Lung Lining Fluid Glutathione Attenuates IL-13-Induced Asthma

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GGT^{enu1} mice, deficient in γ -glutamyl transferase and unable to metabolize extracellular glutathione, develop intracellular glutathione deficiency and oxidant stress. We used intratracheal IL-13 to induce airway inflammation and asthma in wild-type (WT) and GGT^{enu1} mice to determine the effect of altered glutathione metabolism on bronchial asthma. WT and GGT^{enu1} mice developed similar degrees of lung inflammation. In contrast, IL-13 induced airway epithelial cell mucous cell hyperplasia, mucin and mucin-related gene expression, epidermal growth factor receptor mRNA, and epidermal growth factor receptor activation along with airway hyperreactivity in WT mice but not in GGT^{enu1} mice. Lung lining fluid (extracellular) glutathione was 10-fold greater in GGT^{enu1} than in WT lungs, providing increased buffering of inflammation-associated reactive oxygen species. Pharmacologic inhibition of GGT in WT mice produced similar effects, suggesting that the lung lining fluid glutathione protects against epithelial cell induction of asthma. Inhibiting GGT activity in lung lining fluid may represent a novel therapeutic approach for preventing and treating asthma.

Keywords: glutathione; lung lining fluid; asthma

Oxidant stress is believed to be important in asthma pathogenesis (1). Nrf2 (nuclear factor, erythroid derived 2, like 2) null mice display increased susceptibility to airway inflammation and airway hyperreactivity in an ovalbumin model of experimental asthma (2). Although this transcription factor regulates an array of antioxidant genes, at least part of this antioxidant imbalance seems to involve glutathione because glutathione content and the ratio of glutathione/oxidized glutathione (redox ratio) increase in the ovalbumin-sensitized lung of normal mice but not in that of Nrf2-null mice. Conversely, wild-type (WT) mice display decreased susceptibility to airway inflammation and airway hyperreactivity in the same experimental model when supplemented with membrane-permeable γ -glutamylcysteinylethyl ester. This glutathione precursor, delivered via the peritoneum, augments lung glutathione content, increases the glutathione redox ratio, and alters Th1/Th2 imbalance to decrease eosinophil migration (3).

Glutathione is normally abundant in lung cells and in the extracellular lining fluid that bathes the gas exchange surface (4).

CLINICAL RELEVANCE

We identify gamma-glutamyl transferase (GGT) as a target to treat asthma. GGT regulates glutathione metabolism in lung lining fluid. Inhibition of GGT augments lining fluid glutathione, buffers oxidants derived from inflammation, and attenuates asthma.

Extracellular glutathione is metabolized by the enzyme gamma-glutamyl transferase (GGT) (EC 2.3.2.2, which is present in the lung lining fluid (5). In the absence of GGT, extracellular pools of glutathione in the blood, urine (6, 7), and lung lining fluid (8) enlarge due to decreased turnover. However, intracellular glutathione is decreased because cysteine availability, derived from glutathione breakdown, becomes limiting for intracellular glutathione synthesis (7). Cellular glutathione deficiency causes intracellular oxidant stress in GGT-deficient GGT^{enu1} mouse lung in normoxia and hyperoxia (8). We used intratracheal IL-13 to induce experimental asthma (9) in the GGT^{enu1} mouse to test the hypothesis that the GGT^{enu1} mutant would be more susceptible to asthma than the WT mouse due to cellular glutathione deficiency. We show, however, that GGT^{enu1} mice were protected from IL-13-induced asthma, suggesting that increasing lung lining fluid glutathione protects airway epithelial cells from inflammation-induced oxidant stress and thus may provide a new approach to preventing and treating asthma.

MATERIALS AND METHODS

Mouse Model

GGT^{enu1} mice (C57Bl/6J) were bred and genotyped in the Laboratory and Animal Science Center according to protocols approved by the Institutional Utilization and Animal Care Committee at Boston University School of Medicine (10). Instillation of IL-13 was used as a cytokine-driven model of experimental asthma (9). Mice were transiently anesthetized with Metofane and received 5 μ m of IL-13 delivered in 100 μ l of saline via the trachea and dosed for three successive days. Controls received saline alone. Airway physiology was assessed on the fourth day under appropriate anesthesia via an intratracheal catheter using the Scireg flexivent apparatus (SCIREQ, Montreal, PQ, Canada). The lungs were exposed to graded doses of methacholine (0, 5, 10, and 15 μ g/ml), and airway resistance was measured. In separate experiments, lung lining fluid GGT activity was inhibited in WT mice by intratracheal delivery of acivicin, an irreversible GGT antagonist (11).

Bronchoalveolar Lavage Analysis

The lung was lavaged once with 500 μ m of ice-cold saline and aspirated to recover bronchoalveolar lavage fluid (BALF) (99% recovery). Cells were counted on a hemocytometer, and cytospin slides were prepared and stained with Diff-Quik for differential cell counts (8). Cell-free BALF was assessed for IL-13 protein plus eotaxin protein and leukotriene

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C4 and E4 contents by ELISA using the R&D Systems mouse Quantikine kit and the Cayman Chemical kit (Cayman, Ann Arbor, MI), respectively, using an internal standard curve according to the manufacturer's instructions. Cytokine proteins were assayed using the RayBio32-mouse cytokine array (RayBio, Norcross, GA). Signal intensity was determined by densitometry, and values were normalized to internal standards on each blot and then to the saline-treated WT lung (12). Glutathione, cysteine, and GSNO were assayed by HPLC (8, 13). Protein carbonyl content was assayed by ELISA using a kit from Cell Biolabs, Inc. (San Diego, CA) according to manufacturer's instructions using an internal standard curve. Samples were assayed in duplicate or triplicate and normalized to the WT saline-treated lung.

Histology and Immunohistochemistry

Lung tissue was inflation fixed with freshly prepared 4% paraformaldehyde (5, 8). Mucin accumulation was assessed with Alcian blue/periodic acid-Schiff (PAS) stain (14). Native EGF receptor was localized in lung using peptide-specific rabbit antisera from Cell Signaling Technology (Catalogue #2232; Cell Signaling Technology, Beverly, MA) at a dilution of 1:25 and incubation at 4°C overnight. For antigen retrieval, slides were exposed to 50 mM glycine at pH 3.5 and

10 mM EDTA for 10 minutes at 95°C. Activation of EGF receptor was assessed using phospho-specific antisera raised against phosphorylated EGFR from the same company at a dilution of 1:25 and incubation at 4°C overnight (Catalogue #2231; Cell Signaling Technology). For antigen retrieval, slides were exposed to Proteinase K (Zymeda preparation) at a dilution of 1:16 for 8 minutes at 42°C. In each case, signal specificity was confirmed in a competition experiment with the corresponding peptide antigen obtained from Cell Signaling Technology. Sections were stained for peroxidase activity with the Vectabond ABC kit according to the manufacturer's instructions.

Versican was localized with 8 mg/ml of a rabbit anti-mouse versican (GAG b domain) polyclonal antibody (Chemicon International, Temecula, CA). This antibody was raised against a GST fusion protein containing amino acids 1360 through 1439 of mouse versican and recognizes a large molecular weight band by Western blot in aortic and cardiac tissue from mouse and media from mouse smooth muscle cells. All sections were photographed in a Leitz Orthoplan microscope (5, 8).

Real-Time RT-PCR

Lung RNA was isolated with Tri-Reagent, treated with DNase, and quantified by spectrophotometry. Versican mRNA analysis was per-

Figure 1. Lung inflammatory milieu induced by IL-13. (A) Profile of cytokine proteins induced by IL-13 in wild-type (WT) and GGT^{enu1} mouse bronchoalveolar lavage fluid (BALF) compared with WT saline control. (B) Cell differentials in BALF from saline-treated and IL-13-treated WT and GGT^{enu1} mice. (C) Immunohistochemistry for versican protein shows the absence of signal in saline-treated WT (top left) and GGT^{enu1} lung (bottom left) but abundant signal surrounding the airways after IL-13 treatment in WT (top right) and GGT^{enu1} lung (bottom right). Original magnifications: $\times 400$.

formed in the laboratory of Dr. Wight using 2 μ m of total RNA and reverse transcribed in a 40- μ l reaction mix with random primers and the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Relative quantification was performed using the Taqman Gene Expression Assay Mm00490179_m1 to detect all versican splice variants by amplifying across the exon junction of the last C-type lectin domain and the complement control protein module or SUSHI domain in the G3 domain. Gene expression was normalized to eukaryotic 18S rRNA Endogenous Control part no. 4333760 (Applied Biosystems). Messenger RNA analyses for the mucin genes *Muc1*, *Muc2*, *Muc3*, *Muc4*, *Muc5ac*, *Muc5b*, and the mucous granule protein *Clca3* (*gob-5*) were performed in a similar fashion at the Pulmonary Center of Boston University using intron-spanning primer sets with Taqman probes from Applied Biosciences. Target cDNA was quantified using Taqman probes and the standard curve method, and determinations were done with three replicates.

EGF receptor mRNA induction in response to IL-13 was assessed with SYBR green using primers purchased from Superarray Bioscience Corporation (Frederick, MD). Fifty nanograms of cDNA were used in a 50- μ l reaction volume with SYBR Green PCR master mix from Applied Biosciences. Forty cycles of amplification, data acquisition, and data analysis were performed on the ABI Prism 7700 Sequence detector (Applied Biosystems). EGFR was normalized to actin mRNA.

Statistics

Nominal data are presented as means with SE and analyzed by ANOVA using STATISTICA release 4.5 (StatSoft, Inc, 1993). *Post hoc* comparison was done with the Newman-Keuls test, and critical ranges and significant differences among groups are denoted in the figures. *P* values < 0.05 were considered significant.

RESULTS

IL-13 Induces Airway Inflammation in WT and GGT^{enu1} Mice

Bronchoalveolar lavage cytokines. IL-13 protein assayed by ELISA was barely detectable in saline-treated mice, but levels

rose to $\sim$ 1200 pg/ml in IL-13-treated mice of both genotypes. Proinflammatory cytokines assayed by RayBio cytokine protein array were increased by IL-13 to similar levels in WT and GGT^{enu1} mice (Figure 1A). IL-6 was induced on average 15-fold (*P* = 0.017) and IL-12 by 5-fold (*P* = 0.014). This array detected the increase in IL-13 content (*P* = 0.016). Eotaxin protein, an eosinophil chemotactic factor, was also significantly induced in IL-13-treated mice of both genotypes (*see* Table E1 in the online supplement).

Inflammatory cells in bronchoalveolar lavage. IL-13 increased inflammatory cells by 3- to 4-fold in WT and GGT^{enu1} mice, and differential cell counts were similar in both genotypes (Figure 1B). Macrophages predominated in BALF from saline-treated mice (*n* = 4) of both genotypes. Eosinophils predominated after IL-13 treatment (*P* = 0.000006, *dagger* in Figure 1B, even more so in GGT^{enu1}), macrophages declined (*P* = 0.000005), and lymphocytes were unchanged.

IL-13 induction of inflammation-associated proteoglycan. Versican was examined as a proteoglycan that is known to accumulate in the subepithelial layer of human airways in association with inflammation in asthma (15). IL-13 induced versican mRNA by 5- to 6-fold in mice from both genotypes. No immunohistochemical signal for versican was detected in saline-treated mice (Figures 1C and 1E), but an intense signal surrounded the airways (Figures 1D and 1F) and vasculature in IL-13-treated mice of each genotype.

GGT Deficiency Attenuates the Airway Epithelial Cell Response to IL-13

Accumulation of PAS-positive material and *Muc5ac* mRNA. An abundance of PAS-positive material was evident in airway epithelial cells of IL-13-treated WT mice (Figure 2A). In contrast, only sparse amounts of PAS-positive material were present in

Figure 2. IL-13 induces lung mucus accumulation plus mucin and mucin-related gene expression. (A) Periodic acid-Schiff (PAS)-positive signal in airway epithelial cells from IL-13-treated WT mice (top left). (B) Absence of PAS-positive material in IL-13-treated GGT^{enu1} lung. No PAS-positive cells were identified in saline-treated WT (C) or GGT^{enu1} (D) lung. Original magnifications: $\times$ 100. (E) Compared with saline-treated WT control mice, *Muc5ac* mRNA was significantly induced after IL-13 treatment but only in WT lung (asterisk). (F) Chloride channel 3 mRNA, *Clca3* (*gob-5*), was significantly induced after IL-13 treatment but only in WT lung (asterisk).

airway epithelial cells of IL-13-treated GGT^{enu1} mice (Figure 2B), whereas no signal was evident in saline-treated WT (Figure 2C) or GGT^{enu1} (Figure 2D) mice. The accumulation of PAS-positive material in epithelial cells after IL-13 treatment was associated with a 35-fold induction of Muc5ac mRNA in WT mice ($P < 10^{-6}$; $n = 6$), but this induction was 4-fold less in GGT^{enu1} mice ($n = 6$) (Figure 2E). The increase in Muc5ac mRNA was specific because mRNA for Muc1, Muc2, Muc3, Muc4, or Muc5b were not induced by IL-13 in mice of either genotype (Figure E1).

Expression of *Clca3*. Messenger RNA for the chloride channel *Clca3*, a gene that is induced at the onset of asthma

(16), was induced 281-fold in IL-13-treated WT mice ($P = 0.002$; $n = 6$) but was 4-fold less in IL-13-treated GGT^{enu1} mice ($n = 6$) (Figure 2F).

EGFR induction and activation. The effect of IL-13 on EGF receptor was examined because Muc5ac gene induction in asthma is mediated by oxidant-dependent activation of the EGFR (17–19).

EGFR Immunohistochemistry

EGFR protein was identified on the apical surface of ciliated airway epithelial cells in saline-treated and IL-13-treated WT and GGT^{enu1} lung. Signal from WT lung is shown in Figures 3A

and 3B. Specificity was confirmed in a competition experiment coincubating EGFR antisera with the EGFR peptide antigen, which abolished signal (Figure 3C).

Expression of Activated EGFR

EGFR is known to translocate to the nucleus when activated (20). EGFR cellular localization was assessed with peptide-specific antisera raised against a phosphorylated domain of EGFR (21, 20). No nuclear signal was detectable in saline-treated GGT^{enu1} (Figure 3D) or WT (Figure 3F) mice or in IL-13-treated GGT^{enu1} mice (Figure 3E), but an intense nuclear signal was present in scattered nuclei of airway epithelial cells of IL-13-treated WT mice. This signal predominated in epithelial cells from larger airways (Figure 3G) and was inhibited by coincubation with peptide (Figure 3H).

Induction of EGF Receptor mRNA

EGFR receptor activation is associated with induction of its mRNA. Real-time PCR (Figure 3I) showed that IL-13 treatment did not induce EGFR mRNA in GGT^{enu1} lung over that of the saline-treated GGT^{enu1} control (0.65 ± 0.08 ; $n = 4$) but significantly induced EGFR mRNA by 1.7 ± 0.2 -fold in WT lung compared with its saline-treated control ($P = 0.003$; $n = 4$).

GGT Deficiency Prevents IL-13–Induced Airway Hyperreactivity in GGT^{enu1} Mice

Airway resistance measured during graded exposure to methacholine was significantly higher (asterisks in Figure 4A) in IL-13-treated WT mice (WT_{IL13}, $n = 4$) at 5 ($P = 0.0002$), 10 (ANOVA; $P = 0.0002$), and 15 ($P = 0.02$) $\mu\text{g/ml}$ of methacholine compared with saline-treated WT (WT_{sal}, $n = 5$), saline-treated GGT^{enu1} (GGT^{enu1}_{sal}, $n = 7$), and IL-13-treated GGT^{enu1} (GGT^{enu1}_{IL13}, $n = 8$) mice (Figure 4A).

GGT Deficiency Increases Glutathione Content and Decreases Protein Carbonyl Content in Lung Lining Fluid after IL-13

Bronchoalveolar lavage glutathione and protein carbonyls. We have previously shown that lung lining fluid glutathione is 2-fold greater in GGT^{enu1} mice compared with WT control mice (8). Compared with saline-treated WT mice, IL-13 treatment increased protein content in bronchoalveolar lavage (BAL) by 35-fold in mice of each genotype (asterisks in Figure 5) ($n = 4$; $P = 0.0004$). Total cysteine content did not differ significantly among the three groups of mice, suggesting that lung cysteine supply was able to meet demand. Compared with saline-treated WT mice, BAL glutathione content did not increase significantly in IL-13-treated WT mice but increased by 10-fold in IL-13-treated GGT^{enu1} mice (asterisks in Figure 5, $n = 4$; $P = 0.03$). Compared with saline-treated WT mice ($n = 3$), protein carbonyl content increased significantly in IL-13-treated WT mice by 2.7-fold ($P = 0.005$; $n = 7$). In contrast, this marker of protein oxidation increased only 1.4-fold in IL-13-treated GGT^{enu1} mice ($n = 4$; $P = 0.17$) (Figure 6) compared with saline-treated GGT^{enu1} mice ($n = 3$).

BAL glutathione-related products. BALF was examined for accumulation of glutathione-related molecules, including nitroglutathione (GSNO), an endogenous bronchodilator, and leukotrienes C4 and E4, bronchoconstrictors induced during inflammation (22). There was no significant accumulation of either in IL-13-treated WT or GGT^{enu1} mice (GSNO data not shown; leukotriene data shown in Table E2).

Plasma. IL-13 treatment increased total plasma protein content in both genotypes compared with saline-treated WT control but did not significantly alter the total cysteine content or affect the level of glutathionemia present at baseline in GGT^{enu1} mice ($n = 4$) (Figure E2).

Figure 4. Airway hyperreactivity. (A) Induction of airway hyperreactivity was measured by assaying airway resistance during graded methacholine (MC) exposure. At each methacholine dose, airway resistance was significantly higher (asterisks) in IL-13-treated WT mice compared with saline-treated mice of either genotype and IL-13-treated GGT^{enu1} mice. (B) Airway resistance measured during graded exposure to methacholine was significantly higher in IL-13-treated WT mice (WT_{IL13}) at all doses compared with saline-treated (WT_{sal}) and acivicin-treated WT mice (WT_{acivicin}) (asterisks). The response at 15 $\mu\text{g/ml}$ was significantly attenuated when IL-13 treatment was combined with acivicin (dagger).

Inhibition of GGT Activity in Lung Lining Fluid of WT Mice Attenuates EGFR mRNA Induction and Airway Hyperreactivity

Seventy-eight percent of lung lining fluid GGT activity in WT mice was inactivated for up to 24 hours with intratracheal delivery of the irreversible inhibitor acivicin at 1 to 2.5 μM (Figure E3). Acivicin treatment significantly attenuated IL-13-induced EGFR mRNA induction (0.99 ± 0.06 ; $n = 3$) (see Figure 3I). Airway resistance measured during graded exposure to methacholine was significantly higher in IL-13-treated WT mice (WT_{IL13}; $n = 5$) at all doses compared with saline-treated (WT_{sal}; $n = 6$) and acivicin-treated (WT_{acivicin}; $n = 10$) WT mice (see asterisks in Figure 4), and the response at 15 $\mu\text{g/ml}$ was significantly attenuated when IL-13 treatment was combined with acivicin (ANOVA; $P = 0.003$; $n = 4$) (see Figure 4B, dagger).

DISCUSSION

Asthma is characterized by inflammation, and oxidants derived from inflammatory cells contribute to the oxidant-antioxidant

Figure 5. Total protein, cysteine, and glutathione content in BALF. Compared with saline-treated WT mice, total protein content was elevated 35-fold in IL-13-treated WT and GGT^{enu1} mice (*asterisks*). Total nonprotein cysteine content was similar in all three groups. Total glutathione content was significantly elevated 10-fold in IL-13-treated GGT^{enu1} mice (*asterisk*).

imbalance and oxidant stress that are associated with asthma pathogenesis (1). Whether attenuation of oxidant stress alone can affect the phenotype of asthma is unclear (23). Our studies, exposing GGT^{enu1} mice to IL-13, were designed to explore the selective role of glutathione metabolism and oxidant stress in an experimental model of asthma (9). Glutathione metabolism is regulated by the ectoenzyme GGT. Loss of GGT enzyme activity impairs glutathione metabolism, limits cysteine availability, and causes deficiency of intracellular glutathione pools. This is particularly evident in endothelial cells, alveolar macrophages, and bronchiolar Clara cells in the GGT^{enu1} lung even in normoxia. Upon exposure to hyperoxia, where metabolism of oxygen increases the level of intracellular reactive

oxygen species and induces oxidant stress, these cells are injured more rapidly so that the onset of pulmonary edema, hemorrhage, and death is accelerated in GGT^{enu1} mice compared with WT mice (8).

We hypothesized that deficiency of cellular glutathione pools would increase oxidant stress and accentuate the asthma phenotype in the GGT^{enu1} mouse lung after IL-13 treatment. However, our experiments show that the asthma phenotype is attenuated in these mice despite IL-13 delivery, cytokine activation, and inflammatory cell and matrix-associated protein accumulation to levels seen in WT mouse lung. The attenuated asthmatic response occurred specifically in airway epithelial cells as decreased levels of airway mucous cell hyperplasia, mucin protein,

Figure 6. Protein carbonyl content in BALF. BAL protein carbonyl content was assayed, and data were normalized to the lowest value of the WT saline-treated control mice ($n = 3$). This marker of protein oxidation accumulated significantly by 2.7-fold in IL-13-treated WT mice compared with saline-treated WT control mice (*asterisk*) but not in IL-13-treated GGT^{enu1} mice compared with GGT^{enu1} saline-treated control mice.

and Muc5ac gene induction and EGFR gene induction and receptor activation.

Airway hyperreactivity, one of the hallmarks of asthma, was prevented in the GGT^{enu1} mice, suggesting that hyperreactivity is linked to oxidant-induced airway epithelial cell signaling rather than airway inflammation and cytokine release. Pharmacologic inhibition of GGT activity in lung lining fluid of WT mice also attenuated IL-13-induced epithelial cell activation and airway hyperreactivity, demonstrating that lung lining fluid GGT activity, rather than other metabolic consequences of the GGT^{enu1} mutation, prevented IL-13-induced asthma.

GGT regulates extracellular glutathione pools in the plasma (6) and the lung lining fluid (8). In the absence of turnover, the plasma glutathione pool enlarges 5-fold (6, 7) and the lung lining fluid pool 2-fold (8) in the GGT-deficient GGT^{enu1} mouse compared with WT mice at baseline. This abundance of lung lining fluid glutathione in GGT^{enu1} mice increased after IL-13 treatment, and the lack of accumulation of protein carbonyls in BALF confirms an augmentation of antioxidant buffering capacity against the reactive oxidant species generated by inflammatory cells.

Studies in humans suggest that lung lining fluid glutathione is dynamic in patients with asthma. Total glutathione increases in BALF (24) and in supernatant from induced sputum (25). Extracellular glutathione protects human airway epithelial cells against toxicity from hexamethylene diisocyanate, an *in vitro* model of isocyanate-induced asthma (26). There is an inverse correlation between BALF glutathione and airway hyperreactivity to methacholine (24). Our results in the GGT^{enu1} mouse lung support these human data.

We considered that the lack of IL-13-induced airway hyperreactivity in the GGT^{enu1} mouse lung could involve the accumulation of glutathione-related molecules such as the nitrosothiol GSNO, an endogenous bronchodilator (27). However, we were unable to detect any difference in GSNO content in BALF between IL-13-treated GGT^{enu1} or WT mice. Enhanced lung lining fluid glutathione content alone could explain our result in the GGT^{enu1} mouse because its antioxidant activity affects airway tone (28).

Although GGT may be involved in the production of the bronchoconstrictive cysteinyl leukotriene LTD₄, recent mouse data show that LTC₄ to LTD₄ metabolism is mainly regulated by gamma-glutamyl leukotrienase (Gglt1), a GGT-related enzyme that is encoded by a separate gene (22). Loss of Gglt1 activity, but not GGT activity, increases airway hyperreactivity as LTC₄ accumulates without metabolism. Absence of IL-13-induced airway hyperreactivity in the GGT^{enu1} mouse lung is unlikely to be related to altered cysteinyl leukotriene metabolism. Our data concur with the hypothesis that the major function of GGT in the mouse is glutathione metabolism.

Our results support a role for enhanced antioxidant activity in the extracellular glutathione pool as a mechanism for attenuation of IL-13-mediated asthma in the GGT^{enu1} mouse lung. This extracellular pool does not affect the IL-13-induced inflammatory response, which was similar in WT and GGT^{enu1} mice. Rather, we propose that it protects airway epithelial cells by buffering the oxidizing milieu created by the inflammatory response induced by IL-13. These data directly implicate the epithelial cell in the process that leads to airway hyperreactivity because the absence of EGFR activation is associated with absence of airway hyperreactivity.

Previously glutathione aerosols have been used to alter lung lining fluid glutathione in asthma (29), but these had limited success and induced bronchoconstriction (30). Ingestion of the prodrug N-acetylcysteine failed to elevate lung or lung lining fluid glutathione content (31, 32). Pharmacologic inhibition of

lung lining fluid glutathione metabolism seems to be a robust strategy to enhance lung lining fluid glutathione content in the presence of oxidant stress associated with inflammation. A class of γ -phosphono diester glutamate analogues is available as novel GGT inhibitors with greater potency and specificity than acivicin (33). We believe that this mechanism should be studied further as a means of preventing or treating asthma inasmuch as lung lining fluid glutathione has been proposed as an antioxidant shield that protects the airway epithelium (4) and EGFR is a signaling system that is affected by epithelial barrier integrity (34). Although experiments with allergen challenged GGT^{enu1} mice will be important to extend these results, augmentation of lung lining fluid glutathione by inhibiting metabolism, combined with supplementation of cellular glutathione pools with γ -GCS (3), may be a relevant strategy to protect airway epithelium while modulating immune cell function in asthma and other inflammatory lung diseases, inasmuch as oxidant stress associated with inflammation is emerging as a common factor in the pathogenesis of lung injury (35).

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.

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