Redox Biology 8 (2016) 375–382

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research paper

Glutathione S-transferase pi modulates NF-κB activation and pro-inflammatory responses in lung epithelial cells

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ARTICLE INFO

Article history:
Received 15 March 2016
Accepted 22 March 2016
Available online 26 March 2016

Keywords:
NF-κB
GSTP
S-glutathionylation
Asthma
Inflammation
Lung

ABSTRACT

Nuclear Factor kappa B (NF-κB) is a transcription factor family critical in the activation of pro- inflammatory responses. The NF-κB pathway is regulated by oxidant-induced post-translational modifications. Protein S-glutathionylation, or the conjugation of the antioxidant molecule, glutathione to reactive cysteines inhibits the activity of inhibitory kappa B kinase beta (IKKβ), among other NF-κB proteins. Glutathione S-transferase Pi (GSTP) is an enzyme that has been shown to catalyze protein S-glutathionylation (PSSG) under conditions of oxidative stress. The objective of the present study was to determine whether GSTP regulates NF-κB signaling, S-glutathionylation of IKK, and subsequent pro-inflammatory signaling. We demonstrated that, in unstimulated cells, GSTP associated with the inhibitor of NF-κB, IκBα. However, exposure to LPS resulted in a rapid loss of association between IκBα and GSTP, and instead led to a protracted association between IKKβ and GSTP. LPS exposure also led to increases in the S-glutathionylation of IKKβ. SiRNA-mediated knockdown of GSTP decreased IKKβ-SSG, and enhanced NF-κB nuclear translocation, transcriptional activity, and pro-inflammatory cytokine production in response to lipopolysaccharide (LPS). TLK117, an isotype-selective inhibitor of GSTP, also enhanced LPS-induced NF-κB transcriptional activity and pro-inflammatory cytokine production, suggesting that the catalytic activity of GSTP is important in repressing NF-κB activation. Expression of both wild-type and catalytically-inactive Y7F mutant GSTP significantly attenuated LPS- or IKKβ-induced production of GM-CSF. These studies indicate a complex role for GSTP in modulating NF-κB, which may involve S-glutathionylation of IKK proteins, and interaction with NF-κB family members. Our findings suggest that targeting GSTP is a potential avenue for regulating the activity of this prominent pro-inflammatory and immunomodulatory transcription factor.

1. Introduction

Nuclear Factor kappa B (NF-κB) is a family of transcription factors involved in the regulation of pro-survival, pro-inflammatory, and immune regulatory pathways. Dysregulation of NF-κB has been linked to a variety of chronic inflammatory diseases including cancer, sepsis, and asthma. NF-κB activity is elevated in lung epithelial cells of asthmatic patients in comparison to healthy controls [1], and activation of classical NF-κB in the lung epithelium is not only sufficient, but also necessary to regulate airway inflammation in mice [2–4]. At least two parallel NF-κB pathways exist: the classical (canonical) pathway and an alternative pathway both of which contribute to inflammatory responses in lung epithelial cells [5]. In the classical pathway, activation of Inhibitory kappa B kinase beta (IKKβ) induces the phosphorylation and subsequent degradation of inhibitory kappa B alpha (IκBα), translocation of RelA/p50 dimers to the nucleus, and transcriptional activation of over 100 target genes [6]. In the alternative pathway IKKα phosphorylates p100, which is then partially degraded into p52.

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http://dx.doi.org/10.1016/j.redox.2016.03.005

RelB/p52 dimers then translocate to the nucleus and initiate transcription of distinct NF-κB target genes [7].

Given the importance of NF-κB in the regulation of numerous biological functions, multiple mechanisms exist which tightly regulate its activation, including oxidant-dependent modifications. Thiol groups on protein cysteines with a low pKa are typically considered susceptible to oxidative modifications such as S-nitrosylation, sulfenic acid formation, disulfide bond formation, and S-glutathionylation [8]. Previous work from our laboratory and others has demonstrated that NF-κB is modified via S-glutathionylation, the conjugation of glutathione (GSH) to cysteines via mechanisms that remain incompletely understood. S-glutathionylation of IKKβ inhibits its kinase activity and downstream pro-inflammatory responses in response to lipopolysaccharide (LPS). Additionally, S-glutathionylation prevents the ubiquitination and subsequent degradation of IκBα, as well as the DNA binding of RelA/p50 dimers [9–12], and is therefore a crucial mechanism of to regulate the activation of NF-κB.

Our laboratory has previously demonstrated that hydrogen peroxide-induced S-glutathionylation of IKKβ can be reversed by the oxidoreductase, glutaredoxin-1 (Glrx1) [9]. We also demonstrated that overexpression of Glrx1 decreases LPS-induced IKKβ S-glutathionylation and enhances NF-κB activation, and pro-inflammatory cytokine production in lung epithelial cells [13]. While it is known that Glrx1 can deglutathionylate IKKβ under physiological conditions, the potential enzymatic mechanisms that catalyze IKKβ S-glutathionylation have not been determined. Glutathione S-transferase pi (GSTP), an enzyme traditionally linked to phase II drug metabolism, is a putative catalyst of protein S-glutathionylation reactions [14–16], and has previously been linked to asthma [17]. GSTP also associates with TRAF2, a known regulator of NF-κB [18]. Given the link between GSTP and S-glutathionylation, the known role for epithelial NF-κB in regulating pulmonary inflammation, and the susceptibility of NF-κB to redox-based regulation, we sought to explore the role of GSTP in the regulation of activation of NF-κB in lung epithelial cells exposed to LPS. Our studies demonstrate a prominent role of GSTP in the regulation of LPS-driven NF-κB activation and pro-inflammatory mediator production, which may involve both protein-protein interactions as well as the catalytic activity of GSTP.

2. Materials and methods

2.1. Cell culture and reagents

A spontaneously transformed type II mouse lung alveolar epithelial cell line [19] (C10) was cultured as described previously [20]. A C10 cell line stably expressing NF-κB luciferase was generated as previously described [21]. Briefly, C10 cells were transfected with the plasmid, 6 κB-tk-luc, in the presence of the plasmid PSV-2Neo (Promega), conferring a resistance to neomycin. Following incubation with the antibiotic, geneticin, antibiotic-resistant colonies were propagated. C10 cells were starved in medium containing 0.5% FBS for 16 h prior to exposure of 1 μg/mL LPS (List Biological Laboratories, Inc).

2.2. Plasmids and siRNA

C10 cells were incubated with Dharmacon SMARTpool non-targeting siRNA or Dharmacon SMARTpool siRNA against murine GSTP1 (100 nM) (Dharmacon, Lafayette, CO). Plasmid transfections were performed with Lipofectamine (Invitrogen, Carlsbad, CA). Wild-type mouse GSTP, Tyrosine 7 Phenylalanine (Y7F) mutant mouse GSTP, were cloned into PCMV. All transfections utilized a total of 1 μg/mL DNA unless otherwise indicated.

2.3. TLK117 synthesis and treatment

TLK117 is the most specific GSTP inhibitor to date with a binding affinity greater than GSH itself and a selectivity for GSTP over 50-fold greater than the GSTM and GSTA classes (Ki = 0.4 μM) [22,23]. TLK117 (γ-glutamyl-S-(benzyl)cysteinyl-R-(−)-phenyl glycine) was generated by Fmoc solid-state peptide synthesis with HBTU as previously described [24], and was confirmed to be > 98% pure by HPLC. TLK117 was reconstituted in 0.375 M Tris–HCl, pH = 7.4, containing 0.02% DMSO. C10 cells were pretreated with 50 μM of TLK117 24 h, and a second time one hour prior to treatment with LPS. A separate group of cells received 0.375 M Tris–HCl, pH = 7.4, containing 0.02% DMSO as the vehicle control.

2.4. Western blotting and antibodies

Protein concentration was determined with the Bio-Rad DC Protein Assay kit (Bio-Rad, Hercules, CA), and 20 μg protein were used for Western Blot analysis. RelA, RelB, and β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against IκBα, IKKβ, and phosphoserine RelA 536 were from Cell Signaling Technology (Danvers, MA). IKKα antibody was purchased from Upstate (Lake Placid, NY), and GSTP antibody was purchased from MBL (Woburn, MA). Cytosolic and nuclear extracts were prepared as previously described [25].

2.5. Enzyme-linked immunosorbent assay (ELISA)

C10 cells were treated and/or transfected as indicated, and CCL-20 and GM-CSF were detected in cell supernatants with Duoset ELISA kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

2.6. GSTP activity assay

GSTP activity was determined as previously described [14,24]. C10 cells were harvested in a potassium phosphate buffer containing 0.1% Triton X-100 and protease and phosphatase inhibitors. Immunoprecipitation of GSTP from cell lysates was performed with an anti GSTP antibody (MBL) and protein G agarose beads (Invitrogen). Immunoprecipitated samples were incubated with 5 mM GSH (Fluka, St. Louis, MO) and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) (Sigma) and vortexed. Following a 10 min incubation, samples were again vortexed and centrifuged briefly at 14,000 rpm, and supernatant was measured at an absorbance of 340 nm to detect GSH-conjugated CDNB. Results are expressed as nmol substrate/mg protein/min, wherein one unit represents the consumption of CDNB per 1 mg of GSTP per minute.

2.7. Detection of protein S-glutathionylation

C10 cell lysates were subjected to immunoprecipitation of GSH-bound proteins as previously described [26]. Briefly, protein was isolated with an SDS lysis buffer containing 20 mM N-ethyl maleimide (NEM) (Sigma). 250 μg protein was incubated with 1 μg/mL anti-PSSG antibody (Virogen, Watertown, MA) and recombinant protein G agarose beads, and subjected to Western Blot analysis. Prior to immunoprecipitation, select reagent control samples were incubated with 50 mM DTT for 30 minutes as previously described [24].

2.8. Assessment of luciferase activity

A C10 cell line stably expressing NF-kB-luciferase construct was utilized to detect NF-kB transcriptional activity. Luciferase units were normalized to protein content, and results are expressed as relative luciferase units (RLU) [21].

2.9. Statistical analysis

Statistical significance was determined with GraphPad Prism v5.0 using either one-way analysis of variance (ANOVA) with a Bonferroni posttest or an unpaired Student's *t*-test as indicated. *P*-values below 0.05 were considered statistically significant. All experiments were repeated at least two times.

3. Results

3.1. Impact of lipopolysaccharide (LPS) on the interaction between GSTP, IKKβ and IκBα

In addition to its role in phase II drug metabolism, early characterization of GST family members ascribed the term "ligandin" to reflect the capacity of GST to bind to heme or bilirubin [27]. More recently GSTP has been found to form multiple protein interactions, which in many cases regulate the activity of the target proteins it binds to [18,28,29]. We therefore first sought to investigate whether an interaction was present between GSTP and the inhibitor of NF-κB, IκBα, and whether this was affected in cells stimulated with the NF-κB activator, LPS. In unstimulated cells a strong interaction was detected between GSTP and IκBα which was rapidly lost following stimulation of cells with LPS (Fig. 1A). The LPS-induced loss of interaction between IκBα and GSTP occurred prior to the degradation of IκBα, which is required for NF-kB activation, and was maintained for at least 24 h following stimulation with LPS (Fig. 1A). Conversely, LPS exposure led to an interaction between GSTP and IKKβ which first appeared between 1 and 2 h and further increased throughout the 24 h exposure period, and was barely detectable in unstimulated cells (Fig. 1B). Despite the functional role of IKKα for LPS-induced pro-inflammatory responses in C10 lung epithelial cells [5], an association of GSTP with IKKα was not detected in response to LPS (Fig. 1B).

3.2. GSTP knockdown or inhibition promotes increased NF-κB activation and pro-inflammatory cytokine production

Given the interactions between GSTP and IκBα and IKKβ which were affected following activation of NF-kB by LPS, we next examined a potential role for GSTP in regulating NF-κB activation and downstream inflammatory responses. As expected, siRNA-mediated GSTP knockdown decreased GSTP protein content and overall enzymatic activity in cells (Fig. 2A). LPS-induced nuclear RelA content was noticeably increased in C10 cells transfected with GSTP siRNA relative to controls (Fig. 2B). LPS-induced NF-κB luciferase activity was also increased in GSTP siRNA-transfected cells compared to control siRNA transfected cells (Fig. 2C). Furthermore, levels of GM-CSF and CCL-20, two NF-κB-driven cytokines in lung epithelial cells, also increased following LPS exposure upon siRNA-mediated GSTP knockdown (Fig. 2D and E). Altogether these findings demonstrate that decreases in GSTP protein lead to an enhanced activity of NF-kB, and resultant increases in pro-inflammatory mediators following exposure to LPS. We next evaluated the impact of inhibition of GSTP catalytic activity for LPS-induced activation of NF-kB. C10 cells pretreated with the isotype-selective GSTP inhibitor, TLK117, which resulted in attenuation of GSTP activity (Fig. 3A). Treatment with TLK117 resulted in heightened LPS-induced NF-κB luciferase activity and GM-CSF and CCL-20 levels relative to controls (Fig. 3B–D), consistent with findings following siRNA-mediated knock down of GSTP (Fig. 2). Overall, these data suggest that endogenous GSTP is important in the suppression of LPS-induced NF-κB nuclear translocation, transcriptional activity, and production of pro-inflammatory mediators.

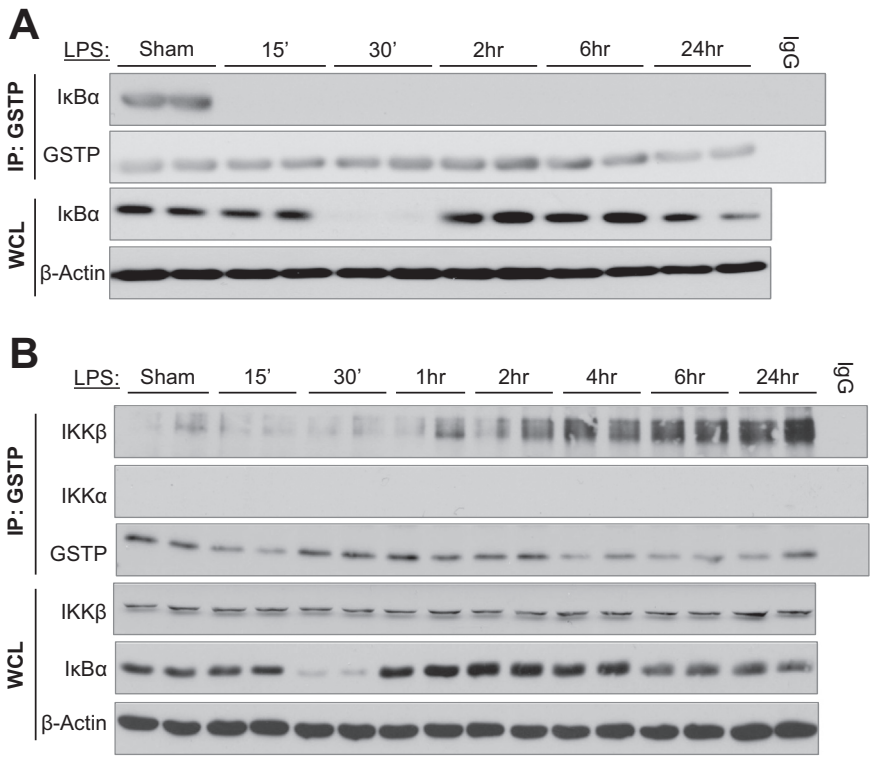

Fig. 1. Interactions between GSTP, IKKβ, and IκBα are affected by the NF-κB activating stimulus, lipopolysaccharide (LPS). C10 lung epithelial cells were treated with 1 μg/mL LPS for the times indicated and GSTP was immunoprecipitated (IP) followed by Western Blot analysis of IκBα (A) or, IKKβ and IKKα (B). WCL: Whole cell lysates. β-Actin: Loading control.

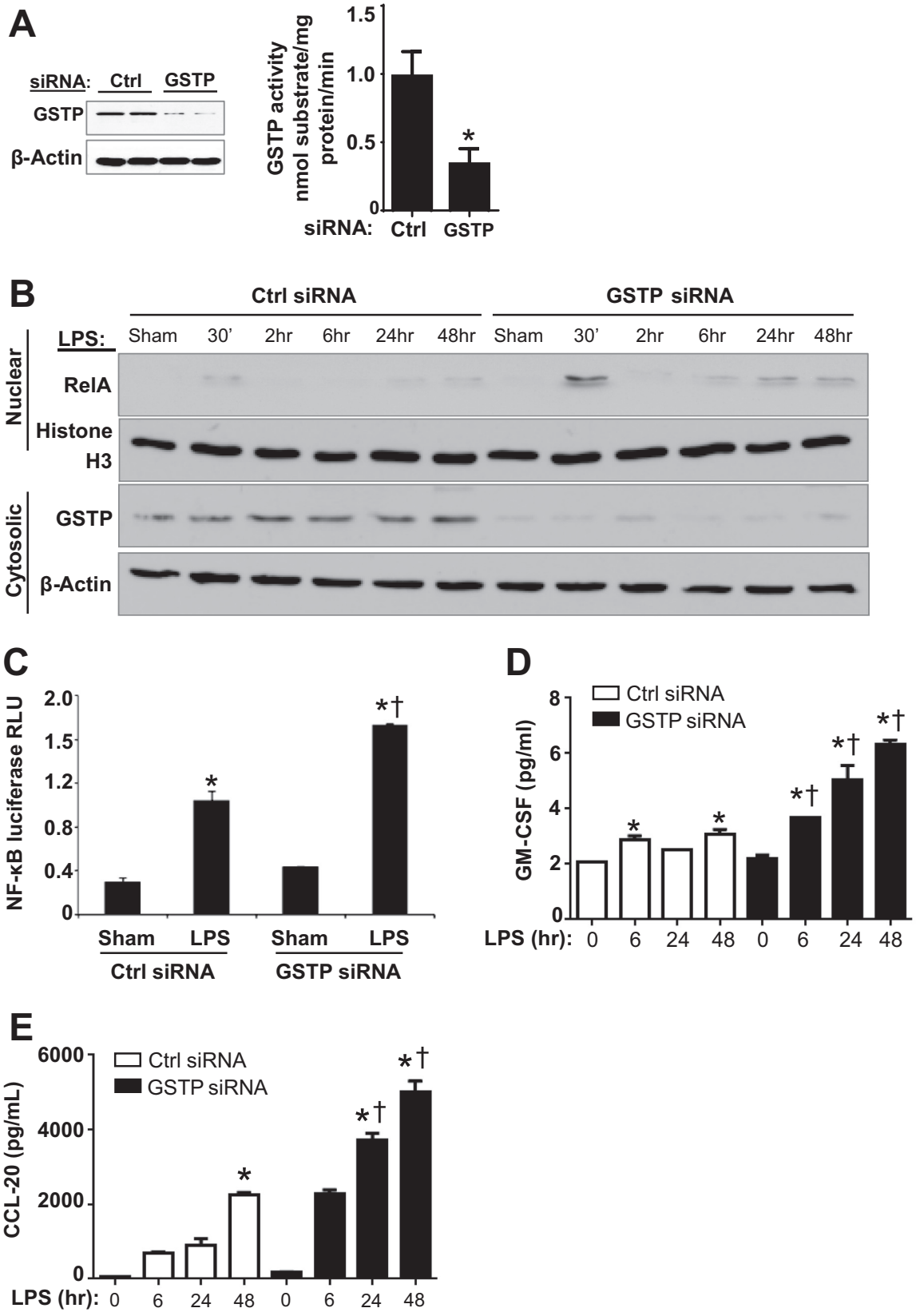

Fig. 2. SiRNA-ablation of GSTP promotes NF-κB activation and pro-inflammatory cytokine production. C10 cells were transfected with either control or GSTP siRNA and thereafter exposed to 1 μg/mL LPS for the times indicated. A) Confirmation of decreased GSTP protein content and enzymatic activity following siRNA-mediated ablation of GSTP. *$p < 0.05$ by unpaired Student's *t*-test ($n=6$ per group). B) Top panels: Assessment of nuclear RelA content by Western blot analysis. Bottom panels: Cytosolic extracts were analyzed by Western Blotting to confirm knockdown of GSTP. C) Assessment of basal or LPS-induced NF-κB luciferase activity following siRNA-mediated ablation of GSTP. Results are expressed as relative luciferase units (RLU). *$p < 0.05$ relative to LPS control group, †$p < 0.05$ relative to siRNA control group by one-way ANOVA ($n=3$ per group, data are representative of three independent experiments). Assessment of GM-CSF (D) and CCL-20 (*E*) levels in cell supernatants by ELISA. *$p < 0.05$ relative to the non-LPS exposed control group, †$p < 0.05$ relative to the respective control (ctrl) siRNA group by one-way ANOVA ($n=3$ per group). Data are representative of three experiments.

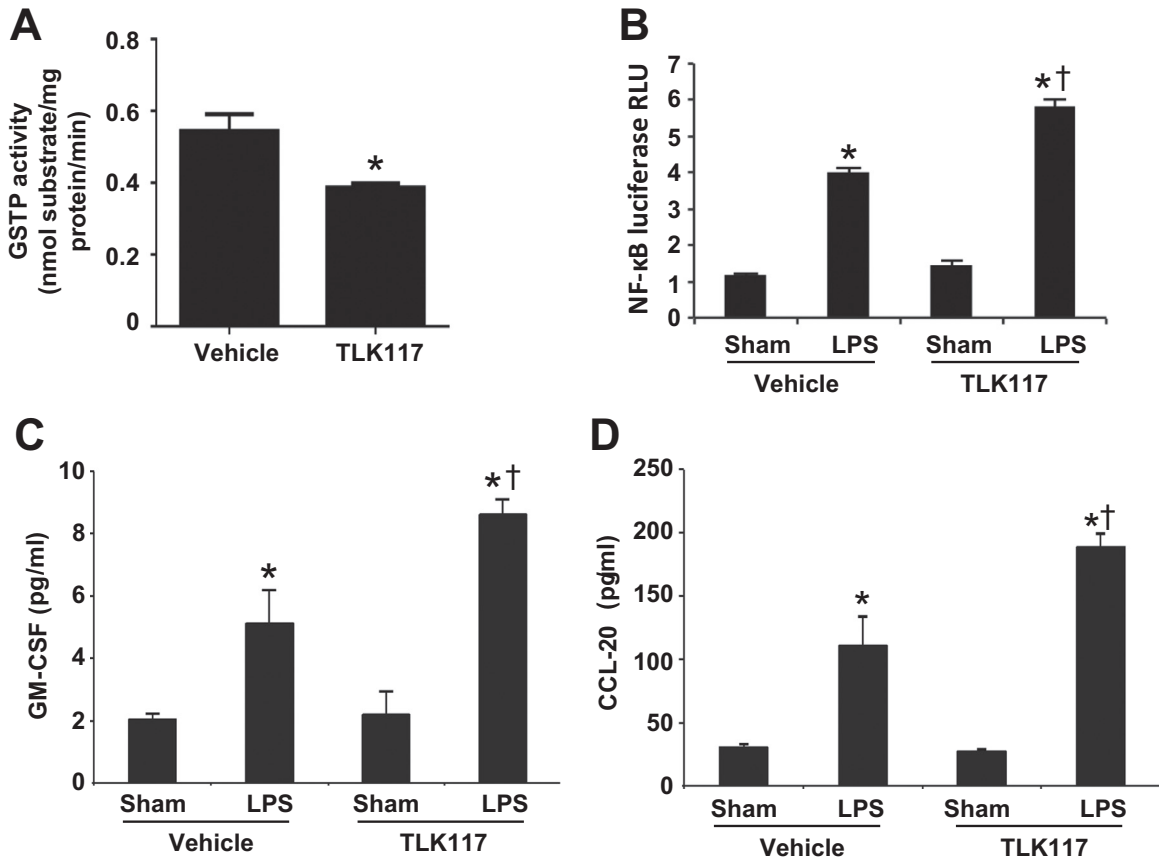

Fig. 3. Parmacological inhibition of GSTP with TLK117 promotes increased NF-κB activation and pro-inflammatory cytokines. C10 cells were pretreated with 50 μM of TLK117 24 h, and a second time one hour prior to treatment with LPS, or 0.375 M Tris–HCl solution, pH=7.4, with 0.02% DMSO as a vehicle control. A) Confirmation of decreased GSTP activity upon administration of TLK117. $^{*}p < 0.05$ by unpaired Student's *t*-test ($n=6$ per group). B) Assessment of NF-κB luciferase activity in C10 cells following TLK117 mediated inhibition of GSTP and subsequent exposure to LPS for 6 h. $^{*}p < 0.05$ relative to non-LPS exposed control group, $\dagger p < 0.05$ relative to the respective vehicle-exposed (-) group by one-way ANOVA ($n=3$ per group). Data are representative of 3 independent experiments. Assessment of GM-CSF (C) and CCL-20 (D) levels in cell supernatants by ELISA. $^{*}p < 0.05$ relative to non LPS-exposed control group, $\dagger p < 0.05$ relative to the respective vehicle-exposed (-) group by one-way ANOVA ($n=3$ per group). Data are representative of three independent experiments.

3.3. SiRNA-mediated ablation of GSTP decreases S-glutathionylation of IKKβ

Given the role for GSTP in regulating NF-κB and pro-inflammatory cytokine production, and in the catalysis of protein S-glutathionylation [14], we next determined whether S-glutathionylation of IKKβ (IKKβ-SSG) was also dependent on GSTP. Small increases in IKKβ-SSG were detected 6 h following exposure to LPS, a timepoint that co-incides with the association between GSTP and IKKβ. Following siRNA-mediated knockdown of GSTP,

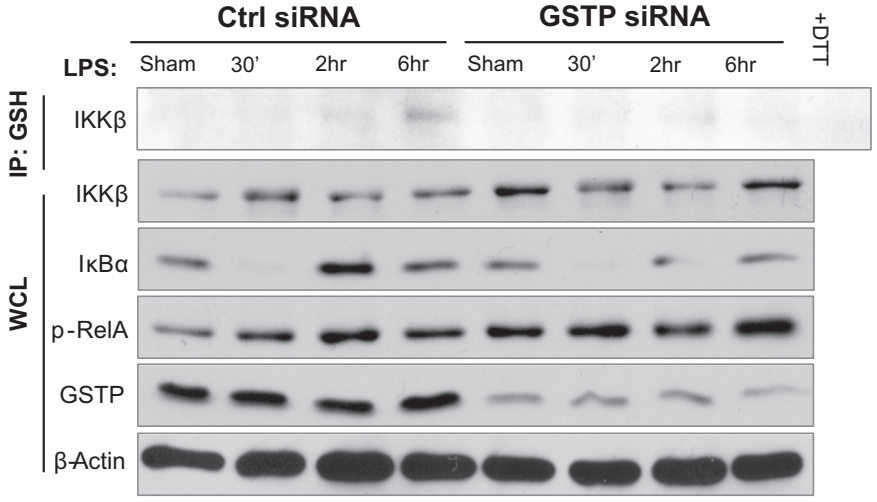

Fig. 4. SiRNA-mediated ablation of GSTP decreases S-glutathionylation of IKKβ. C10 cells were transfected with either control or GSTP siRNAs, treated with LPS for the times indicated, and lysates were subjected to immunoprecipitation of S-glutathionylated proteins using an anti-GSH antibody. S-glutathionylation of IKKβ was assessed by Western blot analysis. Bottom panels: Whole cells lysates (WCL) for assessment of total IKKβ, IκBα, phosphorylated RelA and GSTP, by Western blot analysis.

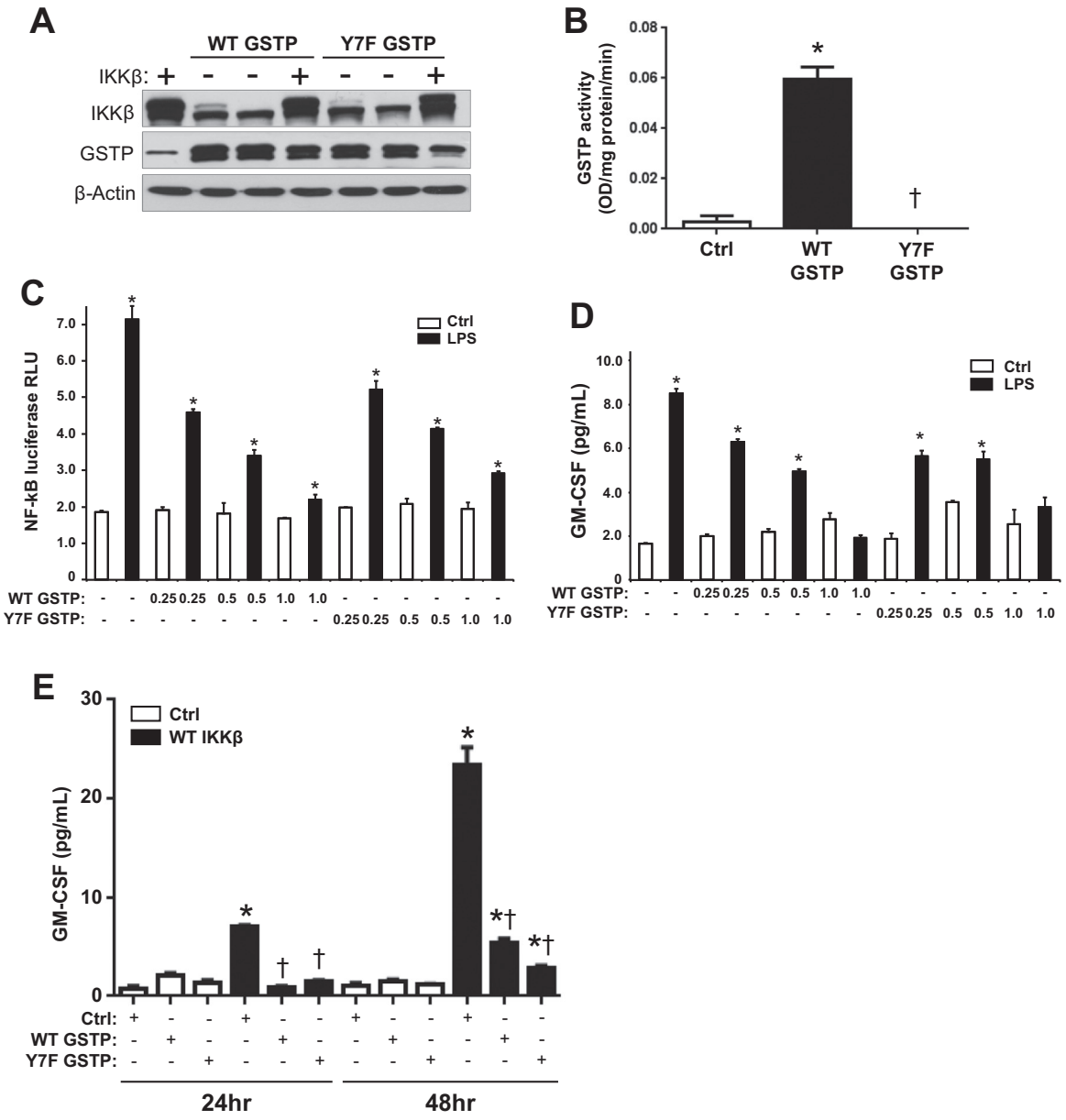

Fig. 5. Role of GSTP in LPS- or IKKβ- mediated activation of NF-κB and production of pro-inflammatory cytokines: (A) C10 lung epithelial cells were either transfected with WT HA-IKKβ or empty vector (EV) alone or in the presence of either FLAG-tagged WT GSTP or FLAG tagged Y7F mutant GSTP. Overexpression of IKKβ and GSTP were assessed via Western Blotting. (B) Assessment of GSTP enzymatic activity in cells overexpressing either WT FLAG-GSTP or Y7F FLAG-GSTP, or empty vector (-). GSTP enzymatic activity was assessed by detection of GSH conjugated CDNB. Assessment of basal or LPS-induced NF-κB luciferase activity (C) and GM-CSF levels in cell supernatants (D) following transfection with WT GSTP or Y7F GSTP. *$p < 0.05$ relative to non LPS-exposed control group, †$p < 0.05$ relative to the respective empty vector groups (-). (*E*) Evaluation of GM-CSF content in C10 cells following transfection with WT HA-IKKβ, WT FLAG-GSTP, Y7F FLAG-GSTP individually, or following co-overexpression of WT HA-IKKβ in the presence or absence of WT GSTP, or Y7F GSTP. GM-CSF was evaluated in the medium 24 or 48 h post transfection. *$p < 0.05$ (ANOVA) compared the empty vector group (ctrl), † $p < 0.05$ (ANOVA) compared WT-IKKβ-transfected groups.

LPS-induced S-glutathionylation of IKKβ was decreased relative to the control siRNA transfected cells (Fig. 4). Decrease in S-glutathionylation of IKKβ was associated with increases in phospho-RelA and prolonged decreases in the content of IκBα (Fig. 4), indicative of protracted IKK activation.

3.4. GSTP attenuates LPS-induced or IKKβ-dependent NF-kB activation and production of pro-inflammatory mediators

Given the observed enhancement of LPS-induced NF-kB activation by GSTP knockdown or inhibition, we next examined whether GSTP directly regulates LPS-induced pro-inflammatory responses and whether the catalytic activity of GSTP is important in this regulation. C10 cells stably expressing NF-kB-luciferase construct were transfected with increasing amounts of FLAG-tagged WT-GSTP, or a version of GSTP containing a Y7F mutation which is catalytically inactive, or empty vector (PCMV) control (Fig. 5A and B). Transfection with WT GSTP attenuated LPS-induced NF-κB luciferase activity (Fig. 5C) and GM-CSF content in the medium (Fig. 5D) in a dose-dependent manner. Surprisingly, overexpression of Y7F-GSTP mutant also attenuated NF-κB luciferase and GM-CSF (Fig. 5C and D). We further tested the regulatory role of GSTP for IKKβ-dependent NF-kB activation. C10 cells were transfected with hemeagglutinin (HA)-tagged IKKβ along with either FLAG-tagged WT-GSTP, or Y7F-GSTP mutant, or PCMV control (Fig. 5A). Following transfection with WT-IKKβ,

GM-CSF content in the medium was significantly increased 24 and 48 h post transfection, compared to cells transfected with control vector (Fig. 5E). Similar to LPS-induced pro-inflammatory responses, transfection with either WT GSTP or the catalytically inactive variant of GSTP, Y7F-GSTP, almost completely ablated IKKβ-induced GM-CSF (Fig. 5E). Overall, these results suggest that catalytic activity of GSTP is not required to attenuate LPS- or IKKβ-induced NF-κB transcriptional responses.

4. Discussion

The airway epithelium plays an important role in inflammatory lung diseases, based upon its roles in barrier function and its interface with cells of the innate and adaptive immune system. GSTP is expressed in lung epithelial cells [30], which is a prominent location of NF-κB activation in inflammatory lung disease [1,3,31]. In the present study we therefore explored the role of GSTP in regulating NF-κB activation, S-glutathionylation, transcriptional activity, and pro-inflammatory cytokine production in epithelial cells exposed to LPS. Our results demonstrate that siRNA-mediated knockdown of GSTP attenuated S-glutathionylation of IKKβ, and enhanced NF-κB activation and transcription. Similarly, the isotype selective inhibitor of GSTP, TLK117, also enhanced NF-κB transcriptional activity. Collectively these findings demonstrate a role for endogenous GSTP in attenuating NF-κB transcriptional activity and production of pro-inflammatory mediators in response to LPS. Results from the present study also suggest that GSTP may predominantly regulate the canonical NF-κB pathway due to its association with IκBα and IKKβ, but not IKKα. Additional studies will be required to test the impact of GSTP on the alternative NF-κB pathway, and to address the role of GSTP for the activation of NF-κB in response to other stimuli.

In addition to its ability to catalyze protein S-glutathionylation reactions, GSTP can also interact with certain target proteins through a number of structural motifs. c-Jun N-terminal kinase (JNK), for example, has been shown to be negatively regulated by GSTP via direct interaction. Following exposure to either radiation or exposure to hydrogen peroxide, GSTP oligomerizes and releases JNK, thus facilitating its activation [29]. GSTP also associates with and inhibits the function of the adaptor protein, TRAF2. The association between GSTP and TRAF2 occurred independent of the catalytic activity of GSTP, suggesting that GSTP can regulate cellular responses independently of its catalytic activity [18]. It is therefore plausible that GSTP physically associates with NF-κB proteins and regulate their function independent of its catalytic activity. In support of this possibility, both wild-type and catalytically-inactive Y7F mutant GSTP suppressed LPS or IKKβ-induced levels of GM-CSF and NF-κB transcriptional activity, suggesting that GSTP can suppress the NF-κB pathway independent of its catalytic activity.

In the present study, we demonstrated a strong constitutive association between GSTP and IκBα in unstimulated cells, which was rapidly lost upon stimulation of cells with LPS, at a time that preceded IκBα degradation. The constitutive interaction between GSTP and IκBα could potentially prevent the phosphorylation and ubiquitination of IκBα, thus preventing NF-κB activation. Our findings demonstrate that LPS-induced nuclear content of RelA, and RelA phosphorylation were increased in cells following siRNA-mediated GSTP knockdown. Similarly, both GSTP knockdown and TLK117-mediated GSTP inhibition promoted heightened LPS-induced NF-κB luciferase activity and cytokine production, suggesting a potential regulatory function of GSTP in preventing IκBα phosphorylation and/or degradation. Previous studies have demonstrated that cysteine 189 of IκBα can be S-glutathionylated and that this decreases phosphorylation by IKK and attenuates ubiquitination in vitro [10], in association with attenuation of its degradation and subsequent activation of NF-κB [11]. Future studies therefore will be aimed at unraveling whether associations between GSTP and IκBα are linked to S-glutathionylation of IκBα, and whether these events controls activation and or assembly of the IKK signalsome. As GSTP does not affect IKKβ-SSG until 6 h post LPS exposure, a time at which NF-κB transcriptional activity is beginning to subside (data not shown), S-glutathionylation of IKK proteins may reflect a mechanism whereby GSTP attenuates NF-κB activity. This putative scenario wherein in the absence of stimulus GSTP prevents degradation of endogenous IκBα, and a regulatory mechanism involving GSTP-mediated S-glutathionylation to shut down IKK activity (graphical abstract) reflects a versatile regulatory mechanism of regulation of NF-κB by GSTP. Additional studies will be needed to unravel how an NF-κB activating stimulus regulates the protein-protein interactions between GSTP and various proteins within the NF-κB family, how this functionally controls NF-κB activation versus suppression and whether these events require S-glutathionylation. The oxidant-producing enzymes that facilitate GSTP-linked S-glutathionylation in epithelial cells stimulated with LPS also remain to be elucidated. It is now well accepted that activation of NF-κB involves multiple pathways that encompass multiple kinases, adaptor proteins, other regulatory post-translational modifications including ubiquitination, chromatin remodeling events, among others, in order to regulate the transcriptional output of NF-κB. The full spectrum of interactions between GSTP and these NF-κB pathway components and GSTP-targeted S-glutathionylation of these proteins will require additional redox proteomics studies in order to determine the precise mechanisms whereby GSTP restricts basal NF-κB activity, controls the duration of NF-kB activation, and the repertoire of NF-κB -dependent target genes affected.

GSTP has been linked to a number of chronic lung diseases, including asthma, chronic obstructive lung disease (COPD), and lung cancer [32–34]. The GSTP gene is located on chromosome 11q13, a known locus for asthma-associated genes. Human polymorphisms of GSTP, have been linked to atopic asthma; however, the functional contribution of polymorphic variants disease pathogenesis remain unclear [35,36]. It remains unclear whether the different GSTP variants differentially control NF-κB activation and whether this involves protein S-glutathionylation. Of direct relevance to the present study are findings demonstrating that GSTP inhibits inflammation in the ovalbumin model of allergic airways disease in mice in a strain-dependent manner [37]. However, it remains unclear whether this inhibitory effect of GSTP on allergic inflammation involved NF-κB or S-glutathionylation chemistry.

In addition to GSTP, other GSTs have been linked to inflammatory lung diseases and NF-κB activation. In addition to GSTP, GSTA and GSTM are also expressed in lung epithelial cells [38]. A recent study demonstrated that GSTM positive humans display higher NF-κB activity and airway neutrophilia following allergen challenge, as compared to GSTM deficient humans [39]. These findings are contradictory to the present study which demonstrates that endogenous GSTP represses NF-κB activity. While GSTM may have limited S-glutathionylating activity, there are fewer indications that it possesses the chaperone binding properties of GSTP[40] Nonetheless, additional studies will be required to elucidate whether GSTM affects S-glutathionylation of NF-κB pathway components, and whether absence of GSTM affects the expression and/or function of GSTP. Similarly, GSTO also has been linked to the COPD [41] and can be active in the process of de-glutathionylation [42], yet its role in the regulation of NF-κB also remains unclear, and future studies will be needed to address this question.

Herein, we have demonstrated that GSTP modulates NF-κB activation and pro-inflammatory cytokine production in lung epithelial cells. One potential mechanism by which this occurs is

via GSTP-catalyzed S-glutathionylation of IKKβ; however, we also provide evidence that GSTP associates with NF-κB proteins, notably IKKβ and IκBα. These data suggest multiple mechanisms by which GSTP regulates NF-κB. Given the reported relevance of GSTP polymorphisms in allergic asthma, the present findings which demonstrate a strong impact of GSTP on the activation of NF-κB studies provide new insights into the mechanisms by which GSTP can regulate pro-inflammatory signaling in inflammatory lung diseases.

Acknowledgments

This work was supported by Grants from the National Institutes of Health, R01 HL060014 and HL085464 (to Y J-H), HL085646 (to A vd V) and the National Cancer Institute, CA85660 (to KDT) and the National Institute of General Medical Sciences, 5P20GM103542 (to KDT).

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