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ORIGINAL RESEARCH COMMUNICATION

Glutathione Peroxidase 1 Deficiency Attenuates Allergen-Induced Airway Inflammation by Suppressing Th2 and Th17 Cell Development

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Abstract

Engagement of T cell receptor (TCR) triggers signaling pathways that mediate activation, proliferation, and differentiation of T lymphocytes. Such signaling events are mediated by reactive oxygen species (ROS), including hydrogen peroxide and lipid peroxides, both of which are reduced by glutathione peroxidase 1 (GPx1). We have now examined the role of GPx1 in the activation, differentiation, and functions of $CD4^+$ T helper (Th) cells. TCR stimulation increased the intracellular ROS concentration in Th cells in a time-dependent manner, and such TCR-induced ROS generation was found to promote cell proliferation. GPx1-deficient Th cells produced higher levels of intracellular ROS and interleukin-2 than wild-type Th cells and proliferated at a faster rate than did wild-type cells. Moreover, differentiation of GPx1-deficient Th cells was biased toward Th1, and Th17 cell development was also impeded by GPx1 depletion. Consistent with these findings, GPx1-null mice were protected from the development of ovalbumin-induced allergic asthma. Eosinophil infiltration, goblet cell hyperplasia, collagen deposition, and airway hyperresponsiveness were thus all attenuated in the lungs of GPx1-null mice. These data indicate that GPx1-dependent control of intracellular ROS accumulation is important not only for regulation of Th cell proliferation but for modulation of differentiation into Th1, Th2, and Th17 cells. *Antioxid. Redox Signal.* 13, 575–587.

Introduction

With regard to the immune system, reactive oxygen species (ROS) have been studied mostly in relation to their capacity as antimicrobial agents produced as part of the innate immune response. However, evidence suggests that ROS are also produced by cells of the adaptive immune system to serve as mediators of signaling cascades initiated by cell surface receptors. Stimulation of the T cell receptor (TCR), for example, induces the generation of ROS that function as important mediators of signal transduction underlying regulation of gene expression (6, 14). NADPH oxidase (Nox), which was originally identified as the enzyme responsible for the production of bactericidal ROS by phagocytic cells, has also been detected in small amounts in T cells, in which it generates ROS in response to TCR activation (6, 20). As in many other types of cells, lipoxygenases (31, 50) and mitochondria (14) have also been shown to produce ROS in activated T cells.

Early indications for a regulatory function of ROS in T cell activation were provided by experiments with diphenyliodonium (DPI), an inhibitor of flavoprotein oxidoreductases that include NADPH oxidase (6), or with broad-spectrum antioxidants such as *N*-acetyl-L-cysteine (NAC) or butylated hydroxyanisole (10–14). These pharmacological agents were found to inhibit TCR-dependent T cell proliferation or cytokine production. TCR activation was shown to result in the discrete generation of both the superoxide anion and hydrogen peroxide (6), and these two species of ROS appear to regulate distinct signaling pathways activated by TCR stimulation. Overexpression of superoxide dismutase (SOD) thus inhibited TCR-induced activation of Fas ligand expression, whereas overexpression of the peroxide-specific enzymes catalase or peroxiredoxin (Prx) had no such effect (6).

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Conversely, TCR-induced activation of extracellular signal-regulated kinase was augmented by overexpression of catalase or Prx but not by that of SOD (27). Signaling by peroxides is mediated predominantly by targeted oxidation of cysteine residues in many types of proteins, including protein tyrosine phosphatases, caspases, the tumor suppressor PTEN, transcription factors (NF-κB, AP1, Nrf2-Keap), and thioredoxins (22, 26). As in many other cell types, ROS in T cells cause oxidative damage, promote apoptosis, and mediate signaling cascades that affect cell proliferation and differentiation. The final outcome of the modulation of ROS levels in T cells appears to depend on the cell system and activation status, as exemplified by thymocytes, naïve T cells, activated T cell blasts, and T cell lines (51).

Glutathione peroxidase 1 (GPx1) is an abundant antioxidant enzyme that is expressed in most cell types and catalyzes the reduction of hydrogen peroxide or lipid peroxides to water or the corresponding lipid alcohol with the use of electrons provided by reduced glutathione. GPx1-deficient mice are healthy and fertile, indicating that the enzyme plays a limited antioxidant role under normal physiological conditions (15). However, angiogenesis was found to be impaired and the frequency of ROS-induced apoptosis was increased in GPx1-deficient endothelial cells (11). Conversely, expression of human GPx1 in mice resulted in a marked reduction both in the extent of tissue damage resulting from cerebral ischemia (49) as well as in the incidence of myocardial infarction and dysfunction (45), suggesting that GPx1 protects against peroxide toxicity (10, 30, 34, 45, 49).

Engagement of the TCR and CD28 receptor on naïve T cells results in their enlargement and transformation into blast cells that begin both repeated rounds of cell division as well as the production of the cytokine interleukin (IL)-2. Interaction of the secreted IL-2 with the IL-2 receptor further promotes proliferation of the activated T cells followed by their differentiation (9). $CD4^+$ T helper (Th) cells differentiate into distinct effector Th cells, including Th1, Th2, and Th17 cells (4, 13, 28, 47, 53). Whereas IL-12 and IL-4 induce differentiation into Th1 and Th2 cells, respectively, the combined influence of transforming growth factor (TGF)-β and IL-6 promotes Th17 cell development. Th1 and Th2 cells, which manifest distinct patterns of cytokine production [interferon (IFN)-γ and tumor necrosis factor-α for Th1 cells; IL-4, IL-5, and IL-13 for Th2 cells], contribute to the elimination of both intracellular and extracellular pathogens (12, 38, 48). Th17 cells secrete IL-17 and have been found to contribute to autoimmune disorders such as arthritis (2, 19), Crohn's disease (42, 43), ulcerative colitis (25), psoriasis (39), and multiple sclerosis (23).

We have now examined the possible role of ROS in the proliferation and differentiation of $CD4^+$ Th cells with the use of cells derived from GPx1-null mice. We found that ROS generation was essential for T cell proliferation induced by TCR engagement. Ablation of GPx1 resulted in increased ROS levels and IL-2 production in Th cells. Moreover, GPx1 deficiency attenuated Th2 and Th17 development *in vitro* and was associated with attenuation of allergen-induced airway inflammation *in vivo*. The extents of eotaxin-induced eosinophil infiltration, goblet cell hyperplasia, collagen deposition, and airway hyperresponsiveness were thus all markedly reduced in the airways of GPx1-deficient mice.

Materials and Methods

Reagents

All cytokines and antibodies to cytokines were obtained from BD Pharmingen (San Diego, CA) unless indicated otherwise. NAC and DPI were from Sigma-Aldrich (St. Louis, MO). Antibodies to GPx1, catalase, Mn^{2+}-dependent SOD (MnSOD), PrxI, Prx II or PrxIII were obtained from Ab-Frontier (Seoul, Korea).

Mice

C57BL/6 wild-type (WT) and GPx1 knockout (KO) mice on the C57BL/6 background (15) were housed under specific pathogen-free conditions at Ewha Womans University. Animal handling and experiments were performed in accordance with the guidelines of the institutional Animal Care and Use Committee.

Isolation of $CD4^+$ Th cells and TCR stimulation

Single-cell suspensions were prepared from the lymph node and spleen of WT or GPx1 KO mice and were incubated for 30 min on ice with anti-CD4 microbeads (Miltenyi Biotech, Auburn, CA). The isolated CD4+ Th cells (2×10^6/ml) were stimulated with plate-bound anti-CD3 (1 μg/ml, BD Pharmingen) and anti-CD28 (1 μg/ml, BD Pharmingen) antibodies in the additional presence of recombinant human IL-2 (10 U/ml; R&D Systems, Minneapolis, MN).

Determination of intracellular ROS concentration

TCR-stimulated $CD4^+$ Th cells were harvested and incubated for 30 min at 37°C with 5 μM chloromethyl dichlorofluorescein diacetate (CM-DCFDA, Invitrogen, Carlsbad, CA). The cells were then washed with phosphate-buffered saline (PBS), after which CM-DCF fluorescence was measured immediately with the use of a FACSCalibur flow cytometer and CellQuest software (BD Biosciences, San Diego, CA).

Staining for surface markers and intracellular cytokines

For staining of surface marker, single cell suspensions were isolated from thymus and lymph node and incubated with allophytocyanin (APC)-conjugated anti-CD4 and phycoerythrin (PE)-conjugated anti-CD8 Abs, followed by flow cytometry. For intracellular cytokine staining, TCR-triggered cells were incubated for 3 h at 37°C with 4 μM monensin (Sigma-Aldrich), fixed with 4% paraformaldehyde, washed with permeabilization buffer (0.1% saponin, 0.1% NaN_3, and 1% fetal bovine serum in PBS), and incubated with PE-conjugated antibodies to mouse IL-2, IL-4, IFN-γ, or IL-17. The cells were then washed twice with 1% fetal bovine serum in PBS and analyzed with a FACSCalibur flow cytometer.

ELISA

Culture supernatants of Th cells were incubated in enzyme-linked immunosorbent assay (ELISA) plates (Nunc, Naperville, IL) coated with capture antibodies to mouse cytokines. The plates were then incubated consecutively with biotinylated antibodies to mouse cytokines and alkaline phosphatase-conjugated streptavidin (BD Pharmingen), after which the absorbance of each well was measured at 405 nm with an ELISA plate reader (Molecular Devices, Palo Alto, CA).

RT and real-time PCR analysis

Total RNA was isolated from cells or lung tissue with the use of an RNeasy Mini Kit (Qiagen, Hilden, Germany) and was treated with DNase to remove any remaining genomic DNA. The RNA (2 μg) was then subjected to reverse transcription (RT) with a Superscript First-Strand Synthesis System (Invitrogen), and the resulting cDNA was subjected to real-time polymerase chain reaction (PCR) analysis with the use of SYBR Premix and an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). The abundance of target mRNAs was normalized by the corresponding amount of β-actin mRNA. The sequences of the PCR primers (forward and reverse, respectively) were as follows: β-actin, 5′-aagcaggagtatgacgagtccg-3′ and 5′-cggaactaagtca tagtccgcc-3′; Nox1, 5′-ctccctttgcttccatcttg-3′ and 5′-gcaaagg cacctgtctctcta-3′; Nox2, 5′-ccaactgggataacgagttca-3′ and 5′-gagagtttcagccaaggcttc-3′; Nox4, 5′-ttgcctggaagaacccaagt-3′ and 5′-tccgcacaataaaggcacaa-3′; IFN-γ, 5′-agcaacagcaaggcga aaa-3′ and 5′-ctggacctgtgggttgttga-3′; IL-2, 5′-ctcctgagcag gatggagaatt-3′ and 5′-cgcagaggtccaagtgtagct-3′; IL-4, 5′-ggca ttttgaacgaggtcaca-3′ and 5′-aggacgtttggcacatccat-3′; IL-5, 5′-agcacagtggtgaaagagacctt-3′ and 5′-tccaatgcatagctggtgattt-3′; IL-13, 5′-agaccagactcccctgtgca-3′ and 5′-tgggtcctgtagatgg cattg-3′; IL-17, 5′-caggacgcgcaaacatga-3′ and 5′-gcaacagcatcag agacacagat-3′; eotaxin, 5′-cagatgcaccctgaaagccata-3′ and 5′-tgctttgtggcatcctggac-3′; and RORγt, 5′-ccgctgagagggcttcac-3′ and 5′-tgcaggagtaggccacattaca-3′.

CFSE labeling and cell proliferation assay

Isolated CD4$^+$ Th cells were incubated for 10 min at 37°C with 10 μM carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes, Eugene, OR), washed with PBS, and stimulated with plate-bound antibodies to CD3 or to CD28 for the indicated times. The cells were then harvested for analysis of CFSE fluorescence by flow cytometry. The number of cells in each division cycle was determined with the use of CellQuest software. Cells stimulated for 24 h were also incubated for an additional 24 h with 10 mM NAC before analysis of dye dilution by flow cytometry.

Murine asthma model and BALF analysis

WT or GPx1 KO mice were sensitized by intraperitoneal injection of 50 μg of ovalbumin (OVA) absorbed into aluminum hydroxide (2 mg) on days 0 and 14. They were subsequently challenged eight times by intranasal administration of 1% OVA in PBS every other day, beginning on day 28. The animals were sacrificed 44 days after the first injection, the lungs were washed via the tracheal tube with 1 ml of PBS, and total and differential cell counts in the bronchoalveolar lavage fluid (BALF) were determined in a blinded fashion by counting at least 200 cells on cytocentrifuged preparations (Cytospin 3; Shandon Ltd, Runcorn, UK) stained with Leukostat (Fisher Diagnostics, Fair Lawn, NJ).

Histological analysis

Lungs were fixed in formalin (Sigma-Aldrich) and embedded in paraffin. Tissue sections with a thickness of 10 μm were stained with hematoxylin-eosin, periodic acid-Schiff (PAS) solution, or Masson's trichrome solution and were examined with the use of an Eclipse E200 microscope (Nikon, Japan). The number of PAS$^+$ cells per millimeter of epithelial basement membrane was determined.

Determination of airway hyperresponsiveness (AHR)

Airway responsiveness was determined from peak pulmonary resistance (R_L, $cmH_2O/ml/sec$) and compliance using the FlexiVent system (10 ml/kg tidal volume at 160 breaths/min, SCIREQ, Montreal, Quebec, Canada) after delivery of aerosolized methacholine (MCh, Sigma-Aldrich). MCh aerosol (3.1–100 mg/ml) was administrated for 10 sec, after which airway resistance was continuously monitored and recorded.

Statistical analysis

Quantitative data are presented as means $\pm$ SEM and were analyzed by the unpaired Student's t-test or analysis of variance (ANOVA). A p value of <0.05 was considered statistically significant.

Results

TCR-induced ROS generation in GPx1-deficient CD4$^+$ Th cells

We first examined the generation of ROS induced by stimulation with anti-CD3, anti-CD28, or both anti-CD3 and anti-CD28 in CD4$^+$ Th cells isolated from the lymph nodes and spleen of WT mice. Loading of the cells with CM-DCFDA revealed that anti-CD3 alone induced an increase in the intracellular concentration of ROS similar to that induced by both anti-CD3 and anti-CD28, whereas anti-CD28 alone had no such effect (Fig. 1A), indicating the importance of TCR stimulation for ROS production. The intracellular ROS level increased in a time-dependent manner for up to 24 h in cells stimulated with anti-CD3 (Fig. 1B). Previous studies on TCR-induced ROS production in T cells suggested Nox2 is responsible for ROS generation (6, 20). Indeed, the expression of Nox 2 in CD4$^+$ Th cells was confirmed by real-time PCR, while Nox 1 and Nox 3 were not detectable (Fig. 1C). Nox 2 transcript in Th cells substantially decreased at 24 h after TCR activation (Fig. 1C). The decrease of Nox 2 expression 24 and 48 h after TCR stimulation was also seen immunoblot analysis of Nox 2 (Fig. 1D). Immunoblot analysis of various antioxidant molecules that regulate the intracellular ROS level revealed that CD4$^+$ Th cells express catalase, MnSOD, and various Prx isoforms (PrxI, PrxII, PrxIII) but that the abundance of these proteins was not altered by TCR stimulation (Fig. 1E). In contrast, the expression of GPx1 in the cells decreased gradually during TCR stimulation for up to 48 h (Fig. 1E, F). ROS levels were higher in CD4$^+$ Th cells from GPx1 KO mice at 24 h and 48 h after the onset of TCR stimulation than in those from WT mice (Fig. 1G). The ROS levels of unstimulated cells did not differ between the two genotypes (Fig. 1G). These results thus indicated that the TCR-induced increase in intracellular ROS level is limited by GPx1 in CD4$^+$ Th cells.

Enhanced IL-2 production and proliferation in GPx1-deficient Th cells

At first, we confirmed that there was no difference of T cell maturation and development between WT and GPx1 KO mice, as evidenced by the comparable expression of T cell

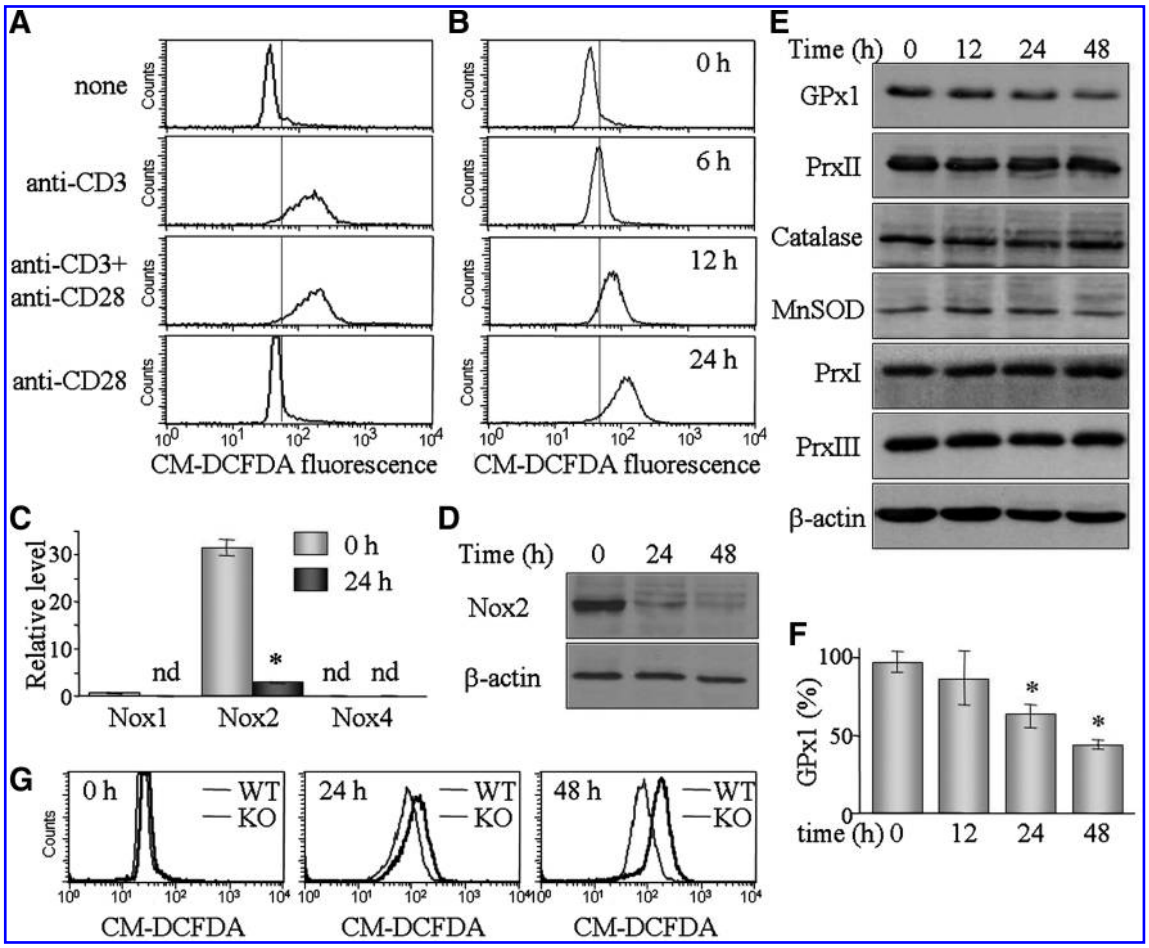

FIG. 1. TCR-induced ROS generation in GPx1-deficient CD4$^+$ Th cells. (**A**) CD4+ Th cells isolated from WT mice were incubated for 24 h in the absence or presence of plate-bound anti-CD3, anti-CD28, or both anti-CD3 and anti-CD28. The cells were then harvested, incubated with CM-DCFDA for 30 min, and monitored for CM-DCF fluorescence by flow cytometry. (**B**) WT CD4$^+$ Th cells were stimulated with anti-CD3 for the indicated times and then analyzed as in (**A**). (**C**) Total RNA was prepared from 0 h and 24 h-stimulated cells and used to perform RT and real-time PCR for measuring Nox1, Nox2, and Nox4 expression. nd, not determined; $^*p < 0.05$. (**D, E**) WT CD4$^+$ Th cells stimulated with anti-CD3 for the indicated times were lysed and subjected to immunoblot analysis with antibodies to the indicated proteins. (**F**) The immunoblot density of GPx1 was scanned and expressed as a percentage relative to that of 0 h. $^*p < 0.05$. (**G**) CD4$^+$ Th cells isolated from WT or GPx1 KO mice were stimulated with anti-CD3 for 0, 24, or 48 h and then analyzed as in (**A**). All data are representative of three independent experiments.

markers in thymocytes (Fig. 2A). We next examined the effect of the increased ROS level in TCR-stimulated GPx1-deficient CD4$^+$ Th cells on the production of IL-2. Although the T cell populations between WT and KO mice were comparable (Fig. 2A), both the proportion of cells producing IL-2 (Fig. 2B) and the concentration of IL-2 in culture supernatants (Fig. 2C) after cell stimulation were greater for GPx1 KO cells than for WT cells. RT and real-time PCR analysis also revealed that the amount of IL-2 mRNA was greater in the GPx1 KO cells than in the WT cells (Fig. 2D). These results thus suggested that the increased accumulation of ROS associated with GPx1 deficiency augments IL-2 production in response to TCR stimulation. Moreover, monitoring of cell proliferation by loading of CD4$^+$ Th cells with CFSE revealed that the proportion of cells that had undergone five rounds of division after stimulation with anti-CD3 for 72 h was 27.8% for GPx1 KO cells but only 15.4% for WT cells (Fig. 2E), showing that GPx1 deficiency promoted cell proliferation.

Essential role of ROS in IL-2 production and subsequent Th cell expansion

To determine whether ROS indeed affect IL-2 production, we exposed Th cells to NAC or DPI, commonly used antioxidants. CD4$^+$ Th cells from WT and KO mice were first stimulated with anti-CD3 and anti-CD28 for 24 h and then exposed to NAC for an additional 24 h in order to avoid the direct reduction of the intrachain disulfide bonds located in the extracellular domain of TCR subunits before TCR stimulation. NAC treatment inhibited IL-2 production by the Th cells of both WT and KO mice (Fig. 3A), and consequently decreased cell proliferation (Fig. 3B). The release of IL-2 from WT and KO Th cells was also inhibited to a similar extent when treated with DPI for 24 h; the extents of inhibition by DPI were greater than those by NAC (Fig. 3A). In addition, DPI substantially increased apoptotic cell death, while NAC had no influence on cell apoptosis (Fig. 3C). These results thus

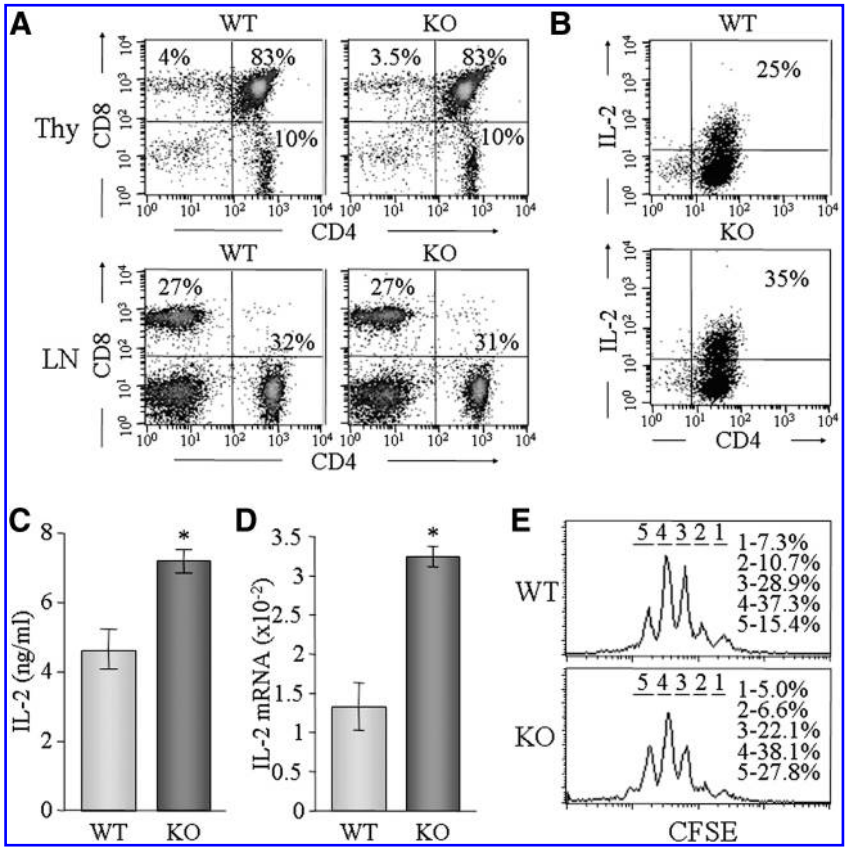

FIG. 2. Enhanced IL-2 production and proliferation in GPx1-deficient Th cells. (**A**) Thymocytes (Thy) and lymphocytes (LN) were isolated from WT or GPx1 KO mice and stained with APC-conjugated anti-CD4 and PE-conjugated anti-CD8 Abs. Cells were washed and subsequently analyzed by flow cytometry. (**B**) $CD4^+$ Th cells isolated from WT or GPx1 KO mice were stimulated with plate-bound anti-CD3 for 72 h, further stimulated with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 μg/ml ionomycin for 4 h, and then processed for staining of intracellular IL-2 of $CD4^+$ cells and analysis by flow cytometry. The percentage of IL-2-producing cells is indicated. (**C**, **D**) $CD4^+$ Th cells from WT or GPx1 KO mice were stimulated with anti-CD3 for 48 h, after which the concentration of IL-2 in the culture supernatants was measured by ELISA (**C**) and the amount of IL-2 mRNA in the cells was determined by RT and real-time PCR analysis and normalized by the amount of β-actin mRNA (**D**). (**E**) $CD4^+$ Th cells from WT or GPx1 KO mice were labeled with CFSE for 10 min, stimulated for 72 h with anti-CD3, and analyzed for CFSE fluorescence by flow cytometry. The percentage of cells in each division cycle is indicated. Data in (**A**), (**B**), and (**E**) are representative of three independent experiments, and those in (**C**) and (**D**) are means $\pm$ SEM from three independent experiments. $^*p < 0.05$.

indicated that ROS are required for IL-2 production and consequent proliferation in $CD4^+$ Th cells, and probably also for cell survival.

Preferred differentiation of GPx1-deficient Th cells into Th1 cells

We next examined the effect of GPx1 deficiency on Th cell differentiation. $CD4^+$ Th cells isolated from WT or GPx1 KO mice were stimulated with anti-CD3 and anti-CD28 in the presence of excess recombinant IL-2 in order to compensate for the different amounts of this cytokine produced by the WT and GPx1-deficient cells. Under such conditions, both WT and GPx1 KO cells divided at similar rates, as revealed by the similar numbers of cells in each division cycle observed after 72 h (Fig. 4A). The cells were then allowed to differentiate into effector Th cells under nonskewing conditions before restimulation with anti-CD3 for 24 h. The amount of IL-2 released by the Th cells during this secondary stimulation did not differ between the two genotypes (Fig. 4B). The amounts of IL-2 mRNA in the WT and GPx1 KO cells were similar (Fig. 4C). We also measured the abundance of the mRNAs for IFN-γ and IL-4, signature cytokines of Th1 and Th2 effector cells, respectively. The ratio of IFN-γ to IL-4 mRNA levels measured in differentiated GPx1-deficient Th cells was three times that for the differentiated WT cells (Fig. 4D). In addition, intracellular cytokine staining verified increased production of IFN-γ in GPx1-null Th cells greater than that in WT (Fig. 4E), suggesting that GPx1-deficient Th cells show an increased tendency to differentiate into Th1 cells compared with WT cells. Interestingly, a Th1-specific transcription factor, T-bet was augmented in GPx1-deficient Th cells at 48 h after TCR stimulation (Fig. 4F), elucidating that increased T-bet expression may cause Th1-biased differentiation of GPx1 KO Th cells.

The preferential differentiation of GPx1-deficient Th cells into Th1 cells was also tested under Th1- or Th2-skewing conditions. While effector Th1 cells lacking GPx1 produced slightly more IFN-γ than did WT Th1 cells (Figs. 5A and 5B), GPx1-null Th2 cells produced a smaller amount of IL-4 than did WT Th2 cells (Figs. 5C and 5D). Moreover, the attenuated Th2 development of GPx1 KO Th cells was proven by the less abundance of mRNAs for the Th2 cytokines IL-4, IL-5, and IL-13 in GPx1 KO than in WT (Fig. 5E). These results thus further suggested that the ablation of GPx1 in Th cells promotes their differentiation into Th1 cells rather than into Th2 cells.

Attenuated differentiation of GPx1-deficient Th cells into Th17 cells

The effect of GPx1 ablation on the development of Th17 cells, a third lineage of effector Th cells, was evaluated. $CD4^+$ Th cells from WT or GPx1 KO mice were induced to differentiate into Th17 cells by exposure to TGF-β and IL-6, anti-IL-4 Ab, and anti-IFN-γ Ab concurrent with TCR stimulation. The amounts of IL-17 released by (Fig. 6A) and of IL-17 mRNA in (Fig. 6B) GPx1 KO cells at 48 h after stimulation-differentiation were substantially less than those apparent for WT cells. Even after prolonged differentiation under Th17-skewing

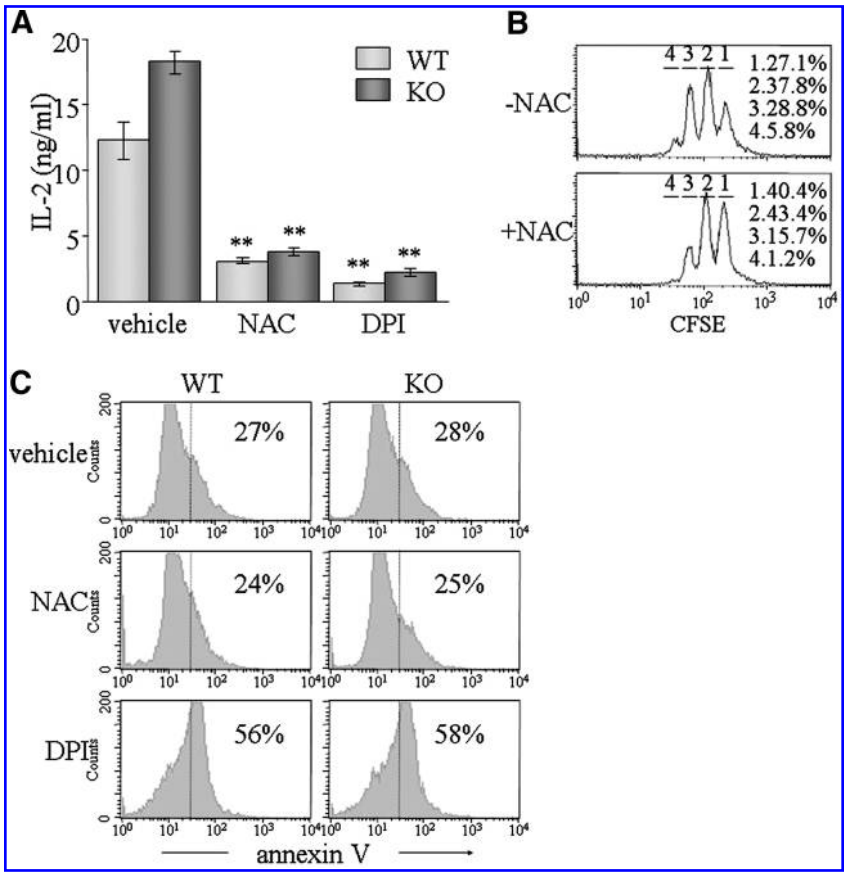

FIG. 3. Essential role of ROS in IL-2 production and subsequent Th cell expansion. WT and KO $CD4^+$ Th cells were stimulated with plate-bound anti-CD3 and anti-CD28 for 24 h and then incubated in the additional presence of 10 m*M* NAC or 5 μM DPI for 24 h. (**A**) The concentration of IL-2 in culture supernatants was then determined by ELISA. Data are means ± SEM from three independent experiments. $^{**}p < 0.005$. (**B**) $CD4^+$ Th cells from WT mice were labeled with CFSE for 10 min, stimulated with anti-CD3 and anti-CD28 for 24 h, incubated in the additional absence or presence of 10 m*M* NAC for 24 h, and then subjected to flow cytometry for determination of the number of cells in each division cycle. The percentage of cells in each division cycle is indicated. (**C**) Cells as in (**A**) were incubated with PE-conjugated annexin V and subjected to flow cytometry analysis. The percentage of apoptotic cells was determined by CellQuest program. Data in (B) and (C) are representative of three independent experiments.

conditions and restimulation with either anti-CD3 Ab or PMA plus ionomycin, the expression of IL-17 in GPx1 KO cells was only ~50% of that observed in WT cells (Figs. 6C and 6D). Given that Th17 cell differentiation is controlled by the Th17-specific transcription factor RORγt, we examined the effect of GPx1 deficiency on RORγt expression. The expression of RORγt mRNA was attenuated in GPx1 KO cells (Fig. 6E). These results thus indicated that ablation of GPx1 suppresses RORγt-mediated Th17 cell development. Furthermore, in order to certify whether IL-17 production was directly affected by ROS, fully differentiated Th17 cells were treated with DPI for 4 h. IL-17 expression was significantly increased by treatment with DPI, as confirmed by real-time PCR (Fig. 6F), supporting the notion that optimal level of ROS may be required for the suppression of RORγt-induced IL-17 expression.

Attenuation of allergen-induced eosinophil infiltration and AHR in GPx1 KO mice

Mouse models of allergic airway inflammation have revealed that Th17 cells not only induce infiltration of neutrophils into the airway but also promote the Th2 cell-dependent infiltration of eosinophils (46). Given that GPx1 ablation was found to hinder the development of Th2 and Th17 cells, we examined the effects of GPx1 deficiency in the OVA-induced model of airway inflammation. OVA challenge in sensitized mice results in an increase in the number of immune cells including macrophages, lymphocytes, neutrophils, and eosinophils in the lungs. We found that the total number of cells in BALF after OVA challenge in sensitized animals was significantly decreased in GPx1 KO mice than in WT mice (Fig. 7A). In addition, the numbers of macrophages, lymphocytes, neutrophils, and eosinophils in BALF of GPx1 KO mice were significantly smaller than those in WT mice (Fig. 7B). The expression level of chemokines such as macrophage inflammatory protein-1α (MIP-1α), monocyte chemotactic protein-1 (MCP-1), and TNF-α were substantially declined in the lung of GPx1-deficient mice (Figs. 7C–7E). The amount of the mRNA for eotaxin, a potent chemokine for eosinophils, was also decreased in the lung tissue of GPx1 KO mice compared with that observed in WT mice (Fig. 7F). Moreover, AHR to increasing doses of methacholine was enhanced in OVA-injected WT mice, whereas GPx1 KO mice challenged with OVA revealed markedly diminished AHR to methacholine (Fig. 7G). These results demonstrated that signature features of airway inflammation including eosinophilic infiltration, chemokine production, and AHR were apparently attenuated in the absence of GPx1.

Attenuation of goblet cell hyperplasia, collagen deposition, and inflammatory cytokine expression in GPx1 KO mice

Excess production of mucus, increased goblet cell differentiation, and extensive and confluent deposits of collagen are characteristic of chronic pulmonary diseases such as asthma. We examined lung histopathology to evaluate the number of mucus-secreting goblet cells and collagen deposition in the airways of GPx1 KO mice sensitized and challenged with

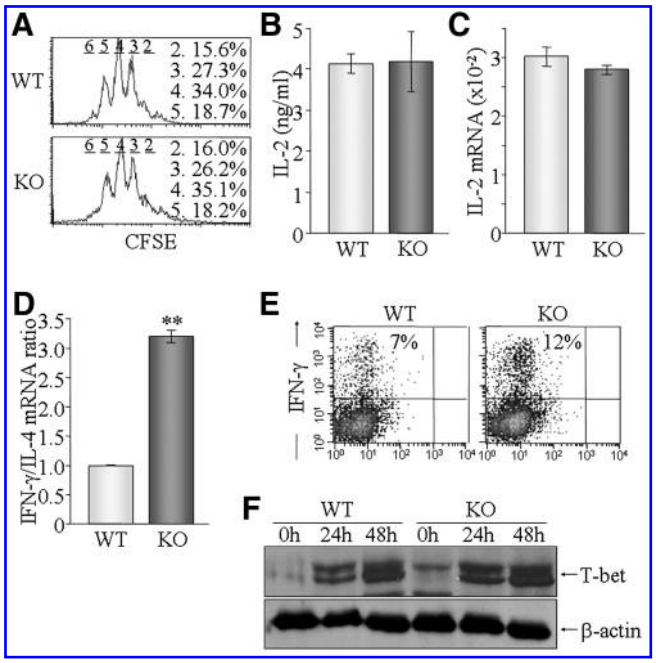

FIG. 4. Preferred differentiation of GPx1-deficient Th cells into Th 1 cells. (**A**) $CD4^+$ Th cells from WT or GPx1 KO mice were labeled with CFSE for 10 min, stimulated for 72 h with plate-bound anti-CD3 and anti-CD28 in the presence of recombinant human IL-2, and then subjected to flow cytometry for determination of the number of cells in each division cycle. Data are representative of three independent experiments. (**B**–**E**) WT and KO $CD4^+$ Th cells were stimulated with anti-CD3 and anti-CD28 in the presence of recombinant human IL-2, allowed to differentiate for 5 to 6 days, and then restimulated with plate-bound anti-CD3 (1 μg/ml) for 24 h. The concentration of IL-2 in the final culture supernatants was measured by ELISA (**B**). Relative transcription levels of IL-2 (**C**) as well as of IFN-γ and IL-4 (**D**) in the cells were determined by real-time PCR analysis and normalized by the amount of β-actin mRNA; the ratio of the normalized amounts of IFN-γ and IL-4 mRNAs was calculated. Data are means $\pm$ SEM from four independent experiments. $^{**}p < 0.005$. Restimulated Th cells were pretreated with monensin and incubated with PE-conjugated IFN-γ Ab (**E**). (**F**) $CD4^+$ Th cells were obtained from WT and KO mice and stimulated for 48 h as in (**A**). Whole cell extracts were harvested from the cells and resolved by SDS-PAGE, followed by immunoblot analysis using anti-T-bet or β-actin Ab.

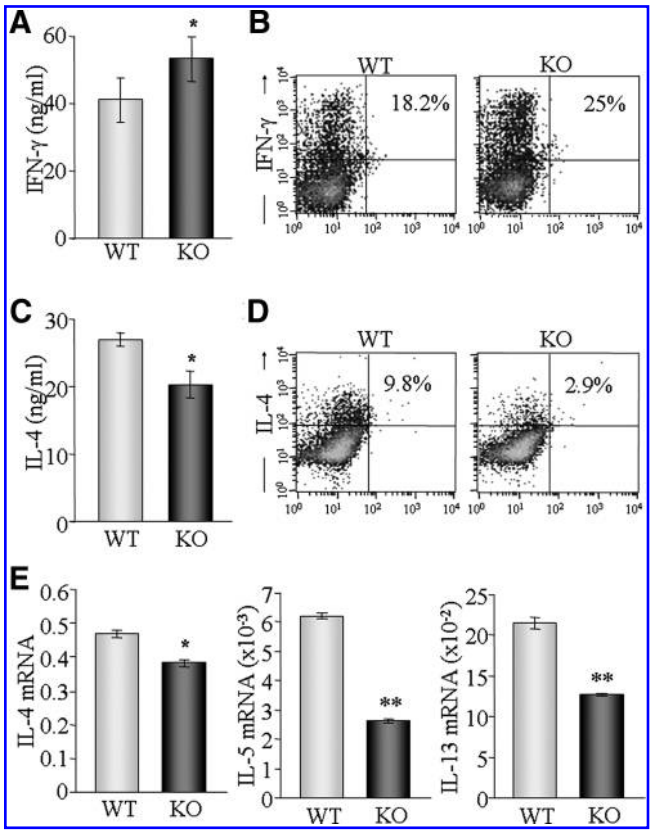

FIG. 5. Differentiation of GPx1-deficient $CD4^+$ Th cells under Th1- or Th2-skewing conditions. $CD4^+$ Th cells from WT or GPx1 KO mice were stimulated with plate-bound anti-CD3 and anti-CD28 for 24 h. The cells were then treated either with IL-12 (2 ng/ml) and anti–IL-4 (5 μg/ml) in order to induce them to differentiate into Th1 cells (**A**, **B**) or with IL-4 (10 ng/ml) and anti–IFN-γ (5 μg/ml) to differentiate into Th2 cells (**C, D, E**). After incubation for 4 days, the differentiated cells were stimulated with 10 ng/ml PMA and 1 μg/ml ionomycin for 4 h before processing for intracellular staining of IFN-γ (**B**) or IL-4 (**D**) and analysis by flow cytometry. Alternatively, after differentiation for 6 days, the cells were restimulated with anti-CD3 (1 μg/ml) for 24 h, after which the concentrations of IFN-γ (**A**) or IL-4 (**C**) in the culture supernatants were determined by ELISA. (**E**) Cells allowed to differentiate under Th2-skewing conditions as in (**C**) were assayed for the amounts of IL-4, IL-5, and IL-13 mRNAs by RT and real-time PCR analysis. The amount of each mRNA was normalized by the corresponding amount of β-actin mRNA. All data are means $\pm$ SEM from four independent experiments with the exception of those in (**B**) and (**D**), which are representative of four independent experiments. $^{*}p < 0.05$, $^{**}p < 0.005$.

OVA. The number of PAS-positive goblet cells was significantly smaller in GPx1 KO mice than in WT mice (Figs. 8A and 8B). In addition, Masson's trichrome staining revealed that collagen deposition was also diminished in the lungs of GPx1 KO mice compared with that in WT animals (Fig. 8C). Moreover, the amount of allergy- and inflammation-associated cytokines IL-5, IL-13 and IL-17 were smaller in GPx1 KO mice, as revealed by ELISA using lung homogenates and by RT and real-time PCR analysis of total RNA isolated from lung tissue (Figs. 8D and 8E). Finally, *ex vivo* analysis of cells isolated from draining lymph nodes and stimulated with anti-CD3 revealed that the amounts of the allergy-related cytokines IL-5 and IL-13 released into the culture supernatants were significantly reduced for cells derived for GPx1 KO mice compared with those derived from WT animals (Fig. 8F). These results thus supported the notion that GPx1 KO mice are protected against allergen-induced airway inflammation as a result of inhibition of allergic Th2 and inflammatory Th17 cell development.

Alterations of Th cell differentiation in the deficiency of PrxIII

Enzymes responsible for the elimination of hydroperoxides include catalase, GPx, and Prx. Mammalian cells express six

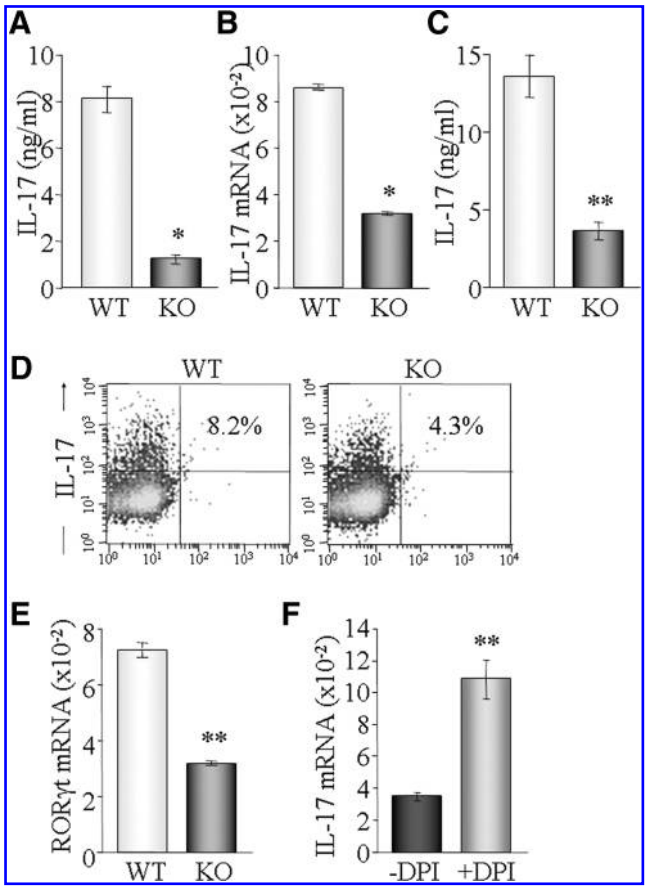

FIG. 6. Attenuated differentiation of GPx1-deficient Th cells into Th17 cells. $CD4^+$ Th cells from WT or GPx1 KO mice were stimulated with plate-bound anti-CD3 and anti-CD28 in the presence of TGF-β (5 ng/ml), IL-6 (10 ng/ml), anti–IFN-γ (5 μg/ml), and anti–IL-4 (5 μg/ml) to induce differentiation into Th17 cells. (**A**) Cell supernatants were collected from 48 h-stimulated cells and used for measuring IL-17 by ELISA. (**B**) Cells were harvested at 48 h after differentiation for preparing total RNA and the relative amount of IL-17 mRNA was determined by RT and real-time PCR analysis. (**C**, **D**) Cells stimulated as in (**A**) were differentiated for an additional 5–6 days and restimulated with anti-CD3 (1 μg/ml). The concentration of IL-17 in culture supernatants was measured by ELISA (**C**), and the cells were processed for intracellular IL-17 staining by flow cytometric analysis (**D**). (**E**) Total RNA prepared as in (**B**) was analyzed to determine the relative expression level of RORγt mRNA using real-time PCR analysis. (**F**) WT $CD4^+$ Th cells were cultured under Th17-skewing conditions for 5 days and restimulated with anti-CD3 for 24 h. Restimulated Th17 cells were additionally treated with or without DPI for 4 h before harvest. Relative expression level of IL-17 was calculated by RT and real-time PCR analysis. All data are means $\pm$ SEM from four independent experiments with the exception of those in (**D**), which are representative of four independent experiments. $^*p < 0.05$, $^{**}p < 0.005$.

different Prx enzymes. Whereas Prx I and Prx II are found in the cytosol, Prx III is exclusively present in the mitochondria. An examination of the role of PrxI in allergic asthma revealed that unlike the GPx1-null mice examined in the present study, PrxI null mice were more sensitive to OVA-induced allergic airway inflammation than were WT animals (17). In addition, PrxII-deficient Th cells do not manifest the same preference for Th1 differentiation shown by GPx1-deficient Th cells (unpublished results). It appears that only GPx1-deficient T cells exhibit an increased tendency to differentiate into Th1 cells after TCR activation. This selectivity might be related to the fact that PrxI and PrxII are cytosolic enzymes, whereas GPx1 is located in both the cytosol and mitochondria. We examined the development of Th cells in Prx III-deficient mice. TCR stimulation increased significantly more ROS in PrxIII-null Th cells compared with WT Th cells (Fig. 9A). Moreover, subsequent differentiation of Th cells under Th1-skewing conditions exhibited increase of IFN-γ production in PrxIII-deficient mice (Fig. 9B), while considerable reduction in Th2 and Th17 development was observed (Figs. 9C–9E).

Discussion

We have shown that ligation of the TCR in Th cells results in the intracellular accumulation of ROS for periods up to 48 h. ROS have previously been shown to be produced by NADPH oxidase (Nox), lipoxygenases, and mitochondria in activated T cells (6, 20). NADPH oxidase and lipoxygenases are likely responsible for the immediate generation of ROS in response to TCR stimulation, and many TCR-induced signaling events, including the tyrosine phosphorylation of linker for activation of T cells, phospholipase C–γ1, and mitogen-activated protein kinases as well as the activation of transcription factors such as c-Fos, c-Jun, NFAT, and NF-κB, are likely augmented as a result of the increase in intracellular ROS level (6). Prolonged TCR stimulation results in the enlargement of T cells into blasts and the onset of repeated rounds of cell division, which are associated with an increased rate of cellular metabolism and consequent ROS production by mitochondria. ROS production observed at the later times of TCR stimulation in the present study is likely attributable to mitochondria instead of the sustained Nox activity. Previous studies with T cell blasts derived from Nox 2-deficient mice indicated that Nox 2 is responsible for TCR-induced ROS production and that the Nox 2-dependent ROS production reaches a plateau within 1 h (19). We found that Th cells express Nox2 but not Nox 1 and 3 and that Nox 2 decreased significantly at 24 h after TCR stimulation (Figs. 1C and 1D). This observation is in line with the notion that Nox2 is responsible for the early generation of ROS following TCR stimulation.

Our results obtained by exposure of Th cells to ROS scavenger NAC or DPI indicated that ROS production is critical for TCR-stimulated IL-2 production and cell proliferation. Examination of the expression of various antioxidant enzymes in TCR-stimulated Th cells by immunoblot analysis revealed that the amounts of catalase, Prxs, and MnSOD remained unchanged during stimulation for up to 48 h. However, the amount of GPx1 was found to decrease gradually with time of stimulation. This downregulation of GPx1 would be expected to promote the accumulation of ROS and the production of IL-2 by reducing the cellular capacity to remove ROS generated by mitochondria. In support of this notion, we found that Th cells isolated from GPx1-deficient mice accumulated higher levels of ROS, produced more IL-2, and proliferated faster than did those isolated from WT mice. In addition, subsequent differentiation of GPx1-deficient Th cells exhibited attenuated Th2 and Th17 cells and rather prominent

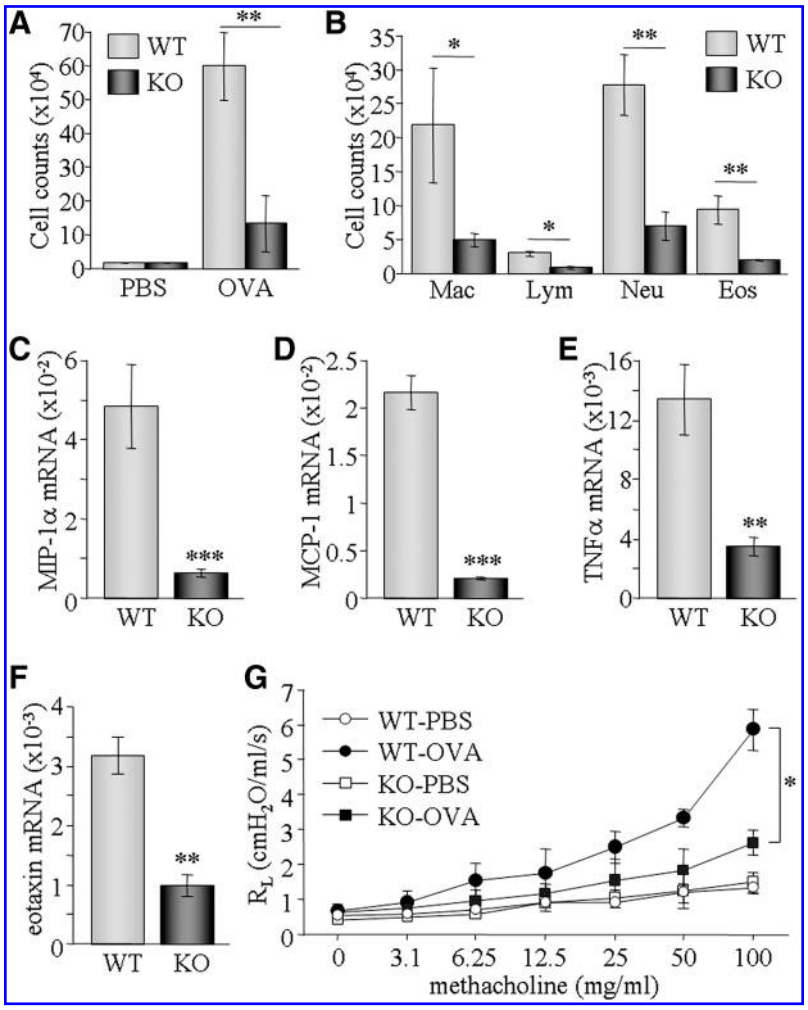

FIG. 7. Attenuation of allergen-induced eosinophil infiltration and airway hyperresponsiveness in GPx1 KO mice. WT and GPx1 KO mice were sensitized and challenged with OVA as described in Materials and Methods, after which the total cell count (**A**) as well as the numbers of macrophages (Mac), lymphocytes (Lym), neutrophils (Neu), and eosinophils (Eos) (**B**) in BALF were determined. The amount of MIP-1α (**C**), MCP-1 (**D**), TNFα (**E**), and eotaxin mRNA (**F**) in lung tissue was also determined by RT and real-time PCR analysis and normalized by the amount of β-actin mRNA. (**G**) Peak pulmonary resistance (R_L) in response to increasing doses of methacholine was measured in PBS- or OVA-challenged WT and GPx1 KO mice ($n = 6$). All data are means ± SEM from six mice of each genotype. *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$.

Th1 cells than WT Th cells, implying the important functions of intracellular ROS in Th cell differentiation. Further analysis of the mice lacking mitochondria-specific PrxIII demonstrated enhanced IFN-γ production and ameliorated IL-5, IL-13, and IL-17 expression as compared with WT Th cells, strongly supporting the notion that augmented intracellular and mitochondrial ROS may by critical for the modulation of Th cell development.

The differentiation of naïve $CD4^+$ Th cells into effector Th subsets (Th1, Th2, and Th17) is controlled by cytokines and subsequent activation of transcription factors such as T-bet, GATA-3, and RORγt (38, 48, 53). Greater tendency to differentiate into Th1 subset of GPx1 KO $CD4^+$ Th cells was mediated by the increased expression of T-bet, while a reduced tendency for Th17 differentiation was due to a decreased RORγt. We previously showed T-bet is decreased in Th cells by the presence of quercetin, a potent antioxidant, and its degradation is blocked by MG132 (52). These findings suggest that the optimal concentration of intracellular ROS is required for the maintenance of T-bet level. On the contrary, RORγt is diminished in GPx1-deficient Th cells under Th17-skewing conditions. Temporal treatment of DPI in effector Th17 cells rather increased RORγt-induced IL-17 gene transcription. Although it is largely unknown how ROS control Th cell development, our results suggest that transcription factors, T-bet, and RORγt are directly or indirectly controlled by intracellular ROS through the regulation of gene transcription and protein stability.

A long-term increase in the intracellular ROS level resulting from ablation of PrxI and PrxII also promoted T cell proliferation (17, 36). However, T cells deficient in the two cytosolic antioxidant enzymes did not appear to exhibit an increased tendency to differentiate into Th1 cells after TCR activation (unpublished result). In contrast, ablation of a mitochondrial enzyme PrxIII promoted Th1 cell development and suppressed Th2 and Th17 cell development. GPx1 is located in both the cytosol and mitochondria. Thus, it appears that Th cell differentiation is affected by mitochondrially produced ROS, whereas Th cell proliferation is enhanced by global increase of intracellular ROS.

Likely as the result of reduced numbers of Th2 and Th17 cells, GPx1 KO mice were found to be protected from the development of OVA-induced allergic asthma, which is largely dependent on these Th cell subsets. Asthma is primarily an airway inflammatory disease, and oxidative damage inflicted by ROS produced by phagocytic cells is one of the causes or facilitators of inflammation in various tissues (8, 40). Previous studies have shown that ROS generation is increased in bronchoalveolar lavage cells (24) and in the exhaled breath condensates (5) of stable asthmatics, that the bronchial airways are particularly susceptible to oxidant-induced tissue damage, and that various markers of oxidative stress are

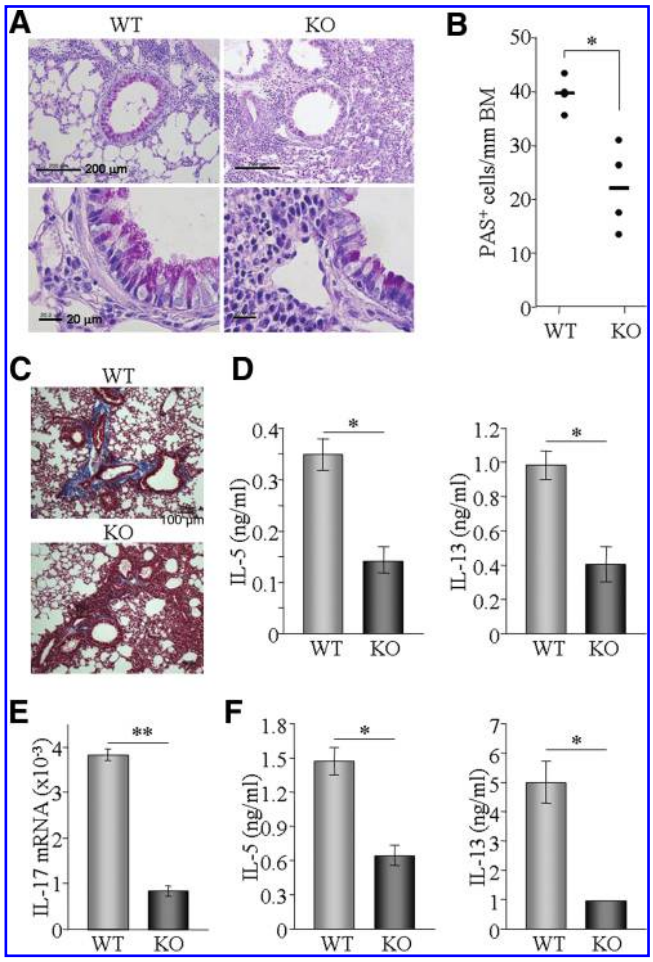

FIG. 8. Attenuation of goblet cell hyperplasia, collagen deposition, and inflammatory cytokine expression in GPx1 KO mice. WT and GPx1 KO mice were sensitized and challenged with OVA as in Figure 7. (**A**) PAS staining of lung tissue isolated from WT and GPx1 KO mice. Scale bars represent 200 and 20 μm in the *upper* and *lower panels*, respectively. (**B**) The number of PAS-positive cells per millimeter of basement membrane was determined in sections similar to those shown in (**A**). Each data point represents the value determined from one mouse, with the horizontal bars indicating the mean ($n = 4$). (**C**) Tissue sections as in (**A**) were also stained for collagen (*blue*) with Masson's trichrome solution. Scale bar, 100 μm. (**D**) Total lung homogenates were harvested and used for measuring IL-5 and IL-13 by ELISA. (**E**) Total RNA was isolated from lung tissues and was subjected to RT and real-time PCR analysis of IL-17 mRNA, the amounts of which were normalized by the corresponding abundance of β-actin mRNA. (**F**) Single-cell suspensions were isolated from draining lymph nodes of OVA-challenged mice and were stimulated overnight with plate-bound anti-CD3. Culture supernatants were then assayed for IL-5 and IL-13 by ELISA. Data in (**D**) through (**F**) are means ± SEM from 6 mice of each genotype. $^*p < 0.05$, $^{**}p < 0.005$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

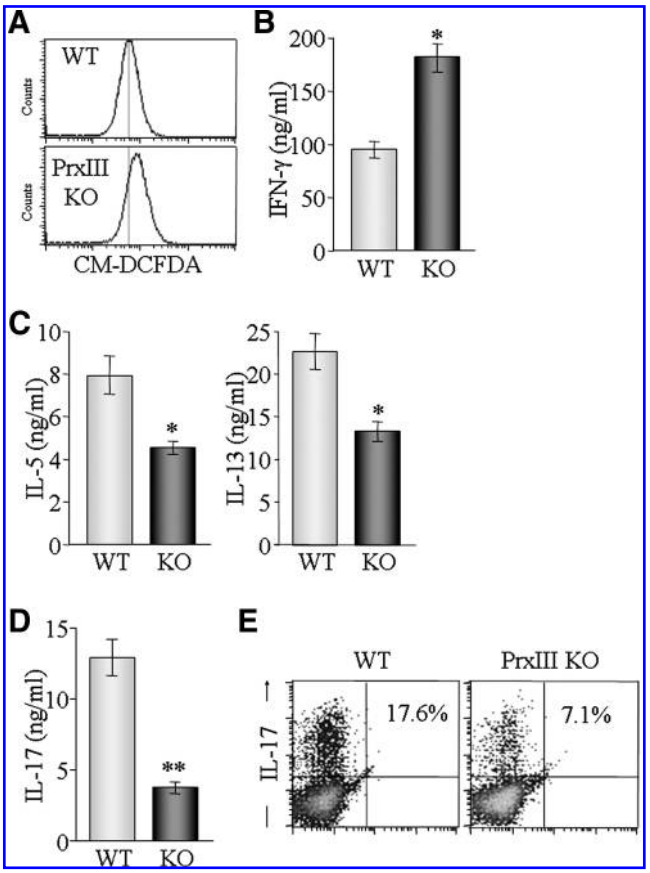

FIG. 9. Attenuation of Th2 and Th17 cell differentiation by the increased mitochondrial ROS. (**A**) $CD4^+$ Th cells were obtained from WT and PrxIII KO mice and were triggered with anti-CD3 and anti-CD28 for 48 h and harvested for analyzing CM-DCFDA fluorescence. (**B–E**) WT and PrxIII KO $CD4^+$ Th cells were induced to differentiate into Th1, Th2, and Th17 cells for 5 days and were restimulated with anti-CD3 for 24 h. Cell supernatants from Th1, Th2, and Th17 cells were respectively used for measuring IFN-γ (**B**), IL-5, IL-13 (**C**), IL-17 (**D**) by ELISA. (**E**) Th17 cells were stained with PE-conjugated anti-IL-17 Ab and analyzed by flow cytometry. All data are means ± SEM from four independent experiments with the exception of those in (**A**) and (**E**), which are representative of three independent experiments. $^*p < 0.05$, $^{**}p < 0.005$.

increased in asthmatics (1, 7, 8, 37). Endogenous antioxidants such as GPxs, catalase, Prxs, and MnSOD have been shown to provide protection against oxidative damage and to inhibit inflammation in several tissues (32, 33, 41, 44). In particular, GPx1 expression is induced in response to Ag challenges within the lung (16), whereas blood GPx1 activity is lower in asthmatic patients than in nonasthmatic subjects (35). It seems likely that the expression and activity of GPx1 in blood may be important for the control of asthma via the modulation of Ag-responsive immune system. In addition, several reports reveal that GPx1 KO mice are more susceptible to virus-induced myocarditis by the impaired humoral immune response (3) and liver injury induced by lipopolysaccharide and galactosamine (21). Oppositely, GPx1 transgenic mice are more resistant to renal ischemia/reperfusion injury by less neutrophil infiltration (18). Our present studies suggest that depletion of GPx1 attenuates asthma, likely because the effect of GPx1 on Th cell differentiation is of greater consequence than is the protection this enzyme affords against oxidative damage in lung tissue. We found that the number of macrophages and eosinophils in BALF from OVA-sensitized and -challenged mice was smaller for GPx1-null mice than for

WT mice. In addition, AHR, goblet cell hyperplasia and collagen deposition were significantly decreased in GPx1-lacking mice. In the current study, we could not test if Prx III-deficient mice also exhibit attenuated responses to allergen-induced inflammation because Prx III knockout mice are difficult to breed, which was attributed to the crucial role of Prx IIII in placental antioxidant defense (29).

Collectively, our results provide the first functional connection between specific antioxidant enzymes and Th cell differentiation. The more detailed mechanism by which intracellular and mitochondrial ROS modulates the expression of transcription factor T-bet and RORγt remain to be elucidated.

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Author Disclosure Statement

No potential conflicts of interest were disclosed.

References

1. Al–Muhsen S and Casanova JL. The genetic heterogeneity of mendelian susceptibility to mycobacterial diseases. *J Allergy Clin Immunol* 122: 1043–1051, 2008.
2. Annunziato F, Cosmi L, Liotta F, Maggi E, and Romagnani S. Type 17 T helper cells. Origins, features and possible roles in rheumatic disease. *Nat Rev Rheumatol* 5: 325–331, 2009.
3. Beck MA, Esworthy RS, Ho YS, and Chu FF. Glutathione peroxidase protects mice from viral-induced myocarditis. *FASEB J* 12: 1143–1149, 1998.
4. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, and Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441: 235–238, 2006.
5. Corradi M, Folesani G, Andreoli R, Manini P, Bodini A, Piacentini G, Carraro S, Zanconato S, and Baraldi E. Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation. *Am J Respir Crit Care Med* 167: 395–399, 2003.
6. Devadas S, Zaritskaya L, Rhee SG, Oberley L, and Williams MS. Discrete generation of superoxide and hydrogen peroxide by T cell receptor stimulation: selective regulation of mitogen-activated protein kinase activation and fas ligand expression. *J Exp Med* 195: 59–70, 2002.
7. Dinakar C. Exhaled nitric oxide in the clinical management of asthma. *Curr Allergy Asthma Rep* 4: 454–459, 2004.
8. Dunstan JA, Breckler L, Hale J, Lehmann H, Franklin P, Lyons G, Ching SY, Mori TA, Barden A, and Prescott SL. Associations between antioxidant status, markers of oxidative stress and immune responses in allergic adults. *Clin Exp Allergy* 36: 993–1000, 2006.
9. Fidelus RK, Ginouves P, Lawrence D, and Tsan MF. Modulation of intracellular glutathione concentrations alters lymphocyte activation and proliferation. *Exp Cell Res* 170: 269–275, 1987.
10. Furling D, Ghribi O, Lahsaini A, Mirault ME, and Massicotte G. Impairment of synaptic transmission by transient hypoxia in hippocampal slices: Improved recovery in glutathione peroxidase transgenic mice. *Proc Natl Acad Sci USA* 97: 4351–4356, 2000.
11. Galasso G, Schiekofer S, Sato K, Shibata R, Handy DE, Ouchi N, Leopold JA, Loscalzo J, and Walsh K. Impaired angiogenesis in glutathione peroxidase-1-deficient mice is associated with endothelial progenitor cell dysfunction. *Circ Res* 98: 254–261, 2006.
12. Glimcher LH. Lineage commitment in lymphocytes: Controlling the immune response. *J Clin Invest* 108: s25–s30, 2001.
13. Harrington LE, Mangan PR, and Weaver CT. Expanding the effector CD4 T-cell repertoire: The Th17 lineage. *Curr Opin Immunol* 18: 349–356, 2006.
14. Hildeman DA, Mitchell T, Teague TK, Henson P, Day BJ, Kappler J, and Marrack PC. Reactive oxygen species regulate activation-induced T cell apoptosis. *Immunity* 10: 735–744, 1999.
15. Ho YS, Magnenat JL, Bronson RT, Cao J, Gargano M, Sugawara M, and Funk CD. Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. *J Biol Chem* 272: 16644–16651, 1997.
16. Hoffmann PR, Jourdan–Le Saux C, Hoffmann FW, Chang PS, Bollt O, He Q, Tam EK, and Berry MJ. A role for dietary selenium and selenoproteins in allergic airway inflammation. *J Immunol* 179: 3258–3267, 2007.
17. Inoue K, Takano H, Koike E, Warabi E, Yanagawa T, Yanagisawa R, and Ishii T. Peroxiredoxin I is a negative regulator of Th2-dominant allergic asthma. *Int Immunopharmacol* 9: 1281–1288, 2009.
18. Ishibashi N, Weisbrot–Lefkowitz M, Reuhl K, Inouye M, and Mirochnitchenko O. Modulation of chemokine expression during ischemia/reperfusion in transgenic mice overproducing human glutathione peroxidases. *J Immunol* 163: 5666–5677, 1999.
19. Iwanami K, Matsumoto I, Tanaka–Watanabe Y, Inoue A, Mihara M, Ohsugi Y, Mamura M, Goto D, Ito S, Tsutsumi A, Kishimoto T, and Sumida T. Crucial role of the interleukin-6/interleukin-17 cytokine axis in the induction of arthritis by glucose-6-phosphate isomerase. *Arthritis Rheum* 58: 754–763, 2008.
20. Jackson SH, Devadas S, Kwon J, Pinto LA, and Williams MS. T cells express a phagocyte-type NADPH oxidase that is activated after T cell receptor stimulation. *Nat Immunol* 5: 818–827, 2004.
21. Jaeschke H, Ho YS, Fisher MA, Lawson JA, and Farhood A. Glutathione peroxidase-deficient mice are more susceptible to neutrophil-mediated hepatic parenchymal cell injury during endotoxemia: Importance of an intracellular oxidant stress. *Hepatology* 29: 443–450, 1999.
22. Janssen–Heininger YM, Mossman BT, Heintz NH, Forman HJ, Kalyanaraman B, Finkel T, Stamler JS, Rhee SG, and van der Vliet A. Redox-based regulation of signal transduction: Principles, pitfalls, and promises. *Free Radic Biol Med* 45: 1–17, 2008.
23. Kebir H, Kreymborg K, Ifergan I, Dodelet–Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, and Prat A. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med* 13: 1173–1175, 2007.
24. Kelly C, Ward C, Stenton CS, Bird G, Hendrick DJ, and Walters EH. Number and activity of inflammatory cells in bronchoalveolar lavage fluid in asthma and their relation to airway responsiveness. *Thorax* 43: 684–692, 1988.
25. Kobayashi T, Okamoto S, Hisamatsu T, Kamada N, Chinen H, Saito R, Kitazume MT, Nakazawa A, Sugita A, Koganei K, Isobe K, and Hibi T. IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. *Gut* 57: 1682–1689, 2008.

26. Kondo N, Ishii Y, Son A, Sakakura–Nishiyama J, Kwon YW, Tanito M, Nishinaka Y, Matsuo Y, Nakayama T, Taniguchi M, and Yodoi J. Cysteine-dependent immune regulation by TRX and MIF/GIF family proteins. *Immunol Lett* 92: 143–147, 2004.
27. Kwon J, Devadas S, and Williams MS. T cell receptor-stimulated generation of hydrogen peroxide inhibits MEK-ERK activation and lck serine phosphorylation. *Free Radic Biol Med* 35: 406–417, 2003.
28. Lee YK, Mukasa R, Hatton RD, and Weaver CT. Developmental plasticity of Th17 and Treg cells. *Curr Opin Immunol* 21: 274–280, 2009.
29. Li L, Shoji W, Oshima H, Obinata M, Fukumoto M, and Kanno N. Crucial role of peroxiredoxin III in placental antioxidant defense of mice. *FEBS Lett* 582: 2431–2434, 2008.
30. Liu J, Hinkhouse MM, Sun W, Weydert CJ, Ritchie JM, Oberley LW, and Cullen JJ. Redox regulation of pancreatic cancer cell growth: Role of glutathione peroxidase in the suppression of the malignant phenotype. *Hum Gene Ther* 15: 239–250, 2004.
31. Los M, Schenk H, Hexel K, Baeuerle PA, Droge W, and Schulze–Osthoff K. IL-2 gene expression and NF-kappa B activation through CD28 requires reactive oxygen production by 5-lipoxygenase. *EMBO J* 14: 3731–3740, 1995.
32. Manevich Y, Sweitzer T, Pak JH, Feinstein SI, Muzykantov V, and Fisher AB. 1-Cys peroxiredoxin overexpression protects cells against phospholipid peroxidation-mediated membrane damage. *Proc Natl Acad Sci USA* 99: 11599–11604, 2002.
33. Mann H, McCoy MT, Subramaniam J, Van Remmen H, and Cadet JL. Overexpression of superoxide dismutase and catalase in immortalized neural cells: Toxic effects of hydrogen peroxide. *Brain Res* 770: 163–168, 1997.
34. Mirochnitchenko O, Palnitkar U, Philbert M, and Inouye M. Thermosensitive phenotype of transgenic mice overproducing human glutathione peroxidases. *Proc Natl Acad Sci USA* 92: 8120–8124, 1995.
35. Misso NL, Powers KA, Gillon RL, Stewart GA, and Thompson PJ. Reduced platelet glutathione peroxidase activity and serum selenium concentration in atopic asthmatic patients. *Clin Exp Allergy* 26: 838–847, 1996.
36. Moon EY, Noh YW, Han YH, Kim SU, Kim JM, Yu DY, and Lim JS. T lymphocytes and dendritic cells are activated by the deletion of peroxiredoxin II (Prx II) gene. *Immunol Lett* 102: 184–190, 2006.
37. Murata Y, Amao M, Yoneda J, and Hamuro J. Intracellular thiol redox status of macrophages directs the Th1 skewing in thioredoxin transgenic mice during aging. *Mol Immunol* 38: 747–757, 2002.
38. Murphy KM and Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2: 933–944, 2002.
39. Pene J, Chevalier S, Preisser L, Venereau E, Guilleux MH, Ghannam S, Moles JP, Danger Y, Ravon E, Lesaux S, Yssel H, and Gascan H. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J Immunol* 180: 7423–7430, 2008.
40. Rahman I. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J Biochem Mol Biol* 36: 95–109, 2003.
41. Robertson RP, Tanaka Y, Takahashi H, Tran PO, and Harmon JS. Prevention of oxidative stress by adenoviral overexpression of glutathione-related enzymes in pancreatic islets. *Ann NY Acad Sci* 1043: 513–520, 2005.
42. Sakuraba A, Sato T, Kamada N, Kitazume M, Sugita A, and Hibi T. Th1/Th17 immune response is induced by mesenteric lymph node dendritic cells in Crohn's disease. *Gastroenterology* 137: 1736–1745, 2009.
43. Seiderer J, Elben I, Diegelmann J, Glas J, Stallhofer J, Tillack C, Pfennig S, Jurgens M, Schmechel S, Konrad A, Goke B, Ochsenkuhn T, Muller–Myhsok B, Lohse P, and Brand S. Role of the novel Th17 cytokine IL-17F in inflammatory bowel disease (IBD): Upregulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p.His161Arg polymorphism in IBD. *Inflamm Bowel Dis* 14: 437–445, 2008.
44. Shen X, Zheng S, Metreveli NS,and Epstein PN. Protection of cardiac mitochondria by overexpression of MnSOD reduces diabetic cardiomyopathy. *Diabetes* 55: 798–805, 2006.
45. Shiomi T, Tsutsui H, Matsusaka H, Murakami K, Hayashidani S, Ikeuchi M, Wen J, Kubota T, Utsumi H, and Takeshita A. Overexpression of glutathione peroxidase prevents left ventricular remodeling and failure after myocardial infarction in mice. *Circulation* 109: 544–549, 2004.
46. Wakashin H, Hirose K, Maezawa Y, Kagami S, Suto A, Watanabe N, Saito Y, Hatano M, Tokuhisa T, Iwakura Y, Puccetti P, Iwamoto I, and Nakajima H. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 178: 1023–1032, 2008.
47. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, and Murphy KM. Th17: an effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 24: 677–688, 2006.
48. Weaver CT, Hatton RD, Mangan PR, and Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 25: 821–852, 2007.
49. Weisbrot-Lefkowitz M, Reuhl K, Perry B, Chan PH, Inouye M, and Mirochnitchenko O. Overexpression of human glutathione peroxidase protects transgenic mice against focal cerebral ischemia/reperfusion damage. *Brain Res Mol Brain Res* 53: 333–338, 1998.
50. Williams MS and Henkart PA. Role of reactive oxygen intermediates in TCR-induced death of T cell blasts and hybridomas. *J Immunol* 157: 2395–2402, 1996.
51. Williams MS and Kwon J. T cell receptor stimulation, reactive oxygen species, and cell signaling. *Free Radic Biol Med* 37: 1144–1151, 2004.
52. Yu ES, Min HJ, An SY, Won HY, Hong JH, and Hwang ES. Regulatory mechanisms of IL-2 and IFNgamma suppression by quercetin in T helper cells. *Biochem Pharmacol* 76: 70–78, 2008.
53. Zhou L, Chong MM, and Littman DR. Plasticity of CD4+ T cell lineage differentiation. *Immunity* 30: 646–655, 2009.

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Abbreviations Used

Ag = antigen
ANOVA = analysis of variance
BALF = bronchoalveolar lavage fluid
CFSE = carboxyfluorescein succinimidyl ester
CM-DCFDA = chloromethyl dichlorofluorescein diacetate
DPI = diphenyliodonium
ELISA = enzyme-linked immunosorbent assay
GPx1 = glutathione peroxidase 1
IFN = interferon
IL = interleukin
KO = knockout
NAC = *N*-acetyl-L-cysteine
OVA = ovalbumin
PAS = periodic acid-Schiff
PBS = phosphate-buffered saline
PCR = polymerase chain reaction
PMA = phorbol 12-myristate 13-acetate
Prx = peroxiredoxin
ROS = reactive oxygen species
RT = reverse transcription
SOD = superoxide dismutase
TCR = T cell receptor
TGF = transforming growth factor
Th = T helper
WT = wild-type

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2. Zhi Huang , Aaron H. Rose , Peter R. Hoffmann . 2012. The Role of Selenium in Inflammation and Immunity: From Molecular Mechanisms to Therapeutic Opportunities. *Antioxidants & Redox Signaling* **16**:7, 705-743. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
3. Robert L. Norton, Peter R. Hoffmann. 2011. Selenium and asthma. *Molecular Aspects of Medicine* . [CrossRef]
4. Phil-Dong Moon, Hyung-Mim Kim. 2011. Antiinflammatory Effects of Traditional Korean Medicine, JinPi-tang and Its Active Ingredient, Hesperidin in HaCaT cells. *Phytotherapy Research* n/a-n/a. [CrossRef]
5. Fernando Lopes, Fernanda M. Coelho, Vivian V. Costa, Érica L. M. Vieira, Lirlândia P. Sousa, Tarcília A. Silva, Leda Q. Vieira, Mauro M. Teixeira, Vanessa Pinho. 2011. Resolution of neutrophilic inflammation by H2O2 in antigen-induced arthritis. *Arthritis & Rheumatism* **63**:9, 2651-2660. [CrossRef]
6. Qing-qing Wang, Hong-zhen Yang, Han-zhi Liu, Su Mi, Xiao-wei Zhang, Hui-min Yan, Yong-gang Ma, Xiao-xing Wang, Zhuo-wei Hu. 2011. Interleukin-17A is involved in development of spontaneous pulmonary emphysema caused by Toll-like receptor 4 mutation. *Acta Pharmacologica Sinica* . [CrossRef]
7. K. Lee, H. Y. Won, M. A. Bae, J.-H. Hong, E. S. Hwang. 2011. Spontaneous and aging-dependent development of arthritis in NADPH oxidase 2 deficiency through altered differentiation of CD11b+ and Th/Treg cells. *Proceedings of the National Academy of Sciences* **108**:23, 9548-9553. [CrossRef]
8. Eun Sook Hwang . 2011. Response to Meyer et al.. *Antioxidants & Redox Signaling* **14**:1, 170-171. [Citation] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
9. Hellmuth A. Meyer , Anna Maria Dittrich , Eckard Hamelmann . 2011. Different Isoforms of Glutathione Peroxidase Cause Opposing Effects During the Development of Allergic Asthma in Mice. *Antioxidants & Redox Signaling* **14**:1, 169-170. [Citation] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
10. Hee Yeon Won, Eun Jung Jang, Hyun Jung Min, Eun Sook Hwang. 2011. Enhancement of Allergen-induced Airway Inflammation by NOX2 Deficiency. *Immune Network* **11**:3, 169. [CrossRef]