmental exposures, cigarette smoke and air pollutants such as ozone, diesel exhaust particles, particulate matters, sulfur dioxide, volatile organic compounds and polycyclic aromatic hydrocarbons (PAHs) are considered to provoke the asthma symptoms. Parental smoking is likely to be causally related to childhood asthma and wheezing.[26] There are sufficient evidences to suggest that air pollutants decrease lung function, trigger exacerbations of asthma and increase rates of hospitalization for asthma.[27]

The reactive oxygen species are superoxide, hydrogen peroxide,

hydroxyl radicals, ozone, and peroxynitrite. They readily react with other molecules, such as proteins, lipids, and DNA. Excessive exposure to reactive oxygen and nitrogen species leads to damage of proteins, lipids, and DNA.[2,4] Direct exposure to such environmental air results in reactive oxygen species in the airways. Cigarette smoke inhalation results in increased exposure to both superoxide and hydrogen peroxide.[28,29] Cigarette smoke-mediated lung damage might be a result of increased exposure to nitric oxide and nitrites.[30,31]

All organisms have evolved elaborate cellular defenses collectively termed antioxidants to overcome this toxicity.[2] The antioxidant defenses within the airway are endogenous antioxidants which can be subdivided into enzymatic and nonenzymatic categories.[2] The enzymatic antioxidants include GST, GPx, SOD, catalase, and thioredoxin. The nonenzymatic antioxidants include low-molecular-weight compounds, such as GSH, ascorbate, urate, bilirubin, α-tocopherol, and lipoic acid. Concentrations of these antioxidants vary depending on both subcellular and anatomic location.

GST enzymes use a wide variety of products of oxidative stress as substrates and thereby have an important role in preventing the damage by ROS.[7] GSTs catalyze the conjugation of GSH on a wide

variety of toxic substrates found in air pollution, cigarette smoke, and mold.[8-10] The products of GST catalysis are more water soluble, promoting ROS detoxification and thereby protecting tissues from oxidative damage.[11,12] Wide variations in the GST activities are thought to play a role in the pathophysiology of asthma. GSTP, GSTM, and GSTA are possibly expressed in the lung and may play a role in protecting ROS.[7,32] However the expression and regulation of GST subtypes against ROS in the airways of asthmatics are poorly understood.

In the present study, total GST activity in BAL fluids was increased in OVA murine asthma model. GSTP and GSTA expression was highly increased in the inflammatory cells of airway after allergen challenges of mice. GSTP1 mRNA expression was significantly increased in the murine lung of OVA-treated mice. Moreover GSTA1 mRNA expression in induced sputum cells was increased in asthmatics. However GSTM was mainly expressed in the epithelial cells and not increased in inflammatory cells. GSTM1 and GSTT1 mRNA were not increased in murine lung and human sputum cells. These findings suggest that GST expressions and activity might be enhanced to protect against increased oxidative stress in airways of asthmatics. GSTA and GSTP may have key roles among GST subtypes in the lung, although GSTA might be

more important in humans rather than GSTP. These are consistent with the findings of the previous studies.[32,33] Antilla et al. showed that GSTA and GSTP were the most abundant GSTs in human lungs, found in the bronchial and bronchiolar epithelium by immunohistochemistry of surgically resected lung tissue.[32] GSTA was also expressed in alveolar macrophage as well as epithelial cells. Harju et al. showed that GSTP and GSTA expressions are increased in sputum supernatants and macrophages of mild to moderate COPD, which also increases oxidative stress in airway.[33] They suggested that the presence of GSTs in the airway secretions can play a protective role as intracellular and extracellular mediators in the lung. GSTP are widely thought to have a role in asthma by epidemiologic, genetic and animal studies.[15,25,34] In GSTP knock-out mice, airway hyperresponsiveness, eosinophilia, airway remodeling, and goblet cell hyperplasia were enhanced.[34] Moreover there is a correlation between GST activity and the level of GSTP mRNA expressions.[35] In this study, GSTP mRNA expression and GST activity are increased in murine lung and BAL fluids. However, Schroer et al. showed that GST mRNA expressions and activity were decreased in lung of house dust mite and aspergillus-treated mice model. This decrease of GST mRNA expressions returned to basal levels after the repetitive allergen challenges. They suggested

that downregulation of GSTP after allergen challenge might contribute to the asthma phenotype because of disruption of redox homeostasis and increased oxidative stress.[36] We assumed that the murine models and interval of challenges might cause the difference between two studies. Moreover they evaluated GST regulation in the lung of mice and did not check them in the airway. In the present study, the GST expressions were increased in murine peribronchial inflammatory cells and human sputum cells and the GST activity was increased in the BAL fluids of mice. This finding suggested that GSTs can be exported or secreted from the lung to airways or be induced in airway cells, to reduce the oxidative stress in the airway.[33] Respiratory - tract lining fluid (RTLF) contains more than 140 - fold higher levels of glutathione (GSH) compared to plasma,[37] and has also enzymatic antioxidants such as GST.[38] This suggests a critical role in protecting airway epithelium from oxidative injury of airway. Antioxidants may be induced in the front line against oxidative injury in the airway after allergen and air pollution exposures. It is well known that the oxidative stress is increased in airway of asthma.[39] Oxidized glutathione (GSSG) is increased and reduced glutathione (GSH) is decreased in the airway. GSTs are enzymes that can detoxify a wide variety of toxic substrates formed by radical attack using the high concentrations of

reduced glutathione (GSH) found in RTLF.[38] GST genes are inducible by oxidative stress and exposure to ROS in air pollution induced decreases in GSH that increased transcription of GSTs.[40] Increased non-enzymatic and enzymatic antioxidants may protect airway from ROS in asthma. N-acetylcysteine amide, a GSH precursor, has been shown to attenuate airway inflammation and hyperresponsiveness by increasing GSH and reducing ROS in OVA-inhaled allergic mice.[41] AEOL 10113, a SOD minetics, was shown to reduce airway hyperresponsiveness and inflammation in OVA challenged murine model.[42] Moreover our data showed that GSTP1 and GSTA1 mRNA were increased with airway hyperresponsiveness. Oxidative stress is associated with development of airway hyperresponsiveness.[43,44] GSTs and other antioxidants enzymes like SOD, GPx might be upregulated to support the antioxidant defense in the airway of asthma. Further study of this issue is required.

GST expressions in the murine lung were low and not increased in this OVA murine asthma model. The change in protein expressions of GSTs analyzed by means of Western blotting were not observed. These findings are in accordance with the previous studies.[34,36] Schroer et al. analyzed the protein expressions of GSTP with the same method and found no change between two groups in murine

asthma model. It was suggested that Western blotting used for measuring GSTP protein expression in the lung might not be sensitive enough to detect modest changes in protein levels and it was possible that one subtype is conserved or even upregulated relative to the other because the antibody does not distinguish between GSTP1 and GSTP2.[36]

The GST regulations in the airway of asthma are poorly understood. The present study shows the upregulation of GST expressions and activity in airway and lung of asthma. These results combined with the previous studies suggest that GST might be induced or secreted in the airway to protect against oxidative stress in asthma. GSTP and GSTA were thought to be important enzymes in this role. Further studies will be needed to confirm these observations.

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국문초록

천식에서 glutathione S-transferase: 천식환자의 기도에서 발생하는 산화스트레스에 대한 GSTP와 GSTA의 보호작용

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천식은 기도의 지속적인 염증이 주요한 특징이다. 활성 산소종(reactive oxygen species, ROS)은 천식의 기도 염증의 악화인자로 잘 알려져 있다. Glutathione S-transferases(GST)는 흡연, 공해 등 환경에서 노출되는 활성 산소종과 독성 화합물의 위해독성을 글루타티온 의존기전을 통해 제거한다. 하지만 천식 질환에서 GST 조절 및 GST 아형 각각의 발현에 대하여는 잘 알려져 있지 않다. 이 연구의 목적은 천식동물모델 및 천식환자에서 GST의 발현 및 활성을 연구하여 천식의

병인 및 악화에서 GST의 기능을 이해하고자 하였다. 마우스 천식동물모델을 이용하여 천식이 유도된 마우스 폐에서 GST 활성도 및 발현을 관찰하였고 천식 환자의 유도객담 세포를 이용하여 천식환자의 기도에서 GST 발현을 조사하였다. 연구 결과는 다음과 같다. 마우스 천식동물모델에서 측정한 총 GST 활성도가 OVA를 처리한 마우스 천식군에서 PBS를 처리한 대조군에 비하여 의미있는 증가를 보였다. 마우스 천식동물모델의 폐조직병리검사에서 GSTP, GSTA가 마우스 천식군에서 세기관지주변에 침윤된 단핵염증세포와 기관지상피세포에서 높게 발현되었고, 대조군에서는 기관지상피세포에서만 발현이 관찰되었다. GSTM은 주로 기관지상피세포에서 발현되었으며 마우스 천식군과 대조군에서 차이가 없었다. 마우스 천식군의 폐조직에서 측정한 GST는 GSTP1 mRNA가 대조군에 비하여 높게 발현되었고 GSTA1, GSTM1, GSTT1 mRNA의 발현은 두 군간의 차이가 없었다. 하지만 GST 단백질은 모두 낮게 발현되거나 두 군간의 차이가 없었다. 천식환자의 유도객담 세포에서 GST의 발현을 관찰한 결과 GSTA1 mRNA가 천식환자에서 정상 대조군에 비하여 높게 발현되었다. GSTP1, GSTM1, GSTT1 mRNA의 발현은 두 군간에 통계적인 차이가 없었다. 또한 천식환자를 기도과민성에 따라 분류한 결과 기도과민성이 높은 군에서 유도객담 세포의 GSTP1, GSTA1 mRNA의 발현이 기도과민성이 낮은 군에 비하여 통계적으로 높게 발현되었다. 결론으로, 본 연구에서 천식 질환의 기도 및 폐조직에서 GST의 발현 및 기능이 증가함을 관찰하였다. 이는 GST가 천식 악화인자인 산화스트레스에

대하여 보호작용을 하는 것으로 생각된다. 이 과정에서 GSTP, GSTA가 중요한 역할을 함을 확인하였다.

주요어: 천식, Glutathione S - transferase, 산화스트레스, 활성 산소종

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