Oxidative stress potentially enhances FcεRI-mediated leukotriene C4 release dependent on the late-phase increase of intracellular glutathione in mast cells

Kaori Seki a, Takeshi Hisada a, Tadayoshi Kawata a, Yosuke Kamide a, Kunio Dobashi a, Masanobu Yamada a, Masatomo Mori a, Fumikazu Okajima b, Tamotsu Ishizuka a,c,*

a Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi 371-8511, Japan

b Laboratory of Signal Transduction, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371-8512, Japan

c Third Department of Internal Medicine, Faculty of Medical Sciences, University of Fukui, Fukui 910-1193, Japan

*Corresponding author

Tamotsu Ishizuka, M.D., Ph.D.

Address: Third Department of Internal Medicine, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuoka-Shimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan. Fax +81-776-61-8111

E-mail address: tamotsui@u-fukui.ac.jp (T. Ishizuka)
Abstract

Cysteinyl leukotrienes (cysLTs), which include leukotriene C₄ (LTC₄), are the predominant class of LTs synthesized by mast cells. CysLTs can induce many of the abnormalities seen in asthma. LTC₄ is generated by the conjugation of LTA₄ with reduced glutathione (GSH) by LTC₄ synthase. During screening of the effects of prostanoids on high-affinity IgE receptor (FcεRI)-mediated LTC₄ release from mast cells, we realized that some prostanoids, including ONO-AE1-259-01 and ONO-AE-248, inhibited LTC₄ 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 LTC₄ release. In addition, cell-permeable GSH, the glutathione reduced form ethyl ester (GSH-OEt), enhanced LTC₄ 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 LTB₄ release in contrast to LTC₄. 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 (H₂O₂) 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 hrs after exposure), which was accompanied by an increase in LTC₄ release. In conclusion, FcεRI-mediated LTC₄ 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 LTC₄ release from mast cells.

Key words

mast cell, glutathione, leukotriene C₄, oxidative stress

Abbreviations: FcεRI, high-affinity IgE receptor; LT, leukotriene; EP, prostaglandin E₂ receptor; BSO, L-buthionine-S,R-sulfoximine; GSH, glutathione; GSSG, oxidized glutathione; GSH-OEt, glutathione reduced form ethyl ester
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 C4 (LTC4) and its derivatives, LTD4 and LTE4, 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 LTA4. LTA4 is either hydrolyzed by LTA4 hydrolase (LTA4H) to form LTB4 or conjugated to glutathione (GSH) by LTC4 synthase (LTC4S) to form LTC4 [1,2,4]. LTC4 synthesis may be regulated by both LTC4S activities and the amount of intracellular GSH as a substrate for LTC4S [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 LTC₄ synthesis has not been examined. During experiments in which the effects of prostanoids on FcεRI-mediated LTC₄ release were examined, we found that some prostanoids inhibited LTC₄ release independent of stimulation of prostaglandin E₂ receptors (EPs). Our results suggested that FcεRI-mediated LTC₄ 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 LTC₄ 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 EP3 agonist, sulprostone [9,10], prostaglandin E2 (PGE2), and dimethyl PGE2 were purchased from Cayman Chemical (Ann Arbor, MI). ONO-DI-004 (an EP1 agonist) [9,10,11], ONO-AE1-259-01 (an EP2 agonist) [9,11], ONO-AE-248 (an EP3 agonist) [9,10,11,12], and ONO-AE1-329 (an EP4 agonist) [9,10,11] were kindly provided by ONO Pharmaceutical Co., Ltd. (Osaka, Japan). Hydrogen peroxide (H2O2) 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 hr. 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 LTC₄ and LTB₄

LTC₄ or LTB₄ was assayed with an LTC₄ EIA kit or an LTB₄ 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′-ACCAAAATTCACATCCTCAAGCA-3′

5′-CTTGTGGCATTGCGCATCAAT-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 LTC₄ release from MC/9 cells

Incubation with ONO-AE-248 or ONO-AE1-259-01 for 6 hrs inhibited FcεRI-mediated LTC₄ release from MC/9 cells in a dose-dependent manner. The mean value of FcεRI-mediated LTC₄ release from MC/9 cells without prostanoids was 7.95 ng/10⁶ cells (Fig. 1A). On the other hand, neither PGE₂, dimethyl PGE₂, ONO-DI-004, sulprostone, nor ONO-AE1-329 blocked FcεRI-mediated LTC₄ 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. EP₃ mRNA and EP₄ mRNA were strongly expressed in MC/9 cells, but expression of EP₁ and EP₂ 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
hrs. Incubation with ONO-AE-248 did not affect mRNA expression of 5-LO, FLAP, LTC₄S, or LTA₄H in MC/9 cells (Fig. 1D).

3.3. Effects of prostanoids or GSH modulators on the amount of intracellular GSH and FcεRI-mediated LTB₄/LTC₄ release from MC/9 cells

Incubation with 1 μM ONO-AE-248 for 6 hrs significantly enhanced FcεRI-mediated LTB₄ release from MC/9 cells, but not LTC₄ release. The mean value of FcεRI-mediated LTB₄ release without ONO-AE-248 was 125 pg/10⁶ cells (Fig. 2A). ONO-AE1-259-01 strongly decreased the amount of intracellular total GSH after incubation for 6 hrs, although EP₂ mRNA expression was low in MC/9 cells. Incubation with ONO-AE-248 for 6 hrs also decreased the amount of intracellular total GSH in accordance with inhibition of LTC₄ release. However, sulprostone decreased neither the amount of intracellular GSH nor LTC₄ release. The mean value of intracellular total GSH in MC/9 cells without prostanoids was 3.51 nmol/10⁷ cells (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 LTC₄ from MC/9 cells (data not shown). Thus, we investigated the effects of incubation for 24 hrs 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 LTC₄ release in the presence of 10% FBS. Incubation for 24 hrs with 5 mM GSH-OEt increased the amount of intracellular total GSH and FcεRI-mediated LTC₄ release in MC/9 cells. On the contrary, incubation with 50 μM BSO for 24 hrs significantly decreased both the amount of intracellular total GSH and the FcεRI-mediated LTC₄ release (Fig. 2C). Similar to ONO-AE-248 (Fig. 2A), BSO significantly enhanced FcεRI-mediated LTB₄ release (Fig. 2D).

We examined the effects of GSH-OEt on ONO-AE-248-induced inhibition of LTC₄ release. Incubation with 5 mM GSH-OEt for 6 hrs blocked the decrease in intracellular total GSH and LTC₄ 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 LTC₄ release. Incubation with 1 mM cysteine for 6 hrs had similar protective effects on the ONO-AE-248-induced suppression of intracellular total GSH and LTC₄ release (Fig. 3B).

3.4. Effects of GSH modulators on LTC₄ release from human cultured basophils
We examined whether LTC₄ release from human cells also depended on intracellular GSH levels. Incubation with 5 mM GSH-OEt for 6 hrs significantly enhanced FcεRI-mediated LTC₄ release from human cultured basophils. The mean value of FcεRI-mediated LTC₄ release from human cultured basophils was 2.30 ng/10⁶ cells. Incubation with 5 mM BSO for 24 hrs significantly decreased FcεRI-mediated LTC₄ release from human cultured basophils (Fig. 3C).

3.5. Effects of exogenous H₂O₂ and diamide on the amount of intracellular total or reduced GSH, the ratio of reduced GSH to GSSG (GSH:GSSG ratio) and LTC₄ release from MC/9 cells

As a model of oxidative stress, we added 300 μM H₂O₂ [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 LTC₄ at several points: 0, 6, 24 and 48 hrs after the treatment with H₂O₂ or diamide. Neither agent appreciably affected GSH:GSSG ratio, or the total and reduced GSH content 6 hrs after exposure to the cells, although mRNA expression of LTC₄S in the treated cells was significantly decreased compared with that in non-treated cells (27% decrease in H₂O₂-treated cells
and 42% decrease in diamide-treated cells; \( P<0.05 \), mean from six experiments).

Unexpectedly, either \( \text{H}_2\text{O}_2 \) or diamide significantly increased the amount of intracellular reduced GSH as well as total GSH at 24 or 48 hrs (Fig. 4B, 4E). FceRI-mediated LTC\(_4\) release was significantly enhanced by these agents (Fig. 4A, 4D). GSH:GSSG ratio within cells is often used as a marker of oxidative stress [20]. The ratio in \( \text{H}_2\text{O}_2 \) or diamide-treated cells did not change 6 or 48 hrs after the treatment compared with that in non-treated cells. Interestingly, GSH:GSSG ratio in \( \text{H}_2\text{O}_2 \) or diamide-treated cells was significantly increased 24 hrs after the treatment (Fig. 4C, 4F). However, the increase of GSH:GSSG ratio did not affect mRNA expression of LTC\(_4\)S 24 hrs after either treatment (LTC\(_4\)S mRNA expression in \( \text{H}_2\text{O}_2 \)-treated cells was 99% and that in diamide-treated cells was 113% compared with that in non-treated cells (mean from six experiments).

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 LTC₄ release in MC/9. We realized that GSH modulators such as GSH-OEt and BSO influenced intracellular GSH levels and affected FcεRI-mediated LTC₄ release from MC/9 cells and human cultured basophils. LTB₄ 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 LTB₄ release from MC/9 cells was enhanced when both intracellular GSH and LTC₄ release were decreased. Thus, conversion of LTA₄ may be shifted to LTB₄ synthesis from LTC₄ synthesis because GSH depletion inhibits the generation of LTC₄.

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 EP₃ and EP₂ 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 DP₂ receptor, 15R-methyl PGD₂, inhibited LTC₄ generation by reducing intracellular GSH
through mechanisms unrelated to DP₂ 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 LTC₄ reduction was attenuated in association with inhibition of GSH reduction. Moreover, exogenous H₂O₂ or diamide unexpectedly increased the total or reduced GSH content 24 or 48 hrs after addition of these agents in association with enhanced FcεRI-mediated LTC₄ release. These results suggest that oxidative stress may elicit overproduction of reduced GSH, although stress may transiently decrease LTC₄S expression or activities, leading to increased LTC₄ generation in inflammatory lesions. We cannot deny the possibility that the increase of GSH:GSSG ratio enhanced LTC₄ release through other mechanisms because LTC₄ release was enhanced 24 hrs after the treatment in accordance with the increase of GSH:GSSG ratio in H₂O₂ or diamide-treated cells. However, we speculate that the amount of intracellular reduced
GSH regulates LTC₄ 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 H₂O₂ is known to stimulate GCL activity without changing the amount of the enzyme [25,26,27].

In conclusion, FceRI-mediated LTC₄ 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 LTC₄.

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.

References

[10] H. Tanaka, S. Kanako, S. Abe, Prostaglandin E2 receptor selective agonists E-prostanoid 2 and E-prostanoid 4 may have therapeutic effects on


[22] J. Fujii, J.I. Ito, X. Zhang, T. Kurahashi, Unveiling the roles of the glutathione...
redox system in vivo by analyzing genetically modified mice, J Clin Biochem Nutr 49 (2011) 70-78.


[27] T. Ochi, Menadione causes increases in the level of glutathione and in the activity of gamma-glutamylcysteine synthetase in cultured Chinese hamster V79 cells, Toxicology 112 (1996) 45-55.
Figure Legends

Figure 1. Effects of prostanoids on Fc\varepsilon RI-mediated LTC\textsubscript{4} release from MC/9 cells.

(A) MC/9 cells sensitized with anti-DNP IgE (1 \times 10^6/ml) were incubated for 6 hrs 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\textsubscript{4} was normalized to that released from stimulated cells after treatment with 0.1% DMSO. (B) PGE\textsubscript{2}, dimethyl PGE\textsubscript{2} (dmPGE\textsubscript{2}), ONO-DI-004, sulprostone, and ONO-AE1-329 did not significantly inhibit Fc\varepsilon RI-mediated LTC\textsubscript{4} release from MC/9 cells at 1 \mu M. The data were normalized as described above. (C) mRNA expression of PGE\textsubscript{2} receptors was normalized to that of GAPDH. EP\textsubscript{3} mRNA and EP\textsubscript{4} mRNA were predominantly expressed in MC/9 cells. (D) mRNA expression of 5-LO, FLAP, LTC\textsubscript{4}S, and LTA\textsubscript{4}H in MC/9 cells was analyzed with quantitative RT-PCR after incubation for 6 hrs with ONO-AE-248 (1 \mu M) or control vehicle. Data were normalized to GAPDH expression. The data are expressed as mean ± SD from at least six experiments (**, \( P < 0.01 \)).

Figure 2. Effects of prostanoids or GSH modulators on the amount of intracellular GSH and Fc\varepsilon RI-mediated LTC\textsubscript{4}/LTB\textsubscript{4} release from MC/9 cells.
(A) After MC/9 cells were sensitized with anti-DNP IgE (1 × 10⁶ cells/ml), cells were incubated with ONO-AE-248 (1 μM) or control vehicle in 0.1% BSA-DMEM for 6 hrs. The cells were stimulated with DNP-HSA (10 ng/ml) for 30 min. LTBD₄ in the supernatants was normalized to that obtained from cells treated with control vehicle. (B) After MC/9 cells (1 × 10⁶/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 hrs, 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 × 10⁶/ml) were incubated in complete medium with 5 mM GSH-OEt, 50 μM BSO, or without agents for 24 hrs. The cells were passively sensitized with anti-DNP IgE (0.5 μg/ml) for at least 1 hr and washed well with 0.1% BSA-DMEM for the LTC₄ experiments. Sensitized MC/9 cells were stimulated with DNP-HSA (10 ng/ml) in 0.1% BSA-DMEM for 30 min. LTC₄ in the supernatant was normalized to that from non-treated cells. LTC₄ 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 hrs. The cells were passively sensitized with anti-DNP IgE for at least 1 hr. After incubation for 24 hrs 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. LTB₄ in the supernatants was assayed and normalized to that in the supernatant of the cells treated without BSO. LTB₄ 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).

Figure 3. FcεRI-mediated LTC₄ 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 hrs (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 hrs (B). The cells were then stimulated with DNP-HSA (10 ng/ml) for 30 min. LTC₄ 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 × 10⁶/ml) were incubated in 0.1% BSA-RPMI for 6 hrs 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 hrs. The cells were then stimulated with anti-human IgE (1 μg/ml) in 0.1% BSA-RPMI for 30 min. LTC₄ 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).

Figure 4. Effects of oxidative stress on FccRI-mediated LTC₄ release from MC/9 cells.

MC/9 cells (1 × 10⁶/ml) were cultured in complete medium in the absence or presence of H₂O₂ (300 μM) for 6, 24 or 48 hrs (A, B, C). MC/9 cells (1 × 10⁶/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 hrs after the treatment with diamide (D, E, F). The cells were sensitized with anti-DNP IgE for at least 1 hr. 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.
LTC₄ in the supernatants was assayed and normalized to that obtained from cells treated without H₂O₂ or diamide (A, D). The amounts of intracellular total GSH, reduced GSH and GSSG were assayed and normalized to those in cells treated without H₂O₂ or diamide. Total or reduced GSH was increased significantly 24 or 48 hrs after the treatment (B, E). GSH:GSSG ratio was significantly increased 24 hrs after the treatment with H₂O₂ 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).
Fig. 1
Fig. 2
Fig. 3