

Downregulation of glutathione S-transferase pi in asthma contributes to enhanced oxidative stress

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Background: Glutathione S-transferase pi (GSTPi) is the predominant redox regulator in the lung. Although evidence implicates an important role for GSTPi in asthma, the mechanism for this has remained elusive.

Objectives: We sought to determine how GSTPi is regulated in asthma and to elucidate its role in maintaining redox homeostasis.

Methods: We elucidated the regulation of GSTPi in children with asthma and used murine models of asthma to determine the role of GSTPi in redox homeostasis.

Results: Our findings demonstrate that GSTPi transcript levels are markedly downregulated in allergen- and IL-13-treated murine models of asthma through signal transducer and activator of transcription 6-dependent and independent pathways. Nuclear factor erythroid 2-related factor 2 was also downregulated in these models. The decrease in GSTPi expression was associated with decreased total glutathione S-transferase activity in the lungs of mice. Examination of cystine intermediates uncovered a functional role for GSTPi in regulating cysteine oxidation, whereby GSTPi-deficient mice exhibited increased oxidative stress (increase in percentage cystine) compared with wild-type mice after allergen challenge. GSTPi expression was similarly downregulated in children with asthma.

Conclusions: These data collectively suggest that downregulation of GSTPi after allergen challenge might contribute to the asthma phenotype because of disruption of redox homeostasis and increased oxidative stress. Furthermore, GSTPi might be an important therapeutic target for asthma, and evaluation of GSTPi expression might prove beneficial in identifying patients who would benefit from therapy targeting this pathway. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Glutathione S-transferase pi, asthma, oxidative stress, redox homeostasis, gene

Asthma, a chronic inflammatory disorder of the airways, affects more than 9 million children (13%) in the United States.¹ A recent review of nearly 500 asthma gene association studies identified 25 genes associated with asthma phenotypes in 6 or more populations.² Among those consistently associated with asthma is the glutathione S-transferase (GST) family of genes. 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,³ cigarette smoke,⁴ and mold.⁵ Each of these environmental factors leads to the generation of reactive oxygen species (ROS) and has been implicated in the development of asthma.⁶⁻⁸ The products of GST catalysis are more water soluble, promoting ROS detoxification and thereby protecting tissues from oxidative damage.⁹

In human subjects GSTs are divided into 8 families: alpha, kappa, mu, omega, pi, sigma, theta, and zeta.¹⁰ A single gene in the pi subfamily, glutathione S-transferase P1 (*GSTP1*), is the predominant cytosolic GST expressed in lung epithelium.¹¹ *GSTP1* is a 2.8-kb gene located on chromosome 11q13, a known “hot spot” for asthma-related genes,^{12,13} and studies by our group and others have demonstrated that an association of mutations in this gene has been associated with asthma.¹⁴⁻¹⁸ Many epidemiologic studies have implicated the *GSTP1* Ile105Val polymorphism (rs1695) as a predictor for asthma. Although the contribution of the *GSTP1* Val¹⁰⁵ allele is not fully understood, the allele has been reported to have significantly lower GST enzyme activity.¹⁹ In addition, we have previously shown that exposure to diesel exhaust particles, environmental tobacco smoke, and mold each conferred an increased risk for wheezing in children who were carriers of the Val¹⁰⁵ allele.¹⁷

Glutathione S-transferase pi (GSTPi) has been shown to play a unique and nonredundant role in total pulmonary GST activity.^{4,20} A recent study with mice deficient in both GSTPi genes (*GSTP1/GSTP2*-deficient mice²⁰) reported an increase in lung resistance compared with that seen in wild-type mice in response to ovalbumin treatment, further supporting a role for GSTPi in allergic airway disease.²¹ Despite the compelling evidence supporting a strong role for GSTPi in asthma, very little is known about the regulation of GSTPi expression in patients with asthma or the molecular mechanism underlying its role in asthma. The aim of this investigation was to determine how GSTPi expression and total GST activity are regulated in asthma and to determine the effect GSTPi on redox homeostasis in murine models of asthma.

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Abbreviations used

AHR: Airway hyperresponsiveness
 APTI: Airway pressure–time index
 Cys: Cysteine
 CySS: Cystine
 GSH: Reduced glutathione
 GST: Glutathione S-transferase
 HDM: House dust mite
 Nrf2: Nuclear factor erythroid 2–related factor 2
 ROS: Reactive oxygen species
 STAT: Signal transducer and activator of transcription

METHODS**Subjects**

Recruitment, nasal mucosal sampling, and RNA isolation from children with and without asthma were previously described.²² Briefly, after institutional review board approval was obtained, healthy and asthmatic children (age 5–18 years old) presenting to Cincinnati Children's Hospital Medical Center were invited to participate in the study. Asthma was diagnosed in accordance with American Thoracic Society criteria.^{7,8} All the children had positive skin prick test responses to at least 1 aeroallergen from an environmental panel that included dust mite, molds, cat, dog, feathers, weeds and ragweed, tree pollens, and grass allergen extracts (Hollister-Stier Laboratories, Spokane, Wash). Healthy control children had no history of chronic illnesses and negative responses to the environmental skin test prick panel indicated above. Exclusion criteria to participate in the study included age of 18 or greater, use of nasal or systemic steroids within the last 30 days, nasal malformations/tumors, and evidence of acute infectious disease. The use of inhaled steroids was not interrupted for this study. Skin prick testing was performed with DermaPiks (Greer Laboratories, Lenoir, NC). Histamine (1 mg/mL) and normal saline (0.9% NaCl) were used as positive and negative controls. Reactions were considered positive if there was an erythematous base with a wheal of 3 mm or more in diameter. Nasal mucosal sampling and RNA isolation were performed with a CytoSoft Brush (Medical Packaging Corp, Camarillo, Calif), and the sample was immediately taken to the laboratory for processing and RNA isolation, as previously described.²² The sex (male/female) and race (African American/white) ratios were 7:3 and 8:2 for the control group and 7:3 and 7:3 for the asthma group. The children in the asthma group were predominantly African American male subjects, which agrees with published data regarding the racial and sex distributions of childhood asthma in urban environments.^{15–17}

Animals and care

Animals were maintained in a pathogen-free vivarium under institutional animal care by using committee-approved procedures.

Allergen treatment of mice

Wild-type C57Bl/6 and BALB/c mice (Jackson Laboratory, Bar Harbor, Me), IL-13^{−/−} mice kindly provided by Andrew McKenzie (Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom),²³ signal transducer and activator of transcription–deficient (STAT6^{−/−}) mice (Jackson Laboratory), and GSTPi^{−/−} mice (kindly provided by Colin J. Henderson, University of Dundee, Dundee, Scotland)²⁰ backcrossed for at least 6 generations with C57Bl/6 mice were immunized with house dust mite (HDM) extract (*Dermatophagoides pteronyssinus*; Greer Laboratories), as previously described.²⁴ For the kinetic experiments, intratracheal challenges were performed at 1-week increments by using 100 µg of HDM. One day after the last challenge, airway hyperresponsiveness (AHR) to acetylcholine (50 µg/kg) was measured as the airway pressure–time index (APTI).²⁵ Blood, bronchoalveolar lavage fluid, and lung tissues were harvested.

Wild-type BALB/c mice were intratracheally challenged with 100 µg of *Aspergillus fumigatus* crude protein extract (Greer Laboratories), as previously described.^{26,27}

IL-13 treatment of mice

Wild-type C57Bl/6 and BALB/c, STAT6^{−/−}, and GSTPi^{−/−} mice were intratracheally challenged with 5 µg of human IL-13 (Peprotech, Rocky Hill, NJ) on days 0, 3, and 6. On day 7, APTI was used to measure AHR; blood, bronchoalveolar lavage fluid, and lung tissues were harvested.

Quantitative RT-PCR

Total lung RNA isolation and real-time PCR normalized to glyceraldehyde-3-phosphate dehydrogenase or 18S rRNA²⁸ were performed as previously described.²⁹ cDNA was generated with SuperScript First-strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, Calif). Primers for murine GSTPi (forward, 5'-ATCTTGAGACACCTTGGC-3'; reverse, 5'-CCTTCACGTAGTCATTCTTACC-3') were designed by using LightCycler Probe Design Software 2.0 (Roche Diagnostics, GmbH, Mannheim, Germany). Remaining primers were previously published.^{30–33}

Total GST activity in murine lung

Ten micrograms of lung protein processed in 0.1 mol/L sodium phosphate buffer (pH 6.5) containing 2 mmol/L EDTA quantified with Coomassie Plus Protein Assay Reagent (Thermo Scientific) was used to assess GST activity for 6 minutes by using the GST assay kit (linear range of detection, 0–0.25 mg/mL GST; Sigma-Aldrich, St Louis, Mo). Experimental OD₃₄₀ values fell within the range of the manufacturer's GST control.

Sample collection and analysis of cysteine and cystine

Cysteine (Cys) and cystine (CySS) levels were measured by using HPLC, as previously described.³⁴

GSTPi Immunohistochemistry

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 1:150 dilution of rabbit anti-GSTPi antibodies (kindly provided by Colin J. Henderson). Sections were washed and incubated with biotinylated secondary antibody. Slides were developed with a peroxidase-labeled avidin detection system (Vector Laboratories, Burlingame, Calif).

Statistical analysis

Statistical significance between 2 groups was determined by using a 2-tailed *t* test and between multiple groups by using 1-way ANOVA followed by a Tukey–Kramer posttest. Significance was determined as a *P* value of .05 or less, as determined with PRISM software (GraphPad Software, Inc, La Jolla, Calif).

RESULTS**GSTPi expression and total GST activity are decreased in allergen-challenged mice**

We examined GSTPi expression and total GST activity in several different murine models of asthma. Challenge with HDM or *A fumigatus* induced a significant increase in AHR, as measured by means of APTI after allergen challenge, as previously expected (data not shown). Contrary to expectations, we observed a marked decrease in the level of GSTPi mRNA expression in the lungs of mice after HDM or *A fumigatus* challenge compared with that seen in control mice (Fig 1, A and C). We also observed a concomitant decrease in total GST activity in the lungs after allergen challenge (Fig 1, B and D). This was not due to a direct inhibitory effect of allergen on GST activity because the addition of up to 100 µg of HDM to the assay had no effect on GST activity (data not shown).

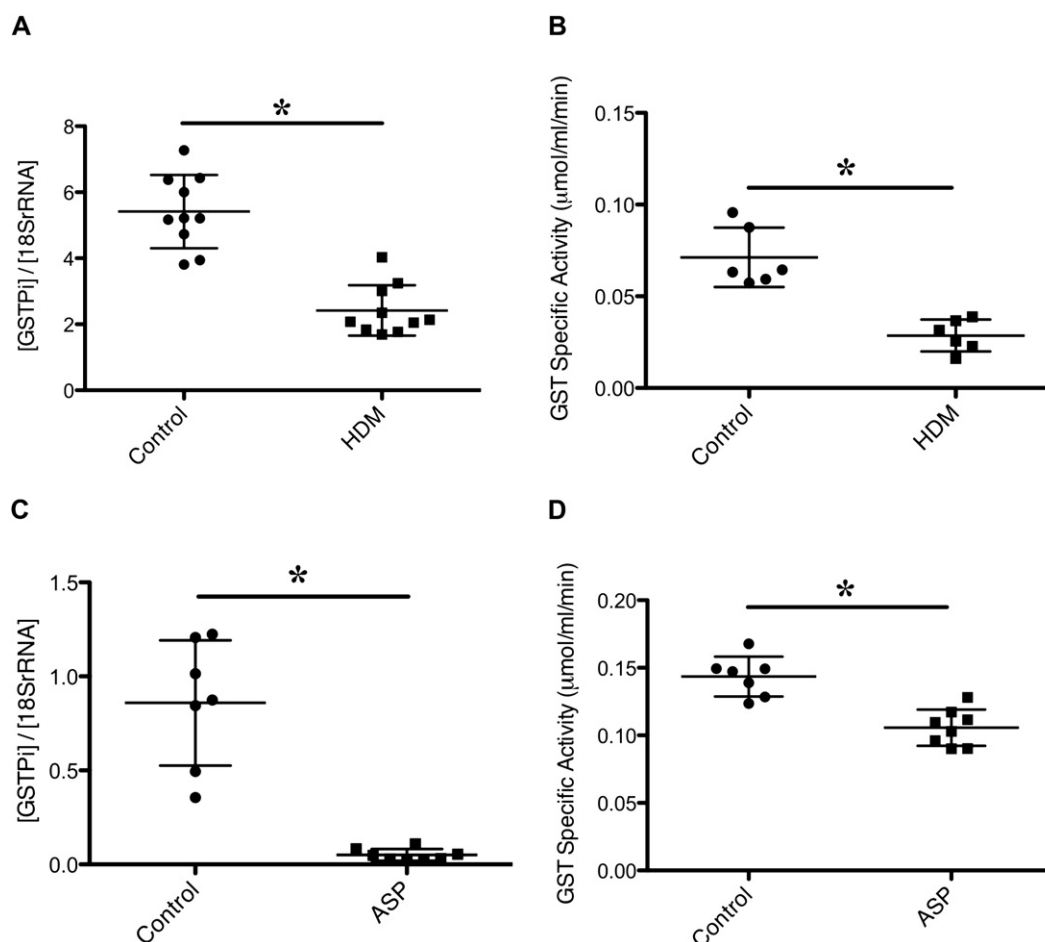


FIG 1. Downregulation of GSTPi expression and total GST activity in the lungs of mice after allergen challenge. BALB/c mice were sensitized intraperitoneally twice and challenged intratracheally twice with HDM (A and B) or challenged intratracheally 9 times with *A fumigatus* (ASP; C and D). Fig 1, A and C, Total lung RNA was analyzed for GSTPi expression by means of real-time RT-PCR. Fig 1, B and D, GST activity in total lung protein. * $P < .05$, mean $\pm$ SD. Data are representative of at least 3 experiments.

Expression of GST family members (GSTK1, GSTM1, and GSTT1) is decreased in allergen-challenged mice

Although it has been reported that GSTPi accounts for more than 90% of the GST activity toward 1-chloro-2,4-dinitrobenzene in the lung,¹¹ other GST family members might also contribute to the total activity. Thus we evaluated the effect of allergen exposure on the expression of the other GST family members also expressed in the lung (GSTK1, GSTM1, and GSTT1). The mu and theta classes (GSTM1 and GSTT1), like the pi class (GSTPi), are cytosolic GSTs, whereas the kappa GSTs (GSTK1) are mitochondrial.³⁵ Similar to GSTPi, lung transcript levels of all the GST family members examined were also significantly attenuated after HDM and *A fumigatus* (Fig 2, A-C).

Kinetics of downregulation of GSTPi expression and total GST activity after allergen challenge

To determine the kinetics of the observed decrease in GSTPi expression and total GST activity after acute and chronic allergen treatment, we analyzed the lungs of mice either after HDM sensitization alone or HDM sensitization followed by weekly

intratracheal challenges for up to 11 weeks. There were no significant differences in either GSTPi expression or total GST activity after intraperitoneal sensitization. However, after the first intratracheal challenge, there was a significant decrease in GSTPi expression (Fig 2, D). Total GST activity was significantly decreased after the second intratracheal challenge (Fig 2, E). After only a single intratracheal challenge without prior sensitization, there was no significant difference in GSTPi expression between HDM- and PBS-treated mice (data not shown). GSTPi expression and total GST activity remained low up to 7 weeks of intratracheal challenge and then returned to control levels. Collectively, these data suggest that the systemic presence of HDM is insufficient to induce downregulation of GSTPi expression and total GST activity. However, sensitization is required for the transient downregulation of GSTPi expression and total GST activity.

IL-13 is sufficient to suppress GSTPi mRNA expression but is not required for allergen-induced downregulation of GSTPi expression and total GST activity downregulation

IL-13 is a key mediator in the pathogenesis of allergic asthma.³⁶ Similar to our observations after allergen treatment,

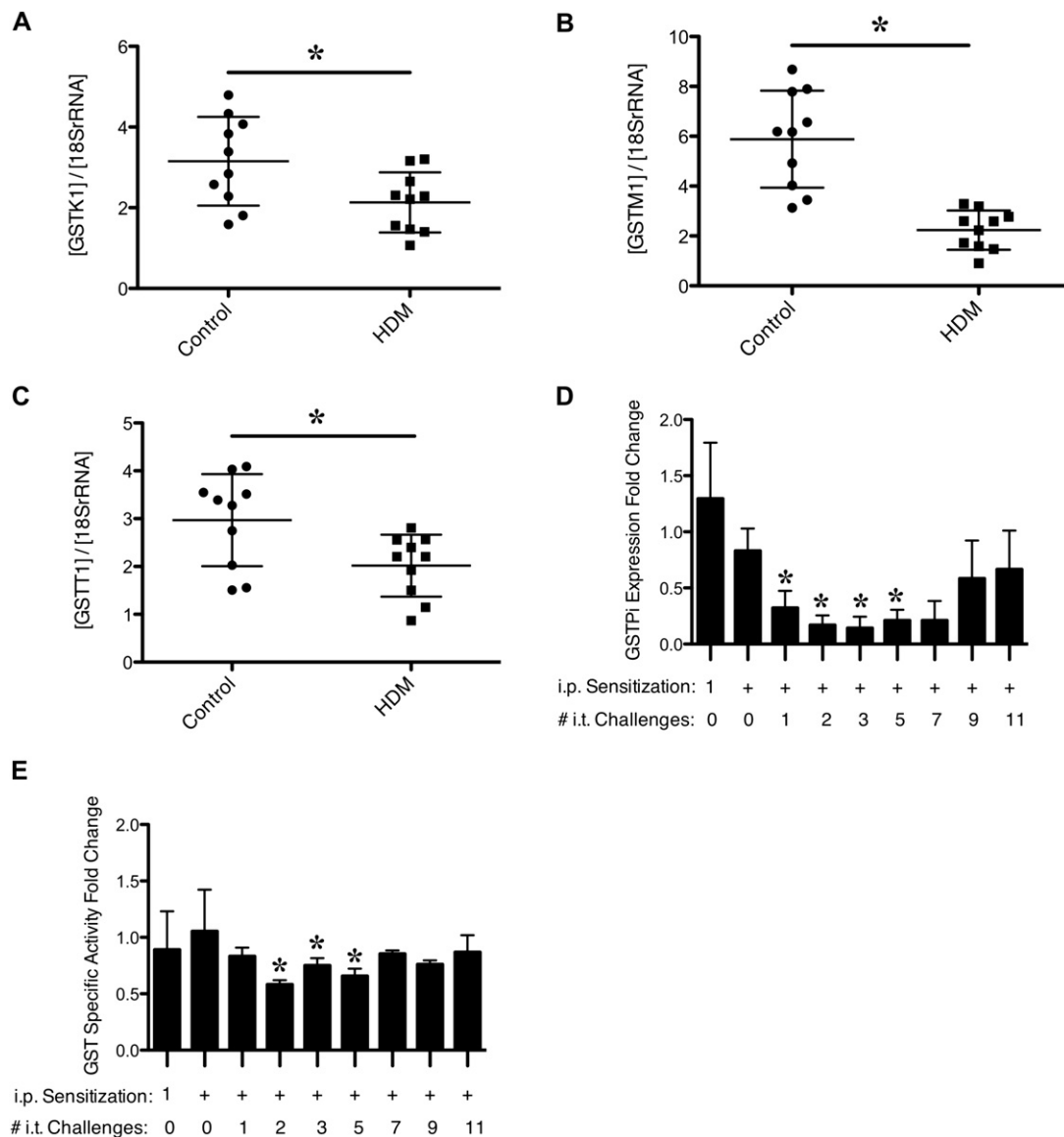


FIG 2. HDM exposure attenuates murine lung expression of GSTK1, GSTM1, and GSTT1. **A-C**, BALB/c mice were sensitized intraperitoneally twice and challenged intratracheally twice with HDM. GSTK1 (Fig 2, A), GSTM1 (Fig 2, B), and GSTT1 (Fig 2, C) RNA expression was determined by means of real-time RT-PCR. * $P < .05$. **D** and **E**, C57BL/6 mice were sensitized intraperitoneally (*i.p.*) once or twice or sensitized intraperitoneally twice and challenged intratracheally (*i.t.*) 1, 2, 3, 5, 7, 9, or 11 times with HDM. Data are represented as fold changes in GSTP1 expression (Fig 2, D) or total GST activity (Fig 2, E) in HDM-treated mice relative to PBS-treated mice. * $P < .05$, HDM- versus PBS-treated mice. By using Bonferroni correction ($P < .004$), there are significant differences between HDM- and PBS-treated mice in both GSTP1 expression and total GST activity after 2 and 3 intratracheal challenges. Values are presented as means $\pm$ SDs ($n = 3$ -5 mice per group). Data are representative of at least 3 experiments.

IL-13 treatment resulted in marked downregulation of GSTP1 expression and total GST activity (Fig 3, A and B).

IL-13-deficient mice were treated with HDM to determine whether IL-13 was necessary for the observed decrease in GSTP1 expression and GST activity. Even in the absence of IL-13, HDM treatment resulted in decreased GSTP1 expression and total GST activity (Fig 3, C and D), indicating that IL-13 is sufficient but not necessary for downregulation of GSTP1 expression.

Allergen-induced GSTP1 mRNA expression and total GST activity downregulation is STAT6 independent

STAT6-deficient mice were treated with HDM to elucidate whether HDM downregulation of GSTP1 was dependent on STAT6. As expected, HDM-exposed wild-type mice displayed increased AHR measured by means of APTI, whereas STAT6-deficient mice treated with HDM did not demonstrate an increase in AHR (data not shown).³⁷ GSTP1 expression and total GST activity were similarly decreased after HDM treatment in wild-type

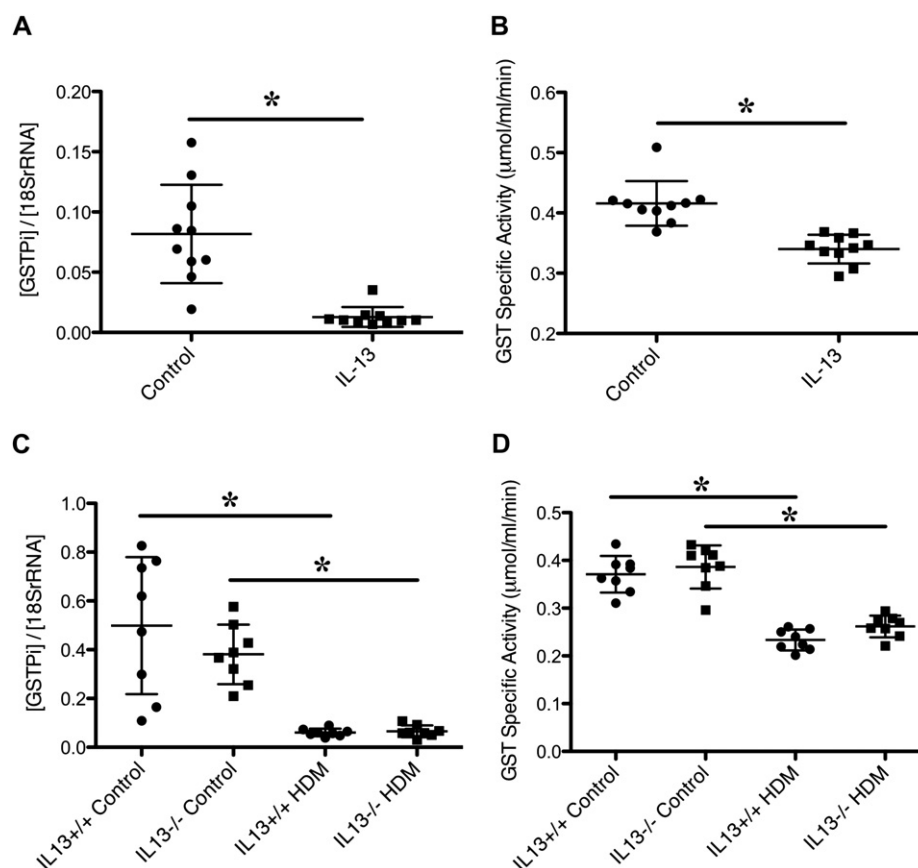


FIG 3. IL-13 is sufficient but not necessary for HDM-induced downregulation of GSTPi expression and total GST activity. **A** and **B**, BALB/c mice were challenged intratracheally 3 times with IL-13. **Fig 3, A**, GSTPi RNA levels in total lung normalized to 18SrRNA. **Fig 3, B**, Total GST activity. **C** and **D**, IL-13^{+/+} and IL-13^{-/-} mice were intratracheally challenged 3 times with HDM and analyzed for GSTPi expression (**Fig 3, C**) and total GST activity (**Fig 3, D**). Data are represented as fold changes in total GST activity in IL-13-treated mice relative to PBS-treated mice. **P* < .05, mean ± SD. Data are representative of 3 experiments.

and STAT6-deficient mice (**Fig 4, A** and **B**). Thus STAT6 is not required for the observed decrease in GSTPi expression and total GST activity after HDM treatment. Interestingly, neither GSTPi expression nor total GST activity changed after IL-13 treatment in STAT6-deficient mice (**Fig 4, C** and **D**). Thus IL-13-induced downregulation of GSTPi expression and total GST activity is STAT6 dependent in contrast to HDM-induced GST downregulation.

Nuclear factor erythroid 2-related factor 2 expression is decreased in allergen- and IL-13-challenged mice

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor involved in the transcriptional regulation of many antioxidant genes, including those involved in the GST pathway.³⁸ In Nrf2-deficient mice GSTPi expression is reduced, demonstrating the obligatory role that Nrf2 plays in GSTPi expression.³⁹ Because Nrf2 is upstream of GSTPi, we examined whether Nrf2 was also downregulated in murine models of asthma. HDM treatment in mice resulted in decreased Nrf2 mRNA levels (**Fig 5, A**). Similar to GSTPi expression, the observed decrease in Nrf2 transcript levels was not allergen specific and was evident after HDM or *A fumigatus* treatment in wild-type

mice (**Fig 5, B**). IL-13 treatment was sufficient to decrease Nrf2 expression levels (**Fig 5, C**). Furthermore, Nrf2 expression levels were significantly depleted in both IL-13^{-/-} and STAT6-deficient mice treated with HDM (**Fig 5, D** and **E**). Together, the data suggest that the decrease in GSTPi expression is a consequence of decreased Nrf2 expression.

Lungs of GSTPi-deficient mice have increased oxidative stress after HDM challenge

Our data revealed that GSTPi expression and total GST activity were significantly downregulated in a murine model of asthma after allergen challenge. One mechanism by which environmental exposures can result in lung injury is by inducing inflammatory cells to generate ROS, leading to oxidative injury.^{40,41} Thus we next determined whether a decrease in GSTPi activity might contribute to increased oxidative stress in an asthma model. GSH, the substrate used by GSTs to catalyze detoxification reactions, is produced from Cys, glycine, and glutamate. Cys availability is often a limiting factor for the rate of GSH synthesis.⁴² Cys and its oxidized disulfide form, CySS, represent the major extracellular thiol/disulfide redox control system in mammals.⁴³ In models of lung injury, Cys/CySS was selectively oxidized early in inflammation, supporting the notion that it is a sensitive marker of redox

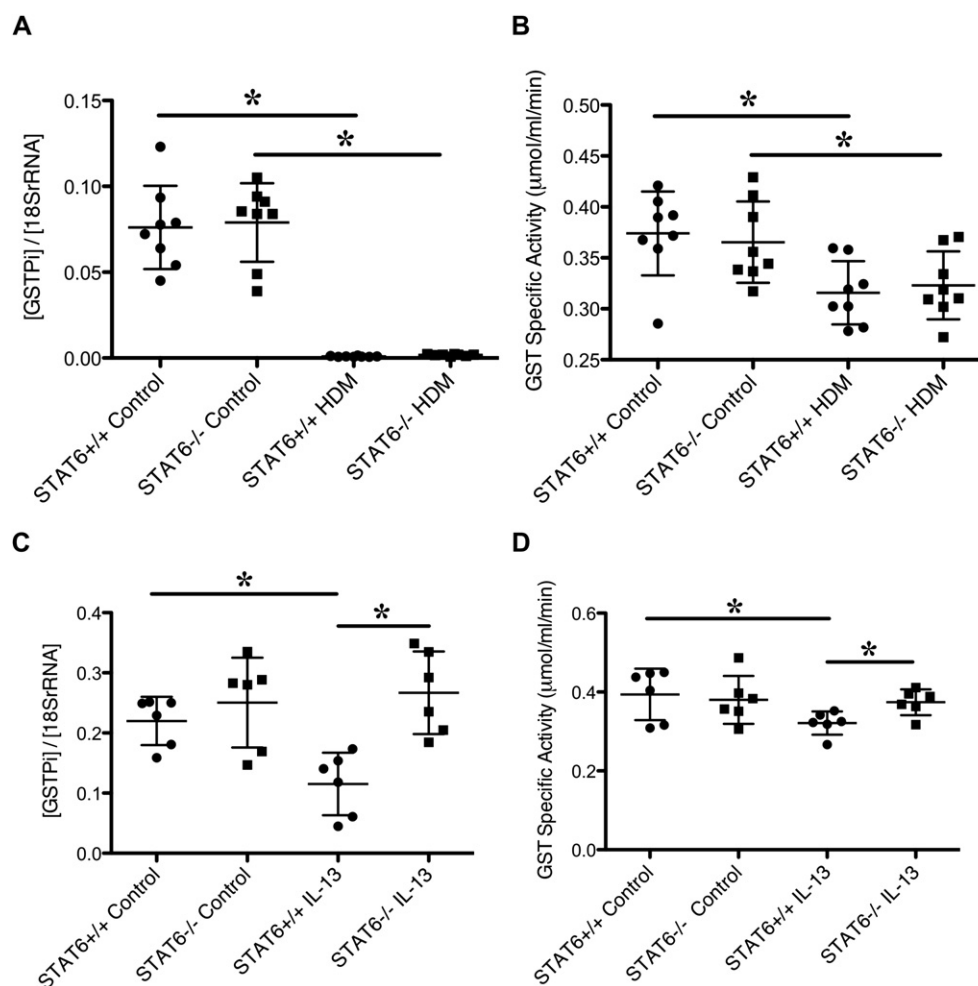


FIG 4. HDM-induced downregulation of GSTPi expression and total GST activity is STAT6-independent, but IL-13-induced downregulation is STAT6 dependent. **A** and **B**, STAT6^{+/+} and STAT6^{-/-} mice were intraperitoneally sensitized twice and intratracheally challenged twice with HDM. **Fig 4, A**, GSTPi RNA expression. **Fig 4, B**, Total GST activity. **C** and **D**, STAT6^{+/+} and STAT6^{-/-} mice were intratracheally challenged 3 times with IL-13 and analyzed for GSTPi expression (**Fig 4, C**) and total GST activity (**Fig 4, D**). **P* < .05, mean ± SD.

homeostasis.⁴⁴ An increase in the percentage of CySS is indicative of oxidative stress. The percentage of CySS (CySS/[Cys + CySS]) was analyzed in the lungs of wild-type and GSTPi-deficient mice after HDM challenge in an asthma model. Wild-type mice did not exhibit any increase in the percentage of CySS after HDM challenge (**Fig 6**), supporting that in the presence of GSTPi, the mice were able to neutralize oxidative stress efficiently. In contrast, the lungs of GSTPi-deficient mice had significantly increased percentages of CySS compared with those of wild-type mice after HDM challenge (*P* < .05, **Fig 6**). Thus a loss in GSTPi activity significantly altered the ability of the lungs to handle the increased oxidative stress burden after allergen challenge.

Localization of GSTPi in the lung

GSTPi immunohistochemistry was performed on murine lungs treated with PBS, HDM, and IL-13 to determine the localization of GSTPi expression in the lung and the relevant cell type or types in which it is regulated. GSTPi expression was expressed

predominantly in epithelial cells, including some expression in type II pneumocytes (**Fig 7, A**). Interestingly, GSTPi expression appeared to be absent in goblet cells induced by either HDM or IL-13 in wild-type mice (**Fig 7, B and C**). No staining was detected in GSTPi-deficient mice (**Fig 7, D-F**). In contrast to wild-type mice, STAT6-deficient mice treated with HDM or IL-13 do not have goblet cell hyperplasia. In these mice GSTPi was mainly expressed in epithelial cells, which is similar to wild-type mice treated with PBS (**Fig 7, G-I**).

GSTPi expression is decreased in nasal epithelial cells of asthmatic children

Our data support a decrease in GSTPi expression after allergen challenge in a murine model of asthma. To evaluate whether GSTPi was similarly downregulated in children with asthma, we quantified GSTPi expression in RNA isolated from nasal epithelial cells of children with asthma, as well as control (nonatopic and nonasthmatic) children. There were no significant differences in age or sex between the 2 groups (data not shown). Similar to the

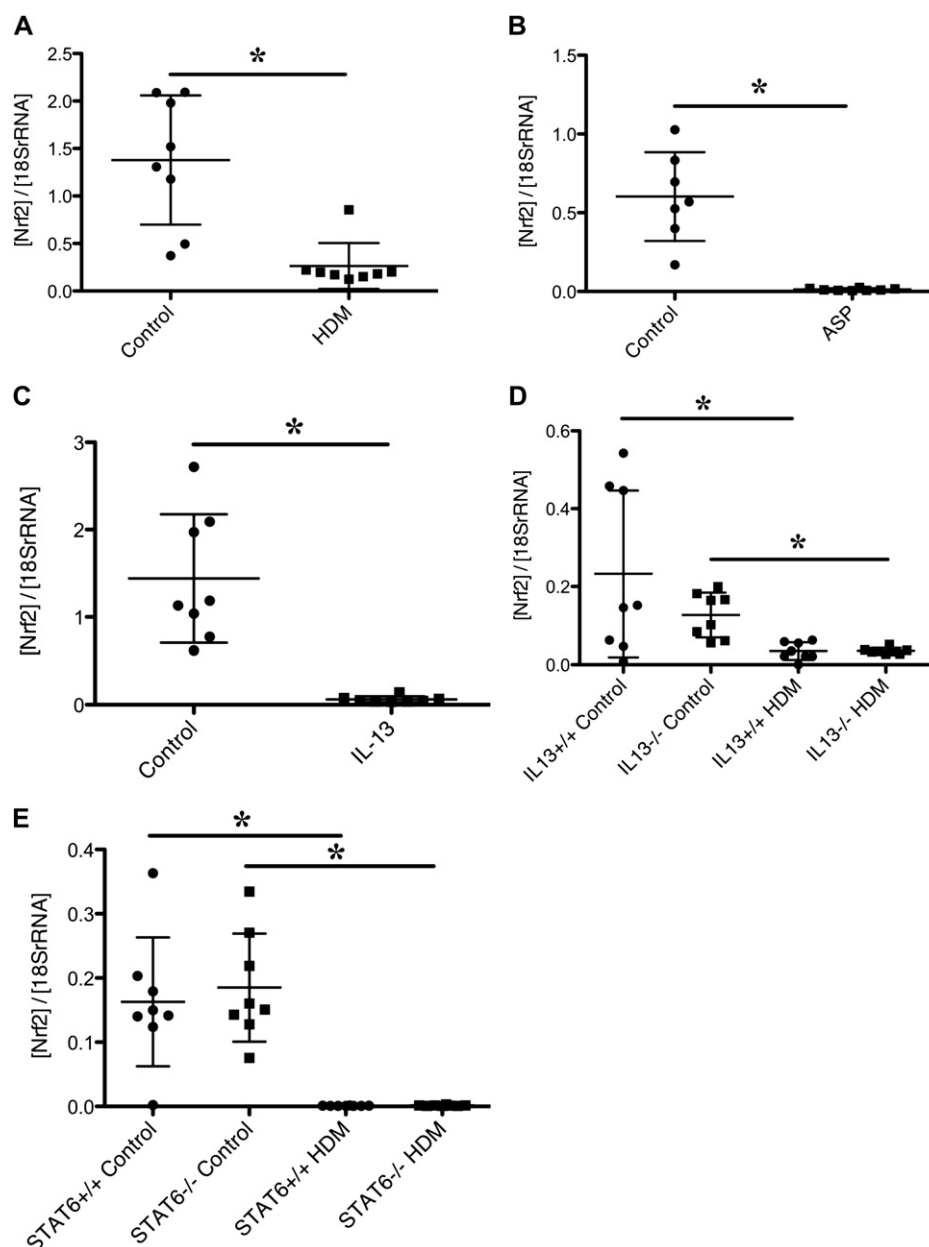


FIG 5. Expression of Nrf2 in murine models of asthma. **A-C**, Total lung RNA was isolated to determine expression levels of Nrf2 in BALB/c mice sensitized intraperitoneally twice and challenged intratracheally twice with HDM (**A**), challenged intratracheally 9 times with *A fumigatus* (ASP; **B**), or challenged intratracheally 3 times with IL-13 (**C**) and in IL-13^{+/+} and IL-13^{-/-} mice (**D**) or STAT6^{+/+} and STAT6^{-/-} mice (**E**) sensitized intraperitoneally twice and challenged intratracheally twice with HDM. **P* < .05, mean ± SD. Data are representative of 3 experiments.

observed downregulation of GSTP α expression in murine models of asthma, GSTP α expression was also attenuated in asthmatic children compared with that seen in nonatopic nonasthmatic control subjects (Fig 8).

DISCUSSION

The contribution of GSTP α to asthma has been supported by epidemiologic, genetic, and animal studies by our group and others,^{14-17,21} although the mechanism for this has remained

elusive. Our findings provide important novel insights into the contribution of GSTP α to asthma in human subjects and mice. Given that several environmental exposures associated with asthma result in enhanced oxidative stress, one might predict that GST enzymes would be induced to increase the capacity to handle the increased electrophilic load and restore redox homeostasis. However, we found that GSTP α transcript levels are markedly downregulated in murine models of asthma after allergen challenge through STAT6-dependent and independent pathways. The observed downregulation was not unique to a specific

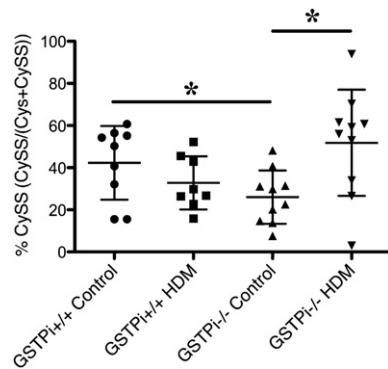


FIG 6. Oxidative stress is increased in GSTP1^{-/-} but not wild-type mice in an allergen-induced asthma model. Wild-type C57Bl/6 and GSTP1^{-/-} mice were sensitized intraperitoneally twice and challenged intratracheally twice with HDM. Lung tissue was collected for HPLC analysis of Cys and CySS. * $P < .05$, mean $\pm$ SD.

allergen. The decrease in GSTP1 expression was associated with decreased total GST activity in the lungs of mice. Levels of GSTP1 mRNA have been shown to correlate to GST activity in other studies ($R^2 = 0.77$, $P < .001$).⁴⁵ GSTP1 was downregulated in BALB/c and C57Bl/6 strains of mice, supporting that the observation was not restricted to a given strain. Furthermore, GSTP1 expression was similarly downregulated in children with asthma. Our findings also uncovered a functional role for GSTP1 in regulating Cys oxidation, revealing that GSTP1 plays an important role in neutralizing oxidative stress in asthma. These data collectively suggest that downregulation of GSTP1 after allergen challenge might result in disruption of redox homeostasis and increased oxidative stress. Interestingly, patients with severe asthma have been found to have lower airway GSH levels with increased oxidized glutathione levels, which is consistent with enhanced oxidative stress.⁴⁶

Perturbations in the extracellular thiol/disulfide redox environment have been shown to correlate with the progression and severity of lung injury.⁴⁷ Cys and its disulfide, CySS, constitute the most abundant, low-molecular-weight thiol/disulfide redox couple in the plasma, and Cys homeostasis is adversely affected during the inflammatory response. In models of lung injury, Cys/CySS was selectively oxidized early in inflammation, supporting that it is a sensitive marker of redox homeostasis.⁴⁴ In the absence of GSTP1, the lungs of GSTP1-deficient mice had significantly increased percentages of CySS after allergen challenge, supporting that a loss in GSTP1 activity significantly altered the ability of the lungs to handle the increased oxidative stress burden after allergen challenge. Key pathways relevant to allergic inflammation have been shown to be sensitive to Cys redox homeostasis,⁴⁸ and thus an increase in the percentage of CySS might have an effect on cytokine signaling in addition to the other effects of ROS.

Because GSTP1 is an enzyme that plays a critical role in cellular detoxification of endogenous and xenobiotic substrates and protection against oxidative stress, it is not surprising that evidence exists supporting a substantial role for GSTP1 in allergic asthma. We had predicted that there would be an increased necessity for GSTP1-driven detoxification in the lung marked by an increase in GSTP1 expression and total GST activity after allergen exposure. Therefore, it was surprising to observe a

decrease in GSTP1 expression and total GST activity after allergen exposure. One potential explanation for this apparent paradox is that the observed GSTP1 downregulation is transient. Downregulation of GSTP1 expression was evident after sensitization and a single intratracheal challenge and remained low for several weeks. However, GSTP1 levels and activity normalized. Because asthma is a chronic disease, the effect of the downregulation might not be as evident in a more chronic model of exposure. The surprising decrease in GSTP1 expression and total GST activity could also indicate that GSTP1 is maladaptive in response to allergen exposure.

The mechanism by which downregulation of GSTP1 expression and total GST activity occurs might involve Nrf2. HDM- and IL-13-treated mice displayed decreased Nrf2 expression in the lungs. IL-13-deficient and STAT6-deficient mice also display decreased Nrf2 expression after HDM exposure. Thus although IL-13 contributes to HDM-induced Nrf2 downregulation, neither IL-13 nor STAT6 is necessary for allergen-induced downregulation of Nrf2. There is an alternate pathway downstream of allergen treatment that results in the observed downregulation in an IL-13- and STAT6-independent manner. Similarly, IL-13-induced, but not HDM-induced, GSTP1 downregulation was STAT6 dependent. Thus HDM can induce downregulation of GSTP1 expression and total GST activity by IL-13- and STAT6-autonomous pathways that involve Nrf2. Furthermore, Nrf2 was found to be an important modulator for mounting an appropriate innate immune response during experimental sepsis.⁴⁹ Thus transient downregulation of Nrf2 and GSTP1 after allergen exposure could signal and increase in proinflammatory cytokine and chemokine levels or the initiation of downstream detoxification pathways.

Our findings are consistent with those of previous studies that have demonstrated a correlation between GST activity and the level of GSTP1 mRNA expression ($R^2 = 0.77$, $P < .001$).⁴⁵ We observed a decrease in GSTP1 gene expression after sensitization and 1 intratracheal challenge, whereas the total GST activity did not decrease until after 2 intratracheal challenges. This suggests that the observed decrease in GSTP1 gene expression precedes the decrease in total GST activity. Surprisingly, we did not observe a change in GSTP1 protein expression analyzed by means of Western blotting (data not shown). One reason for this might be that the assay used for measuring GSTP1 protein expression in the lung might not be sensitive enough to detect modest changes in protein levels. Because the antibody does not distinguish between GSTP1 and GSTP2, it is also possible that one subtype is conserved or even upregulated relative to the other.

The observed decrease in GSTP1 expression and total GST activity is not likely due to the diluting effect of migratory cells infiltrating the lung in response to allergen exposure. STAT6-deficient mice have marked attenuation of lung inflammation, AHR, and mucus production.^{50,51} Because STAT6-deficient mice have diminished airway inflammation, a decrease in GSTP1 expression and total GST activity in STAT6-deficient mice treated with HDM indicates that this decrease is likely due to resident cells and not migratory cells.

In summary, GSTP1 expression and total GST activity are dysregulated in murine models of asthma. GSTP1 is similarly dysregulated in human asthma. GSTP1 plays an important role in neutralizing oxidative stress. The antioxidant and detoxification capacity of GSTP1 and its potential role in GSH homeostasis suggest that the GSTP1 pathway could be a critical

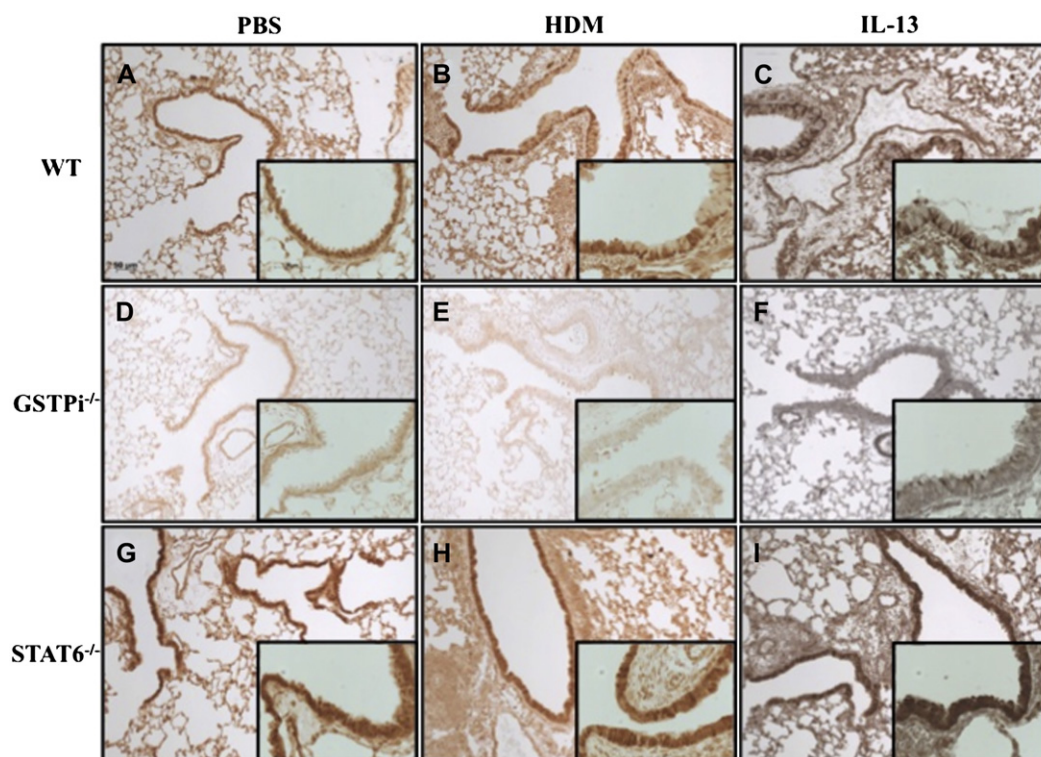


FIG 7. Localization of GSTPi in the lung. Wild-type, GSTPi^{-/-}, and STAT6^{-/-} mice were treated with PBS, HDM, or IL-13 and analyzed for GSTPi expression in the lung by means of immunohistochemistry. **A**, Wild-type mice treated with PBS displayed positive GSTPi expression predominantly in epithelial cells. **B** and **C**, GSTPi expression was not detected in goblet cells of wild-type mice treated with HDM or IL-13. **D-F**, No staining was detected in GSTPi^{-/-} mice treated with either PBS, HDM, or IL-13. **G-I**, GSTPi expression in STAT6^{-/-} mice treated with PBS, HDM, or IL-13 was mainly found in epithelial cells. All images were captured at $\times 100$ magnification, and inset images were captured at $\times 400$ magnification. WT, Wild-type.

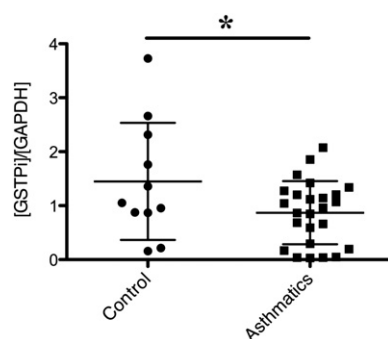


FIG 8. GSTPi expression is decreased in nasal epithelial cells from children with asthma. Children were divided into 2 groups: control subjects (non-atopic nonasthmatic children) and children with asthma (*Asthmatics*). Values are presented as means $\pm$ SDs. * $P < .05$.

therapeutic target for asthma, especially in response to environmental exposures. In fact, GSH has already been shown to alleviate IL-13-induced asthma in mice.⁵² Evaluation of GSTPi expression or GSH homeostasis might prove beneficial in identifying patients who would benefit from therapy targeting this pathway.

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Key messages

- In children with asthma and in a murine model of asthma, GSTPi expression was markedly downregulated.
- This downregulation of GSTPi might contribute directly to the asthma phenotype by disruption of redox homeostasis, resulting in increased oxidative stress.

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