Nrf2 activation by sulforaphane restores the age-related decrease of T_H1 immunity: Role of dendritic cells

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Background: The decrease in cellular immunity with aging is of considerable public health importance. Recent studies suggest that the redox equilibrium of dendritic cells (DCs) is a key factor in maintaining protective cellular immunity and that a disturbance of this homeostatic mechanism could contribute to immune senescence.

Objectives: We sought (1) to elucidate the role of DC redox equilibrium in the decrease of contact hypersensitivity (CHS) and T_H1 immunity during aging and (2) to determine how restoration of glutathione (GSH) levels by the Nrf2-mediated antioxidant defense pathway affects this decrease.

Methods: We assessed the effect of Nrf2 deficiency and boosting of GSH levels by the Nrf2 agonist sulforaphane or the thiol precursor N-acetyl cysteine (NAC) on the CHS response to contact antigens in old mice. We studied the effect of SFN and NAC on restoring T_H1 immunity by treating DCs *ex vivo* before adoptive transfer and *in vivo* challenge.

Results: Aging was associated with a decreased CHS response that was accentuated by Nrf2 deficiency. Systemic SFN treatment reversed this decrease through Nrf2-mediated antioxidant enzyme expression and GSH synthesis. Adoptive transfer of DCs from old animals induced a weakened CHS response in recipient animals. Treatment of DCs from old animals with SFN or NAC *ex vivo* restored the *in vivo* challenge response.

Conclusion: SFN and NAC upregulate T_H1 immunity in aging through a restoration of redox equilibrium. (J Allergy Clin Immunol 2008;121:1255-61.)

Key words: *Aging, redox equilibrium, cellular immunity, dendritic cells, Nrf2, glutathione, N-acetyl cysteine, sulforaphane*

Abbreviations used

APC:	Antigen-presenting cell
ARE:	Antioxidant response element
BM-DC:	Bone marrow–derived dendritic cell
CHS:	Contact hypersensitivity
DC:	Dendritic cell
DNBS:	2,4-Dinitrobenzene sulfonic acid
DNFB:	2,4-Dinitro-1-fluorobenzene
γ-GCL:	γ-Glutamylcysteine ligase
γ-GCLR:	γ-GCL regulatory subunit
GPx:	Glutathione peroxidase
GSH:	Glutathione
GSSG:	Oxidized GSH
LC:	Langerhans cell
MBB:	Monobromobimane
NAC:	N-acetyl cysteine
NQO1:	Reduced nicotinamide adenine dinucleotide phosphate–quinone oxidoreductase
OXA:	Oxazolone
p2E:	Phase II enzyme
ROS:	Reactive oxygen species
SFN:	Sulforaphane

Immune senescence is an important topic from the perspective of aging demographics and the associated increase in infectious disease episodes. Although functional changes in cellular immunity, such as a decrease of naive T cells, aberrant signal transduction by lymphocyte antigen receptors, and a change in cytokine profiles, have been described,[1] an overarching molecular hypothesis to explain these findings is lacking. Harman's original free radical theory suggested that aging could be attributed to the deleterious effects of reactive oxygen species (ROS).[2] Although it is known that ROS can damage structural cellular components and can induce a state of oxidative stress by means of glutathione (GSH) depletion, it is not intuitive how disrupting redox equilibrium could induce immune effects. We are beginning to understand, however, that oxidative stress is not just confined to oxidant injury, but we also have to consider antioxidant defense mechanisms that could determine whether ROS will induce oxidant injury. In fact, the coordinated antioxidant defense that is initiated by the Nrf2 pathway is the most sensitive oxidative stress response.[3] Our hierarchic oxidative stress hypothesis posits that lower levels of oxidative stress induce a protective and adaptive antioxidant defense that allows oxidant injury to become manifest only when this defense is overcome by high levels of ROS production.[4]

Nrf2 regulates the transcriptional activation of more than 200 antioxidant and protective genes that constitute the so-called phase II response. Examples of phase II enzymes (p2Es) include the rate-limiting enzyme in the GSH synthesis pathway, γ-glutamylcysteine ligase (γ-GCL), as well as glutathione peroxidase (GPx), heme oxygenase 1, superoxide dismutase, glutathione S-transferase, and reduced nicotinamide adenine dinucleotide phosphate–quinone oxidoreductase (NQO1).[3] We propose that the dynamic equilibrium between the Nrf2 pathway and injurious oxidant stress responses could determine the effect of aging in the immune system. This is compatible with the tendency toward

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a generalized decrease in GSH levels and γ-GCL expression with aging.[5] Aging also leads to a decrease in Nrf2 activity and p2E expression in parallel with increased markers of oxidative stress.[6] Although the exact reason for decreased Nrf2 activity is unknown, aging leads to decreased binding of this transcription factor to the antioxidant response element (ARE), which regulates the transcriptional activation of p2E gene promoters.[5] Moreover, the decrease in antioxidant activity is exaggerated during aging of female *nrf2* knockout mice.[7] In spite of this decrease in Nrf2 activity, it is noteworthy that p2E expression and GSH production in old rats is correctable by the Nrf2 agonist, α-lipoic acid.[5] Thus the fact that the Nrf2 pathway remains responsive in old animals could also be of benefit to elderly human subjects. This could include the use of even more potent agonists, such as the broccoli chemical sulforaphane (SFN).[8]

Nrf2 protects memory T cells from age-related oxidant injury, including protection against the decrease in mitochondrial function and phenotypic changes in the T-cell compartment with aging.[9] There is increasing evidence that Nrf2 also regulates the function of the innate immune system. Knockout of this gene leads to exaggerated cytokine production by innate cellular elements.[10] This includes our own demonstration that Nrf2 is important in regulating the antigen-presenting cell (APC) activity of dendritic cells (DCs). Thus exposure of myeloid DCs to exogenous oxidative stress stimuli (eg, pro-oxidative chemicals) has been shown to interfere in IL-12 production and T_H1 immunity.[11] There is also growing evidence that the opposite might be true, namely that boosting of GSH levels at the APC level might favor T_H1 skewing of the immune response.[12,13]

We hypothesized that Nrf2 plays a critical role in the decrease of T_H1 immunity and contact hypersensitivity (CHS) during aging. Moreover, we propose that this effect is, in part, explicable by the effect of the Nrf2 pathway on DC function. We assessed the effect of Nrf2 deficiency and boosting of GSH levels by SFN on the CHS response to contact-sensitizing chemicals in old mice. We also made use of adoptive transfer of antigen-pulsed DCs, which were treated *ex vivo* with SFN to test CHS responses *in vivo*. We demonstrate that Nrf2 activation by *in vivo* or *ex vivo* SFN administration reverses the decrease of T_H1 immunity in aged mice.

METHODS

Mice

Young (2-4 months) and old (19-22 months) female C57BL/6 (B6) mice were obtained from the Jackson Laboratory and the National Institute of Aging colony (Bethesda, Md), respectively. $Nrf2^{+/+}$ and $nrf2^{-/-}$ mice, which were initially obtained from Dr Y. Kan,[14] were backcrossed onto a C57BL/6 background for 7 generations.

Reagents

For more information, see the Online Repository at www.jacionline.org.

CHS testing with contact-sensitizing agents

Oxazalone (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 means of epicutaneous application of 20 μL of a 1% OXA solution.[15] 2,4-Dinitro-1-fluorobenzene (DNFB) sensitization was accomplished by the application of 0.5% of the chemical dissolved in 4:1 acetone/olive oil onto the shaved abdomen (days 0 and 1). On day 5, mice were challenged by means of epicutaneous application of 0.2% DNFB on both ears.[15] Ear thickness was measured before and 24 and 48 hours after challenge by using a dial thickness gauge (Mitutoyo, Japan). Mice were killed 48 hours after challenge, and ear tissues were removed for RNA extraction and cytokine message expression, as well as for hematoxylin and eosin staining.

SFN oral administration

SFN (9 μmol/d per mouse) in 0.2 mL of corn oil was administered by means of gavage on consecutive days. The control group received corn oil alone. Pretreatment with SFN or corn oil commenced 5 days before and was carried through until the performance of the antigen challenge (ie, 11 days total).

RNA isolation and real-time RT-PCR

For more information, see Table E1 in the Online Repository.

Generation of bone marrow–derived DCs

Bone marrow–derived DCs (BM-DCs) were prepared as previously described.[13] See the Online Repository for more details.

Surface staining, monobromobimane staining, and flow cytometry

Cells were surface stained with phycoerythrin-labeled anti-CD11c. Monobromobimane (MBB) was used to stain intracellular thiol, followed by conducting flow cytometry, as previously described.[13] See the Online Repository for more details.

Magnetic bead separation of $CD11c^+$ cells

Magnetic cell sorting was performed by using microbead-labeled anti-CD11c (Miltenyi Biotec, Bergisch Gladbach, Germany), as previously described.[9,11] See the Online Repository for more details.

Eliciting CHS responses by means of adoptive DC transfer

CHS was induced by *in vivo* inoculation of antigen-pulsed DCs.[13] Cultured BM-DCs were incubated with or without N-acetyl cysteine (NAC) (20 mM for 1 hour) or SFN (5 μM for 24 hours) and then washed and resuspended in PBS containing 100 μg/mL 2,4-dinitrobenzene sulfonic acid (DNBS) for 30 minutes. For sensitization (day 0), 0.5×10^6 DNBS-treated DCs were injected subcutaneously with 100 μL of saline into the flanks of recipient mice. Five days later, mice were challenged by means of DNFB application to the ear. Mice injected with the same number of unmodified DCs or mock treated and challenged with vehicle alone served as negative controls.

Hematoxylin and eosin staining

For more information, see the Online Repository.

Statistical analysis

Results were expressed as means ± SD and analyzed by using the Student *t* test. *P* values of less than .05 were considered significant.

RESULTS

SFN restores the age-related decrease in the CHS and T_H1 immunity

We have previously shown that aging leads to a decrease of the CHS to contact antigens placed on the skin.[13] Although a number of mechanisms might explain the increase in oxidant stress during