

Glutathione Transferase P1

An Endogenous Inhibitor of Allergic Responses in a Mouse Model of Asthma

Jiansheng Zhou¹, C. Roland Wolf², Colin J. Henderson², Yeping Cai¹, Philip G. Board¹, Paul S. Foster^{1,3}, and Dianne C. Webb¹

¹Division of Molecular Bioscience, John Curtin School of Medical Research, Australian National University, Canberra, Australia; ²Cancer Research UK, Molecular Pharmacology Unit, Ninewells Hospital and Medical School, Dundee, United Kingdom; ³Priority Research Centre for Asthma and Respiratory Disease, Faculty of Health and Hunter Medical Research Institute, University of Newcastle, Newcastle, Australia

Rationale: Although epidemiological studies have linked asthma susceptibility and severity to polymorphisms in human glutathione transferase Pi (GSTP) 1, there is no direct evidence for a functional involvement of GSTP1 in processes that are pathognomic of asthma. **Objectives:** To examine the role of GSTP1 in modulating the development of allergic airways disease.

Methods: Allergic airways disease was induced in wild-type (WT) and *Gstp*-null mice employing both acute and chronic models. Eosinophilia, goblet cells, and remodeling were quantified by histological assessment; respiratory function was determined using invasive methods. ELISA was used to evaluate Th2 cytokines, eotaxin, and phospho-c-Jun. *Gstp1/2* expression was quantified by reverse transcriptase-polymerase chain reaction.

Measurements and Main Results: Compared with allergic WT mice, eosinophilia, goblet cell hyperplasia, airway remodeling, lung resistance, and IL-5 were enhanced in allergic *Gstp*-null mice. However, the protective efficacy of GSTP1 was mouse-strain dependent, and associated with inherent variation in expression of *Gstp1*. Although elevated levels of phospho-c-Jun were detected in *Gstp*-null mice, treatment of WT mice with a GSTP/c-Jun N-terminal kinase (JNK) inhibitory peptide enhanced phospho-c-Jun and significantly attenuated allergic responses.

Conclusions: GSTP1 attenuates the severity of allergic airways disease. However, the efficacy of GSTP1 correlated with mouse strain-dependent variation in *Gstp1* expression. Although GSTP1 attenuated c-Jun phosphorylation, treatment with a GSTP/JNK inhibitory peptide revealed an inverse relationship between c-Jun phosphorylation and allergic responses, indicating that the mechanism by which GSTP attenuates allergic responses is not dependent on the JNK/c-Jun axis. Our data, together with epidemiological evidence, suggest variation in expression and/or function of this protein is an important determinant in asthma pathophysiology.

Keywords: asthma; oxidative stress; Th2 cytokines; JNK

The bronchial epithelium, which is a key mediator of allergic inflammation and airway remodeling (1–3), is more susceptible to oxidant-induced apoptosis in subjects with asthma (4, 5), suggesting an inherent inability of these cells to efficiently metabolize reactive oxygen species (ROS) and electrophilic metabolites. The importance of oxidative stress in augmenting allergic inflammation was recently demonstrated by the observa-

AT A GLANCE COMMENTARY

Scientific Knowledge on This Subject

Although many epidemiological studies have linked polymorphisms in glutathione transferase Pi (GSTP) 1 with the development and severity of asthma, there is no direct evidence that this enzyme participates in the pathophysiological processes that underlie the clinical symptoms of asthma.

What This Study Adds to the Field

GSTP1 attenuates allergic inflammation, respiratory dysfunction, and remodeling. However, the effects are dependent on the level of *Gstp1* expression, suggesting that factors that influence the function of GSTP1 contribute to the severity of asthma.

tion that intrinsic reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases in pollen greatly enhances its allergy-inducing potential by inducing a ROS-mediated innate response that acts in concert with an allergen-induced adaptive response (6). Eosinophils in the asthmatic lung are also potent sources of ROS that are generated and amplified by endogenous NADPH oxidases and eosinophil peroxidases (7). Therefore, it is not surprising that subjects with asthma secrete higher levels of lipid peroxidation derivatives than those without asthma, especially during periods of exacerbation (8–10).

As generation of ROS metabolites is associated with asthma, it is likely that antioxidant defense mechanisms are critical in neutralizing the deleterious effects of oxidative stress in the lung. Glutathione transferase Pi (GSTP) 1, which can function as a detoxificant of ROS metabolites, is highly expressed in bronchial epithelial cells (11). *GSTP1* maps to chromosome 11q13 (12), a locus linked to the development of asthma (13), adding support to the possibility that *GSTP1* is a candidate asthma-related gene. Indeed, analyses of diverse population groups suggest that a polymorphic Val105Ile substitution is linked to the development asthma (14–18), particularly in children living in areas of high pollution (18). However, whether Val or Ile at position 105 confers the greater protective efficacy is controversial (19, 20). Interestingly, challenge of patients sensitive to ragweed with allergen alone or in combination with diesel exhaust particles showed these oxidative pollutants preferentially potentiate IgE and histamine production in patients expressing the GSTP1 Ile105 variant (21).

Distinct from its role in detoxification, GSTP1 can also negatively regulate signaling processes that protect against oxidative stress-induced cell death (22). The ligand-binding partners identified so far include c-Jun N-terminal kinase (JNK)

(Received in original form January 29, 2008; accepted in final form September 10, 2008)

Supported by National Health and Medical Research Peter Doherty Training Fellowship no. 179,841 (D.C.W.), National Health and Medical Research Council Project Grant 366,765 (D.C.W.), and National Health and Medical Research Council Program Grant 224,207 (P.S.F.).

Correspondence and requests for reprints should be addressed to Dianne C. Webb, Ph.D., Division of Molecular Bioscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia. E-mail: dianne.webb@anu.edu.au

Am J Respir Crit Care Med Vol 178, pp 1202–1210, 2008

Originally Published in Press as DOI: 10.1164/rccm.200801-178OC on September 11, 2008
Internet address: www.atsjournals.org

and tumor necrosis factor receptor–associated factor 2, which also activates p38 and JNK (23–25). The ability of GSTP1 to modulate JNK has relevance, as JNK-activated signaling cascades are considered important mediators of allergen-induced pathophysiology (26–28).

Although epidemiological studies suggest a link between variants of GSTP1 and asthma, there is no direct evidence for a functional involvement of GSTP1 in specific pathophysiological processes that underlie the clinical features of asthma. The aim of the current investigation was to employ *Gstp*-null mice to investigate the influence of GSTP on the development of allergic airways disease. We show that GSTP1 plays a critical role in attenuating allergic inflammation, respiratory dysfunction and airway remodeling in a mouse strain-dependent manner. Our data predict that variation in expression levels or function of GSTP1 will significantly modulate the severity of asthma. Components of this study were previously reported in the form of an abstract (29).

METHODS

Mice

In contrast to a single human *GSTP1* gene, two homologous *Gstp1* and *Gstp2* genes have been identified in mice (30, 31). *Gstp*-null mice (deficient in *Gstp1* and *Gstp2*) were generated on a 129/MF1 background (32) and crossed for six generations with either BALB/c or C57BL/6 mice. Wild-type (WT) mice were similarly generated from the 129/MF1 strain.

Induction of Allergic Airways Disease

Acute allergic airways inflammation was induced in equal numbers of 6- to 8-week-old male and female mice by intraperitoneal sensitization, and, after 12 days, the mice were aerosol challenged with ovalbumin (OVA) for 8 days (33). For the chronic model of antigen exposure, mice were sensitized with OVA on Days 0 and 12, followed on Day 18 by a series of OVA aerosols for 5 weeks (34). At 24 hours after the last challenge, mice were killed by cervical dislocation, and lungs and bronchoalveolar lavage fluid (BALF) were collected (33). Mice were treated according to Australian National University Animal Welfare guidelines (protocol JMB18/04).

Quantification of Eosinophils, Goblet Cells, and Collagen Deposition

Lung sections were stained with Carbol's chromotrope-hematoxylin (BDH, Poole, UK) or Alcian blue–periodic-acid Schiff (Anatech Ltd., Battle Creek, MI) for enumeration of peribronchial eosinophils and goblet cells, respectively (32). Collagen deposition was assessed on Martius scarlet blue stained lung sections using Scion Image analysis software (Scion Corp., Frederick, MD) (34).

Western Blots

Western immunoblots were performed with 1/1,000 rabbit anti-Ym1/2 antibody (35).

Airway Function

Lung resistance (airways hyperresponsiveness [AHR]) was determined in anesthetized mice (167 μ g/g ketamine, 8.3 μ g/g xylazine, 4.2 μ g/g acepromazine administered intraperitoneally) that were intubated and attached to an electronically controlled mouse ventilator. Increasing doses of β -methacholine were nebulized via the endotracheal tube, and lung resistance was calculated from tracheal pressure measurements with BioSystem XA software (Buxco Electronics, Wilmington, NC) essentially as described in detail elsewhere (36).

Cytokine Production

Cells from mediastinal lymph nodes (MLNs) were stimulated with 1 mg/ml OVA in complete mixed lymphocyte culture medium (MLC) (33) for 68 hours at 37°C and 5% CO₂ in 96-well plates with 2×10^6

cells per well. IL-5, IL-13, IFN- γ in MLN supernatants, and eotaxin in lung extracts were quantified by ELISA (33).

Phospho-c-Jun

Quantification of total c-Jun and phospho-c-Jun was performed by sandwich ELISA following the manufacturer's instructions (Cell Signaling Technology, Beverly, MA). Essentially, MLNs were lysed in buffer containing protease and phosphatase inhibitors, and the protein concentration of the lysate was quantified (Pierce BCA, Pierce, IL). Total c-Jun or phospho-c-Jun in 40 μ g of cell lysate protein was captured by solid-phase rabbit anti-total c-Jun or anti-phospho-c-Jun antibody. Captured phospho-c-Jun or total c-Jun was detected with anti-c-Jun antibody and anti-mouse/horseradish peroxidase–conjugated antibody.

Reverse Transcriptase–Polymerase Chain Reaction for GSTP

mRNA specific for *Gstp1/2* was detected in lungs by reverse transcriptase–polymerase chain reaction (PCR) (34) using the forward primer, 5'-ACCATAGATACCTGGATGCAAG, and the reverse primer, 5'-CTGGTCACCCACGATGAAAGC, for 30 cycles at 58°C annealing temperature. Primers were designed from GenBank X53451 and hybridize at identical nucleotide sequences in *Gstp1* and *Gstp2*. PCR products were incubated with *MaeIII*, which digests *Gstp1*, but not *Gstp2*.

Statistical Analysis

Experiments were performed at least twice, and representative data are shown. Significance of differences between experimental groups was analyzed using the Student's unpaired *t* test or analysis of variance with *post hoc* Tukey–Kramer multiple comparisons test. Values are the means ($\pm$ SEM). Differences in means were considered significant if *P* < 0.05.

RESULTS

C57BL/6 *Gstp*-Null Mice Develop Exaggerated Allergic Airway Disease

We directly compared inflammation, goblet cell hyperplasia, expression of the alternatively activated macrophage marker, Ym1/2, and respiratory function in allergic C57BL/6 WT and *Gstp*-null mice to obtain insight into the function of GSTP1/2 in the development of these characteristic features of allergic airways disease. Compared with allergic WT mice, blood, BALF and peribronchial eosinophilia, goblet cell hyperplasia (Figures 1A–1C) and expression of the Ym1/2 lectin (Figure 1D) were significantly higher in allergic *Gstp*-null mice, indicating that GSTP1/2 plays a significant role in attenuating allergic airways disease. The ability of GSTP1/2 to suppress inflammation was eosinophil-specific, as no significant differences in blood or airway lymphocytes, neutrophils, or macrophages were observed between allergic WT and *Gstp*-null mice (Figures 1A–1C). Notably, eosinophilia and goblet cell hyperplasia shown in Figures 1A–1C in both groups of allergic mice were significantly higher relevant to control animals (data not shown). The concentration of BALF proteins were similar in WT and *Gstp*-null mice (data not shown), suggesting that elevated Ym1/2 expression was localized rather than systemically derived. Using the same conditions, Ym1/2 was undetectable in BALF from all nonallergic mice (data not shown). We have previously shown that expression of the Ym1/2 proteins is enhanced in allergic mice by a process dependent on Th2 cytokines (34). Therefore, the observation that Ym1/2 expression was higher in allergic *Gstp*-null mice than in allergic WT mice indirectly suggests that GSTP also plays a role in regulating Th2 cytokine responses in the lung. When we investigated the development of AHR, lung resistance was indistinguishable between allergic and nonallergic C57BL/6 WT mice (Figure 1E).

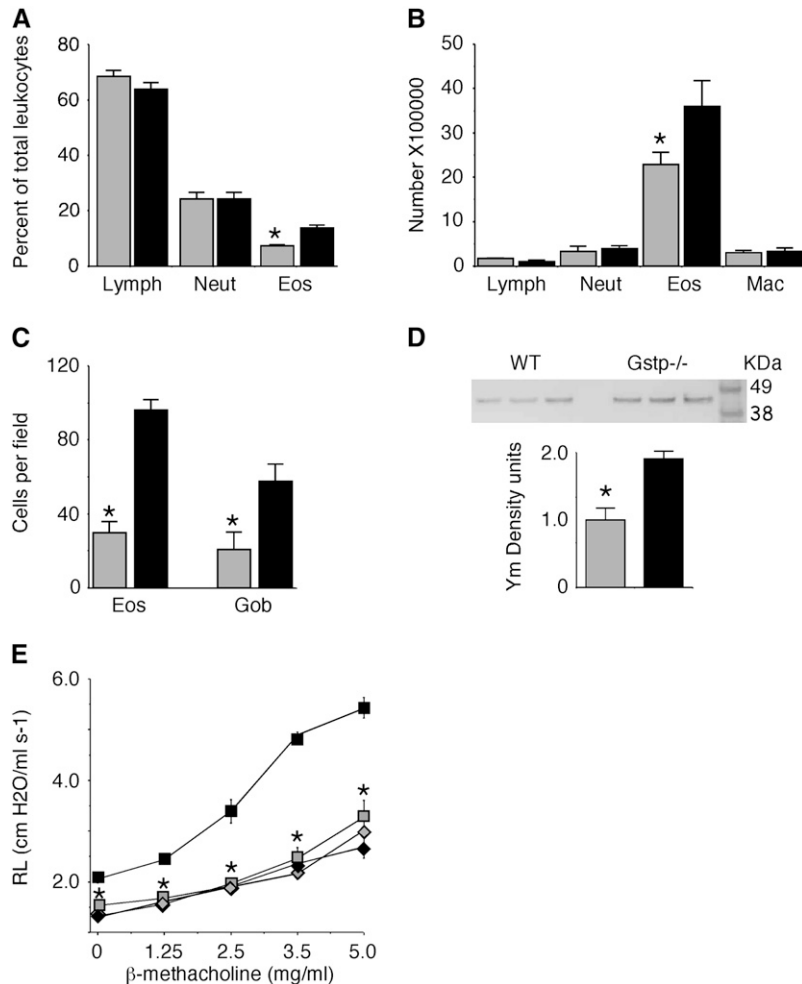


Figure 1. Glutathione transferase Pi (GSTP) 1/2 regulates eosinophilia, goblet cell hyperplasia, Ym1/2 expression, and airway function in allergic C57BL/6 mice. (A) The percentages of blood leukocytes were determined by morphological criteria in May-Grünwald Giemsa-stained smears and (B) the numbers of airway leukocytes were quantified by correlating differential counts of May-Grünwald Giemsa-stained cytopins with the total cell counts in the bronchoalveolar lavage fluid (BALF). (C) Lung sections were either stained with Carbol's chromotrope and the numbers of eosinophils in the peribronchial region of 10 similar fields of 1,000 $\times$ magnification per mouse were counted or (C) stained with Alcian blue-periodic acid Schiff and the numbers of goblet cells in the peribronchial region determined in 10 similar fields of 1,000 $\times$ magnification per mouse. Lymph, lymphocytes; Neut, neutrophil; Eos, eosinophils; Mac, macrophages; Gob, goblet cells. (D) Ym1/2 expression in BALF. Western blots of identical volumes of BALF from three mice per group were probed with rabbit anti-mouse Ym1/2 antibody then anti-rabbit APase-conjugated antibody. The density of bands was determined with a Syngene Bioluminescence System (Syngene, Frederick, MD). (E) Lung resistance was determined in response to increasing doses of β -methacholine. Values are the mean $\pm$ SEM of: (A–C) five or six mice per group; (D) three mice per group; (E) four mice per group. * $P < 0.05$ compared with *Gstp*-null mice. Gray bars/squares, allergic wild-type (WT) mice; black bars/squares, allergic *Gstp*-null mice; gray triangles, nonallergic WT mice; black triangles, nonallergic *Gstp*-null mice.

In direct contrast, the development of allergy-induced AHR was unmasked by depletion of GSTP1/2 (Figure 1E). Collectively, these data demonstrate that GSTP1/2 plays a critical role in regulating allergy-induced eosinophilia, goblet cell hyperplasia, and AHR in C57BL/6 mice.

BALF Eosinophils, but Not Other Allergic Responses, Are Enhanced in Allergic BALB/c *Gstp*-Null Mice

As many mouse models of asthma employ the BALB/c strain, we also crossed *Gstp*-null mice with this background of mice. Compared with WT mice, deficiency in GSTP1/2 significantly enhanced the numbers of allergy-induced eosinophils and macrophages in the BALF (Figure 2B). However, GSTP1/2 played no role in regulating the severity of blood and peribronchial eosinophilia or goblet cell hyperplasia (Figures 2A and 2C). In addition, GSTP1/2 did not influence Ym1/2 expression (Figure 2D) and, apart from the response to the highest dose of methacholine, failed to modulate methacholine-induced lung resistance (Figure 2E). Therefore, the efficacy of GSTP1/2 as an important moderator of allergic inflammation is critically dependent on the genetic context of the host.

C57BL/6 and BALB/c *Gstp*-Null Mice Display Different Patterns of Th2 Biasing and c-Jun Phosphorylation

In order to address the mechanisms underlying disparity in the ability of GSTP1/2 to suppress allergic responses in C57BL/6 and BALB/c mice, we assayed the levels of cytokines produced

by the MLNs and the eosinophil chemokine, eotaxin, in the lungs of WT and *Gstp*-null mice. Compared with WT mice, depletion of GSTP1/2 significantly enhanced IL-5 and IFN- γ production without influencing IL-13 or eotaxin levels in allergic C57BL/6 mice (Figure 3A). In direct contrast, depletion of GSTP1/2 failed to modify the levels of IL-5, IL-13, IFN- γ or eotaxin in allergic BALB/c mice (Figure 3B). The observation that BALB/c *Gstp*-null mice exhibited enhanced numbers of eosinophils in the BALF was intriguing, considering that there were no differences in IL-5 or eotaxin in the WT and *Gstp*-null strains. However, RayBio Array analysis for 62 cytokines (Mouse Cytokine Antibody Array 3; RayBiotech Inc., Norcross, GA) demonstrated that eotaxin-2 was upregulated 2.7-fold in BALB/c *Gstp*-null mice (WT, 58.6 ± 0.1 ; *Gstp*-null, 158.6 ± 0.5 relative spot intensity normalized to positive controls), which may explain why higher numbers of eosinophils were observed in the BALF of BALB/c *Gstp*-null mice. Intriguingly, eotaxin-2 was not similarly upregulated in allergic C57BL/6 *Gstp*-null mice. Collectively, these data show that GSTP1/2 suppresses IL-5 and IFN- γ production in allergic C57BL/6 WT mice, but not in allergic BALB/c WT mice. As GSTP1 is known to complex with the JNK/Jun heterodimer and inhibit the ability of JNK to phosphorylate c-Jun *in vitro* (23, 24) we investigated phosphorylation of c-Jun in the MLN of allergic WT and *Gstp*-null mice. We noted that the level of phospho-c-Jun was significantly lower than total c-Jun in allergic C57BL/6 WT mice, indicating significant levels of c-Jun persisted in the unphosphorylated (inactive) state in these mice (Figure 3C). In contrast, no

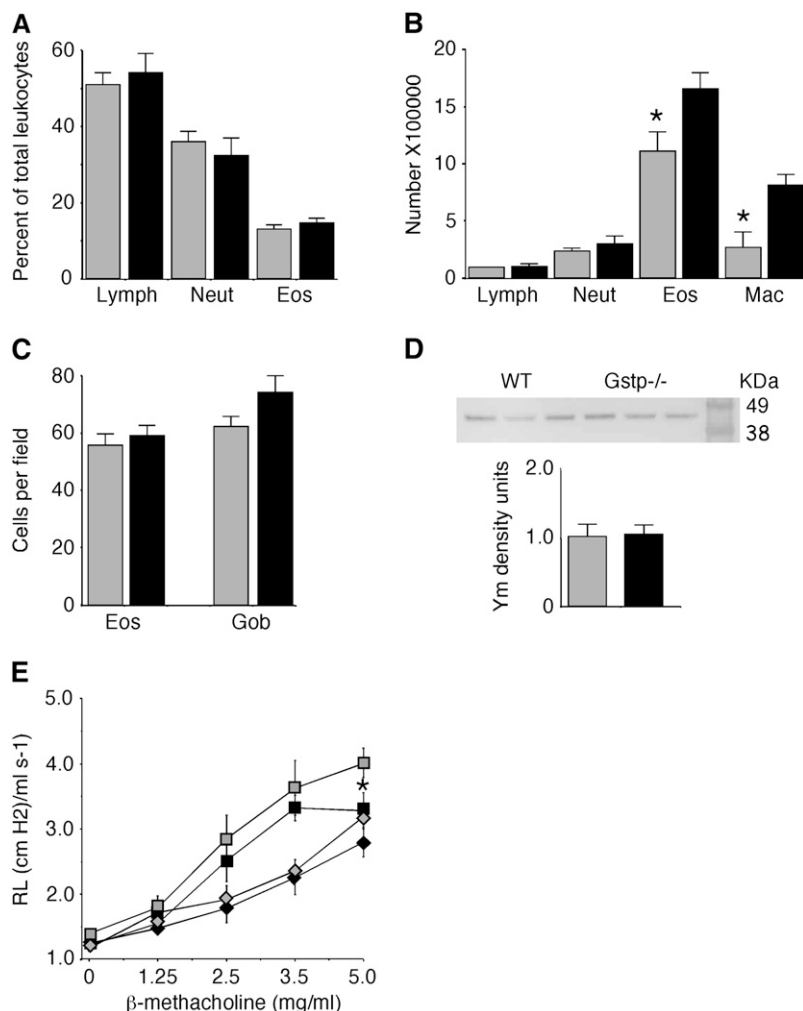


Figure 2. Glutathione transferase Pi (GSTP) 1/2 regulates the numbers of eosinophils and macrophages in bronchoalveolar lavage fluid (BALF), but not tissue eosinophilia, goblet cell hyperplasia, Ym1/2 protein expression, or airway resistance in allergic BALB/c mice. The percentages of (A) peripheral blood leukocytes, (B) BALF leukocytes, (C) peribronchial eosinophils and goblet cells, (D) Ym1/2 expression, and (E) lung resistance were determined as described in the legend for Figure 1. Values are the mean $\pm$ SEM of: (A–C) five–six mice per group; (D) three mice per group; (E) four mice per group. * $P < 0.05$ compared with allergic *Gstp*-null mice. Gray bars/squares, allergic wild-type mice; black bars/squares, allergic *Gstp*-null mice; gray triangles, nonallergic wild-type mice; black triangles, nonallergic *Gstp*-null mice.

significant differences were seen in the levels of phospho-c-Jun and total c-Jun in allergic C57BL/6 *Gstp*-null mice, demonstrating that, in the absence of GSTP1/2, all available c-Jun persisted in the phosphorylated state. We also observed significantly higher levels of total Jun and phospho-c-Jun in allergic C57BL/6 *Gstp*-null mice than in allergic C57BL/6 WT mice, which we assume is reflective of either enhanced cellular activity and/or enhanced cellular recruitment to the MLN in the absence of GSTP1/2. In direct contrast, the levels of phospho-c-Jun and total c-Jun were similar in allergic BALB/c WT and *Gstp*-null mice (Figure 3C), indicating that all c-Jun persisted in the phosphorylated state and that GSTP1/2 plays little role in inhibiting c-Jun phosphorylation in BALB/c mice.

GSTP Inhibits Inflammation, Mucus, Airway Remodeling, and Th2 Cytokines in Chronically Challenged C57BL/6 Mice, but Not BALB/c Mice

In order to examine the role of GSTP1/2 in regulating airway remodeling and to determine whether prolonged antigen challenge could stimulate the ability of GSTP1/2 to suppress allergic responses in BALB/c mice, we employed a previously characterized, chronic model of allergic airways disease (34). Consistent with observations from the acute model, GSTP1/2 significantly suppressed peribronchial eosinophilia, goblet cell hyperplasia, subepithelial collagen deposition, and proliferation of airway smooth muscle cells in allergic C57BL/6 WT mice

(Figures 4A–4D and 5A–5D). In direct contrast, GSTP1/2 did not influence these parameters in allergic BALB/c mice (Figures 4E–4H). Collectively, these data confirm the intriguing strain-dependent dichotomy in the function of GSTP1/2. In allergic C57BL/6 mice, GSTP1/2 functions to attenuate the severity of allergic responses, whereas, in BALB/c mice, even after prolonged antigen exposure, this enzyme plays little role in regulating allergic airways disease.

GSTP Isoforms Are Differentially Expressed in the Lungs of C57BL/6 and BALB/c Mice

In order to obtain insight into the strain-dependent disparity in the ability of GSTP1/2 to regulate pulmonary allergy, we investigated expression of GSTP1 and GSTP2 in C57BL/6 and BALB/c WT mice. As there are no antibodies available that specifically distinguish GSTP1 from GSTP2, we used reverse transcriptase–PCR to determine the relative abundance of *Gstp1* and *Gstp2* gene transcripts in the lungs. *Gstp1* and *Gstp2* were amplified using primers that hybridize to identical regions in both genes, and the resulting PCR products were incubated with *MaeIII*, which cleaves *Gstp1* but leaves *Gstp2* intact. Analysis of the *MaeIII* digestion pattern showed the predominant form of *Gstp* expressed in the lungs of nonallergic C57BL/6 mice was *Gstp1* (Figures 6A, 6B, and 6C). In contrast, the predominant form of *Gstp* expressed in the lungs of nonallergic BALB/c mice was *Gstp2* (Figures 6A, 6B, and 6C). Although induction of allergic responses significantly reduced *Gstp1*

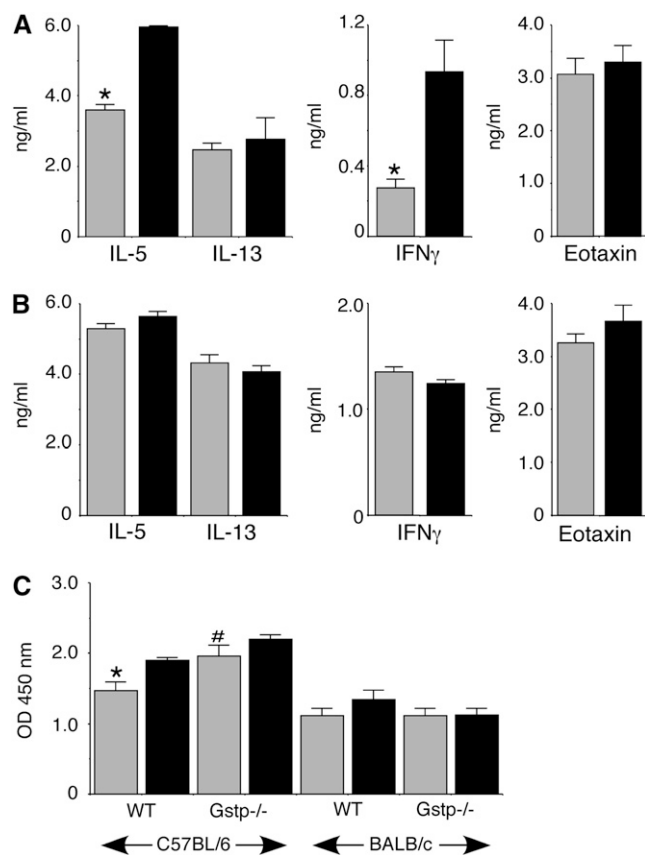


Figure 3. Glutathione transferase Pi (GSTP) 1/2 regulates IL-5, IFN- γ , and c-Jun phosphorylation in allergic C57BL/6 mice, but not in allergic BALB/c mice. Cytokines produced by cultured mediastinal lymph node (MLN) cells and eotaxin in the cell-free supernatant from lung tissue cell suspensions from allergic (A) C57BL/6 and (B) BALB/c mice were assayed by ELISA. (C) Cell extracts were prepared from the MLN of allergic wild-type (WT) and *Gstp*-null mice and total c-Jun and phospho-c-Jun were assayed by ELISA. (A and B) Values are the mean $\pm$ SEM for three assays of a pool of MLN from five or six mice per group or lung extracts from six or seven mice per group. Gray bars, allergic WT mice; black bars, allergic *Gstp*-null mice. * P < 0.05 compared with the *Gstp*-null mice. (C) Values are the mean $\pm$ SEM of MLN from four mice per group. Gray bars, phospho-c-Jun; black bars, total c-Jun. * P < 0.05 for phospho-c-Jun compared with total c-Jun in allergic C57BL/6 mice. # P < 0.05 for phospho-c-Jun in allergic C57BL/6 *Gstp*-null mice compared with phospho-c-Jun in allergic C57BL/6 WT mice.

expression in C57BL/6 mice, the expression of *Gstp1* remained significantly higher than in either nonallergic or allergic BALB/c mice. Therefore, the efficacy of GSTP in attenuating allergic airways disease directly correlates with variation in the expression levels of *Gstp1* in C57BL/6 and BALB/c mice.

The Ability of GSTP1 to Suppress Allergic Responses Independent of c-Jun Phosphorylation

Molecular dynamics and functional studies have shown that a peptide corresponding to residues 194–201 of GSTP1 efficiently blocks this protein's ability to interact with the JNK/Jun heterodimer and inhibit the phosphorylation of c-Jun (37, 38). Because we observed that enhanced allergic responses in C57BL/6 *Gstp*-null mice correlated with elevated levels of c-Jun phosphorylation (Figure 3), we investigated whether blocking the ability of GSTP to inhibit c-Jun phosphorylation *in vivo* influenced the development of allergic responses. The GSTP1 194–201

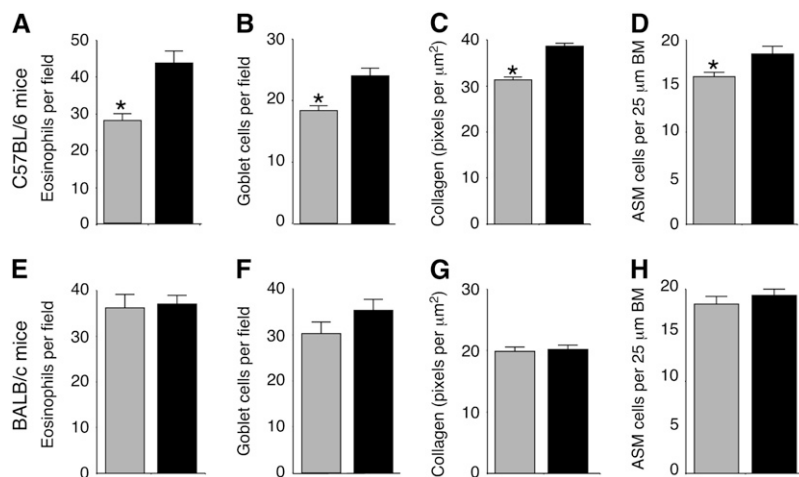
blocking peptide and a scrambled control peptide were synthesized as a fusion with protein transduction domain (PTD) 4, which is a modified HIV-TAT-derived transduction domain that enables transmembrane protein transduction *in vivo* (38). Compared with treatment with the PTD4/scrambled peptide, C57BL/6 WT mice treated with the PTD4/194–201 peptide during aeroallergen challenge exhibited significantly attenuated recruitment of eosinophils to the lungs, attenuated AHR (airway resistance), and reduced Th2 cytokine responses by MLN. (Figures 7A–7E). PTD4/194–201 peptide treatment also significantly enhanced the levels of c-Jun phosphorylation in the lungs (Figure 7F). These data suggest that the ability of GSTP to attenuate allergic responses in the lungs of C57BL/6 mice is unrelated to its ability to attenuate c-Jun phosphorylation.

DISCUSSION

Multiple studies of diverse population groups have linked genetic variation in human *GSTP1* with enhanced susceptibility to asthma and the severity of symptoms (14–20). In order to gain insight into the role this enzyme plays in pathophysiological processes that are pathognomic of asthma, our experiments were aimed to specifically assess the influence of GSTP1/2 on the development of allergic airways disease.

Our findings demonstrate that GSTP1/2 is critically important in attenuating the severity of eosinophilia and goblet cell hyperplasia in allergic C57BL/6 mice. In addition, GSTP1/2 suppressed expression of the alternatively activated macrophage indicator and Th2-dependent marker, Ym1/2 (35), and IL-5 and IFN- γ production by the MLN. Importantly, GSTP1/2 was particularly potent in suppressing allergy-induced respiratory dysfunction in allergic C57BL/6 mice, and depletion of GSTP1/2 allowed the development of AHR in what is a normally hyporesponsive strain of mice. The reasons underlying the strain-dependent genetic propensity for allergen-induced AHR in mice are largely obscure, but may be fundamental to dissecting out regulatory pathways that protect individuals with asthma from inflammation-induced bronchospasm. It is proposed that mouse strain-dependent spatial differences in inflammation may influence the development of AHR, and that eosinophils do not localize in the peribronchial region as efficiently in C57BL/6 mice as in BALB/c mice (40). Although the link between eosinophils and AHR remains controversial, we noted that depletion of GSTP1/2 significantly enhanced peribronchial eosinophilia, and it may be that spatial differences in eosinophil localization in allergic C57BL/6 WT and *Gstp*-null mice contribute to the ability of allergen to induce AHR in *Gstp*-null mice. Notably, the observation that GSTP1/2 influences AHR in mice is in congruence with studies of subjects with asthma showing that the frequency of the GSTP1 Val105 allele inversely correlates with the severity of airflow obstruction and protection against the development of AHR (14, 15).

In direct contrast, GSTP1/2 was much less efficient in attenuating allergic airways disease in BALB/c mice. The numbers of eosinophils and macrophages in the BALF were significantly enhanced in allergic *Gstp*-null mice when compared with allergic WT mice. As such, it seems that GSTP1/2 attenuated infiltration of eosinophils into the BALF in BALB/c mice to a degree, and this may be related to its ability to suppress eotaxin-2. However, blood and tissue eosinophilia, goblet cell hyperplasia, Ym1/2 expression, and AHR were similar in allergic BALB/c WT and *Gstp*-null mice. Therefore, the efficacy with which GSTP1/2 attenuates allergic airways disease is critically dependent on the genetic background of mice in which the *Gstp1/2* genes have been deleted. Comparison of responses in the allergic C57BL/6 and BALB/c WT strains showed that



chaoles of similar diameter per mouse. All values are the mean $\pm$ SEM of four mice per group. * $P < 0.05$ compared with the *Gstp*-null group. Gray bars, allergic WT mice; black bars, allergic *Gstp*-null mice.

BALB/c mice developed significant allergy-induced AHR and more robust peribronchial eosinophilia and goblet cell hyperplasia than allergic C57BL/6 mice. In contrast, the absence of GSTP1/2 in C57BL/6 *Gstp*-null mice pushed allergic responses to levels that approximated those in allergic BALB/c WT mice. These data suggest that BALB/c WT mice develop more robust allergic responses than C57BL/6 WT mice because GSTP1/2 functions less efficiently in BALB/c mice to suppress allergic airways disease and allergen-induced respiratory dysfunction.

In order to mimic the chronic antigen exposure experienced by subjects with asthma and to determine if prolonged antigen exposure could stimulate the ability of GSTP1/2 to protect against allergic responses in BALB/c mice, we employed a pre-

viously characterized chronic model of allergic airways disease (34). Whereas GSTP1/2 attenuated eosinophilia, goblet cell hyperplasia, fibrosis, and airway smooth muscle proliferation in C57BL/6 mice, GSTP1/2 played little role in modulating these processes in allergic BALB/c mice.

IL-5 is well recognized as a central mediator of eosinophil differentiation, activation, trafficking, and survival (41). When we quantified cytokine production by the MLNs, it was apparent that GSTP1/2 significantly attenuated production of IL-5 and IFN- γ , but not eotaxin, in allergic C57BL/6 mice. We have previously shown that IL-5 not only critically regulates eosinophil recruitment to the lung, but also promotes allergen-induced respiratory dysfunction and lung damage in C57BL/6 mice (42). This suggests the ability of GSTP1/2 to attenuate IL-5 contributes to the mechanism by which this enzyme regulates eosinophilia and AHR in C57BL/6 mice. In direct contrast, deletion of GSTP1/2 in allergic BALB/c mice failed to enhance IL-5 or peribronchial eosinophilia.

As GSTP1 can influence the ability of JNK and tumor necrosis factor receptor-associated factor to phosphorylate c-Jun (23–25), we investigated whether GSTP1/2 modulates phosphorylation of c-Jun in the MLN of allergic mice. It is relevant to note that activator protein-1 (comprising c-Fos/c-Jun) is extremely effective at promoting transcription of both mouse and human IL-5 (43, 44). This is consistent with studies showing that treatment of allergic mice with the JNK inhibitor, SP600125, dose-dependently suppressed IL-5 and IFN- γ production (27). Therefore, it seemed likely that the enhanced phosphorylation of c-Jun in allergic C57BL/6 *Gstp*-null mice potentiated IL-5 expression, resulting in enhanced eosinophilic inflammation and allergy-induced AHR. In order to determine whether the ability of GSTP1 to attenuate allergic responses was directly attributable to its inhibition of c-Jun phosphorylation, we treated C57BL/6 WT mice with a peptide comprising the 194–201 region of GSTP1 fused to a modified HIV/TAT PTD4 (39) during the aeroallergen exposure period. Notably, the PTD4 peptide exhibits optimized protein transduction potential, and was recently employed as a delivery system for a dominant-negative STAT6 peptide that, when delivered intranasally, inhibited STAT6 activation in bronchial epithelial cells and ultimately attenuated allergic responses (45). When delivered intranasally, the known capacity of PTD4 to preferentially access bronchial epithelial cells (45) is important, considering that GSTP1 is highly expressed in these cells (11). The

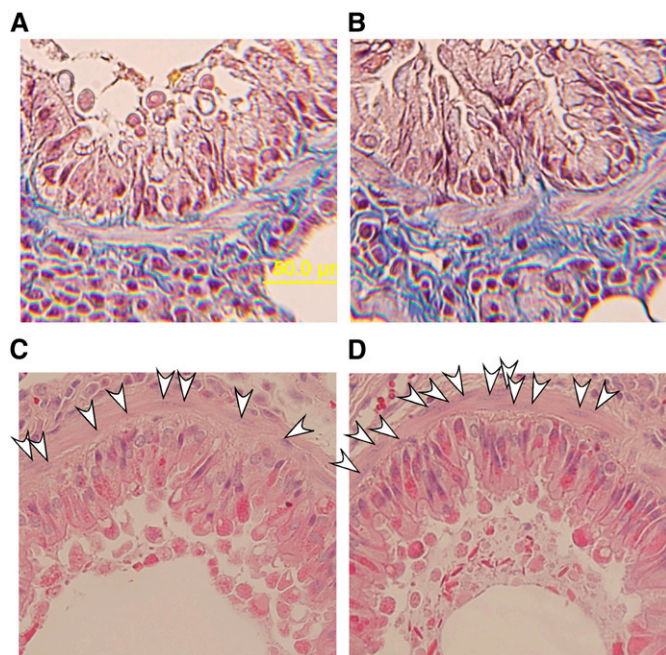


Figure 5. (A and B) Collagen deposition and (C and D) airway smooth muscle proliferation in chronically ovalbumin (OVA)-challenged allergic (A and C) C57BL/6 WT and (B and D) glutathione transferase Pi-null mice. Shown are sections typical for the analysis presented in Figure 4. Arrows in (C) and (D) indicate smooth muscle nuclei.

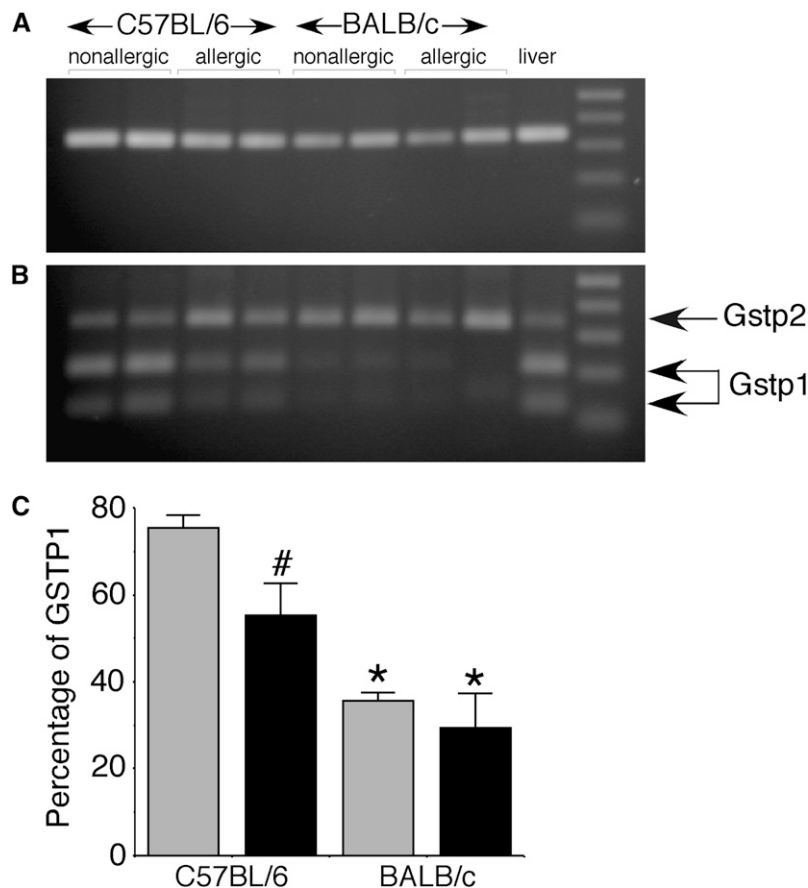


Figure 6. Glutathione transferase Pi (*Gstp1*) and *Gstp2* are differentially expressed in C57BL/6 and BALB/c mice. Reverse transcriptase–polymerase chain reaction (RT-PCR) products for *Gstp1* and *Gstp2* were generated with primers that hybridize to identical regions in both genes and then incubated with *MaeIII*, which digests *Gstp1*, but not *Gstp2*. (A) RT-PCR of total *Gstp* in nonallergic and allergic C57BL/6 and BALB/c mice. For comparison, RT-PCR of liver is shown, which is known to predominantly express *Gstp1*. (B) The same samples digested with *MaeIII*. The right lanes show molecular weight markers ranging from 500 to 100 bp. (C) The relative band density of *Gstp1* expressed as a percentage of the relative band density of total *Gstp* in individual mice. Band density was determined with a Syngene Biolmaging System. Gray bars, nonallergic mice; black bars, allergic mice. * $P < 0.05$ for *Gstp1* in BALB/c mice compared with C57BL/6 mice. # $P < 0.05$ for *Gstp1* in allergic C57BL/6 mice compared with nonallergic C57BL/6 mice.

194–201 GSTP domain was originally identified as one that underwent local structural changes on binding of GSTP inhibitors (36), and a peptide corresponding to this region is known to block the binding of GSTP to the JNK/c-Jun complex (37, 38). It would thus be expected that treatment with the PTD4/194–201 peptide would block the inhibitory effects of GSTP1 and allow enhanced c-Jun phosphorylation when delivered *in vivo*. Intranasal treatment of C57BL/6 WT mice with the PTD4/scrambled peptide potentiated airway resistance, as was previously observed in studies using a PTD4/control peptide (45). However, in comparison to PTD4/scrambled peptide-treated mice, the PTD4/194–201 peptide significantly attenuated the recruitment of eosinophils to the lung, Th2 cytokine responses, and airway resistance. Critically, the PTD4/194–201 peptide enhanced phosphorylation of c-Jun, supporting observations *in vitro* and in cultured cells that the 194–201 peptide blocks the ability of GSTP1 to inhibit c-Jun phosphorylation (38). However, the apparent inverse relationship between c-Jun phosphorylation and allergic responses suggests that the mechanism by which GSTP1 attenuates allergic responses is unrelated to its ability to inhibit the JNK/c-Jun axis. Previous observations, that systemic inhibition of JNK suppressed allergic responses (26, 28), indicate that some aspects of the systemic component of allergic airways disease are positively regulated by JNK/c-Jun activation. Because intranasal delivery of fluorochrome-conjugated PTD4 peptide localizes in bronchial epithelial cells (45), the effects of blocking the GSTP/JNK/c-Jun interaction with the PTD4/194–201 peptide likely occurs specifically in these cells. This suggests a distinct compartmentalized relationship in the requirement for activated c-Jun in promoting allergic responses.

GSTP is proposed to exhibit dual functionality involving a switch between the monomeric and dimeric forms of the protein in response to changing cellular requirements (23). We have shown that, in addition to its ability to block the inhibitory interaction between GSTP and JNK/c-Jun, the PTD4/194–201 peptide clearly inhibits allergic airways inflammation. It is possible that the capacity of the 194–201 peptide to block or dissociate the monomeric form of GSTP1 from the JNK/c-Jun dimer (23) promotes GSTP1 dimerization to the enzymatically active form that is capable of conjugating reduced glutathione to electrophilic metabolites and target these compounds for cellular export via glutathione conjugate transporters. Recent data have also disclosed a role for GSTP1/2 in catalyzing S-glutathionylation of proteins to regulate their function. Oxidation of the catalytic cysteine of 1-cysperoxiredoxin, which protects against lipid peroxidation, results in loss of peroxidase activity. However, heterodimerization with GSTP1 mediates S-glutathionylation of the active cysteine and restoration of function (46, 47). Thus, the process by which S-glutathionylation provides a regulatory switch enabling cells to adapt to changing redox conditions can be controlled directly by GSTP1. The complex nature of the function of GSTP1 is also evident in recent microarray analysis of lung tissue from naive *Gstp*-null mice, demonstrating the diversity of genes that are regulated by GSTP1 (48). Of particular interest to the current investigation was the impact on genes regulating mucus production and inflammation (48).

In order to elucidate the mouse strain-dependent disparity in the ability of GSTP1/2 to attenuate allergic airways disease, we determined the relative expression of *Gstp1/2* in the lungs of C57BL/6 and BALB/c mice. Notably, C57BL/6 mice predom-

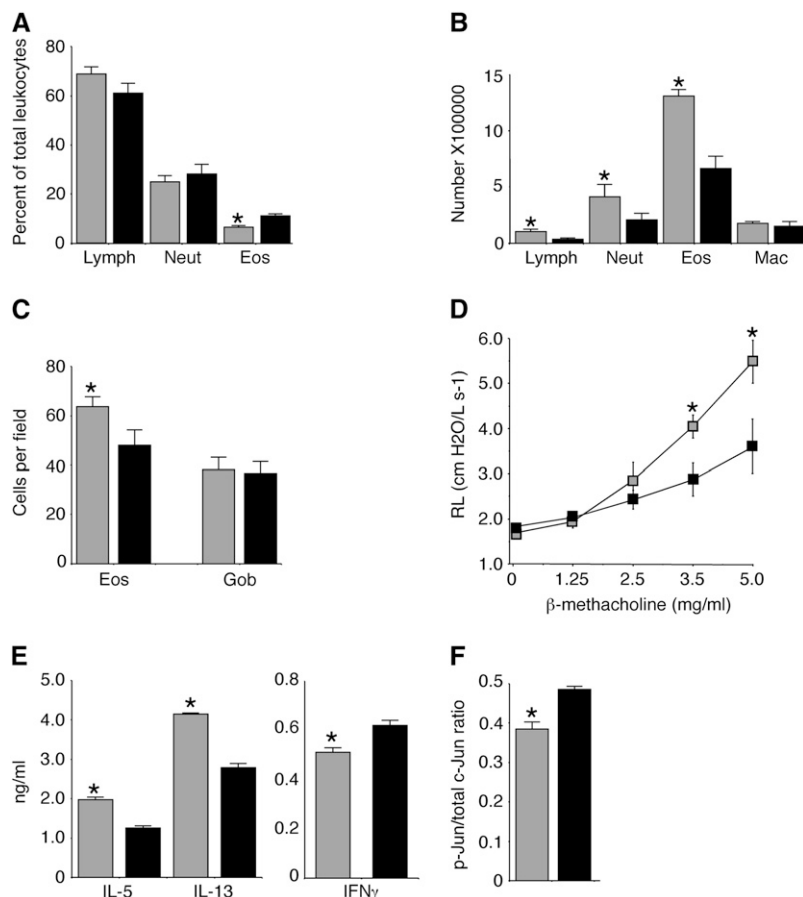


Figure 7. The 194–201 glutathione transferase Pi (GSTP)/ Jun N-terminal kinase (JNK)-inhibitory peptide enhances c-Jun phosphorylation and attenuates allergic responses. C57BL/6 wild-type (WT) mice were treated intranasally with the 40 μ g/mouse PTD4/GSTP194–201 Jun N-terminal kinase (JNK) inhibitory peptide (YARAAAR QARAGGSSPEHVNR) or the PTD4/scrambled peptide (YARAAARQARAGGEPVSHRNS) 2 hours before aeroallergen challenges. These peptides, which were amidated at the carboxy terminus, were purified by reverse-phase chromatography and analyzed by mass spectrometry. The percentages of (A) peripheral blood leukocytes, (B) bronchoalveolar lavage fluid (BALF) leukocytes, (C) peribronchial eosinophils and goblet cells, and (D) lung resistance in peptide-treated allergic mice were determined as described in the legend for Figure 1. Cytokines produced by (E) cultured mediastinal lymph nodes (MLN) cells and the ratio of (F) phospho-c-Jun to total c-Jun were determined by ELISA. Values are the mean $\pm$ SEM of: (A–C) five or six mice per group; (D and F) four mice per group. (E) Values are the mean $\pm$ SEM for three assays of a pool of MLN from five or six mice per group. * $P < 0.05$ for PTD4/scrambled peptide compared with PTD4/GSTP194–201 peptide-treated mice. Gray bars/squares, allergic WT mice treated with PTD4/scrambled peptide; black bars/squares, allergic WT mice treated with PTD4/GSTP194–201.

inantly expressed *Gstp1*, whereas BALB/c mice predominantly expressed *Gstp2*. The mouse GSTP1 and GSTP2 proteins are highly homologous. However, analysis of chimeric constructs and site-directed mutagenesis of amino acids that differ between the two GSTPs have shown that critical amino acid substitutions at positions 11, 12, and 105 render the activity of GSTP2 toward a panel of electrophilic substrates one to three orders of magnitude lower than that of GSTP1 (31, 49). This strongly suggests that GSTP2 would function poorly in detoxification of electrophilic metabolites. Although most studies linking asthma and GSTP1 have focused on allele variation, especially in amino acid 105, it is relevant that individual variation in pulmonary expression of human *GSTP1* has also been noted (50). Epigenetic silencing of human *GSTP1* is known to occur through aberrant methylation in the *GSTP1* promoter in prostate carcinomas (51). However, whether expression levels of *GSTP1* *per se* influence the development of asthma, and whether epigenetic or other factors influence variation in pulmonary expression of human *GSTP1* or interstrain variation in *Gstp1* and *Gstp2* expression in mice, have yet to be investigated.

In summary, our data demonstrate that the ability of GSTP1/2 to attenuate allergic responses directly correlates with the levels of *Gstp1* expression in the mouse lung. Despite a considerable body of epidemiological evidence suggesting a link between the function of GSTP1 and asthma, our study provides the first direct evidence that GSTP1 is important in the pathogenesis and, possibly, the treatment of this disease. Collectively, our data define GSTP1 as an important modulator of allergic airways disease, and indicate that further mechanistic studies beyond linkage analysis are now warranted for enhanced understanding

of what is an important, but complex, asthma-associated molecule.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Knight D. Epithelium-fibroblast interactions in response to airway inflammation. *Immunol Cell Biol* 2001;79:160–164.
2. Reibman J, Hsu Y, Chen LC, Bleck B, Gordon T. Airway epithelial cells release MIP-3 α /CCL20 in response to cytokines and ambient particulate matter. *Am J Respir Cell Mol Biol* 2003;28:648–654.
3. Cookson W. The immunogenetics of asthma and eczema: a new focus on the epithelium. *Nat Rev Immunol* 2004;4:978–988.
4. Davies DE. The bronchial epithelium: translating gene and environment interactions in asthma. *Curr Opin Allergy Clin Immunol* 2001;1:67–71.
5. Buccheri F, Puddicombe SM, Lordan JL, Richter A, Buchanan D, Wilson SJ, Ward J, Zummo G, Howarth PH, Djukanovic R, *et al.* Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. *Am J Respir Cell Mol Biol* 2002;27:179–185.
6. Boldogh I, Bacs A, Choudhury BK, Dharajiya N, Alam R, Hazra TK, Mitra S, Goldblum RM, Sur S. ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflammation. *J Clin Invest* 2005;115:2169–2179.
7. Bowler RP, Crapo JD. Oxidative stress in allergic respiratory diseases. *J Allergy Clin Immunol* 2002;110:349–356.
8. Corradi M, Folesani G, Andreoli R, Manini P, Bodini A, Piacentini G, Carraro S, Zanonato S, Baraldi E. Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation. *Am J Respir Crit Care Med* 2003;167:395–399.
9. Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. *Eur Respir J* 2003;21:177–186.
10. Nadeem A, Raj HG, Chhabra SK. Increased oxidative stress in acute exacerbations of asthma. *J Asthma* 2005;42:45–50.

11. Anttila S, Hirvonen A, Vainio H, Husgafvel-Pursiainen K, Hayes JD, Ketterer B. Immunohistochemical localization of glutathione S-transferases in human lung. *Cancer Res* 1993;53:5643–5648.
12. Board PG, Webb GC, Coggan M. Isolation of a cDNA clone and localization of the human glutathione S-transferase 3 genes to chromosome bands 11q13 and 12q13–14. *Ann Hum Genet* 1989;53:205–213.
13. Moffatt MF, Sharp PA, Faux JA, Young RP, Cookson WO, Hopkin JM. Factors confounding genetic linkage between atopy and chromosome 11q. *Clin Exp Allergy* 1992;22:1046–1051.
14. Spiteri MA, Bianco A, Strange RC, Fryer AA. Polymorphisms at the glutathione S-transferase, GSTP1 locus: a novel mechanism for susceptibility and development of atopic airway inflammation. *Allergy* 2000;55:15–20.
15. Hemmingsen A, Fryer AA, Hepple M, Strange RC, Spiteri MA. Simultaneous identification of GSTP1 Ile105→Val105 and Ala114→Val114 substitutions using an amplification refractory mutation system polymerase chain reaction assay: studies in patients with asthma. *Respir Res* 2001;2:255–260.
16. Mapp CE, Fryer AA, De Marzo N, Pozzato V, Padoan M, Boschetto P, Strange RC, Hemmingsen A, Spiteri MA. Glutathione s-transferase GSTP1 is a susceptibility gene for occupational asthma induced by isocyanates. *J Allergy Clin Immunol* 2002;109:867–872.
17. Aynacioglu AS, Nacak M, Filiz A, Ekinici E, Roots I. Protective role of glutathione S-transferase p1 (Gstp1) val105val genotype in patients with bronchial asthma. *Br J Clin Pharmacol* 2004;57:213–217.
18. Lee YL, Lin YC, Lee YC, Wang JY, Hsiue TR, Guo YL. Glutathione S-transferase p1 gene polymorphism and air pollution as interactive risk factors for childhood asthma. *Clin Exp Allergy* 2004;34:1707–1713.
19. Tamer L, Calikoglu M, Ates NA, Yildirim H, Ercan B, Saritas E, Unlu A, Atik U. Glutathione-S-transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for asthma. *Respirology* 2004;9:493–498.
20. Ercan H, Birben E, Dizdar EA, Keskin O, Karaaslan C, Soyer OU, Dut R, Sackesen C, Besler T, Kalayci O. Oxidative stress and genetic and epidemiologic determinants of oxidant injury in childhood asthma. *J Allergy Clin Immunol* 2006;118:1097–1104.
21. Gilliland FD, Li YF, Saxon A, Diaz-Sanchez D. Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. *Lancet* 2004;363:119–125.
22. Yin Z, Ivanov VN, Habelbah H, Tew K, Ronai Z. Glutathione S-transferase P elicits protection against H₂O₂-induced cell death via coordinated regulation of stress kinases. *Cancer Res* 2000;60:4053–4057.
23. Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, et al. Regulation of JNK signaling by GSTP. *EMBO J* 1999;18:1321–1334.
24. Wang T, Arifoglu P, Ronai Z, Tew KD. Glutathione S-transferase p1-1 (GSTP1-1) inhibits c-Jun N-terminal kinase (JNK1) signaling through interaction with the C terminus. *J Biol Chem* 2001;276:20999–21003.
25. Wu Y, Fan Y, Xue B, Luo L, Shen J, Zhang S, Jiang Y, Yin Z. Human glutathione S-transferase P1-1 interacts with TRAF2 and regulates TRAF2-ASK1 signals. *Oncogene* 2006;25:5787–5800.
26. Eynott PR, Nath P, Leung SY, Adcock IM, Bennett BL, Chung KF. Allergen-induced inflammation and airway epithelial and smooth muscle cell proliferation: role of Jun N-terminal kinase. *Br J Pharmacol* 2003;140:1373–1380.
27. Chialda L, Zhang M, Brune K, Pahl A. Inhibitors of mitogen-activated protein kinases differentially regulate costimulated T cell cytokine production and mouse airway eosinophilia. *Respir Res* 2005;6:36.
28. Nath P, Eynott P, Leung SY, Adcock IM, Bennett BL, Chung KF. Potential role of c-Jun NH₂-terminal kinase in allergic airway inflammation and remodelling: effects of sp600125. *Eur J Pharmacol* 2005;506:273–283.
29. Webb DC, Board PG, Wolf CR, Henderson CJ, Foster PS, Zhou J. The ability of glutathione transferase Pi to suppress eosinophilia, mucus production, remodeling and airways hyperresponsiveness correlates with inhibition of c-Jun phosphorylation and IL-5 in a mouse model of allergic airways disease. *Am J Respir Crit Care Med* 2008;177:A978.
30. Bammler TK, Smith CA, Wolf CR. Isolation and characterization of two mouse Pi-class glutathione S-transferase genes. *Biochem J* 1994;298:385–390.
31. Bammler TK, Driessen H, Finnstrom N, Wolf CR. Amino acid differences at positions 10, 11, and 104 explain the profound catalytic differences between two murine pi-class glutathione S-transferases. *Biochemistry* 1995;34:9000–9008.
32. Henderson CJ, Smith AG, Ure J, Brown K, Bacon EJ, Wolf CR. Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. *Proc Natl Acad Sci USA* 1998;95:5275–5280.
33. Webb DC, Cai Y, Matthaie KI, Foster PS. Comparative roles of IL-4, IL-13, and IL-4 α in dendritic cell maturation and CD4⁺ Th2 cell function. *J Immunol* 2007;178:219–227.
34. McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin Exp Allergy* 2004;34:497–507.
35. Webb DC, McKenzie ANJ, Foster PS. Expression of the Ym2 lectin-binding protein is dependent on interleukin (IL)-4 and IL-13 signal transduction: identification of a novel allergy-associated protein. *J Biol Chem* 2001;276:41969–41976.
36. Siegle JS, Hansbro N, Herbert C, Yang M, Foster PS, Kumar RK. Airway hyperreactivity in exacerbation of chronic asthma is independent of eosinophilic inflammation. *Am J Respir Cell Mol Biol* 2006;35:565–570.
37. Monaco R, Friedman FK, Hyde MJ, Chen JM, Manolatus S, Adler V, Ronai Z, Koslosky W, Pincus MR. Identification of a glutathione-S-transferase effector domain for inhibition of Jun kinase, by molecular dynamics. *J Protein Chem* 1999;18:859–866.
38. Adler V, Pincus MR. Effector peptides from glutathione-S-transferase- π affect the activation of Jun by Jun-N-terminal kinase. *Ann Clin Lab Sci* 2004;34:35–46.
39. Ho A, Schwarze SR, Mermelstein SJ, Waksman G, Dowdy SF. Synthetic protein transduction domains: enhanced transduction potential *in vitro* and *in vivo*. *Cancer Res* 2001;61:474–477.
40. Takeda K, Haczk A, Lee JJ, Irvin CG, Gelfand EW. Strain dependence of airway hyperresponsiveness reflects differences in eosinophil localization in the lung. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L394–L402.
41. Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219–224.
42. Foster PS, Hogan SP, Ramsay AJ, Matthaie KI, Young IG. Interleukin-5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195–201.
43. Wang J, Shannon MF, Young IG. A role for ETS1, synergizing with AP-1 and GATA-3 in the regulation of IL-5 transcription in mouse Th2 lymphocytes. *Int Immunol* 2006;18:313–323.
44. Wang J, Young IG. Eosinophilic inflammation: mechanisms regulating IL-5 transcription in human T lymphocytes. *Allergy* 2007;62:1131–1138.
45. McCusker CT, Wang Y, Shan J, Kinyanjui MW, Villeneuve A, Michael H, Fixman ED. Inhibition of experimental allergic airways disease by local application of a cell-penetrating dominant-negative STAT-6 peptide. *J Immunol* 2007;179:2556–2564.
46. Manevich Y, Feinstein SI, Fisher AB. Activation of the antioxidant enzyme 1-CYS peroxiredoxin requires glutathionylation mediated by heterodimerization with pi GST. *Proc Natl Acad Sci USA* 2004;101:3780–3785.
47. Manevich Y, Fisher AB. Peroxiredoxin 6, a 1-CYS peroxiredoxin, functions in antioxidant defense and lung phospholipid metabolism. *Free Radic Biol Med* 2005;38:1422–1432.
48. Ritchie KJ, Henderson CJ, Wang XJ, Vassieva O, Carrie D, Farmer PB, Gaskell M, Park K, Wolf CR. Glutathione transferase pi plays a critical role in the development of lung carcinogenesis following exposure to tobacco-related carcinogens and urethane. *Cancer Res* 2007;67:9248–9257.
49. Xu X, Stambrook PJ. Two murine GSTPi genes are arranged in tandem and are differentially expressed. *J Biol Chem* 1994;269:30268–30273.
50. Coles B, Yang M, Lang NP, Kadlubar FF. Expression of hGSTP1 alleles in human lung and catalytic activity of the native protein variants towards 1-chloro-2,4-dinitrobenzene, 4-vinylpyridine and (+)-anti benzo[a]pyrene-7,8-diol-9,10-oxide. *Cancer Lett* 2000;156:167–175.
51. Cauchi S, Han W, Kumar SV, Spivack SD. Haplotype-environment interactions that regulate the human glutathione S-transferase P1 promoter. *Cancer Res* 2006;66:6439–6448.