

Glutathione depletion inhibits dendritic cell maturation and delayed-type hypersensitivity: Implications for systemic disease and immunosenescence

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Background: Dendritic cells (DCs) play a key role as antigen-presenting cells in the immune system. There is growing evidence that the redox equilibrium of these cells influences their ability to induce T-cell activation and to regulate the polarity of the immune response. This could affect the outcome of the immune response during systemic diseases and aging.

Objective: Our aim was to elucidate the mechanism by which the redox equilibrium of antigen-presenting DCs affects the delayed-type hypersensitivity (DTH) response during experimental modification of glutathione levels, as well as during aging.

Methods: We looked at the effect of glutathione depletion by diethyl maleate in DCs as well as during systemic administration on the DTH response to the contact-sensitizing antigens, oxazolone, and 2,4-dinitro-1-fluorobenzene. We also determined whether glutathione repletion with N-acetyl cysteine could influence the decline of the DTH response in aged mice.

Results: Glutathione depletion in bone marrow-derived DCs interfered in their ability to mount a DTH response on adoptive transfer into recipient mice. Glutathione depletion interfered in IL-12 production and costimulatory receptor expression in DCs, leading to decreased IFN- γ production in the skin of recipient mice. Systemic diethyl maleate treatment exerted similar effects on the DTH response and IFN- γ production, whereas N-acetyl cysteine administration reversed the decline of the DTH response in aged animals.

Conclusion: Glutathione depletion downregulates T_H1 immunity through a perturbation of DC maturation and IL-12 production.

Clinical implications: These data show that the induction of oxidative stress in the immune system, under disease conditions

and aging, interferes in T_H1 immunity. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Dendritic cells, T_H1, glutathione, delayed-type hypersensitivity, skin dermatitis, aging, antigen-presenting cells, redox

Oxidative stress is a state of redox disequilibrium that is defined as a depletion of intracellular glutathione and an accumulation of glutathione disulfide. A decline of the cellular glutathione/glutathione disulfide ratio initiates a series of biological responses that are relevant to normal physiology as well as the pathophysiology of disease. According to the hierarchical oxidative stress hypothesis, low levels of oxidative stress initiate cellular antioxidant responses through nuclear factor-erythroid 2 (NF-E2)-related factor 2 (Nrf2)-mediated phase II enzyme (eg, catalase and superoxide dismutase) expression.¹ At higher levels of oxidative stress, activation of the mitogen-activated protein kinase and NF- κ B signaling cascades culminate in proinflammatory effects, whereas at the highest level of oxidative stress, interference in mitochondrial function leads to ATP depletion and programmed cell death.¹ This dynamic interplay between protective and injurious oxidative stress responses could modulate the immune response in asthma, AIDS, diabetes mellitus, contact dermatitis, and cancer.²⁻⁶ Moreover, several of the biological effects of aging have been ascribed to altered redox equilibrium and generation of excessive reactive oxygen species (ROSs).⁶

Oxidative stress is of key importance in the effector as well as the induction phases of the immune response.⁵⁻⁷ It is well known that immune activation generates inflammatory states in which ROS production by immune cells contributes to protective as well as tissue damaging effects. Less appreciated is that ROS and oxidative stress also regulate the induction of the immune response by controlling signal transduction pathways in antigen-presenting cells (APCs) and lymphocytes.⁸ ROSs not only regulate T-cell activation but also interfere in the polarity of the immune response as evidenced by the finding that oxidative stress favors T_H2 skewing of the immune response while suppressing T_H1 differentiation.^{8,9} Accordingly, glutathione depletion *in vivo* results in lowered T_H1 but increased T_H2 responses, whereas glutathione repletion has the

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Supported by US Public Health Science funds from the National Institute of Aging, RO1 AG14992, and the National Institute of Allergy and Infectious Diseases, PO1 AI50495.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication August 22, 2006; revised November 13, 2006; accepted for publication January 5, 2007.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2007.01.016

Abbreviations used

APC:	Antigen-presenting cell
BM-DC:	Bone marrow–derived dendritic cell
CON:	Vehicle-treated control
DC:	Dendritic cell
DEM:	Diethyl maleate
DEP:	Diesel exhaust particle
DNBS:	2,4-Dinitrobenzene sulfonic acid
DNFB:	2,4-Dinitro-1-fluorobenzene
DTH:	Delayed-type hypersensitivity
FITC:	Fluorescein isothiocyanate
MBB:	Monobromobimane
MLR:	Mixed-leukocyte reaction
NAC:	N-acetyl cysteine
OXA:	Oxazolone
PE:	Phycoerythrin
ROS:	Reactive oxygen species
Treg:	Regulatory T

opposite effect.⁸ The elucidation of the mechanisms by which oxidative stress changes APC function is of key importance in studying the pathophysiology of disease as well as understanding immune senescence.⁸⁻¹⁰

Although under disease conditions it is difficult to regulate the level of oxidative stress independent from pathological events, it is experimentally possible to obtain glutathione depletion with the thiol-alkylating agent, diethyl maleate (DEM).¹¹ We used this agent to treat dendritic cells (DCs) *ex vivo* as well as deplete glutathione levels in mice to study the effects of oxidative stress on the delayed-type hypersensitivity (DTH) response and cytokine profiles in the skin. Adjustable levels of oxidative stress could be achieved in DCs as well as the lymph nodes and spleens of DEM-treated animals. Glutathione depletion interferes in the expression of costimulatory receptors and IL-12 production in bone marrow–derived DCs (BM-DCs), making these cells less capable of supporting a DTH response and T_H1 immunity after adoptive transfer *in vivo*. DEM also interfered in DTH responses in intact animals, whereas the glutathione precursor, N-acetyl cysteine (NAC), reversed the decline in the DTH response in aged mice.

METHODS

Mice

Young (2–4 months) and old (19–21 months) female C57BL/6 (B6) mice were obtained from the Jackson Laboratory. The UCLA Animal Research Committee approved all the experiments.

Reagents

The reagents are listed in this article's Supplementary [Methods](#) text in the Online Repository at www.jacionline.org.

Single cell preparation from spleen and lymph node tissues

Spleen and lymph nodes were obtained and processed as described in this article's Supplementary [Methods](#) text in the Online Repository at www.jacionline.org.

Monobromobimane staining to assess intracellular thiol content

Intracellular thiol levels were assessed by using monobromobimane (MBB) staining as previously described by us.¹² MBB was added to cell suspension at a final concentration of 40 μ M/L and incubated for 10 minutes at room temperature, followed by conducting flow cytometry. See this article's Supplementary [Methods](#) text in the Online Repository at www.jacionline.org for more detail.

Generation of BM-DCs

Bone marrow was removed from the femurs of mice and cultured in RPMI-1640 supplemented with 10% FCS, 1% penicillin/streptomycin, 1% glutamine, 55 μ M/L 2-mercaptoethanol, GM-CSF (40 ng/mL), and IL-4 (100 pg/mL).¹³ BM-DCs at days 6 to 8 were used for the experiment. See this article's Supplementary [Methods](#) text in the Online Repository at www.jacionline.org for more detail.

Surface staining and flow cytometry procedures

Cell staining was performed by using the following mAbs: anti-CD11c fluorescein isothiocyanate (FITC)/phycoerythrin (PE) plus anti-CD86 FITC, anti-CD80 PE, anti-CD54 FITC, or anti-CD40 PE, followed by analysis in the LSR flow cytometer (Becton Dickinson, Franklin Lakes, NJ). See this article's Supplementary [Methods](#) text in the Online Repository at www.jacionline.org for more detail.

DTH skin testing with contact sensitizing agents

Oxazolone (OXA) or 2,4-dinitro-1-fluorobenzene (DNFB) was used as a contact-sensitizing agent. OXA (3%) dissolved in 100% ethanol was applied on the shaved mouse abdomen on day 0. Control animals were exposed to vehicle alone. Six days after sensitization, mice were challenged on both sides of both ears by epicutaneous application of 20 μ L of a 1% OXA solution.¹⁴ DNFB sensitization was accomplished by the application of 0.5% DNFB dissolved in 4:1 acetone/olive oil onto the shaved abdomen (days 0 and 1). On day 5, mice were challenged by epicutaneous application of DNFB on both ears.¹⁴ Ear thickness was measured before and 24 and 48 hours after challenge by using a dial thickness gauge (Mitutoyo, Kawasaki, Japan). After mice were killed, ear tissue was collected for RNA isolation and immunohistochemistry. To test the effect of glutathione depletion/upregulation on the DTH response, mice were pretreated with 4.5 mmol/kg DEM or 320 mg/kg NAC intraperitoneally 1 hour before sensitization or challenge, as well as every other day.

Hematoxylin and eosin staining

See this article's Supplementary [Methods](#) text in the Online Repository at www.jacionline.org.

RNA isolation

Total RNA was extracted from the right ear using the RNeasy Mini Kit (Qiagen Inc, Valencia, Calif) as described in this article's Supplementary [Methods](#) text in the Online Repository at www.jacionline.org.

Real-time RT-PCR

The primers for *IFN- γ* , *IL-4*, forkhead/winged helix transcription factor 3 (*foxp3*), and β -actin and the procedure for assessing mRNA expression in ear tissue are described in this article's Supplementary [Methods](#) text in the Online Repository at www.jacionline.org.

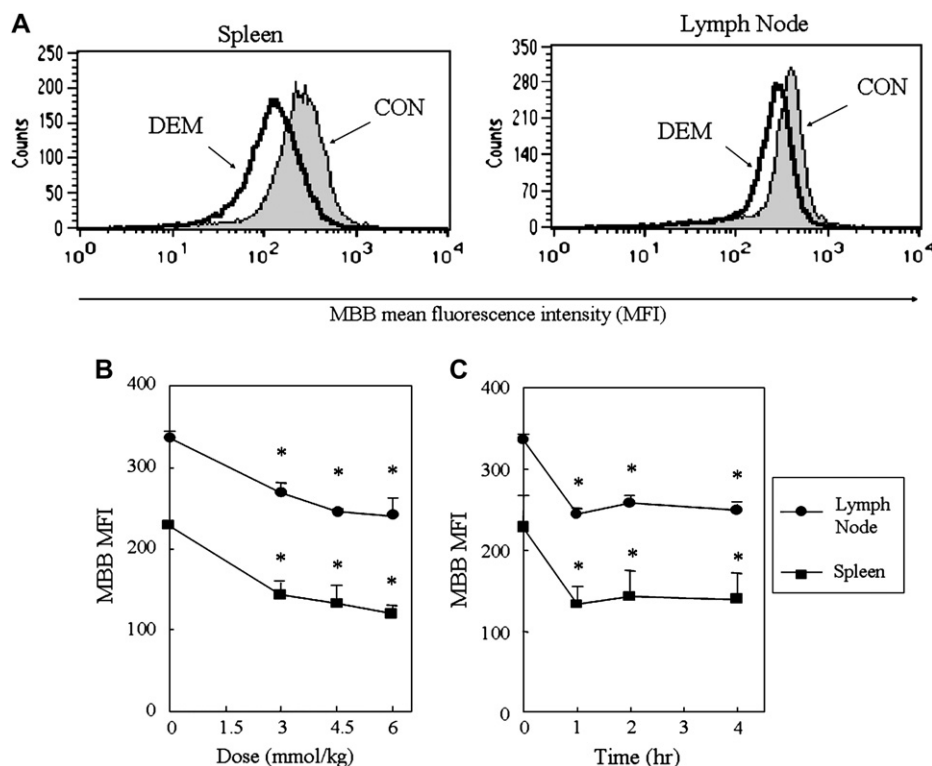


FIG 1. DEM depletes intracellular thiol levels *in vivo*. Mice were injected intraperitoneally with DEM, and cells were stained with MBB for flow cytometry. **A**, Representative histograms (4.5 mmol/kg DEM for 1 hour). **B**, Dose-dependence (1 hour treatment with DEM). **C**, Time-dependence (treatment with 4.5 mmol/kg DEM). Results are presented as means $\pm$ SDs ($n = 3$; $*P < .05$).

Establishing DTH by adoptive transfer

Delayed-type hypersensitivity was induced by *in vivo* inoculation of antigen-loaded DCs.¹⁵ Cultured BM-DCs were incubated with or without 1.6 mmol/L DEM for 15 minutes, then washed and resuspended in PBS containing 100 μ g/mL 2,4-dinitrobenzene sulfonic acid (DNBS) for 30 minutes. For sensitization (day 0), 0.5×10^6 DNBS-treated DCs (in 200 μ L saline) were injected subcutaneously into the flank of recipient mice. Five days later, mice were challenged by the application of 10 μ L 0.2% DNFB in 4:1 acetone/olive oil solution on both sides of the ear. Mice injected with the same number of unmodified DCs or mock-treated and challenged with vehicle alone served as negative controls. Ear thickness was measured as described.

ELISA

ELISA assays for assessing IL-12 p40 and p70, IL-6 and IL-10 levels are described in this article's Supplementary Methods text in the Online Repository at www.jacionline.org.

Mixed-leukocyte reactions

Mixed-leukocyte reaction (MLR) assay to assess the effect of glutathione depletion on T-cell function is described in this article's Supplementary Methods text in the Online Repository at www.jacionline.org.

Statistical analysis

Results were expressed as means $\pm$ SDs and analyzed by the Student *t* test. Differences of $P < .05$ were considered significant.

RESULTS

DEM depletes intracellular glutathione levels in lymphoid tissue obtained from intact mice

Diethyl maleate, the thiol-alkylating agent, was injected intraperitoneally in the amounts of 3, 4.5, and 6 mmol/kg body weight. Animals were killed 1 to 4 hours later, and the spleens and various lymph nodes were removed to assess intracellular thiol content by using MBB fluorescence (Fig 1). Systemic DEM administration induced a dose-dependent and statistically significant decline in MBB fluorescence in splenocytes and lymph node cells (Fig 1, B). This decline in the cellular thiol and glutathione content was sustained for at least 4 hours (Fig 1, C). Interestingly, the thiol content of splenocytes was consistently less than lymph node cells, which could reflect differences in their cellular composition. An intraperitoneal dose of 4.5 mmol/kg DEM, leading to 58% and 73% decreases in the thiol content of splenocytes and lymph node cells, respectively, was used in subsequent experiments. This dose caused a 68% decrease in thiol levels in the CD11c⁺ DC population *in vivo* without affecting cellular viability (Fig 2). This dose, delivered 1 hour before sensitization as well as every second day, did not exhibit noticeable changes in animal well being, allowing us to study the effects of oxidative stress on the immune system *in vivo*.

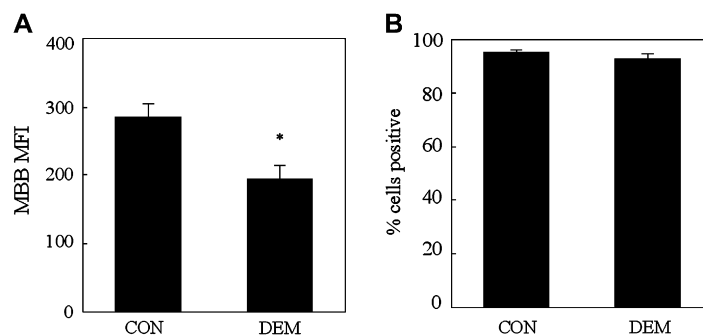


FIG 2. DEM depletes DC thiol levels *in vivo*. Mice were injected intraperitoneally with DEM (4.5 mmol/kg DEM for 1 hour), following which a splenic population was used to conduct flow cytometry. **A**, Mean fluorescence intensity (MFI) for MBB staining. **B**, % 7-amino-actinomycin D positive cells in CD11c-gated cells. Results are presented as means $\pm$ SDs ($n = 3$; * $P < .05$).

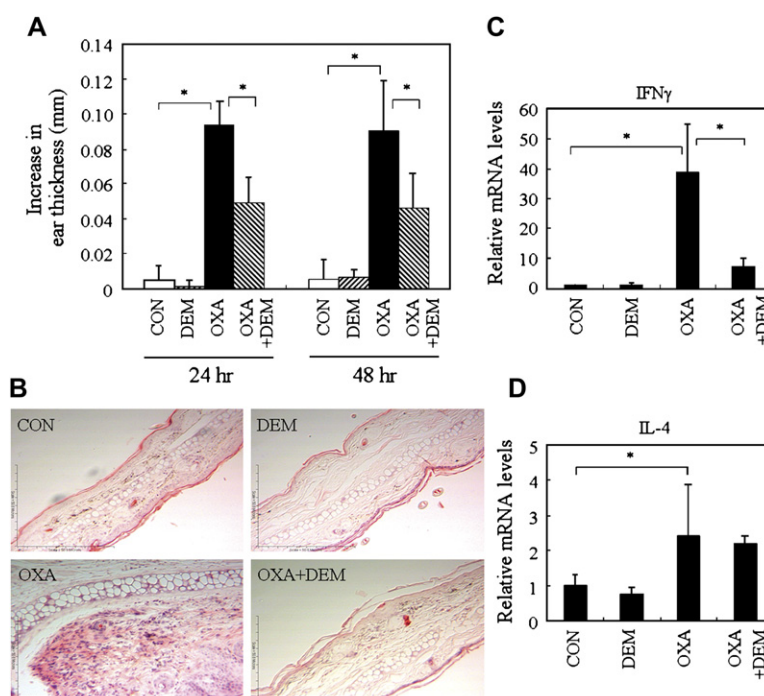


FIG 3. DEM suppresses the DTH response. **A**, Ear swelling response (means $\pm$ SDs). **B**, Hematoxylin and eosin staining of ear tissue. **C** and **D**, IFN- γ and IL-4 mRNA were measured by real-time PCR. Results represent the fold increase (means $\pm$ SDs) compared with the CON group. $n = 6$; * $P < .05$. CON, Vehicle-treated; DEM, DEM-treated; OXA, OXA-sensitized/challenged.

Glutathione depletion suppresses the DTH response as well as T_H1 differentiation

To investigate the effect of glutathione depletion on the DTH response, DEM was administered intraperitoneally to OXA-sensitized as well as DNFB-sensitized mice. As shown in Fig 3, A, there was a significant increase in ear thickness in OXA-sensitized/challenged (OXA) compared with vehicle-treated control (CON) mice. This increase in ear swelling was significantly reduced in the OXA-sensitized mice treated with DEM (OXA+DEM) 1 hour before sensitization and every second day. DEM treatment had no effect in nonsensitized animals. These response differences were maintained over

a period of 48 hours. A similar response pattern was observed when DNFB was used as sensitizing agent (see this article's Fig E1 in the Online Repository at www.jacionline.org).

Tissue sectioning and hematoxylin and eosin staining determined the histologic response in the ear. This showed that mononuclear cell infiltration and intercellular edema were more severe in OXA-sensitized than CON animals (Fig 3, B). Although DEM failed to exert an effect in CON animals, this agent noticeably suppressed the inflammatory infiltrate and intercellular edema in OXA+DEM mice. Similar results were obtained in the DNFB sensitization protocol (not shown).

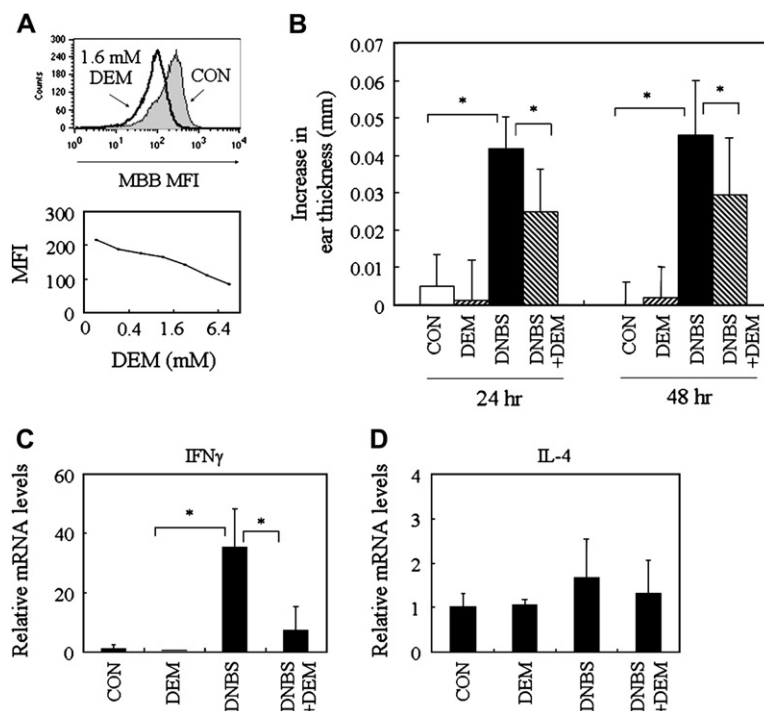


FIG 4. DC exposure to DEM suppresses the DTH response on adoptive transfer. **A**, Intracellular thiol depletion by DEM. **B**, DEM-treated DCs were exposed to DNBS *ex vivo* and adoptively transferred into mice that were challenged with DNFB. Ear swelling response. **C** and **D**, *IFN-γ* and *IL-4* mRNA measurements in the ear. $n = 6$; $*P < .05$. MFI, Mean fluorescence intensity.

To determine whether the induction of the DTH response is accompanied by skewed T-cell differentiation, *IFN-γ* and *IL-4* message levels were measured in the ear of OXA-sensitized mice. Quantitative RT-PCR showed that the OXA-induced expression of *IFN-γ*, a T_H1 cytokine, was significantly suppressed in DEM-treated animals (Fig 3, C). In contrast, the message level of a representative T_H2 cytokine, *IL-4*, was unaffected by DEM (Fig 3, D).

In addition to a change in the cytokine profile, immune regulation can also be achieved by elimination or inactivation of self-reactive lymphocytes. Regulatory T (Treg) cells contribute to the control of the ongoing immune responses and the induction of tolerance. Foxp3 functions as a transcription factor that plays a role in the development of Treg.¹⁶ As shown in this article's Fig E2 and discussed in the Supplementary Methods text in the Online Repository at www.jacionline.org, DEM treatment did not alter the *Foxp3* message expression in the ear, suggesting that glutathione depletion does not directly affect this Treg subset. These data demonstrate that glutathione depletion interferes in T_H1 differentiation along with a decline of the *in vivo* DTH response to contact-sensitizing chemicals.

Glutathione depletion in antigen-pulsed DC interferes in the DTH response on adoptive transfer

Antigen-presenting cells play a key role in determining the polarity of the immune response.^{8,9} To show whether

the redox equilibrium of DC is important in immune response, BM-DCs were generated by culture in GM-CSF and *IL-4*. These cells were treated with DEM for 15 minutes to show that this leads to a dose-dependent decline in MBB fluorescence (Fig 4, A). A dose of 1.6 mmol/L, leading to a 66% decline in the cellular thiol content without affecting cell viability, was used in subsequent studies to investigate the APC capabilities of DCs on adoptive transfer. For this experiment, DNBS was used as a water-soluble DNFB analogue that could be directly added to the tissue culture medium. BM-DCs were previously treated with 1.6 mmol/L DEM for 15 minutes before the addition of 100 μ g/mL DNBS. After washing, the DNBS-pulsed DCs were injected subcutaneously into naive C67BL/6 mice. Five days later, a DTH response was elicited in the recipient mice by DNFB application to the ear. This induced significant ear swelling in recipient mice that received DNBS-pulsed DCs (Fig 4, B). The swelling response was significantly decreased in mice in which the antigen-pulsed DCs were previously treated with DEM (DNBS+DEM). Injection of nonpulsed (CON) DCs did not mount a DTH response.

IFN-γ and *IL-4* mRNA levels were measured in the ear tissue. *IFN-γ* message levels were significantly lower in recipient mice injected with DEM-treated DCs (DNBS+DEM group) than animals receiving antigen-primed DCs alone (DNBS group; Fig 4, C). The *IL-4* message levels were not changed by DEM treatment (Fig 4, D). These data are in agreement with the decreased DTH

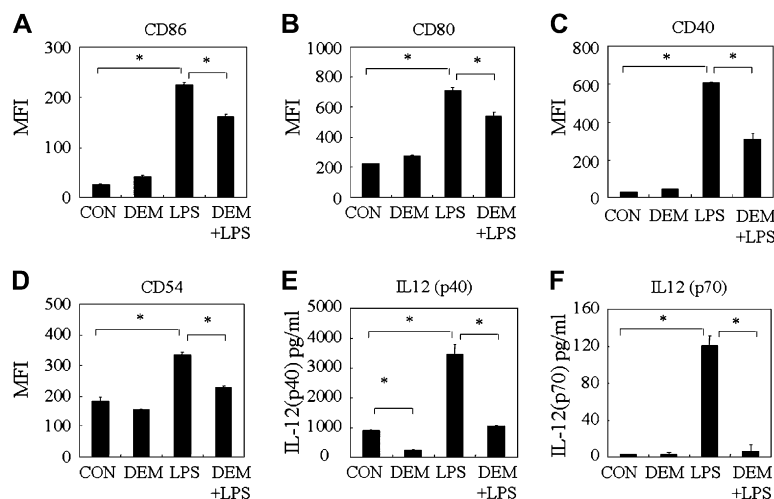


FIG 5. DEM suppresses DC maturation. BM-DCs were treated with 1.6 mmol/L DEM for 15 minutes, washed, and exposed to 5 ng/mL LPS for 24 hours. **A–D**, Flow cytometry was used to assess mean fluorescence intensity (MFI) for CD86, CD80, CD54, and CD40 in CD11c-gated cells. **E** and **F**, IL-12 levels were measured by ELISA in the supernatant. Results represent the means $\pm$ SDs ($n = 3$; $*P < .05$).

response in DEM-treated intact mice (Figs 3 and 4). This demonstrates that glutathione depletion at the APC level can achieve the same outcome as systemic depletion.

Glutathione depletion suppresses costimulatory receptors and IL-12 production in DCs

To determine how glutathione depletion may affect DC function, we used LPS as a potent T_H1 promoting stimulus that induces DC maturation and IL-12 production.¹⁷ We assessed the effect of DEM on LPS-induced expression of (1) CD86, CD80, and CD40; (2) intercellular adhesion molecule 1 (CD54); and (3) IL-12. As expected, LPS induced a significant increase in CD86, CD80, CD40, and CD54 expression in the CD11c⁺ cells compared with untreated controls (Fig 5, A–D). Previous treatment with DEM significantly impaired the expression of these receptors in LPS-treated cells. A similar response pattern was observed when TNF- α was used as the DC maturation stimulus (see this article's Table E1 in the Online Repository at www.jacionline.org). Quantitative analysis of IL-12 levels (p40 and p70) in the culture supernatant further confirmed that the LPS-induced response was suppressed by DEM (Fig 5, E and F). DEM also exerted a weaker but significant effect on IL-6 production but did not significantly suppress LPS-induced IL-10 production (see this article's Fig E3 in the Online Repository at www.jacionline.org). These results show that glutathione depletion affects LPS-induced DC maturation and IL-12 production.

Glutathione depletion reduces the ability of DCs to stimulate allogeneic MLR responses in T cells

A MLR was conducted to see whether DEM exerts an effect on T cells. BM-DCs from C57BL/6 mice were treated with DEM and exposed to allogeneic T cells from

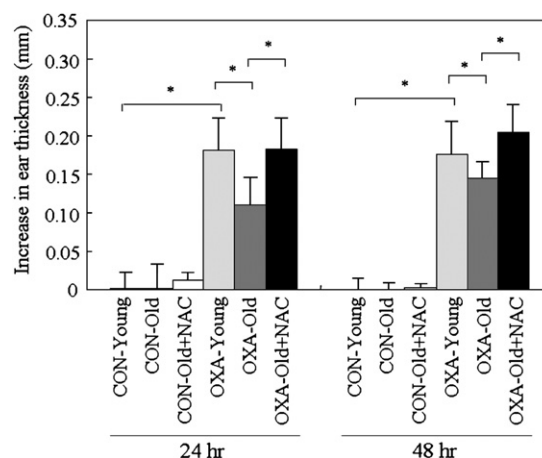


FIG 6. Effect of NAC treatment on the DTH skin response in old mice. The response of young mice to OXA was compared with old mice. In addition, old mice received intraperitoneal NAC before DTH sensitization/challenge. Ear swelling responses were measured 24 hours and 48 hours after challenge. Results represent the means $\pm$ SDs ($n = 6$; $*P < .05$).

Balb/c mice for 5 days. T-cell proliferation was measured by [³H]-thymidine uptake. DEM significantly decreased the ability of DCs to stimulate allogeneic T cells compared with untreated DCs (see this article's Fig E4 in the Online Repository at www.jacionline.org). In reciprocal fashion, MLR was also determined by using T cells exposed to DEM. This demonstrated that DEM-exposed T cells proliferate significantly less when cocultured with untreated allogeneic DCs. Moreover, the T-cell response was synergistically decreased if both DCs and T cells were treated with DEM. A similar response pattern was observed when BM-DCs from Balb/c mice were used together with T cells from C57BL/6 mice (data not shown). These results show that glutathione depletion at the

T-cell level could also contribute to reduced T_H1 immunity *in vivo*.

Aging reduces the DTH response, and this effect is ameliorated by thiol precursor, NAC

Aging is associated with phenotypic changes in the T-cell compartment and a decline in T_H1 immunity.^{12,18} This decline is partially a result of the tendency of cellular elements of the immune system to produce more ROS and develop changes in their redox equilibrium.¹² To investigate the effect of glutathione on the altered DTH response with aging, old mice were treated with a glutathione precursor, NAC. As shown in Fig 6, there was indeed a significant decrease in the ear swelling response of old compared with young OXA-sensitized and challenged mice. In old mice, this reduced response was significantly reversed by NAC administration (Fig 6). These data show that glutathione repletion is capable of reversing decreased T_H1 immunity with aging.

DISCUSSION

In this study, we explored the effects of manipulating systemic glutathione levels on cutaneous DTH responses, with particular emphasis on the effect on DC function, T_H1 immunity, and decline of the cellular immune response with aging. We demonstrate that *in vivo* glutathione depletion inhibits the DTH response to OXA and DNFB, and that this effect can be reproduced when antigen-pulsed DCs are treated *ex vivo* and then adoptively transferred to recipient animals. This study also demonstrates that glutathione depletion interferes in LPS-induced DC maturation and that a glutathione precursor, NAC, can reverse the decreased DTH responses during aging. Taken together, these data show that the state of redox equilibrium in the DCs exerts regulatory effects on the polarity of the immune response.

Various studies have shown that oxidative stress plays an important role in the immune system.^{5,7} Metabolically active cells in the immune system are dependent on ROS as a physiological regulator of immune function.⁷ This includes regulation of T-cell activation by the T-cell antigen receptor.⁷ Another cell type that is significantly affected by oxidative stress is the DC.^{9,13,17} Oxidative stress can change APC function, including their ability to interact with T cells, thereby affecting the polarity of the immune response.^{8,13,18} For instance, a recent study by Kuppner et al¹⁸ showed that glutathione depletion impaired the allostimulatory capacity of DCs, whereas this response could be restored by reconstituting glutathione levels in DCs. This could have an effect on disease progression in the setting of asthma, air pollution exposure, HIV infection, and chronic inflammatory disease processes.^{13,19} Moreover, the redox equilibrium of the immune system also determines the decline of cellular immune function with aging.^{12,20}

A number of DC pathways are affected by oxidative stress, including antigen uptake, processing, and presentation to T cells. Glutathione depletion and oxidative stress

have been shown to downregulate mannose receptor-mediated endocytosis²¹ and also modify the trafficking of endocytic vesicles through an effect on the small GTP-binding protein, Rab5.²² Another possibility is that oxidative stress could exert effects on DC maturation by affecting the signal transduction pathways that are involved in DC maturation and cytokine production. Our study shows that glutathione depletion inhibits LPS-induced CD80, CD86, and CD54 expression (Fig 5). DEM treatment had similar effects on TNF- α -induced responses (see this article's Table E1 in the Online Repository at www.jacionline.org). In addition to the effect on costimulatory receptors, glutathione depletion also interfered in LPS-induced IL-12 production (Fig 5). Because this cytokine is critical for T_H1 generation, this could explain the effect of glutathione depletion on the DTH response and accompanying decrease in IFN- γ production (Figs 3 and 4).

Our data differ from studies showing that ROSs are capable of inducing DC maturation.²¹ For instance, Kantengwa et al²¹ reported that superoxide induced the maturation of human DCs. Another study by Verhasselt et al²³ reported that NAC inhibited LPS-induced costimulatory molecule expression in human DCs as well as the MLR. Although the reason for these differences is unclear, the DC phenotype, their state of maturation, and method of stimulation could play a role in the *in vivo* outcomes. For example, Kantengwa et al²¹ showed that H₂O₂ did not upregulate CD86 in human DCs,²¹ but this contradicts the observation of Rutault et al,²⁴ who reported a 2.5-fold increase in CD86 expression in H₂O₂-treated human DCs. This discrepancy may be attributed to the different basal maturation states at the time DCs were exposed to H₂O₂.

In addition to a direct effect on DCs, ROS and oxidative stress also affect the downstream immune response, including T-cell differentiation and the polarity of the immune response. This is illustrated by the finding that glutathione augmentation *in vivo* favors T_H1 development, whereas thiol depletion shifts the immune response from T_H1 to T_H2 dominance.⁸ Evidence in DTH responses was shown by Schodde et al²⁵ that mice fed a liquid ethanol diet, which depletes glutathione, have impaired DTH responses. These effects could contribute to disease pathogenesis in a number of ways. One possible outcome is a change in the mucosal immune response to common environmental allergens in the lung.¹⁹ Diesel exhaust particles (DEPs) act as an adjuvant that enhances allergic inflammation.^{13,19} In this regard, we have demonstrated that pro-oxidative DEP chemicals exert an effect on DC function and IL-12 production.¹³ Moreover, we have demonstrated that pro-oxidative DEP chemicals are capable of interfering in the activation of NF- κ B cascade, which is responsible for the transcriptional activation of the CD80, CD86, CD54, and IL-12 promoters in DCs.²⁶⁻²⁸ One interpretation of these findings is that interference in IL-12 production could be responsible for decreased IFN- γ production and T_H1 immunity, thereby allowing unopposed T_H2 polarization and enhanced allergic inflammatory responses in the asthmatic airway.¹³ This notion is also

compatible with the demonstration that *in vivo* DEP exposure interferes in IFN- γ production in human beings and animals.^{19,29} The increase in the allergic response of clinical significance, and the accompanying decline in T_H1 immunity could interfere in the protective immune response. Thus, oxidative stress plays a key role in the decline of cellular immunity in HIV-infected individuals.³ In this setting, oxidative stress may affect the DC/APC as well as the T-cell function. The decline in cellular immunity with aging is another example of how a disturbance in redox equilibrium could affect immune function.²⁰

A potential weakness of this study is that DEM may affect a variety of cell types other than DCs. This could include an effect on Treg cells, which control immune responsivity and immune tolerance.¹⁶ To address this possibility, we measured the *Foxp3* message level in the ear and found that DEM does not interfere with its expression (see this article's Fig E2 in the Online Repository at www.jacionline.org). Another possibility is DEM effects on the effector T cells. This possibility is confirmed by our finding that glutathione depletion affects T-cell proliferation in the MLR assay (see this article's Fig E4 in the Online Repository at www.jacionline.org). It is clear, however, from the adoptive transfer experiments (Fig 4) that *ex vivo* treatment of the DCs does have an effect on the immune response *in vivo*. In light of the data in Fig 2 showing that DEM leads to thiol depletion of CD11c⁺ cells *in vivo*, it is reasonable to propose that the generation of oxidative stress at the DC level contributes to an *in vivo* decline of the immune response.

Our study demonstrates the importance of maintenance of redox equilibrium in maintaining T_H1 immunity responses during aging. Although undoubtedly oxidative stress and a perturbation of the redox equilibrium play a role in the decline of T-cell function during aging, increased ROS generation during aging could also affect DC function and APC activity. This could be of particular importance in frail elderly people in whom a change in APC activity could be a major factor in their immune decline.³⁰ The prospect of improving immune function by perturbing redox equilibrium in the immune system provides an attractive means of decreasing morbidity and mortality in old people. Using old mice, we clearly show that treatment with a glutathione precursor could reverse the decrease in the age-related DTH response (Fig 6). This is in keeping with previous observations that vitamin E supplementation can lead to an improvement in the immune response, including strengthening of the DTH response.²⁰ Another means by which antioxidant activity could be boosted in the immune system is through the activation of the Nrf2-mediated antioxidant defense pathway. This pathway mediates the expression of phase II enzymes that play a key role in sustaining DC and T-cell function under conditions of oxidant challenge and aging.^{12,13}

In summary, oxidative stress plays an important role in regulating immune function under physiological conditions but can also play a role in the pathophysiology of disease and immune senescence. Our findings confirm that maintenance of DC redox equilibrium is important for

regulating immune function, providing a possible avenue by which immune function can be therapeutically modified. The University of California, Los Angeles, Asthma and Allergic Disease Clinical Research Center is currently performing a pilot study to determine whether boosting of the phase II antioxidant responses can be used to intervene in the adjuvant effects of air pollutants in allergic disease.

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