

Intracellular glutathione redox status in human dendritic cells regulates IL-27 production and T-cell polarization

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Abstract

Background: Glutathione redox status, changes in intracellular reduced (GSH) or oxidized (GSSG) glutathione, plays a significant role in various aspects of cellular function. In this study, we examined whether intracellular glutathione redox status in human dendritic cells (DCs) regulates the polarization of Th1/Th2 balance.

Methods: Human monocyte-derived DCs (MD-DCs) treated with glutathione reduced form ethyl ester (GSH-OEt) or L-buthionine-(S,R)-sulfoximine (BSO) were stimulated by lipopolysaccharide (LPS), and the levels of polarization cytokines were measured. Next, DCs matured by LPS or thymic stromal lymphopoietin (TSLP) were cocultured with allogeneic CD4⁺ naïve T cells and Th1/Th2 balance was evaluated by cytokine production from the primed T cells.

Results: Monocyte-derived DCs exposed to GSH-OEt and BSO had increased and decreased intracellular GSH contents, respectively. Lipopolysaccharide-induced interleukin (IL)-27 production was enhanced by GSH-OEt and suppressed by BSO, but neither GSH-OEt nor BSO affected the expression of HLA-DR, CD80, CD83, or CD86. Mature GSH-OEt-treated MD-DCs enhanced interferon (IFN)- γ production from CD4⁺ T cells compared with nontreated MD-DCs, and small interfering RNA (siRNA) against IL-27 suppressed the effect of GSH-OEt on IFN- γ production. Additionally, although human myeloid DCs activated by TSLP (TSLP-DCs) prime naïve CD4⁺ T cells to differentiate into Th2 cells, treatment of TSLP-DCs with GSH-OEt reduced IL-13 production and enhanced IFN- γ production by CD4⁺ T cells. Interleukin-27 siRNA attenuated the inhibitory effect of GSH-OEt on Th2 polarization.

Conclusion: Our results reveal that Th1 and Th2 responses are controlled by intracellular glutathione redox status in DCs through IL-27 production.

Cells have an innate reduction mechanism to protect the organism from oxidative stress and to maintain homeostasis. Glutathione is the most important tripeptide for redox control, and it is ubiquitous in almost all mammalian cells.

Abbreviations

BSO, L-buthionine-(S,R)-sulfoximine; GSH, reduced glutathione; GSH-OEt, glutathione reduced form ethyl ester; GSSG, oxidized glutathione; LPS-DCs, LPS-stimulated MD-DCs; MCB, monochlorobimane; mDCs, myeloid DCs; MD-DCs, monocyte-derived DCs; TSLP, thymic stromal lymphopoietin; TSLP-DCs, myeloid DCs activated by TSLP.

Redox cycle consists of the balance of reduced (GSH) and oxidized (GSSG) glutathione (1). A recent study revealed that this redox system controls not only the oxidation-reduction reaction, but also various aspects of cellular functions like gene transcription, expression, cell differentiation, cell proliferation, and cellular death, and thus glutathione is important in immune modulation (2). For instance, we have previously reported that intracellular glutathione redox status regulates interleukin (IL)-12 production (3, 4).

Dendritic cells (DCs) are the only antigen-presenting cells (APCs) that can present an antigen to naïve T cells (5). Dendritic cells activated by the antigen present the antigen to

naïve T cells, which thereafter differentiate into effector T cells (6). This differentiation decision depends on polarizing signals (7). Some polarizing signals are produced by DCs; for example, IL-12 and IL-27 are Th1 polarizing signals (8, 9), and CCL2 is a Th2 polarizing signal (10). In addition, thymic stromal lymphopoietin (TSLP), which is mainly produced by epithelial cells, leads to the transformation of myeloid DCs (mDCs) into Th2-induced DCs (11, 12). Thus, DCs regulate a variety of immune responses by modulating T-cell differentiation.

Interleukin-12 is produced primarily by activated inflammatory cells such as DCs, macrophages, and monocytes (13). This cytokine is considered to be a key regulator of T-cell differentiation, because the cytokine strongly induces Th1 responses (14). Recently, another member of the IL-12 family of cytokines, IL-27, was identified. Interleukin-27 not only increases interferon (IFN)- γ production by T cells through T-bet induction, but also suppresses the expression of GATA-3, a critical Th2-specific transcription factor (15, 16). In addition, IL-27 induces IL-12 receptor $\beta 2$ expression, suggesting that IL-12 is a key inducer of IFN- γ during the inflammatory phase (8), while IL-27 induces IFN- γ during the early inflammatory phase (16).

In the present study, we examined whether intracellular glutathione redox status in human DCs regulates the production of polarizing signals, such as IL-27, IL-12, and Th1/Th2 responses.

Materials and methods

Reagents

Glutathione reduced form ethyl ester (GSH-OEt), L-buthionine(S,R)-sulfoximine (BSO), and lipopolysaccharide (LPS; from *Escherichia coli* serotype 0111:B4) were obtained from Sigma-Aldrich Co.(St. Louis, MO, USA).

Isolation and culture of MD-DCs and blood mDCs

Human peripheral blood mononuclear cells (PBMCs) were obtained from healthy adult volunteers using anti-CD14 magnetic beads (Miltenyi Biotec, Auburn, AL, USA), and monocyte-derived DCs (MD-DCs) were generated from PBMCs as described previously (17, 18). Differentiation into CD1a⁺ CD14[−] immature DCs was confirmed using fluorescence activated cell sorting (FACS) (data not shown). The cells were cultured in RPMI1640 (Invitrogen, Auckland, NZ, USA) containing 10% FBS (Biological Industries, Beit-Haemek, Israel), 50 U/ml penicillin G sodium, and 50 μ g/ml streptomycin sulfate (complete medium; Invitrogen), and maturation of immature MD-DCs was induced by culturing them for 48 h with 100 ng/ml LPS.

CD1c (BDCA-1)⁺ mDCs were isolated from PBMCs using a CD1c Dendritic Cell Isolation kit (Miltenyi Biotec). The purity of the isolated mDCs was > 99%, as determined by flow cytometry (data not shown). The activation of mDCs was induced by culturing them for 24 h with 15 ng/ml human recombinant TSLP (R&D systems, Minneapolis, MN, USA).

Cell exposure to glutathione modulators and measurements of intracellular GSH concentration

Monocyte-derived DCs were incubated at a concentration of 5.0×10^5 cells/well in 12-well plates with 5 mM GSH-OEt or 500 μ M BSO. After 24 h, the concentration of GSH was assayed by the GSSG-reductase 5,5-dithio-bis (2-nitrobenzoic acid) recycling procedure, as described previously (3). In some experiments, cell staining was performed using monochlorobimane (MCB; Invitrogen) as previously reported (19).

Real-time quantitative PCR analysis of cytokine mRNA

We measured cytokine mRNA expression after stimulating immature MD-DCs with 100 ng/ml LPS for 8 h using real-time polymerase chain reaction (PCR) as reported previously (20). The primers and the probes of target genes were TaqMan Assay-on-Demand Gene expression reagents (Applied Biosystems, Foster, CA, USA): IL-27 p28 (Hs00377366_m1), IL-27 EB13 (Hs00194957_m1), IL-10 (Hs00174086_m1), IL-23 p19 (Hs00372324_m1), IL-12 p40 (Hs00233688_m1), IL-12 p35 (Hs01073447_m1), and G3PDH (Hs99999905_m1) was used as a control. Relative expression was calculated using the Δ CT method.

Quantitation of IL-27 and IL-12 p70

Immature MD-DCs were stimulated with 100 ng/ml LPS for 24 h, and the levels of IL-27 and IL-12 p70 protein in the supernatants were measured using DuoSet enzyme-linked immunosorbent assay (ELISA) Development kits purchased from R&D systems.

Analysis of cell surface markers on DCs

Monocyte-derived DCs were stained with FITC-labeled anti-CD80, CD83, CD86, or HLA-DR monoclonal antibodies (mAbs; all from BD Biosciences, San Diego, CA, USA) to analyze the expression of costimulatory molecules. Stained cells were analyzed by FACS Calibur (BD Biosciences) using standard Cell Quest software program (Becton Dickinson, Franklin Lakes, NJ, USA).

DC-T-cell coculture

Naïve CD4⁺ T cells were purified from PBMCs using a naïve CD4⁺ T-cell isolation kit II (Miltenyi Biotec). Immature MD-DCs were seeded at a density of 5.0×10^4 cells/200 μ l of complete medium in 96-well plates and stimulated with 100 ng/ml LPS for 48 h. Matured MD-DCs were washed twice and cocultured with 2.5×10^5 allogeneic naïve CD4⁺ T cells (DC/T ratio, 1 : 5). On day 5, human recombinant IL-2 (40 U/ml) was added.

Myeloid DCs were seeded at a density of 5.0×10^4 cells/200 μ l of complete medium in 96-well plates and stimulated with 15 ng/ml TSLP. After 24 h, mDCs were washed twice and cocultured with 2.5×10^5 allogeneic naïve CD4⁺ T cells (DC/T ratio, 1 : 5), as described previously.

Analysis of T-cell cytokine production

After 12 days of coculture with DCs, the primed CD4⁺ T cells were collected and restimulated with 50 ng/ml PMA, 2 µg/ml ionomycin, and 10 µg/ml Brefeldin A for 4 h. The cells were stained with FITC-labeled IFN-γ/PE-labeled IL-4-conjugated mAbs or a combination of FITC-labeled IFN-γ mAbs and PE-labeled IL-13 mAbs (all from BD Biosciences) using an intracellular cytokine staining kit (BD Pharmingen, San Diego, CA, USA). Stained cells were analyzed using FACS Calibur. For the detection of cytokine production in the culture supernatants, T cells were restimulated with anti-CD3 and anti-CD28 mAbs for 24 h. The levels of IFN-γ and IL-4 were measured using ELISA kits (R&D systems). In some experiments, we used 1 µg/ml anti-human IL-12 antibody (AB-219-NA; R&D systems) for the culture. Normal goat immunoglobulin G was used as a control.

Small interfering RNA against IL-27

We assessed the effect of inducing RNA interference against IL-27 using Ambion Silencer Select small interfering RNA (siRNA) human IL-27 p28 purchased from Applied Biosystems (nos. 48373 and 225997). The control siRNA was an siRNA sequence that does not cause the specific degradation of any cellular message. Immature MD-DCs and mDCs were transfected with 100 nM final concentration of siRNA using a TransIT-TKO transfection reagent (Mirus Bio, Madison, WI, USA) for 1 h and then were stimulated with LPS and TSLP, respectively. After 24 h, cells were collected, and the expression of IL-27 p28 was determined using real-time PCR.

Statistical analysis

All values were expressed as the means ± SEM of the indicated numbers of experiments. In Figs 1, 2C,D and 4B,C, the data were compared by the Student's *t*-test with the Bonferroni correction for multiple comparisons. A value of $P < 0.05/m$ (where *m* is the number of comparisons) was considered statistically significant. In Figs 2A,B and 5A,B, the nonparametric analysis of variance (Kruskal–Wallis method) determined the significance among groups. A value of $P < 0.05$ was considered to be significant.

Results

GSH-OEt and BSO have opposing effects on intracellular GSH concentration in MD-DCs

First, we examined the effects of glutathione modulators, GSH-OEt and BSO, on intracellular GSH content in MD-DCs. Intracellular GSH levels in the MD-DCs were significantly increased by treatment with GSH-OEt for 24 h and were significantly decreased by a 24 h treatment with BSO (Fig. 1A). GSH staining showed the MD-DCs treated with GSH-OEt and BSO to demonstrate strong and weak staining for MCB, respectively, compared to the untreated control cells (Fig. 1B).

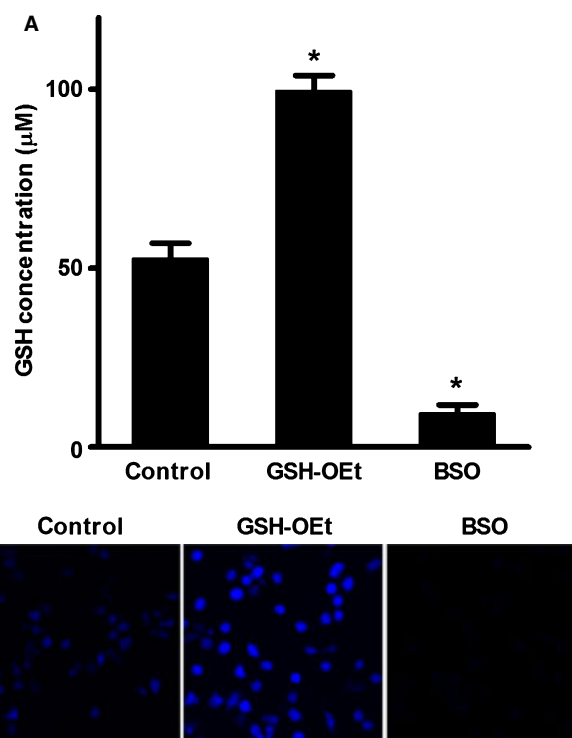


Figure 1 Glutathione reduced form ethyl ester (GSH-OEt) increased while L-buthionine-(S,R)-sulfoximine (BSO) decreased intracellular glutathione level in monocyte-derived DCs (MD-DCs). After incubating MD-DCs with GSH-OEt or BSO for 24 h, intracellular GSH level was determined using an enzyme assay (A) or monochlorobimane staining (B). Values represent means ± SEM of four experiments. * $P < 0.05$ compared with untreated cells. (B) Original magnification $\times 200$.

Intracellular GSH regulates LPS-induced cytokine production in MD-DCs

We next investigated the effect of intracellular GSH modification in MD-DCs on LPS-induced cytokine expression. As shown in Fig. 2A,B, an increase in intracellular GSH content by GSH-OEt and a decrease in intracellular GSH content by BSO enhanced and suppressed the mRNA expression levels of IL-27 p28, EB13, IL-12 p40, and p35 induced in MD-DCs, respectively. The protein production of IL-27 and IL-12 p70 was increased by GSH-OEt, whereas it decreased by BSO ($P < 0.05$, Fig. 2C,D). Conversely, GSH-OEt and BSO did not affect the expression of IL-23 p19 or IL-10 (Fig. 2A,B). In addition, treatment with GSH-OEt or BSO did not affect the viability of MD-DCs, as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (data not shown). Furthermore, treatment with glutathione modulators did not affect the expression of CD14 or TLR4 on MD-DCs, as determined by FACS (data not shown). These findings indicate that intracellular glutathione redox status regulates LPS-induced IL-27 and IL-12 production by MD-DCs.

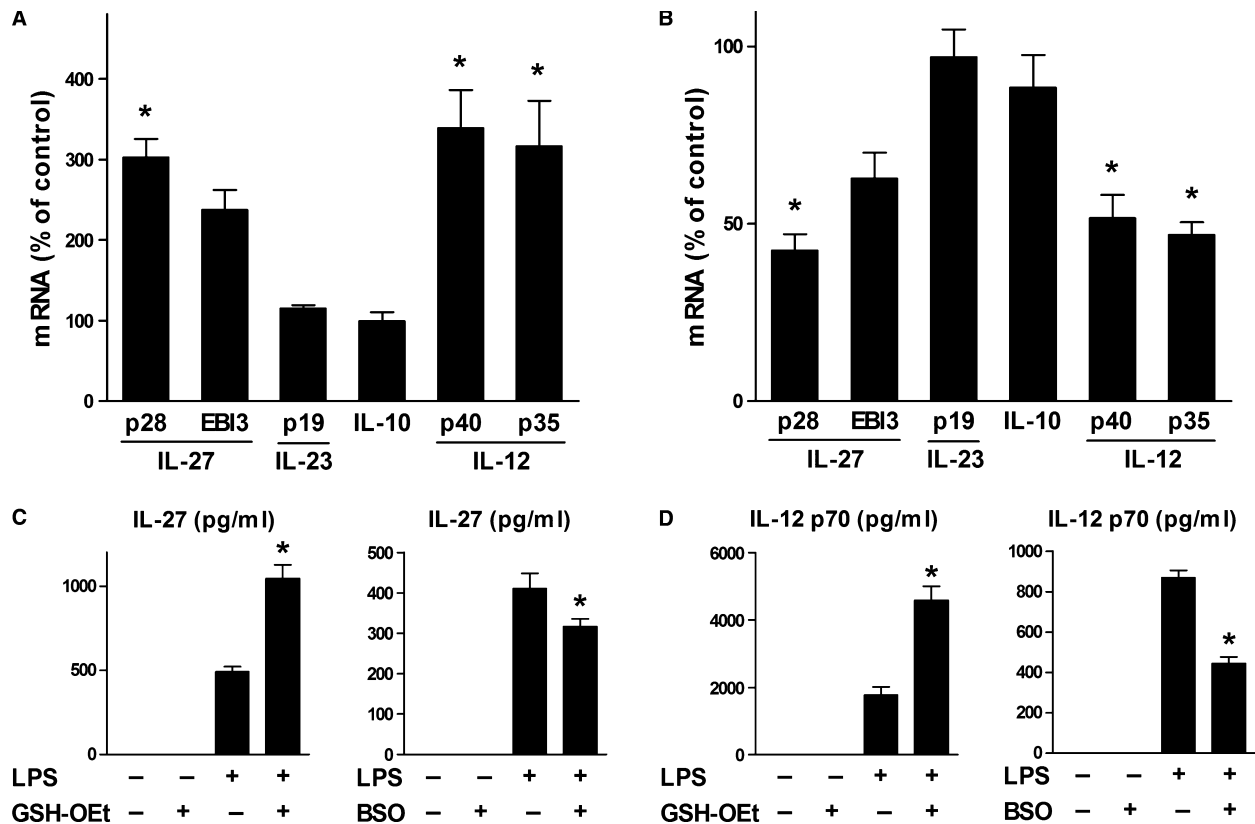


Figure 2 Glutathione reduced form ethyl ester (GSH-OEt) enhanced while L-buthionine-(S,R)-sulfoximine (BSO) suppressed lipopolysaccharide (LPS)-induced interleukin (IL)-12 and IL-27 production in monocyte-derived DCs (MD-DCs). (A, B) MD-DCs were incubated with GSH-OEt (A) or BSO (B) for 24 h and then stimulated with LPS (100 ng/ml) for 8 h. The mRNA expression levels of IL-27 p28, EBI3, IL-23 p19, IL-10, IL-12 p40, and IL-12 p35 were evaluated by a real-time PCR analysis. The results are expressed as

the ratio (%) to the control value. Values represent means $\pm$ SEM of six experiments. * $P < 0.05$ in comparison with LPS-stimulated cells (control cells). (C, D) Cells that were incubated with GSH-OEt or BSO were stimulated with LPS (100 ng/ml) for 24 h. IL-12 p70 (C) and IL-27 (D) protein in culture supernatants was evaluated by enzyme-linked immunosorbent assay. Values represent means $\pm$ SEM of five experiments. * $P < 0.05$ compared with lipopolysaccharide-stimulated cells (control cells).

GSH-OEt and BSO do not affect DC maturation

We next determined the effects of glutathione modulators on the expression of major histocompatibility complex (MHC) and costimulatory molecules on MD-DCs. The stimulation of immature MD-DCs with LPS for 48 h enhanced the expression levels of HLA-DR, CD80, CD83, and CD86 (Fig. 3). However, neither GSH-OEt nor BSO affected the expression of these molecules as enhanced by LPS (Fig. 3).

Intracellular GSH status in MD-DCs influences T-cell differentiation

The regulation of IL-27 and IL-12 production by DCs induced by changes in glutathione redox status prompted us to analyze the phenotype of the primary T-cell responses induced by mature DCs. As shown in Fig. 4A, naive allogeneic CD4⁺ T cells primed by LPS-stimulated MD-DCs (LPS-DCs) produced large amounts of Th1 cytokine (IFN- γ)

and low levels of Th2 cytokine (IL-4). Lipopolysaccharide-stimulated MD-DCs pretreated with GSH-OEt displayed a markedly enhanced capacity to induce IFN- γ -producing Th1 cells and a reduced capacity to induce IL-4-producing Th2 cells (Fig. 4A). Furthermore, IFN- γ secretion by T cells was significantly ($P < 0.05$) higher, while IL-4 secretion was significantly ($P < 0.05$) lower primed in LPS-DCs by GSH-OEt than in the control LPS-DCs (Fig. 4B,C). These results indicate that intracellular glutathione redox status in MD-DCs regulates Th1/Th2 balance: an increase in GSH content in DCs results in Th1 polarization of naive CD4⁺ T cells.

GSH-OEt exposure shifts the MD-DCs polarizing capacity toward Th1 cells through increased production of IL-27 and IL-12

We next investigated the role of IL-27 in the glutathione redox regulation of T-cell differentiation using two different siRNAs against IL-27 p28 subunit. Transfection of MD-DCs

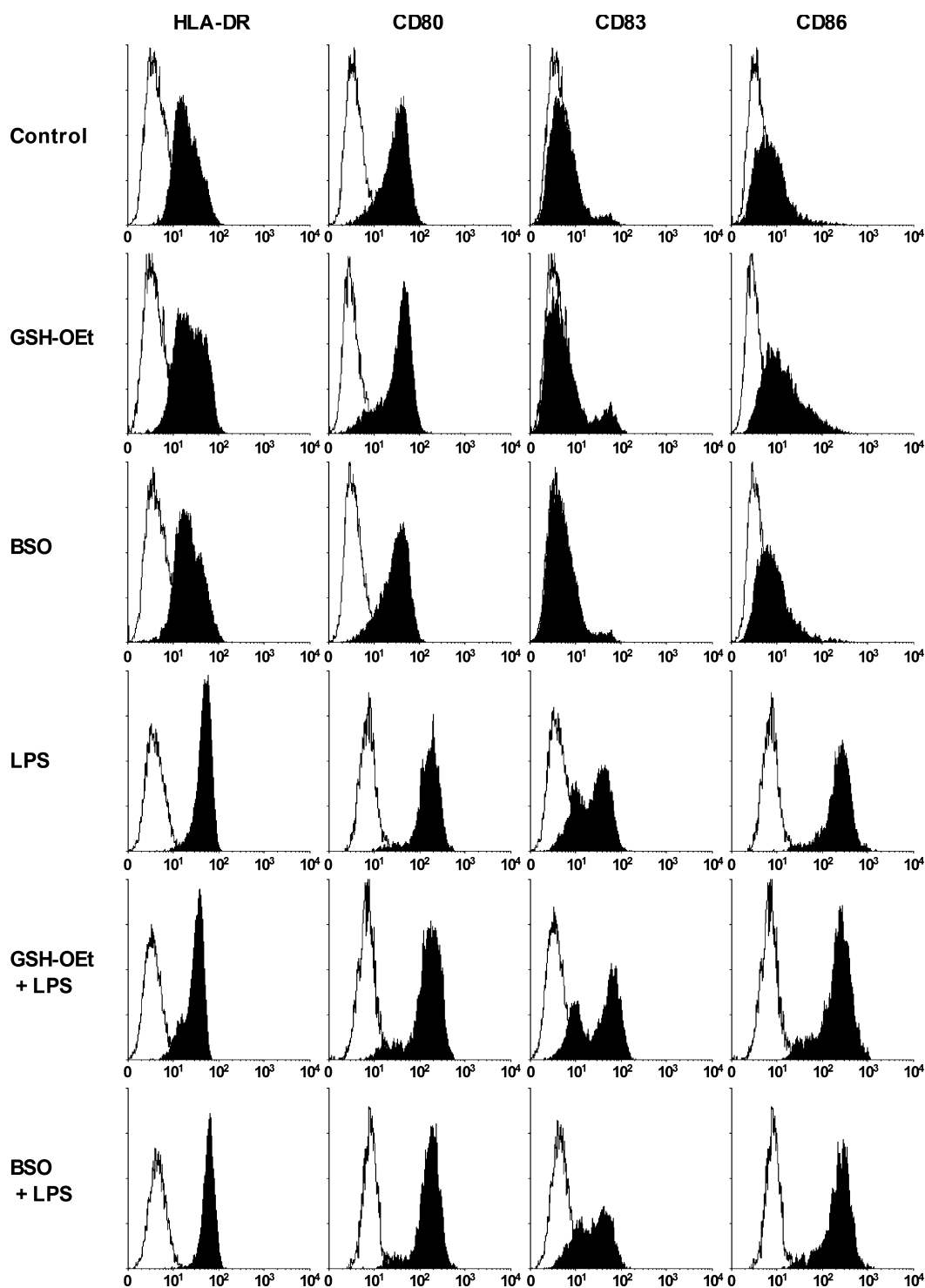


Figure 3 Neither glutathione reduced form ethyl ester (GSH-OEt) nor L-buthionine-(S,R)-sulfoximine (BSO) affected the expression of surface markers in monocyte-derived DCs. Monocyte-derived DCs were incubated with GSH-OEt or BSO for 24 h and then stimulated with lipopolysaccharide (100 ng/ml) for 48 h. Cells were stained

with anti-HLA-DR, CD80, CD83 or CD86 mAbs (shaded area) or isotype-matched control (open area), and the expression levels were analyzed by flow cytometry. Similar results were observed in two independent experiments, and the results of a representative experiment are shown.

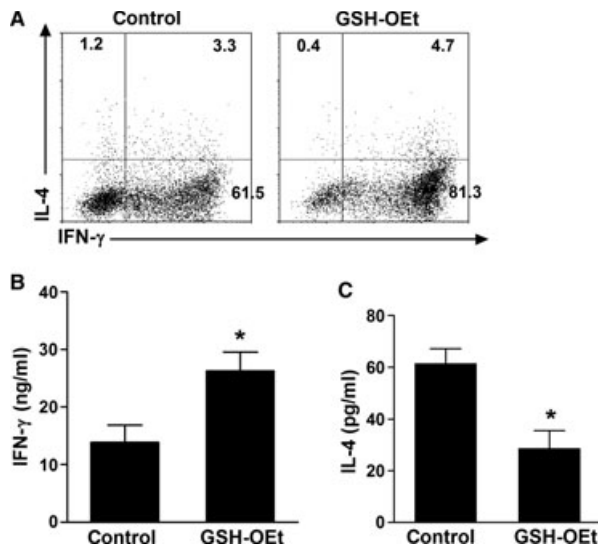


Figure 4 Glutathione reduced form ethyl ester (GSH-OEt)-treated monocyte-derived DCs (MD-DCs) enhanced Th1 response and reduced Th2 response through interleukin-12 induction. Monocyte-derived DCs were cultured with or without GSH-OEt. After 24 h, cells were stimulated by lipopolysaccharide (LPS) for 48 h, and the last 24 h were in the presence or absence of GSH-OEt. Then, MD-DCs were cocultured with allogeneic CD4⁺ naive T cells for 13 days. (A) Cytokine production by T cells was analyzed intracellularly by flow cytometry. Similar results were observed in six independent experiments, and the results of a representative experiment are shown. (B, C) Cytokine production was measured in the supernatants after re-stimulation with anti-CD3 and anti-CD28 mAbs for 24 h by enzyme-linked immunosorbent assay. Values represent means $\pm$ SEM of four experiments. * P < 0.05 compared with LPS-stimulated cells (control cells).

with IL-27 p28 siRNA significantly attenuated IL-27 p28 mRNA expression (Fig. 5A). The expression of IL-12 p40 was not affected by these siRNAs (Fig. 5B), suggesting that these siRNAs are specific for IL-27 p28. Treatment with siRNA did not affect the maturation stage or the viability of MD-DCs, as determined by flow cytometry and MTT assay, respectively (data not shown). The knockdown of IL-27 p28 by siRNAs suppressed IFN- γ production by CD4⁺ T cells enhanced by GSH-OEt-treated LPS-DCs (Fig. 5C). These results indicate that the Th1-polarizing effect of GSH-OEt-treated DCs was dependent on IL-27 production.

In addition to IL-27, IL-12 is a key regulator of Th1 cell differentiation (14). To determine whether IL-12 is involved in glutathione redox regulation of T-cell differentiation, we used anti-IL-12 mAb to block the function of IL-12. The addition of neutralizing anti-IL-12 mAb into the coculture of T cells and GSH-OEt-treated LPS-DCs resulted in a reduced number of IFN- γ -producing Th1 cells (Fig. 5D). Moreover, concurrent knockdown of IL-27 p28 and neutralization of IL-12 strongly inhibited the generation of IFN- γ -producing Th1 cells augmented by GSH-OEt-treated LPS-DCs (Fig. 5E). These results indicate that

both IL-27 and IL-12 are important for the regulation of T-cell differentiation by the intracellular glutathione redox status in MD-DCs.

GSH-OEt-treated mDCs suppress the induction of TSLP-mediated Th2 response through IL-27 and IL-12 production

Lipopolysaccharide stimulation is used to generate experimental models of inflammation, and a number of proinflammatory cytokines are secreted by DCs. For this reason, CD4⁺ T cells primed by LPS-DCs produce low levels of Th2 cytokines. Thymic stromal lymphopoietin strongly activates human mDCs, but not other APCs, without inducing Th1-polarizing cytokines such as IL-12, IL-23, or IL-27, and these TSLP-DCs thereby induce naive CD4⁺ T cells to differentiate into Th2 cells (12, 21). TSLP expression increases in keratinocytes of patients with atopic dermatitis (21) and in the airways of patients with bronchial asthma (22), making it a key player in the physiopathology of allergic disease. As a final investigation into the mechanism underlying the immune regulation by GSH, we investigated the role of glutathione redox status in mDCs on TSLP-induced Th2-polarizing effect. Figure 6A shows that intracellular GSH levels in mDCs were increased by treatment with GSH-OEt, because mDCs treated with GSH-OEt were more strongly stained by MCB than nontreated control cells. CD4⁺ T cells primed by TSLP-DCs produced a large amount of Th2 cytokine IL-13, but lower amounts of Th1 cytokine IFN- γ , whereas TSLP-DCs treated with GSH-OEt induced naive CD4⁺ T cells to differentiate into T cells producing reduced levels of IL-13 and increased levels of IFN- γ compared with control TSLP-DCs (Fig. 6B). In contrast, treatment with GSH-OEt did not affect the expression of TSLP-R on MD-DCs, as determined by FACS (data not shown). These data indicate that intracellular glutathione redox status in TSLP-DCs controls the induction of Th2 polarization primed by TSLP-DCs. Additionally, knockdown of IL-27 p28 by specific siRNAs (Fig. 6C) or neutralization of IL-12 by anti-IL-12 mAb (Fig. 6D) reduced IFN- γ production and promoted IL-13 production by CD4⁺ T cells primed by GSH-OEt-treated TSLP-DCs. Although OX40 ligand (OX40L) is responsible for the induction of Th2-dominant responses by TSLP (21), treatment with GSH-OEt did not affect OX40L expression on TSLP-DCs (data not shown). These results suggest that the induction of Th2-dominant responses is controlled by intracellular glutathione redox status in TSLP-DCs and that the glutathione redox regulation of Th2 polarization is dependent on both IL-27 and IL-12 production, but it is independent of OX40L expression.

Discussion

In this study, we demonstrated a clear link between T-cell polarization and intracellular glutathione redox status in human DCs, which play a crucial role in the differentiation of naive CD4⁺ T cells into effector T cells. The reason for this is the increase in intracellular GSH content in LPS-DCs by GSH-OEt promoted Th1 response and inhibited Th2

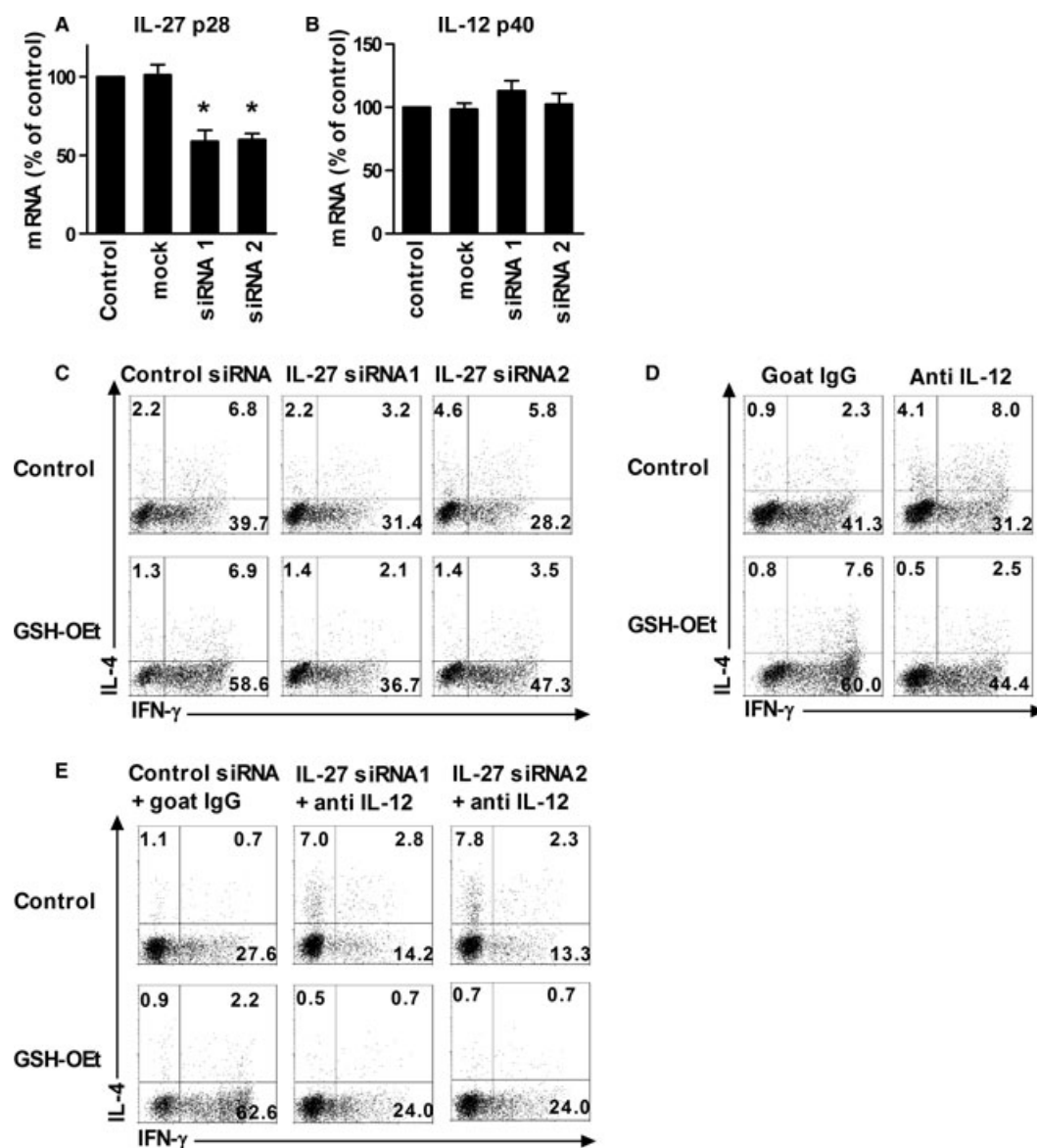


Figure 5 Interleukin (IL)-27 p28 siRNAs and anti-IL-12 antibody negated the augmentative effect of glutathione reduced form ethyl ester (GSH-OEt) on Th1 response. Monocyte-derived DCs (MD-DCs) were transfected with siRNAs for 1 h and then stimulated with lipopolysaccharide (LPS). After 24 h, the mRNA expression levels of IL-27 p28 (A) and IL-12 p40 (B) were evaluated by a real-time PCR analysis. The results are expressed as the ratio (%) to the control value. Values represent means $\pm$ SEM of seven or eight experiments. * $P < 0.05$ in comparison with control siRNA transfected cells. (C-E)

MD-DCs were cultured with or without GSH-OEt. After 24 h, MD-DCs were washed and transfected with siRNAs for 1 h (C, E) and then were stimulated with LPS for 24 h. After washing, cells were treated with or without GSH-OEt for 24 h and then were cocultured with allogeneic CD4⁺ naive T cells for 13 days in the presence of anti-human IL-12 Abs or control Abs (D, E). Cytokine production by T cells was analyzed intracellularly by flow cytometry. Similar results were observed in three independent experiments, and the results of a representative experiment are shown.

response (Fig. 4). The induction of T-cell differentiation by mature DCs mainly requires the following three factors: antigen/MHC class II complex signals, co-stimulatory molecule signals, and polarization signals such as cytokines and chemokines (7). In our study, although the expression of MHC class II and co-stimulatory molecules was not affected by changes in intracellular GSH concentration (Fig. 3), the

LPS-induced production of both IL-27 and IL-12 by MD-DCs was enhanced by GSH-OEt (Fig. 2). Furthermore, the knockdown of IL-27 p28 by siRNAs and/or addition of anti-IL-12 mAb suppressed the effect of GSH-OEt on IFN- γ production by CD4⁺ T cells (Fig. 5). These results indicate that intracellular glutathione redox in MD-DCs regulates T-cell differentiation through the production of IL-27 and IL-12.

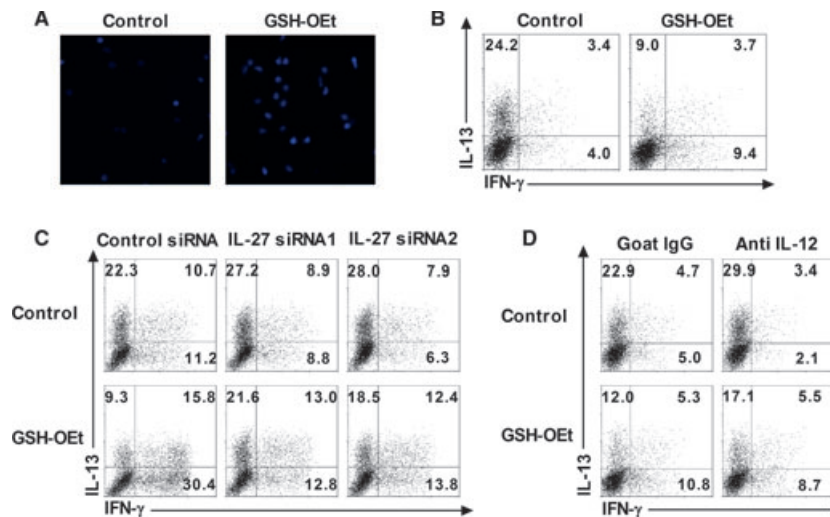


Figure 6 Glutathione reduced form ethyl ester (GSH-OEt) suppressed Th2 response and enhanced Th1 response primed by thymic stromal lymphopoietin (TSLP)-stimulated myeloid DCs (mDCs) through interleukin (IL)-12 and IL-27. (A) After incubating mDCs with GSH-OEt for 24 h, intracellular GSH level was determined using monochlorobimane staining. Original magnification $\times 200$. (B–D) mDCs were transfected with siRNAs for 1 h (C) and then stimulated by TSLP for 24 h in the presence or absence of GSH-OEt.

Then, cells were cocultured with allogeneic CD4⁺ naive T cells in the presence of anti-human IL-12 Abs or control Abs (D). Cytokine production by T cells was analyzed intracellularly by flow cytometry. Similar results were observed in three independent experiments, and the results of a representative experiment are shown.

Some investigators have reported that intracellular glutathione redox status regulates IL-12 production by DCs (23–25). However, in this study we report for the first time that IL-27 production by DCs is controlled by intracellular glutathione redox status and also that glutathione redox regulation of T-cell polarization depends on IL-27 production.

Controlling Th1/Th2 balance is essential for the treatment of allergic diseases such as atopic dermatitis and asthma. Thymic stromal lymphopoietin was recently shown to induce the production of Th2-attracting chemokines, such as TARC/CCL17, and the prime Th2 development (21). This cytokine strongly activates mDCs, which represent the early molecular and cellular components that trigger allergic disease (26). In this study, we demonstrated that TSLP-DCs treated with GSH-OEt inhibited IL-13 production and promoted IFN- γ production by CD4⁺ T cells (Fig. 6B). Interleukin-27 p28 siRNAs or anti-IL-12 mAb attenuated these effects (Fig. 6C,D). These results reveal that IL-27 and IL-12 underlie T-cell polarization regulated by glutathione redox status in TSLP-DCs. Others have demonstrated that TSLP strongly activates human mDCs to secrete Th2-attracting chemokines, but neither IL-12 nor pro-inflammatory cytokines (21). On the other hand, TSLP primes DCs to induce IL-12 production in response to CD40 ligand (27). We therefore believe that the interaction between DCs and naive CD4⁺ T cells induces IL-27 and IL-12 production by TSLP-DCs. Moreover, IL-27 p28 siRNAs restored the reduction of IL-13 production and the enhancement of IFN- γ production by GSH-OEt to a similar extent as anti-IL-12 mAb (Figs 5 and 6) in spite of the

incomplete inhibition of IL-27 p28 expression (Fig. 5). This indicates that IL-27 production is more critical than IL-12 production with regard to the regulation of T-cell polarization by glutathione redox status in TSLP-DCs.

Of interest, TSLP expression increases in keratinocytes of patients with atopic dermatitis and in the airways of patients with bronchial asthma (21, 22), making it a key player in the physiopathology of allergic disease. Our present data indicate that glutathione redox can regulate processes associated with allergic diseases such as atopic dermatitis and bronchial asthma. Additionally, we have demonstrated that an increase in GSH content in lung fibroblasts ameliorates TGF- β -induced fibrogenic effects (28), suggesting that the change in glutathione redox status during inflammation may affect chronic inflammation, such as airway remodeling, as well as T-cell polarization.

We have previously shown that intracellular glutathione redox status regulates IL-12 production from human monocytes (3) and macrophages (4) through c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK). Jarnicki et al. (29) have recently demonstrated that IL-10 production by DCs is mediated by TLR signaling through p38 MAPK, and Liu W et al. (30) have reported that JNK, p38 MAPK, and ERK regulate IL-23 p19 gene expression at the transcriptional level. However, as shown in Fig. 2A,B, GSH-OEt and BSO specifically controlled the expression of IL-27 and IL-12, but did not affect the expression of IL-23 p19 or IL-10. We therefore propose that the regulation of IL-12 and IL-27 production by MD-DCs as a result of treatment with glutathione modulators does not

depend on the JNK or p38 MAPK pathways. Indeed, GSH-OEt did not influence LPS-induced phosphorylation of JNK or p38 MAPK in MD-DCs (data not shown). These findings indicate that intracellular glutathione redox status in DCs regulates IL-27 and IL-12 production through an alternative signaling pathway.

Interleukin-27 is said to be a pleiotropic immunological mediator that has seemingly paradoxical functions in inflammation. Some investigators have revealed that IL-27 has temporally distinct, opposing functions as a supportive factor in Th1 differentiation during early stages of the response and as an immunosuppressive factor during late stages of the response (31–33) by upregulation of IL-10 or via an independent anti-inflammatory mechanism (34). However, the role of IL-27 in the human immune system, especially the immunosuppressive system, is not well understood. The contribution of intracellular glutathione redox in human DCs to the immunosuppressive effect induced by IL-27 remains obscure. Further studies are necessary to clarify this issue.

Under normal conditions, cellular GSH levels are regulated by controlling the rates of its synthesis and of its export from cells. However, GSH levels are also influenced by agents or conditions that alter the thiol redox state, which lead to the formation of glutathione S-conjugates or complexes, and that disrupt the distribution of GSH among various intracellular organelles. In addition, GSH levels are affected by the nutritional status, hormonal/stress levels, developmental or diurnal variations, and are affected by certain physiological states, including pregnancy and exercise (2). On the other hand, we have previously reported that exposure of alveolar macrophages to IFN- γ and IL-4

increased and decreased the GSH/GSSG ratio, respectively, and that LPS-induced secretion of IL-12 in macrophages was enhanced and inhibited by IFN- γ and IL-4, respectively (35). These results suggest that endogenous cytokines such as IFN- γ and IL-4 regulate IL-12 production and T-cell differentiation by controlling intracellular glutathione redox. Therefore, we speculate that endogenous or exogenous factors that affect T-cell differentiation may change intracellular glutathione redox status *in vivo*.

In conclusion, we herein provided the first evidence that the polarization of Th1/Th2 balance is regulated by intracellular redox status in both LPS-DCs and TSLP-DCs. In addition, IL-27 and IL-12 both play critical roles in redox regulation. It is of note that the altered functions of DCs, distinct in their intracellular glutathione redox status, may provide a new therapeutic option for influencing Th1/Th2 balance in antigen-triggered immune responses, such as allergic and autoimmune diseases.

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Conflict of interest

The authors have no conflict of interest to declare.

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