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Review

Dehydroepiandrosterone synergizes with antioxidant supplements for immune restoration in old as well as retrovirus-infected mice

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Production of the antioxidant hormone dehydroepiandrosterone (DHEA) declines as immunosenescence develops in the elderly. Very old C57BL/6 female mice, survivors after 71% had died due to aging, were evaluated after DHEA supplementation for 6 weeks. DHEA significantly increased their T-cell proliferation, restored secretion of Th1 cytokines [interleukin (IL)-2], decreased interferon-γ (IFN-γ) production, and normalized secretion of Th2 cytokine (IL-4 and IL-6) by lowered production. Survival was significantly increased in the 29-month-old mice treated by DHEA. Dehydroepiandrosterone sulfate (DHEAS), the storage form of DHEA, has lowered immune dysfunction caused by increased oxidation during LP-BM5 murine retrovirus infection. To assert the synergistic effect of DHEA + antioxidant nutrients, 17-month-old mice were fed with antioxidants or antioxidants + DHEAS for 16 weeks. DHEAS + antioxidants significantly increased B-cell proliferation and IL-2 secretion, and maintained Th2 cytokine secretion and hepatic vitamin E levels nearer to that of old, uninfected mice than antioxidant supplementation alone. Our study suggests that DHEA alone, and especially DHEAS plus antioxidant nutrients, can prevent immune dysfunction in very old and in old, retrovirus-infected mice. (J. Nutr. Biochem. 9:362–369, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Aging is associated with a global immune dysfunction that leads to an increased incidence of infection, cancer, and autoimmune disease. Age-related changes in immunity primarily involve defects in T-cell function, including a diminished proliferative response of T cells to mitogen and dysregulation of cytokine production. The capacity of activated lymphocytes to synthesize and secrete interleukin (IL)-2, IL-3, and granulocyte/monocyte colony-stimulating factor is significantly reduced in cells derived from aged animals and humans, whereas the capacity of these same cells to produce IL-4, IL-5, IL-6, IL-10, and interferon-γ (IFN-γ) is markedly increased.[1] Altered B-cell function also occurs with aging, as evidenced by a decreased ability to generate antibodies to antigens and decreased effectiveness of vaccines to confer immunity.[2]

Dehydroepiandrosterone (DHEA) is a derivative of androsterone, a weakly androgenic and estrogenic steroid that is synthesized primarily by the adrenal cortex. Dehydroepiandrosterone sulfate (DHEAS) is its storage form. Endogenous DHEA synthesis exhibits an age-related decline in all

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mammalian species.[3] DHEA plays an important role in development of immune responses by its ability to influence T-cell function.[3] Recently, DHEA and DHEAS supplementation of aging mice corrected the age-associated dysregulated production of T-cell lymphokines from various lymphoid organs.[3] In vitro studies have shown that DHEA exerts a stimulatory effect on IL-2 secretion and prevents the age-related increase in IL-6 production.[2] DHEA in vivo enhanced IL-2 production by activated murine T cells,[4,5] perhaps by its powerful antioxidant activity.[6]

Acquired immunodeficiency syndrome (AIDS) and aging involve excessive free radical and reactive oxygen species production, decreasing cellular antioxidants due to excessive consumption with cell damage.[7,8] Oxidation also activates the production of inflammatory cytokines by Th2 cells.[9] Thus, supplementation with antioxidant hormones DHEA or DHEAS should synergize with antioxidant nutrients in preventing immune dysfunction caused by oxidative stress in old animals. LP-BM5 murine retrovirus infection in mice also produces immune dysfunction, antioxidant nutrient deficiencies, and increased oxidation, much as human immunodeficiency virus (HIV) does in humans. Aging and murine retrovirus infection synergize to exacerbate loss of vitamin E. Because antioxidant vitamins and DHEA are very low during human and murine retrovirus infection and aging, their concurrent supplementation may be more effective than either alone. Therefore, supplementation with antioxidant nutrients and DHEA was assessed for immune modulation during oxidation and immune dysfunction produced by a LP-BM5 murine retrovirus in old mice.

Methods and materials

Animals

Study I: Very old mice + DHEA. For the study of very old mice, 16-month-old female C57BL/6 mice were obtained from Charles River Laboratories (Wilmington, DE USA). The 59 mice were housed in transparent plastic cages with stainless wire lids (four per cage) in the animal facility of the Arizona Health Science Center. Animals were cared for as required by the University of Arizona Committee on Animal Research. They were fed AIN 93 M synthetic mouse diet for 13 months until they were 29-months-old. By this time 71% of the mice had died. The housing facility was maintained at 20 to 22°C and 60 to 80% relative humidity, with a 12-hour light:dark cycle. Mice found to have tumors and grossly visible skin lesions were not used. The mice were randomly assigned to the control group (eight mice), which was fed the AIN 93 M synthetic mouse diet, or the supplemented group (nine mice), which was fed the AIN 93 M diet supplemented with 0.06% DHEA for 6 weeks. Surviving mice were sacrificed while under ether anesthesia. Spleens were then dissected, removed, and stored at 4°C.

Study II: Old mice + antioxidants. Data from non-DHEA treated mice, the controls in the present study, were published previously.[17] For the antioxidant nutrients + DHEAS study, 16-month-old female C57BL/6 mice were randomly assigned to one of the following groups: uninfected control mice fed the control AIN 93 M diet; uninfected mice fed the AIN 93 M diet nutrients supplemented antioxidant; retrovirus-infected mice fed the control diet; or infected mice fed antioxidant nutrients. Administration of diet supplemented with the diet antioxidant nutrients was begun 2 weeks after LP-BM5 infection. The 16-month-old mice were fed the AIN 93 M diet for 16 weeks. The control diet was AIN 93 M synthetic, pelleted diet (Dyets Inc., Bethlehem, PA USA). It was supplemented with placebo beadlets for the antioxidant nutrient study. The antioxidant nutrients supplemented diet was the AIN 93 M synthetic, pelleted diet with beta-carotene beadlets (15 mg/g diet [Hoffmann-La Roche, Nutley, NJ USA]), bioflavanoids (300 μg/g diet), coenzyme Q10 (300 μg/g diet), d-alpha-tocopherol (tenfold increase over the control diet, 1.5 mg/g diet), L-ascorbic acid (300 μg/mg diet), L-carnitine (300 μg/g diet), magnesium (fivefold increase over control diet, 4.2 mg/g diet), N-acetylcysteine (300 μg/g diet), retinol (80 μg/g diet), selenium (1.8 μg/g diet), and zinc (289 μg/g diet). DHEAS powder was purchased from Sigma (St Louis, MO USA) and dissolved into the animal drinking water to the final concentration of 0.01%. Half of the mice were in each group (eight mice) fed DHEAS for 16 weeks. Both were given ab libitum. Treatment with the supplemented diet or water was for 16 weeks.

Retroviral infection

Infection of old female C57BL/6 mice with LP-BM5 MuLV leads to the rapid induction of clinical symptoms with virtually no latent phase.[10] The infection period was 18 weeks. LP-BM5 retrovirus was administered intraperitoneally to the 16-month-old mice in the infected groups in 0.1 mL of minimum essential medium with an esotropic titer (XC) of 4.5 $\log_{10}$ plaque forming units $\times 10^{-3}$/L. This induced disease with a time course comparable with that previously published.[10] When murine AIDS had developed, all mice in all groups were sacrificed the same week while under ether anesthesia. Spleens and lymph nodes were then dissected, removed, and stored at 4°C. Livers and hearts for nutritional analysis were collected and stored at −70°C until assayed.

ELISA for cytokines

The production of IL-2, IL-4, IL-6, tumor necrosis factor-α (TNF-α) and IFN-γ from mitogen-stimulated splenocytes was determined as described previously.[11] Briefly, spleens were gently teased with forceps in culture medium (CM, RPMI 1640 containing 10% fetal bovine serum, 2 mmol/L glutamine, 1×10^5 units/L of penicillin and streptomycin), producing suspension of spleen cells. Red blood cells were lysed by the addition of a lysis buffer (0.16 mol/L ammonia chloride Tris buffer, pH 7.2) at 37°C for 3 minutes. Then the cells were washed twice with CM. Cell concentrations were counted and adjusted to 1×10^{10} units/L. Splenocyte viability was greater than 95% as determined by trypan blue exclusion. Splenocytes 0.1 mL/well (1×10^{10} cells/L) were cultured in triplicate on 96-well flat-bottom culture plates (Falcon 3072, Lincoln Park, NJ USA) with CM. The splenocytes were then stimulated with concanavalin A (Con A, 1×10^{-2} g/L, 0.1 mL/well, Sigma) to determine their production of IL-2 after 24 hours incubation and IL-4 after 48 hours incubation in a 37°C, 5% CO_2 incubator. Splenocytes were also incubated for 24 hours after the addition of lipopolysaccharide (LPS, 1×10^{-2} g/L, Gibco, Grand Island, NY USA) to induce IL-6. After incubation, the plates were centrifuged for 10 minutes at 800 × g. Supernatants were collected and stored at −70°C until analysis. The cytokines were determined by sandwich ELISA as described previously.[11] Rat anti-murine IL-2, IL-4, IL-6, TNF-α, and IFN-γ purified antibodies, rat anti-murine IL-2, IL-4, IL-6, TNF-α, and IFN-γ biotinylated antibodies, and recombinant murine IL-2, IL-4, IL-6, TNF-α, and IFN-γ were obtained from Pharmingen (San Diego, CA USA).

Mitogenesis of splenocytes

Splenic T- and B-cell proliferation was determined by ^{3}H-thymidine incorporation as described previously.[11] Briefly, splenocytes in 0.1 mL of CM (1×10^7 cells/L) were cultured in 96-well flat-bottom cultured plates (Falcon) with Con A and LPS (10 μg/mL). They were incubated at 37°C, 5% CO_2 incubator for 20 hours for Con A-induced T-cell proliferation and 44 hours for LPS-induced B-cell proliferation, and then pulsed with ^{3}H-thymidine (0.5 μCi/well, New England Nuclear, Boston, MA USA). After 4 hours, they were harvested by a cell sample harvester (Cambridge Technology, Cambridge, MA USA). Radioactivity was determined by a liquid scintillation counter (Tri-Carb, 2200 CA, Packard, Lagunahills, CA USA). Data were presented as counts per minute (cpm).

Determination of vitamin E

Vitamin E levels in liver were measured by high performance liquid chromatography (HPLC) as described previously.[12] Briefly, approximately 0.1 g of tissues was homogenized in 1 mL of water. Butylated hydroxytoluene was added to prevent oxidation of α-tocopherol. Pentane, ethanol, and sodium dodecyl sulfate were used to extract α-tocopherol from the homogenate. Extracts were evaporated under steady flow of nitrogen gas at 20°C and then redissolved in 0.5 mL of methanol with injection onto a C18 column (3.9 × 150 mm NovaPak, Millipore, Bedford, MA USA). A mobile phase composed of methanol:1 mol/L sodium acetate in the ratio of 98:2 (by volume) at a flow rate of 1.5 mL/min was used. α-Tocopherol, eluting at 6.5 minutes, was monitored by a fluorescence detector (Millipore) at 290-nm excitation and 320-nm emission wavelength.

Statistics

All variables were compared using a one-way analysis of variance (ANOVA), followed by a two-tailed Student's *t*-test for comparison between any two groups. Differences between two groups were considered significant at a *P*-value of less than 0.05.

Results

Study I: Treatment of immune dysfunction in very old mice with DHEA

Body weight and survival. Body and spleen weights were not significantly different between unsupplemented and DHEA supplemented very old mice. The 16-month-old mice were aged 13 months, with 71% dying gradually over that period. Supplementation was begun at 29 months of age. Survival of very old mice treated with DHEA was significantly ($P < 0.05$) higher than the untreated ones (*Figure 1*).

Mitogenesis of splenocytes. T-cell proliferation in spleen was significantly ($P < 0.05$) increased by DHEA supplementation in very old mice (*Figure 2*). No difference of B-cell proliferation between groups was found.

Cytokine production by splenocytes. In aging, production of the mitogen- or antigen-stimulated Th1 cell cytokine IL-2 is suppressed, whereas IFN-γ is increased.[1] In the present study, production of IL-2 was significantly ($P < 0.05$) increased in very old mice by DHEA supplementation

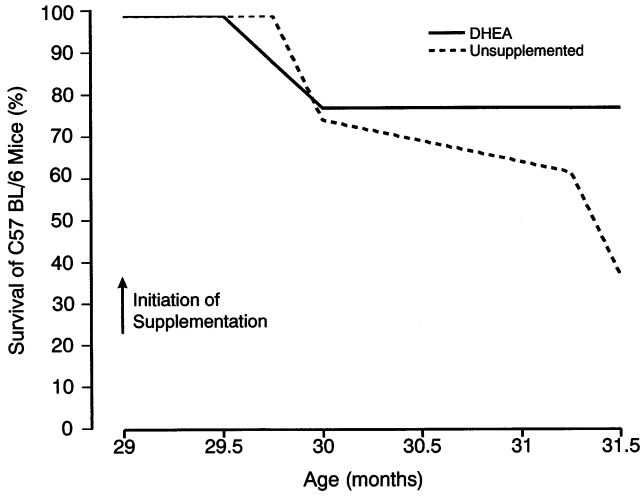

Figure 1 Dihydroepiandrosterone (DHEA) supplementation modification survival of very old mice. In the treated group, seven of nine mice survived, whereas in the control group only three of eight C57BL/6 female mice survived.

(*Figure 3*). DHEA supplementation significantly ($P < 0.05$) decreased production of IFN-γ in old very mice (*Figure 3*).

Th2 cells produce cytokines that stimulate the synthesis and secretion of antibodies. High levels suppress Th1 cells and thus cellular immunity to pathogens and cancers. Release of Th2 cytokines IL-4 and IL-6 by LPS-stimulated splenocytes tended to be lower in very old mice (*Figure 3*) treated with DHEA for 6 weeks, but not significantly so.

Study II: Immune function in old mice fed antioxidant nutrients and/or DHEAS

Body weight. There were no significant changes in food or water consumption due to infection (data not shown). Mice consuming the diet containing the antioxidant nutrients had an intake of 3.8 ± 0.28 g/mouse/day, while controls ate 3.62 ± 0.41 g/mouse/day. Mice also consumed 3.9 mL/mouse/day in water containing 0.01% of DHEAS and there

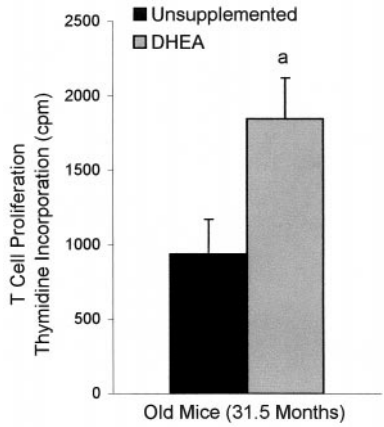

Figure 2 Supplementation of dihydroepiandrosterone (DHEA) on T-cell proliferation. Every sample from each very old mouse was determined in triplicate. Values are means ± SE for each group. *a* indicates significant differences at $P < 0.05$ compared with control mice.

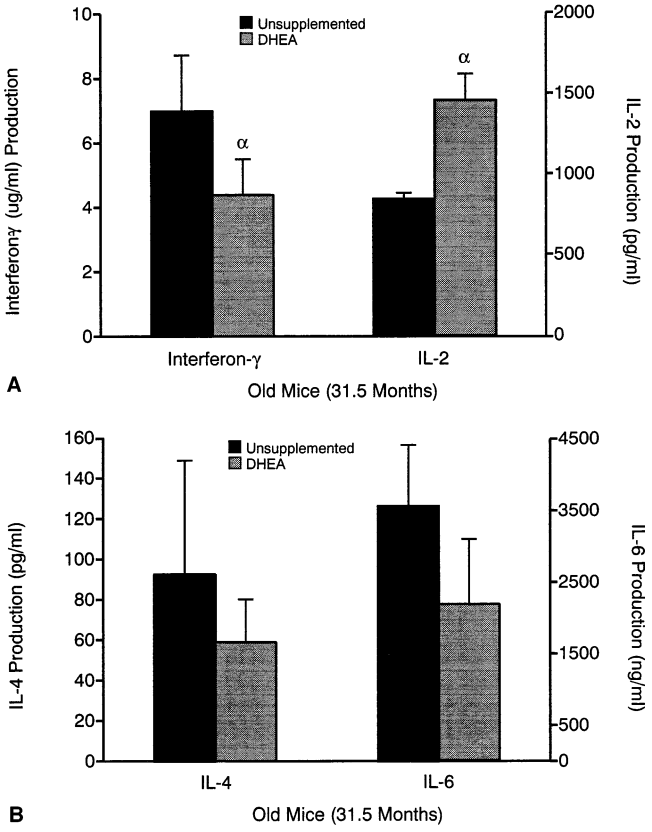

Figure 3 (*Figure 3A*) Supplementation of dihydroepiandrosterone (DHEA) on interferon-γ (INF-γ) and interleukin-2 (IL-2) production by splenocytes from *in vitro*. Every sample from each very old mouse was determined in triplicate. Values are means ± SE for each group. *a* indicates significant differences at $P < 0.05$ compared with control mice. (*Figure 3B*) Supplementation of DHEA on interleukin-4 (IL-4) and interleukin-6 (IL-6) production by splenocytes from in vitro. Every sample from each very old mouse was determined in triplicate. Values are means ± SE for each group.

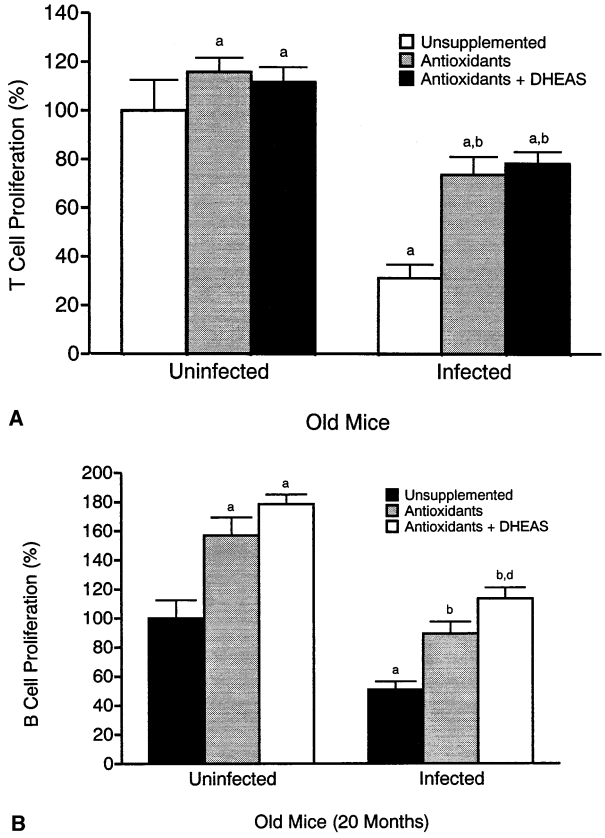

Figure 4 Supplementation of antioxidant nutrients + dihydroepiandrosterone sulfate (DHEAS) on T-cell proliferation (*Figure 4A*) and B-cell proliferation (*Figure 4B*). Supplementation of antioxidant nutrients + DHEAS modified T- and B-cell proliferation of 17-month-old mice by the time they were 20 months old. Every sample was determined in triplicate. Values are means ± SD for each group. Letters indicate significant differences at $P < 0.05$: *a*, compared with uninfected control mice; *b*, compared with retrovirus-infected control mice; *d*, compared with infected and antioxidant nutrient supplemented mice. 100% of Y axis is equal to 3915 cpm for T-cell proliferation and 4009 cpm for B-cell proliferation.

were no significant differences between feeding groups. Spleen and lymph node weights (18 week postinfection) were significantly ($P < 0.05$) elevated in infected mice (data not shown), indicating that the infection had progressed to murine AIDS.

Mitogenesis of splenocytes. Proliferation of Con A- and LPS-induced splenocytes in vitro was significantly ($P < 0.05$) decreased by murine retrovirus infection (*Figure 4*). This was significantly ($P < 0.05$) prevented by the antioxidant nutrients consumed at 16 weeks. Supplementation of retrovirus-infected mice increased T- and B-cell mitogenesis 137% and 76%, respectively. Antioxidant nutrient supplementation for 16 weeks also significantly ($P < 0.05$) increased proliferation of T and B cells from uninfected mice. These treatments partially restored mitogen-stimulated T-cell proliferation and maintained near normal B-cell proliferation in retrovirus-infected mice compared with uninfected, unsupplemented mice. Supplementation of infected mice with antioxidant nutrients plus DHEAS significantly ($P < 0.05$) increased T- and B-cell proliferation compared with infected, unsupplemented mice. However, combined treatment augmented only B-cell but not T-cell proliferation by 127% compared with cells from mice fed antioxidant nutrients only.

Cytokine production by splenocytes. In vitro production of the Th1 cell cytokine IL-2 is vital to stimulate T-cell proliferation and activity. IL-2 secretion by Con A-stimulated splenocytes was significantly ($P < 0.05$) inhibited in retrovirus-infected mice (*Figure 5*). Consumption of the antioxidant diet for 16 weeks significantly ($P < 0.05$) increased IL-2 release by mitogen-stimulated splenocytes from infected mice compared with those from infected, unsupplemented mice. IL-2 release in infected mice fed the antioxidant diet was 0.688 ± 0.04 ng/mL whereas those fed the control diet had 0.271 ± 0.012 ng/mL. Antioxidant + DHEAS significantly normalized IL-2 secretion compared with uninfected, unsupplemented mice and was more effective than antioxidants alone in infected mice.

Th2 cells produce cytokines that stimulate the synthesis and secretion of antibodies while their high levels suppress cytokine production by Th1 cells and cellular immunity. Release of Th2 cytokines, TNF-α, IL-4, and IL-6 by

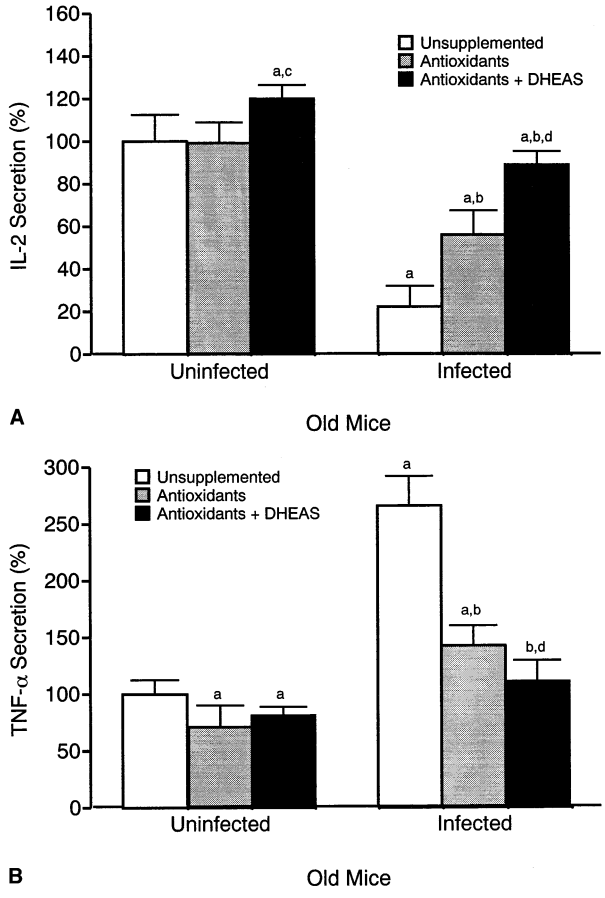

Figure 5 Supplementation of antioxidant nutrients + dihydroepiandrosterone sulfate (DHEAS) on interleukin-2 (IL-2; *Figure 5A*) and tumor necrosis factor-α (TNF-α; *Figure 5B*) production by splenocytes mitogen stimulated in vitro. Every sample from each old mouse was determined in triplicate. Values are means ± SD for each group. Letters indicate significant differences at $P < 0.05$: *a*, compared with uninfected control mice; *b*, compared with retrovirus-infected control mice; *c*, compared with uninfected and antioxidant nutrient supplemented mice; *d*, compared with infected and antioxidant nutrient supplemented mice. 100% of Y axis is equal to 1.223 ng/mL for IL-2 and 2.24 ng/mL for TNF-α.

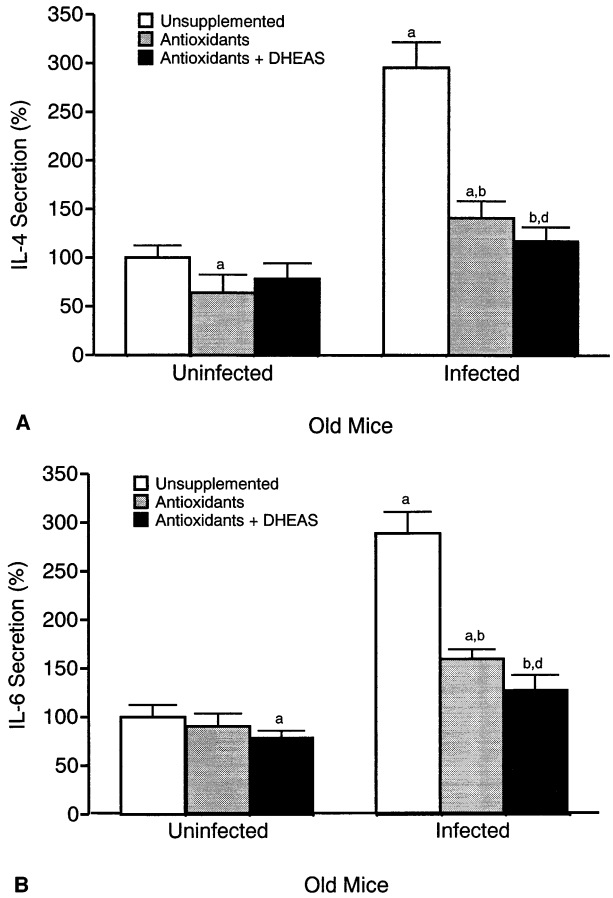

Figure 6 Supplementation of antioxidant nutrients + dihydroepiandrosterone sulfate (DHEAS) on interleukin-4 (IL-4; *Figure 6A*) and interleukin-6 (IL-6; *Figure 6B*) production by splenocytes in vitro. Every sample from each old mouse was determined in triplicate. Values are means ± SD for each group. Letters indicate significant differences at $P < 0.05$: *a*, compared with uninfected control mice; *b*, compared with retrovirus-infected control mice; *d*, compared with infected and antioxidant nutrient supplemented mice. 100% of Y axis is equal to 1.74 ng/mL for IL-4 and 1.599 ng/mL for IL-6.

LPS-stimulated spleen cells was significantly ($P < 0.05$) increased in the retrovirus-infected mice (*Figure 5* and *Figure 6*). Nutrient supplementation for 16 weeks lowered TNF-α, IL-4, and IL-6 release in infected mice. It was significantly ($P < 0.05$) lower than that of cells from infected, unsupplemented mice (*Figure 5* and *Figure 6*). However, antioxidant nutrient supplementation did not significantly affect IL-6 release in uninfected mice (*Figure 6*). Consumption of the antioxidant nutrient supplemented diets + DHEAS in infected mice significantly ($P < 0.05$) reduced all Th2 cytokine secretion, TNF-α, IL-4, and IL-6 to 77.4%, 83.2%, and 79.7%, respectively, compared with nutrient supplementation only. It also maintained Th2 cytokine production by cells from infected mice near that of cells from uninfected, unsupplemented mice.

Hepatic vitamin E. The liver is the major organ studied for tissue vitamin E deficiency in murine AIDS. The concentration of hepatic vitamin E was significantly ($P < 0.05$) reduced by retrovirus infection (*Figure 7*). Hepatic vitamin E levels of retrovirus in infected mice fed the antioxidant diet was 0.163 ± 0.013 μmol/g whereas those fed the control diet had 0.09 ± 0.009 μmol/g. Antioxidant supplementation for 16 weeks significantly ($P < 0.05$) retarded the loss of tissue vitamin E during infection. Uninfected mice that consumed antioxidants also had significantly ($P < 0.05$) increased hepatic vitamin E levels to 0.239 ± 0.016 μmol/g compared with the uninfected control group, which had 0.185 ± 0.013 μmol/g. Hepatic vitamin E levels in infected mice fed antioxidants + DHEAS 0.17 ± 0.014 μmol were essentially the same as uninfected control.

Discussion

Aging is associated with significant immune dysfunction, including a decline in both cell-mediated and humoral immune responses in animals and humans.[13–15] Many of the age-related changes involve defects in the function of T

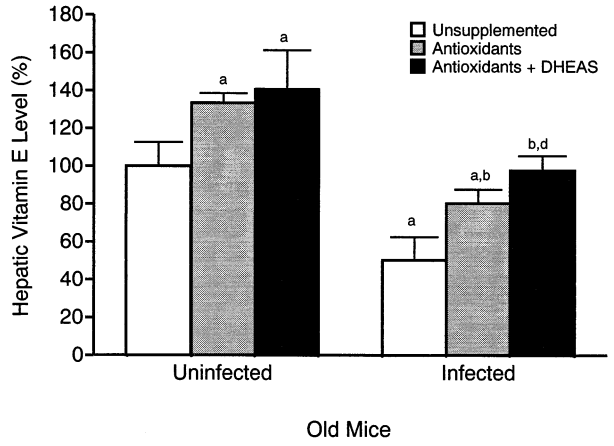

Figure 7 Effect of antioxidant nutrients + dihydroepiandrosterone sulfate (DHEAS) on hepatic vitamin E concentration. Values are means ± SE for each group. Letters indicate significant differences at $P < 0.05$: *a*, compared with uninfected control mice; *b*, compared with retrovirus-infected control mice; d, compared to infected and antioxidant nutrient supplemented mice. 100% of Y axis is equal to 0.185 μmol/mg.

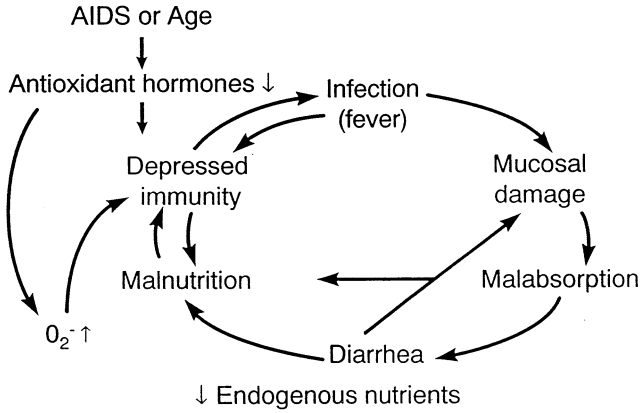

Figure 8 Schematic overview of acquired immunodeficiency syndrome's (AIDS) or age's mechanisms for accentuation of immune dysfunction. The process of AIDS or age decreases antioxidant hormones such as dihydroepiandrosterone or dihydroepiandrosterone sulfate and increases reactive free radicals, causing depressed immunity and cellular antioxidative nutrients. Immune dysfunction and malnutrition promote activation of human immunodeficiency virus or bacterial infection, resulting in mucosal damage, malabsorption, and diarrhea.

cells including diminished proliferative response and altered cytokine production. Immune dysfunction increases autoimmune diseases and death. Recently, DHEA and DHEAS were shown to have immune enhancing properties in retrovirus-infected old mice by reducing oxidation and cytokine dysregulation (*Figure 8*).[16] Our data demonstrate that short-term DHEAS supplementation significantly ($P < 0.05$) increased survival of very old mice, prevented the dysregulation of cytokines, and significantly ($P < 0.05$) restored T-cell proliferation in aging mice. This result is consistent with the finding of other investigations[5,11,21] as well as our recent studies using much younger mice.[17,18]

The production of IL-2 by Th1 cells decreases with age. However, production of autoantibodies and secretion of IL-4 and IL-6 by Th2 cells increases with age. IL-2 release by lymphocytes from elderly humans and animals was decreased as compared with that produced by young controls.[1,19,20] In vivo studies have shown the ability of DHEA to enhance IL-2 production by activated murine T cells.[5,11] Our recent studies[17,18] demonstrated that DHEA or DHEAS partially corrected the retrovirus infection or age-associated depressed production of IL-2. Our current study shows that DHEA significantly prevented age-associated suppression of IL-2 secretion in very old (31.5 months) mice. IL-2 is an important growth factor for T cells. Significant IL-2 release by cells from aging mice during DHEA treatment should facilitate optimum T-cell proliferation upon mitogen exposure. This cytokine effect help explain the increase in T-cell proliferation with DHEA supplementation.

An age-associated loss in the regulation of IL-6 production is vital to pathophysiology of the aging process.[21] IL-6 is a multifunctional cytokine that regulates the generation of the acute phase and inflammatory responses as well as B-cell proliferation and maturation. Production of high levels of IL-6 probably contributes significantly to the pathology of these diseases of aging, as well as to AIDS and its associated leukemia. Supplementation of DHEA may prevent the excessive secretion of IL-6 by Th2 cells in aging human and mice.[21] The results of our study are consistent with a previous one[21] that found that supplementation of DHEA reduced IL-6 secretion in aging mice, even though DHEA supplementation was for a short time (5 weeks). This is the first DHEA treatment in very old mice that significantly slowed their death rate.

Dysfunction in production of IFN-γ, a cytokine with highly pleiotropic activities, also may be a major contributing factor to the pathogenesis of aging.[13] Unstimulated lymphoid cells from aged mice spontaneously produce significant amounts of IFN-γ.[1] Splenic T cells from 26-month-old mice, when compared with published data from young controls, showed increased IFN-γ production. Increased production was accounted for by increased numbers of $CD4^+CD44^{high}$ and $CD8^+CD44^{high}$ T cells in aged mice.[13] IFN-γ can upregulate the expression of class II major histocompatability complex (MHC) molecules in numerous cells[22,23] associated with the pathogenesis of autoimmune diseases, rheumatoid arthritis, and multiple sclerosis.[24] Supplemental DHEAS treatment of 24-month-old mice significantly reduced IFN-γ production compared with the aging control mice.[1] Similarly IFN-γ production changes due to aging were also reduced by DHEA in our study.

Aging is associated with the pathology of many viral diseases.[9] With better treatments more AIDS patients are surviving in old age. With aging more virus is produced with CD4 T cells infected. HIV production is under the control of many proinflammatory cytokines such as IL-6 and TNF-α, which upregulate HIV production.[9] HIV preferentially infects memory T cells containing CD45RO, which is increased with aging,[25] suggesting that older persons are more susceptible to HIV infection. Recently, we asserted that DHEA prevented immune dysfunction caused

by LP-BM5 murine retrovirus infection.[17,18] Therefore uninfected and infected 17-month-old old mice were fed with DHEAS + antioxidants for 16 weeks. DHEAS is water soluble and present in humans at 500 to 1000 times the level of DHEA. DHEA and DHEAS are interconverted in peripheral and adrenal tissues. Antioxidants are lower in older people, so their supplementation may prevent the aging process or may retard HIV progression to AIDS due to blocking the free radical cascade.[26] Our data suggest that DHEAS + antioxidant nutrient supplementation was more effective in minimizing immune dysfunction in old retrovirus-infected mice than antioxidant supplementation alone. Because aging and AIDS cause similar immune dysfunction changes, their combined presence may synergize for more severe immune dysregulation. More people are surviving AIDS longer, so a greater number of older people will be infected and need immune therapy.

Supplementation with antioxidants during the whole course of infection significantly prevented retrovirus-induced suppression of immune responses. It maintained nearly normal cytokine production. This occurred simultaneously with restoration of tissue vitamin E and T- and B-cell proliferation. The combined treatment was more effective than antioxidant nutrient supplementation alone. DHEAS accentuated the effects of antioxidants and maintained cytokine production, T- and B-cell proliferation, and hepatic vitamin E close to the activity levels of uninfected old mice. The concentration of hepatic vitamin E were significantly ($P < 0.05$) reduced by retrovirus infection, whereas multiple antioxidant supplementation significantly ($P < 0.05$) maintained hepatic vitamin E levels near that of uninfected old mice.

In summary, our study provides the basis for future investigation on the use of antioxidants, nutrients, and DHEA for the treatment of immunosenescence in aging or AIDS. Supplementation DHEA, especially with antioxidant nutrients, significantly overcame the immune dysfunction by increasing IL-2, decreasing IL-4, IL-6, and IFN-γ and enhancing T-cell proliferation. Restoration of the immune system by DHEA occurred concomitantly with increased survival of very old mice. DHEAS synergized the effect of multiple antioxidants on preventing immune dysfunction in old mice including those further immunologically damaged by retrovirus infection. Our data suggest that DHEA supplementation is safe and well tolerated in very old mice. This study, which shows the synergistic effects of DHEAS + antioxidants, expands upon our data recently published using antioxidants.[17] Some of those data is, of necessity, repeated.

Acknowledgments

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