Biochemical and Biophysical Research Communications 439 (2013) 357–362

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Oxidative stress potentially enhances FcεRI-mediated leukotriene C_4 release dependent on the late-phase increase of intracellular glutathione in mast cells

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ARTICLE INFO

Article history:
Received 20 August 2013
Available online 30 August 2013

Keywords:
Mast cell
Glutathione
Leukotriene C_4
Oxidative stress

ABSTRACT

Cysteinyl leukotrienes (cysLTs), which include leukotriene C_4 (LTC_4), are the predominant class of LTs synthesized by mast cells. CysLTs can induce many of the abnormalities seen in asthma. LTC_4 is generated by the conjugation of LTA_4 with reduced glutathione (GSH) by LTC_4 synthase. During screening of the effects of prostanoids on high-affinity IgE receptor (FcεRI)-mediated LTC_4 release from mast cells, we realized that some prostanoids, including ONO-AE1-259-01 and ONO-AE-248, inhibited LTC_4 release, which was associated with a decrease in the amount of intracellular total GSH. We ascertained that L-buthionine-*S,R*-sulfoximine (BSO), a selective inhibitor of glutamate-cysteine ligase, inhibited LTC_4 release. In addition, cell-permeable GSH, the glutathione reduced form ethyl ester (GSH-OEt), enhanced LTC_4 release in accordance with the change in intracellular total GSH. Depletion of intracellular total GSH induced by ONO-AE-248 or BSO enhanced FcεRI-mediated LTB_4 release in contrast to LTC_4. Oxidative stress contributes to many pathological conditions including asthma. GSH is a major soluble antioxidant and a cofactor for several detoxifying enzymes including GSH peroxidase. Exposure of mast cells to hydrogen peroxide (H_2O_2) or diamide to mimic oxidative stress unexpectedly increased rather than decreased the intracellular reduced GSH content as well as total GSH in the late phase (i.e., 24 or 48 h after exposure), which was accompanied by an increase in LTC_4 release. In conclusion, FcεRI-mediated LTC_4 release from mast cells is mainly regulated by the amount of intracellular GSH. In some cases, oxidative stress may induce a late-phase increase in intracellular GSH, resulting in enhanced LTC_4 release from mast cells.

1. Introduction

Several lines of evidence support a role for leukotrienes (LTs) in the pathogenesis of asthma [1,2]. The cysteinyl LTs (cysLTs), which include leukotriene C_4 (LTC_4) and its derivatives, LTD_4 and LTE_4, are the predominant class of LTs synthesized by mast cells and eosinophils [3]. Upon FcεRI-mediated activation of mast cells, the intracellular calcium concentration rises, causing translocation of the enzyme 5-lipoxygenase (5-LO) to the perinuclear membrane. Here, 5-LO associates with the helper protein, 5-LO activating protein (FLAP), which presents the membrane phospholipid-derived substrate arachidonate to 5-LO, permitting 5-LO to catalyze the formation of an unstable intermediate known as LTA_4. LTA_4 is either hydrolyzed by LTA_4 hydrolase (LTA_4H) to form LTB_4 or conjugated to glutathione (GSH) by LTC_4 synthase (LTC_4S) to form LTC_4 [1,2,4]. LTC_4 synthesis may be regulated by both LTC_4S activities and the amount of intracellular GSH as a substrate for LTC_4S [1,4,5].

Oxidative stress may have detrimental effects on cellular and tissue function, contributing to the pathogenesis of a wide variety of diseases including asthma [6,7]. GSH, a thiol tripeptide, is an important component of antioxidant enzyme systems, including GSH peroxidase, and modulates the development and function of a variety of immune cells [7,8]. However, the relationship between oxidative stress and FcεRI-mediated LTC_4 synthesis has not been examined. During experiments in which the effects of prostanoids

Abbreviations: FcεRI, high-affinity IgE receptor; LT, leukotriene; EP, prostaglandin E_2 receptor; BSO, L-buthionine-*S,R*-sulfoximine; GSH, glutathione; GSSG, oxidized glutathione; GSH-OEt, glutathione reduced form ethyl ester.

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http://dx.doi.org/10.1016/j.bbrc.2013.08.081

on FcεRI-mediated LTC_4 release were examined, we found that some prostanoids inhibited LTC_4 release independent of stimulation of prostaglandin E_2 receptors (EPs). Our results suggested that FcεRI-mediated LTC_4 release from mast cells mainly depends on intracellular GSH levels and that the oxidative stress-induced late-phase increase in intracellular GSH may enhance airway inflammation through FcεRI-mediated LTC_4 release.

2. Materials and methods

2.1. Cells and reagents

The MC/9 mouse mast cell clone (American Type Culture Collection, Manassas, VA) was maintained by passaging in complete medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) (Sigma–Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) (Equitech-Bio, Inc., Kerrville, TX), 5 ng/ml mouse recombinant interleukin-3 (IL-3) (R&D Systems, Minneapolis, MN), 50 μM 2-mercaptoethanol (2-ME), 50 μg/ml streptomycin, and 50 U/ml penicillin. Human cord blood $CD34^+$ cells were purchased from ALLCELLS (Emeryville, CA) and cultured in complete medium consisting of RPMI1640 (Life Technologies, Carlsbad, CA) supplemented with 10% FBS (HyClone, Logan, Utah), 10 ng/ml human recombinant IL-3 (R&D Systems), 0.5 μg/ml human IgE (Yamasa Corporation, Tokyo, Japan), 50 μM 2-ME, streptomycin, and penicillin. After 2 weeks of culture, alcian blue-stained non-adherent cells were obtained. These cells were referred to as human cultured basophils. Goat anti-human IgE was purchased from Chemicon International (Temecula, CA). Mouse monoclonal anti-dinitrophenyl (DNP) IgE was obtained from Yamasa Corporation. DNP-conjugated human serum albumin (DNP-HSA), L-buthionine-*S,R*-sulfoximine (BSO), glutathione reduced form ethyl ester (GSH-OEt), and diamide were purchased from Sigma–Aldrich. The EP_3 agonist, sulprostone [9,10], prostaglandin E_2 (PGE_2), and dimethyl PGE_2 were purchased from Cayman Chemical (Ann Arbor, MI). ONO-DI-004 (an EP_1 agonist) [9–11], ONO-AE1-259-01 (an EP_2 agonist) [9,11], ONO-AE-248 (an EP_3 agonist) [9–12], and ONO-AE1-329 (an EP_4 agonist) [9–11] were kindly provided by ONO Pharmaceutical Co., Ltd. (Osaka, Japan). Hydrogen peroxide (H_2O_2) was obtained from Wako (Osaka, Japan). Bovine serum albumin (BSA) was purchased from EMD Chemicals (San Diego, CA).

2.2. Sensitization and stimulation of the cells

MC/9 cells (1–5 × 10^6/ml) were cultured with anti-DNP IgE (0.5 μg/ml) in complete medium for 1 h. After washing with 0.1% BSA-DMEM, the cells (1 × 10^6/ml) were stimulated for 30 min with DNP-HSA (10 ng/ml). Human cultured basophils, with cell surface-bound IgE, were washed with RPMI1640 containing 0.1% BSA (0.1% BSA-RPMI), and the cells (1 × 10^6/ml) were stimulated for 30 min with goat anti-human IgE antibody (1 μg/ml).

2.3. Measurement of LTC_4 and LTB_4

LTC_4 or LTB_4 was assayed with an LTC_4 EIA kit or an LTB_4 EIA kit (Cayman Chemical) according to the manufacturer's instructions.

2.4. Measurement of the amount of intracellular GSH

The amount of intracellular GSH was assayed with a Glutathione Assay kit (Cayman Chemical). Briefly, cells (1 × 10^7/sample) were sonicated with an ultra-sound sonicator (Tomy Seiko, Tokyo, Japan) in 50 mM 2-(*N*-morpholino) ethanesulfonic acid containing 1 mM EDTA. The supernatant obtained by centrifugation at 10,000*g* for 15 min at 4 °C was mixed with an equal volume of 10% (w/v) metaphosphoric acid. Total GSH and oxidized glutathione (GSSG) were assayed according to the manufacturer's protocol. The values of reduced GSH for each sample were calculated by subtracting the values of GSSG from those of total GSH.

2.5. Quantitative RT-PCR using real-time TaqMan technology

Total RNA from cells was extracted with an RNeasy Mini kit (QIAGEN, Chatsworth, CA) and converted to cDNA using a High-Capacity cDNA Archive kit (Life Technologies). mRNA expression was quantified with real-time PCR using TaqMan probes (Life Technologies). TaqMan assay-on-demand gene expression assays were utilized except for 5-LO. The primers and the probe for 5-LO were as follows.

5′-FAM-TCGATACTGTTTTTGCCGTGCCTCCA-MBG-3′
5′-ACCAAATTCACATCCTCAAGCA-3′
5′-CTTGTGGCATTTGGCATCAAT-3′

The expression level of the target mRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as described [13].

2.6. Statistical analysis

The data were analyzed using GraphPad Prism software (GraphPAd, La Jolla, CA).

3. Results

3.1. Effects of prostanoids on FcεRI-mediated LTC_4 release from MC/9 cells

Incubation with ONO-AE-248 or ONO-AE1-259-01 for 6 h inhibited FcεRI-mediated LTC_4 release from MC/9 cells in a dose-dependent manner. The mean value of FcεRI-mediated LTC_4 release from MC/9 cells without prostanoids was 7.95 ng/10^6 cells (Fig. 1A). On the other hand, neither PGE_2, dimethyl PGE_2, ONO-DI-004, sulprostone, nor ONO-AE1-329 blocked FcεRI-mediated LTC_4 release at 1 μM (Fig. 1B).

3.2. Expression of EPs and the molecules involved in the biosynthesis of LTs in MC/9 cells

mRNA expression of EPs was analyzed with quantitative real-time PCR. EP_3 mRNA and EP_4 mRNA were strongly expressed in MC/9 cells, but expression of EP_1 and EP_2 was barely detected (Fig. 1C). To examine the effects of ONO-AE-248 on mRNA expression of the molecules involved in biosynthesis of LTs, MC/9 cells were incubated in 0.1% BSA-DMEM with ONO-AE-248 (1 μM) or control vehicle (0.1% DMSO) for 6 h. Incubation with ONO-AE-248 did not affect mRNA expression of 5-LO, FLAP, LTC_4S, or LTA_4H in MC/9 cells (Fig. 1D).

3.3. Effects of prostanoids or GSH modulators on the amount of intracellular GSH and FcεRI-mediated LTB_4/LTC_4 release from MC/9 cells

Incubation with 1 μM ONO-AE-248 for 6 h significantly enhanced FcεRI-mediated LTB_4 release from MC/9 cells, but not LTC_4 release. The mean value of FcεRI-mediated LTB_4 release without ONO-AE-248 was 125 pg/10^6 cells (Fig. 2A). ONO-AE1-259-01 strongly decreased the amount of intracellular total GSH after incubation for 6 h, although EP_2 mRNA expression was low in MC/9 cells. Incubation with ONO-AE-248 for 6 h also decreased the amount of intracellular total GSH in accordance with inhibition

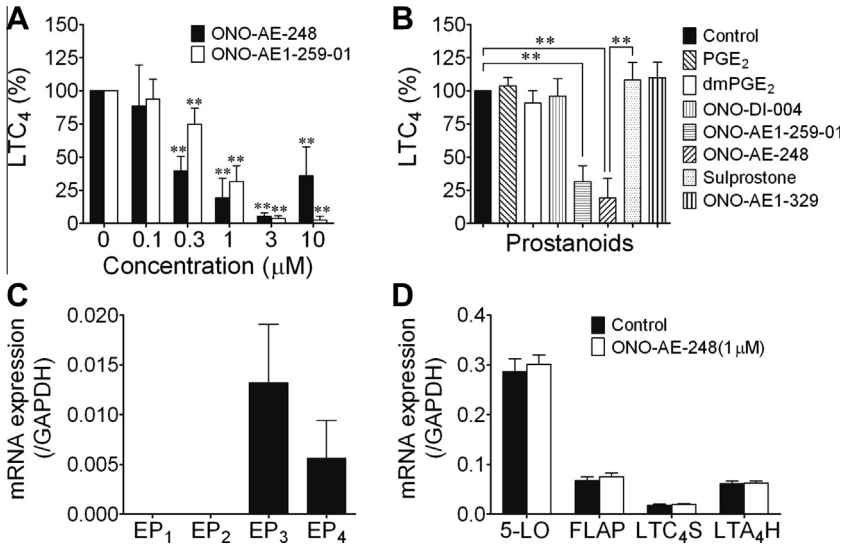

Fig. 1. Effects of prostanoids on FcεRI-mediated LTC_4 release from MC/9 cells. (A) MC/9 cells sensitized with anti-DNP IgE (1×10^6/ml) were incubated for 6 h with ONO-AE-248, ONO-AE1-259-01, or control vehicle (0.1% DMSO) in 0.1% BSA-DMEM, and stimulated with DNP-HSA (10 ng/ml) for 30 min. LTC_4 was normalized to that released from stimulated cells after treatment with 0.1% DMSO. (B) PGE_2, dimethyl PGE_2 ($dmPGE_2$), ONO-DI-004, sulprostone, and ONO-AE1-329 did not significantly inhibit FcεRI-mediated LTC_4 release from MC/9 cells at 1 μM. The data were normalized as described above. (C) mRNA expression of PGE_2 receptors was normalized to that of GAPDH. EP_3 mRNA and EP_4 mRNA were predominantly expressed in MC/9 cells. (D) mRNA expression of 5-LO, FLAP, LTC_4S, and LTA_4H in MC/9 cells was analyzed with quantitative RT-PCR after incubation for 6 h with ONO-AE-248 (1 μM) or control vehicle. Data were normalized to GAPDH expression. The data are expressed as mean ± SD from at least six experiments (**, $P < 0.01$).

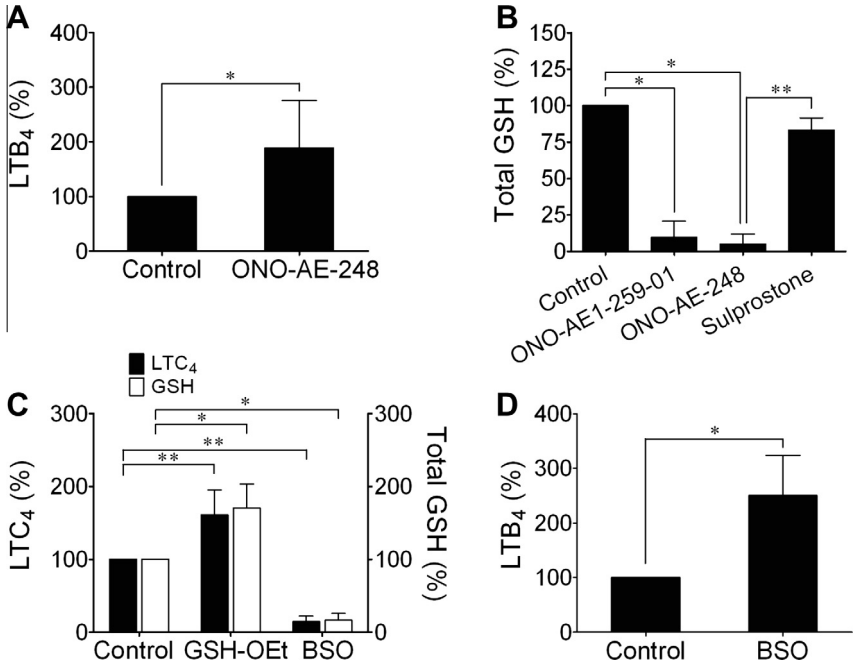

Fig. 2. Effects of prostanoids or GSH modulators on the amount of intracellular GSH and FcεRI-mediated LTC_4/LTB_4 release from MC/9 cells. (A) After MC/9 cells were sensitized with anti-DNP IgE (1×10^6 cells/ml), cells were incubated with ONO-AE-248 (1 μM) or control vehicle in 0.1% BSA-DMEM for 6 h. The cells were stimulated with DNP-HSA (10 ngml) for 30 min. LTB_4 in the supernatants was normalized to that obtained from cells treated with control vehicle. (B) After MC/9 cells (1×10^6/ml) were incubated with ONO-AE1-259-01 (1 μM), ONO-AE-248 (1 μM), sulprostone (1 μM), or control vehicle in 0.1% BSA-DMEM for 6 h, the amount of intracellular total GSH was assayed and normalized to that in cells treated with control vehicle. Incubation with ONO-AE1-259-01 or ONO-AE-248 but not sulprostone strongly decreased the amount of intracellular total GSH. (C) MC/9 cells (1×10^6/ml) were incubated in complete medium with 5 mM GSH-OEt, 50 μM BSO, or without agents for 24 h. The cells were passively sensitized with anti-DNP IgE (0.5 μg/ml) for at least 1 h and washed well with 0.1% BSA-DMEM for the LTC_4 experiments. Sensitized MC/9 cells were stimulated with DNP-HSA (10 ng/ml) in 0.1% BSA-DMEM for 30 min. LTC_4 in the supernatant was normalized to that from non-treated cells. LTC_4 release from GSH-OEt-treated cells was significantly higher, and that from BSO-treated cells was significantly lower than that from non-treated cells. The amount of intracellular total GSH in GSH-OEt-treated cells was significantly higher and that in BSO-treated cells was significantly lower than that in non-treated cells. (D) MC/9 cells (1×10^6/ml) were cultured with or without 50 μM BSO in complete culture medium for 24 h. The cells were passively sensitized with anti-DNP IgE for at least 1 h. After incubation for 24 h with or without 50 μM BSO, MC/9 cells were stimulated with DNP-HSA (10 ng/ml) in 0.1% BSA-DMEM for 30 min. LTB_4 in the supernatants was assayed and normalized to that in the supernatant of the cells treated without BSO. LTB_4 release was significantly enhanced by treatment with BSO. The data are expressed as mean ± SD from at least six experiments (*, $P < 0.05$; **, $P < 0.01$).

of LTC_4 release. However, sulprostone decreased neither the amount of intracellular GSH nor LTC_4 release. The mean value of intracellular total GSH in MC/9 cells without prostanoids was 3.51 nmol/10^7cells (Fig. 2B).

We observed that depletion of serum in the culture medium itself decreased both the amount of intracellular total GSH and the release of LTC_4 from MC/9 cells (data not shown). Thus, we investigated the effects of incubation for 24 h with cell-permeable GSH, GSH-OEt [13], or BSO, a selective inhibitor of glutamate-cysteine ligase (GCL), which is the enzyme in the GSH biosynthesis pathway [14], on intracellular total GSH and LTC_4 release in the presence of 10% FBS. Incubation for 24 h with 5 mM GSH-OEt increased the amount of intracellular total GSH and FcεRI-mediated LTC_4 release in MC/9 cells. On the contrary, incubation with 50 μM BSO for 24 h significantly decreased both the amount of intracellular total GSH and the FcεRI-mediated LTC_4 release (Fig. 2C). Similar to ONO-AE-248 (Fig. 2A), BSO significantly enhanced FcεRI-mediated LTB_4 release (Fig. 2D).

We examined the effects of GSH-OEt on ONO-AE-248-induced inhibition of LTC_4 release. Incubation with 5 mM GSH-OEt for 6 h blocked the decrease in intracellular total GSH and LTC_4 release induced by ONO-AE-248 (Fig. 3A). Exogenous addition of cysteine [15] also increased the amount of intracellular total GSH and enhanced FcεRI-mediated LTC_4 release. Incubation with 1 mM cysteine for 6 h had similar protective effects on the ONO-AE-248-induced suppression of intracellular total GSH and LTC_4 release (Fig. 3B).

3.4. Effects of GSH modulators on LTC_4 release from human cultured basophils

We examined whether LTC_4 release from human cells also depended on intracellular GSH levels. Incubation with 5 mM GSH-OEt for 6 h significantly enhanced FcεRI-mediated LTC_4 release from human cultured basophils. The mean value of FcεRI-mediated LTC_4 release from human cultured basophils was 2.30 ng/10^6 cells. Incubation with 5 mM BSO for 24 h significantly decreased FcεRI-mediated LTC_4 release from human cultured basophils (Fig. 3C).

3.5. Effects of exogenous H_2O_2 and diamide on the amount of intracellular total or reduced GSH, the ratio of reduced GSH to GSSG (GSH:GSSG ratio) and LTC_4 release from MC/9 cells

As a model of oxidative stress, we added 300 μM H_2O_2[16,17] or 500 μM diamide [18,19] to the complete culture medium of MC/9 cells. Diamide can easily penetrate cell membranes and react with intracellular thiols. We measured the amounts of intracellular total GSH, reduced GSH, and GSSG as well as the released LTC_4 at several points: 0, 6, 24 and 48 h after the treatment with H_2O_2 or diamide. Neither agent appreciably affected GSH:GSSG ratio, or the total and reduced GSH content 6 h after exposure to the cells, although mRNA expression of LTC_4S in the treated cells was significantly decreased compared with that in non-treated cells (27% decrease in H_2O_2-treated cells and 42% decrease in diamide-treated cells; $P < 0.05$, mean from six experiments).

Unexpectedly, either H_2O_2 or diamide significantly increased the amount of intracellular reduced GSH as well as total GSH at 24 or 48 h (Fig. 4B and E). FcεRI-mediated LTC_4 release was significantly enhanced by these agents (Fig. 4A and D). GSH:GSSG ratio within cells is often used as a marker of oxidative stress [20]. The ratio in H_2O_2 or diamide-treated cells did not change 6 or 48 h after the treatment compared with that in non-treated cells. Interestingly, GSH:GSSG ratio in H_2O_2 or diamide-treated cells was significantly increased 24 h after the treatment (Fig. 4C and F). However, the increase of GSH:GSSG ratio did not affect mRNA expression of LTC_4S 24 h after either treatment (LTC_4S mRNA expression in H_2O_2-treated cells was 99% and that in diamide-treated cells was 113% compared with that in non-treated cells (mean from six experiments).

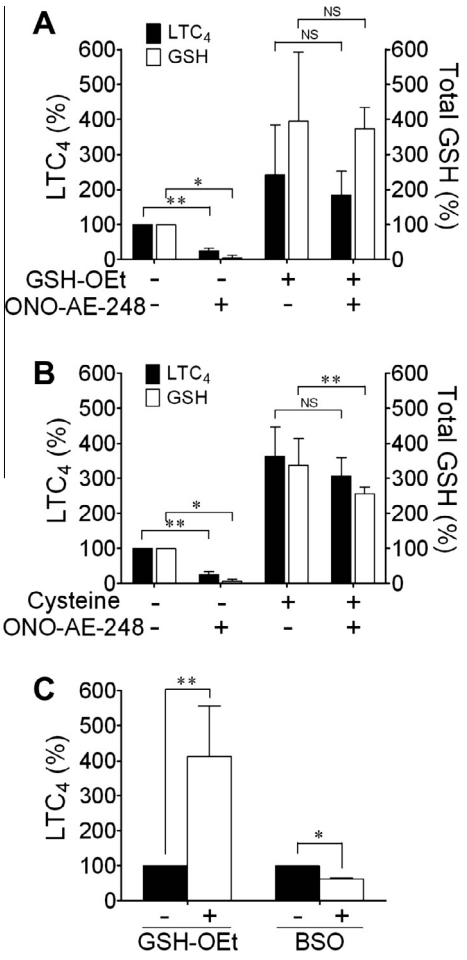

Fig. 3. FcεRI-mediated LTC_4 release depends on the levels of intracellular total GSH. MC/9 cells sensitized with anti-DNP IgE (1×10^6/ml) were incubated with 1 μM ONO-AE-248 or control vehicle (0.1% DMSO) in 0.1% BSA-DMEM in the presence or absence of 5 mM GSH-OEt for 6 h (A) MC/9 cells sensitized with anti-DNP IgE (1×10^6/ml) were incubated with 1 μM ONO-AE-248 or control vehicle (0.1% DMSO) in 0.1% BSA-DMEM in the presence or absence of 1 mM cysteine for 6 h (B). The cells were then stimulated with DNP-HSA (10 ng/ml) for 30 min. LTC_4 in the supernatants was assayed and normalized to that obtained from cells treated without ONO-AE-248, GSH-OEt, or cysteine. Non-sensitized MC/9 cells were used to measure GSH. The amount of intracellular total GSH was assayed and normalized to that in cells treated without ONO-AE-248, GSH-OEt, or cysteine. (C) Human cultured basophils sensitized with human IgE (1×10^6/ml) were incubated in 0.1% BSA-RPMI for 6 h with or without 5 mM GSH-OEt. The cells were then stimulated with anti-human IgE (1 μg/ml) for 30 min. Human cultured basophils were cultured in complete culture medium with or without 5 mM BSO for 24 h. The cells were then stimulated with anti-human IgE (1 μg/ml) in 0.1% BSA-RPMI for 30 min. LTC_4 in the supernatants was normalized to that obtained from cells treated without GSH-OEt or BSO. The data are expressed as mean ± SD from at least six experiments (NS, not significant; $^*P < 0.05$; $^{**}P < 0.01$).

4. Discussion

GSH is a critical player in detoxifying and antioxidant enzyme systems and protects tissues from oxidative stress [7,21,22]. GSSG generated from reduced GSH in oxidative stress conditions is actively exported out of the cell [19]. We noticed that some prostanoids reduced both intracellular GSH and LTC_4 release in MC/9. We realized that GSH modulators such as GSH-OEt and BSO influenced intracellular GSH levels and affected FcεRI-mediated LTC_4

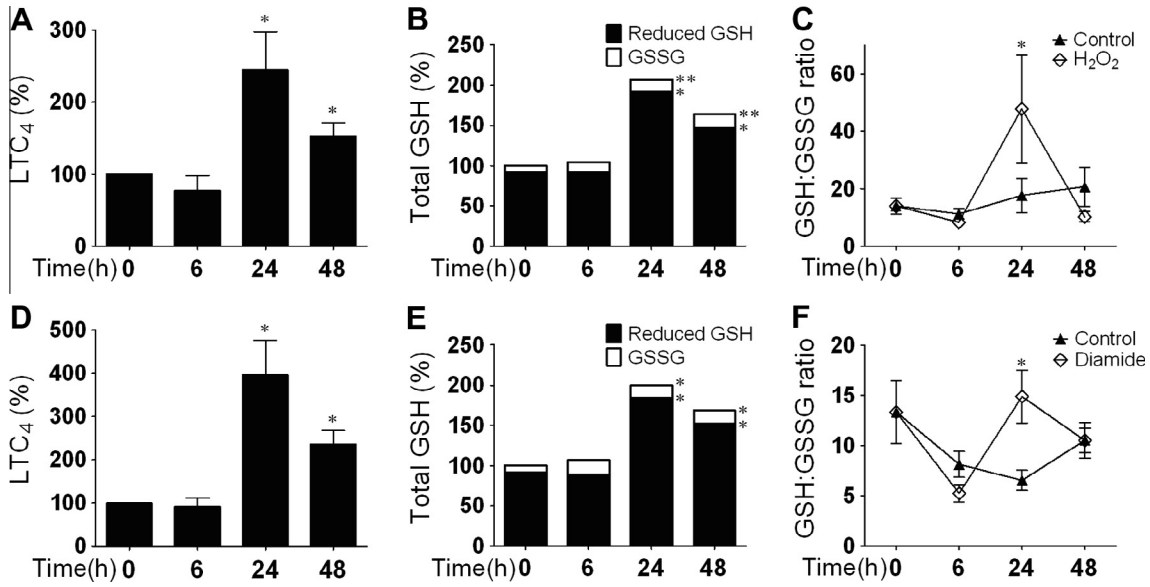

Fig. 4. Effects of oxidative stress on FcεRI-mediated LTC_4 release from MC/9 cells. MC/9 cells (1×10^6/ml) were cultured in complete medium in the absence or presence of H_2O_2 (300 μM) for 6, 24 or 48 h (A, B, C). MC/9 cells (1×10^6/ml) were cultured in complete medium in the absence or presence of diamide (500 μM) for 30 min and washed well to remove diamide. The cells were cultured in complete medium for 6, 24 or 48 h after the treatment with diamide (D, E, F). The cells were sensitized with anti-DNP IgE for at least 1 h. Sensitized MC/9 cells were washed with 0.1% BSA-DMEM, and the cells were stimulated with DNP-HSA (10 ng/ml) for 30 min. LTC_4 in the supernatants was assayed and normalized to that obtained from cells treated without H_2O_2 or diamide (A, D). The amounts of intracellular total GSH, reduced GSH and GSSG were assayed and normalized to those in cells treated without H_2O_2 or diamide. Total or reduced GSH was increased significantly 24 or 48 h after the treatment (B, E). GSH:GSSG ratio was significantly increased 24 h after the treatment with H_2O_2 or diamide (C, F). The data are expressed as mean ± SD (A, B, D, E) or mean ± SEM (C, F) from six experiments ($^*P < 0.05$; $^{**}P < 0.01$).

release from MC/9 cells and human cultured basophils. LTB_4 generated from mast cells seems to be involved in the early airway recruitment of granulocytes [23] and airway hyperresponsiveness [24] in an asthma model. Interestingly, FcεRI-mediated LTB_4 release from MC/9 cells was enhanced when both intracellular GSH and LTC_4 release were decreased. Thus, conversion of LTA_4 may be shifted to LTB_4 synthesis from LTC_4 synthesis because GSH depletion inhibits the generation of LTC_4.

Many agonists and antagonists with selectivity for PG receptor subtypes have been synthesized to characterize their role in pathophysiological processes and as potential therapeutic medicines [9]. ONO-AE-248 and ONO-AE1-259-01 are classified as selective EP_3 and EP_2 receptor agonists, respectively. The present study suggests that these drugs may exert pharmacological actions independent of EPs. While the current study was being conducted, another group reported that a selective agonist of the DP_2 receptor, 15*R*-methyl PGD_2, inhibited LTC_4 generation by reducing intracellular GSH through mechanisms unrelated to DP_2 receptors or PPARγ in calcium ionophore A23187-stimulated mouse bone marrow-derived mast cells [18]. These results are consistent with those of the current study, although the precise mechanism by which these prostanoids reduce intracellular GSH is unknown. We speculate that these compounds themselves or their metabolites may be conjugated to GSH and excreted out of the cell, resulting in reduction of intracellular GSH.

In the presence of excess GSH supplied by exogenous GSH-OEt or cysteine, the prostanoid-induced LTC_4 reduction was attenuated in association with inhibition of GSH reduction. Moreover, exogenous H_2O_2 or diamide unexpectedly increased the total or reduced GSH content 24 or 48 h after addition of these agents in association with enhanced FcεRI-mediaed LTC_4 release. These results suggest that oxidative stress may elicit overproduction of reduced GSH, although stress may transiently decrease LTC_4S expression or activities, leading to increased LTC_4 generation in inflammatory lesions. We cannot deny the possibility that the increase of GSH:GSSG ratio enhanced LTC_4 release through other mechanisms because LTC_4 release was enhanced 24 h after the treatment in accordance with the increase of GSH:GSSG ratio in H_2O_2 or diamide-treated cells. However, we speculate that the amount of intracellular reduced GSH regulates LTC_4 generation more directly.

The mechanism by which intracellular total or reduced GSH increases in response to oxidative stress is undefined. Although we were unable to detect significant increases in mRNA expression of the catalytic subunit of GCL and glutathione synthase, which are involved in GSH biosynthesis (data not shown) [21,25], oxidative stress may enhance GCL activity via post-translational modification because H_2O_2 is known to stimulate GCL activity without changing the amount of the enzyme [25–27].

In conclusion, FcεRI-mediated LTC_4 release from mast cells mainly depends on levels of intracellular reduced GSH. A late-phase increase in intracellular reduced GSH following exposure to agents that can induce oxidative stress may exacerbate airway inflammation by enhancing release of LTC_4.

Acknowledgments

We are grateful to Dr. Masami Narita of ONO Pharmaceutical Co. Ltd. for providing us with EP agonists. We thank Dr. Hisao Imai, Dr. Masakiyo Yatomi, and Dr. Shinichi Matsuzaki for their assistance and helpful discussion. This work was partly supported by Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science (T.I.). None of the authors declare competing financial interests.

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