Activation of Immune Function by Dehydroepiandrosterone (DHEA) in Age-Advanced Men

Omid Khorram, Lan Vu, and Samuel S. C. Yen

Department of Reproductive Medicine, University of California, San Diego School of Medicine.

Background. Substantial data from animal studies have demonstrated a stimulatory effect of dehydroepiandrosterone (DHEA) on immune function. However, little is known about the effects of DHEA on the human immune system. Since aging is associated with a decline in immune function and in DHEA production, we proposed that oral administration of DHEA to elderly men would result in activation of their immune system.

Methods. Nine healthy age-advanced men (mean age of 63 years) with low DHEA-sulfate levels participated in this study. They were treated nightly with an oral placebo for 2 weeks followed by DHEA (50 mg) for 20 weeks. Fasting (0800h-0900h) blood samples were obtained at 4- to 8-week intervals for immune function studies and hormone determinations. Freshly isolated peripheral lymphocytes were used for flow cytometric identification of lymphocyte subsets, cells expressing the IL-2 receptor (IL-2R), mitogen stimulation studies, and for determining natural killer (NK) cell number and cytotoxicity. Levels of interleukin-2 (IL-2) and IL-6 secreted from cultured lymphocytes were determined under basal and mitogen stimulated conditions. Sera were analyzed for soluble IL-2 Receptor (sIL-2R) levels, insulin-like growth factor-1 (IGF-I) and IGF binding protein-1 (IGFBP-1) concentrations.

Results. Baseline levels of serum DHEA sulfate (DHEAS), a stable marker of circulating DHEA levels, were 2 standard deviations below young adult values and increased 3-4 fold within 2 weeks. These levels were sustained throughout the duration of DHEA administration. When compared with placebo, DHEA administration resulted in a 20% increase (p < .01) in serum IGF-I, a decreasing trend in IGFBP-1, and a 32% increase in the ratio of IGF-I/IGFBP-1 (p < .01). Activation of immune function occurred within 2-20 weeks of DHEA treatment. The number of monocytes increased significantly (p < .01) after 2 (45%) and 20 (35%) weeks of treatment. The population of B cells fluctuated with increases (p < .05) at 2 (35%) and 10 (29%) weeks of treatment. B cell mitogenic response increased 62% (p < .05) by 12 weeks unaccompanied by changes in serum IgG, IgA, and IgM levels. Total T cells and T cell subsets were unaltered. However, a 40% increase (p < .05) in T cell mitogenic response, 39% increase in cells expressing the IL-2R (CD25+) (p < .05), and 20% increase in serum sIL-2R levels (p < .01) were found at 12-20 weeks of DHEA treatment, suggesting a functional activation of T lymphocytes occurred. In vitro mitogen stimulated release of IL-2 and IL-6 was enhanced 50% (p < .05) and 30% (p < .01) respectively by 20 weeks of treatment without basal secretion being affected. NK cell number showed a 22-37% increase (p < .01) by 18-20 weeks of treatment with a concomitant 45% increase (p < .01) in cytotoxicity. There were no adverse effects noted with DHEA administration.

Conclusion. Administration of oral DHEA at a daily dose of 50 mg to age-advanced men with low serum DHEAS levels significantly activated immune function. The mechanism(s) to account for the immunoenhancing properties of DHEA are unclear. Consideration is given to the potential role of an increase in bioavailable IGF-1, which by virtue of its mitogenic effects on immune cell function, may mediate the DHEA effects. While extended studies are required, our findings suggest potential therapeutic benefits of DHEA in immunodeficient states.
sus, pharmacologic doses of oral DHEA prevented the formation of circulating auto-antibodies to double-stranded DNA in plasma, prolonged survival (15), and reversed an IL-2 deficient state (16). In vitro studies have shown that DHEA exerts a stimulatory effect on IL-2 secretion, inhibits NK cell differentiation (17), and prevents the age-related increase in IL-6 production (18). In an elegant series of in vivo experiments, Daynes and associates have demonstrated the ability of DHEA, but not DHEAS, to enhance IL-2 production by activated murine T cells (19,20). Arancho et al. found that treatment of burned mice with a single injection of 100 μg of DHEA sc restored their capacity to produce T cell derived lymphokines and to generate a cellular immune response (21). The same group also demonstrated that topical application of DHEA (10 μg) or sc injection (100 μg) of DHEAS in aging mice corrected the age-associated dysregulated production of T cell lymphokines from various lymphoid organs (22). When DHEA was incorporated into the hepatitis B vaccine, the combination produced an enhanced antibody response against hepatitis B surface antigen (22). DHEA has also been shown to increase IL-2 production and cytotoxic effector function in human T cells (23) and inhibit Epstein-Barr virus induced morphologic transformation and stimulation of DNA synthesis in human lymphocytes in vitro (24).

Human studies examining DHEA effects on immune function are limited. In a prospective randomized double-blind study, Casson et al. reported that daily treatment of postmenopausal women with oral DHEA (50 mg) for 3 weeks increased NK cell cytotoxicity, decreased the number of CD4 T helper cells, and inhibited T cell mitogenic response without affecting IL-6 or IL-2 secretion (25). In a Phase I open label study of patients with symptomatic HIV infection, mega oral doses of DHEA (750–2250 mg/day) for 16 weeks failed to induce improvement in immune parameters (26). The therapeutic potential of DHEA in systemic lupus erythematosus (SLE) was tested in an open label, non-controlled trial involving 10 women with mild to moderate disease; oral DHEA (200 mg daily dose) produced a significant symptomatic improvement and decreased their corticosteroid requirements (27). In another study, patients with SLE were found to have very low serum levels of DHEA and administration of DHEA restored the impaired IL-2 production of their T cells in vitro (28). In light of these findings and the lack of information on the effects of DHEA on immune function in age-advanced men we have conducted the following studies.

METHODS

Subjects

Nine healthy age-advanced men, nonsmokers, on no medications, with a mean age of 63.7 years (range 53–69) and body mass index of 26.7 kg/m² (range 22–30) were recruited for this study. Psychiatric and concurrent medical illness and major depression were excluded by history, physical examination, blood chemistry, complete blood count, and Beck depression tests. The protocol was approved by Human Subjects Committee of the University of California, San Diego.

Study Design and Protocol

The study design was a single-blind placebo-controlled trial of 22 weeks duration. Subjects took an oral placebo (50 mg) nightly for the first 2 weeks followed by oral DHEA (50 mg) for 20 weeks. Subjects were instructed not to change their dietary habits or exercise regimen.

Subjects were seen as outpatients at 4- and 8-week intervals. During each visit they filled out an open-ended questionnaire regarding self-reported changes and side effects. Fasting blood samples were drawn between 0800h–0900h for immune studies, hormone measurements, and standard complete blood and chemistry panels. Blood pressure and body weight were monitored at each visit.

Hormone Measurements

All hormone measurements for each individual subject were performed in duplicate in the same assay. DHEAS and cortisol were measured by a specific RIA previously described (29). IGF-I was measured by acid-ethanol extraction by a RIA kit (Nichols Institute, San Juan Capistrano, CA). IGFBP-1 was measured by a time-resolved immunofluorometric assay (30). Assay sensitivities were 0.6 μmol/L, 13.5 ng/ml, 0.1 μg/L and 20 nmol/L for DHEAS, IGF-I, IGFBP-1, and cortisol respectively. Inter- and intraassay coefficients of variation for DHEAS were 5% and 7%, IGF-I were 8% and 3%, IGFBP-1 were 8% and 6%, and cortisol were 6.4 and 5.4 respectively.

Immune Cell Studies

Lymphocytes were isolated from freshly drawn blood by Ficoll-Hypaque centrifugation (Sigma, St. Louis, MO) (31). Cells were then washed three times with PBS buffer, counted in a hemocytometer, and suspended in the appropriate concentration in RPMI 1640 containing 1% glutamine, 1% penicillin-streptomycin and 10% fetal calf serum.

Mitogen assays. — Lymphocytes were incubated at a concentration of 1 x 10⁶ cell/ml in the presence of PHA (Murex Diagnostics, Norcross, GA) at 0.1 and 2 μg/ml, and PWM (Gibco, Gaithersburg, MD) at 0.5 and 5 μg/ml, in 5% CO₂ at 37 °C for 6 days at which time 0.2 μCi of ³H-thymidine was added. The cells were harvested 18 hours later onto filters and counted in a liquid scintillation counter. Control wells contained only buffer. Results were expressed in terms of stimulation index determined as follows: cpm in mitogen containing wells/cpm in control wells.

Flow cytometry. — 2 x 10⁶ cells were incubated with monoclonal antibodies (Beckton-Dickinson, San Jose, CA) against various cell surface antigens listed in Table 1. After 20 minutes of incubation at 4 °C the cells were washed two times with PBS buffer and fixed with 2% paraformaldehyde. Cells were then analyzed in a FACS scan flow cytometer (Beckton-Dickinson Immunocytometry System, San Jose, CA), and data were analyzed by Lysys II software.

NK cell activity. — Target K-562 cells were incubated with 200 μCi of chromium-51 for 2 hours in 5% CO₂ at 37 °C, after which cells were washed and adjusted to 10⁶ cells per ml. Target cells were then incubated with lymphocytes...
isolated from the DHEA treated subjects at effector to target (E:T) ratios of 50:1 and 100:1 in 5% CO₂ at 37 °C for 4 hours. One hundred μl of the well contents was counted in a gamma counter. Target cells were incubated in culture medium with and without 3% SDS for measurement of spontaneous and maximum release. The specific lysis was determined as follows: (experimental release)−(spontaneous release)/(maximum release)−(spontaneous release).

Cytokine secretion and immunoglobulins. — 1 × 10⁶ cells/ml were incubated with and without PHA (20 μg/ml) for 48 hours in 5% CO₂ at 37 °C. After 48 hours the well contents were centrifuged at 600 g for 5 minutes and the supernatant stored at −70 °C for subsequent measurements of IL-2 and IL-6 by ELISA kits (Biosource, Camarillo, CA). The sensitivity of these assays was 8.7 pg/ml and 2 pg/ml, respectively. sIL-2R was measured by ELISA (Genzyme, Boston, MA) with a sensitivity of 100 pg/ml. Immunoglobulins were measured by radial immunodiffusion (The Binding Site, La Jolla, CA).

Statistics
The endocrine data were analyzed by paired Student’s t-test. All other data were analyzed by ANOVA with repeated measure. Post hoc testing was performed by the method of least square deviation. All time points following the 20 weeks of DHEA treatment were compared with baseline and placebo treatment values. Significance was established at p < .05.

RESULTS
Baseline DHEA levels including those from a 53-year-old subject were two standard deviations below the young adult range. DHEA administration restored DHEAS levels to young adult levels (32) within 2 weeks and sustained through the duration of the study (Table 2). Serum cortisol levels were unaltered by DHEA administration and thus the DHEAS/cortisol ratio increased 4-fold (Table 2). When compared to baseline or placebo, DHEA treatment was associated with a significant (p < .01) elevation in serum IGF-I levels and a decreasing trend in IGFBP-1 levels, resulting in a significant (p < .01) increase of the IGF-I/IGFBP-1 ratio (Figure 1). This finding confirmed our earlier studies and suggested an increase in bioavailability of IGF-I to target cells (33). The effect of ex vivo DHEA treatment on lymphocyte subsets is shown in Table 1. In response to DHEA treatment, the number of monocytes (CD14) increased at 2 and 20 weeks (p < .01). B cell numbers (CD20) showed a fluctuating pattern with a transient rise at 2 weeks (p < .01) and an increase by 20 weeks (p < .01). This increase was accompanied by functional activation, as evidenced by a dose-related increase in proliferative response to PWM at 12 weeks with further increases by 20 weeks (Figure 2). Serum IgG, IgM, and IgA levels were not affected over the course of the study (Table 3). Although DHEA treatment did not affect the low levels of basal IL-6 secretion, PHA-induced secretion of IL-6 increased significantly (p < .01) by 20 weeks (Figure 3).

The number of total T lymphocytes (CD3) and T cell subsets (CD4, CD8) were unaffected by DHEA treatment.

| Table 1. Effect of DHEA on Lymphocyte Subsets and Monocytes as Determined by Flow Cytometry; Values Are Expressed as % Lymphocytes ± SEM |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cell Marker | Placebo | 2 | 6 | 10 | 14 | 18 | 20 |
| CD20 (B cells) | 17.9 ± 2.2 | 27.7 ± 3.6** | 18.0 ± 2.3 | 18.6 ± 2.4 | 19.8 ± 2.5 | 20.5 ± 2.7 | 25.2 ± 2.7** |
| CD14 (monocytes) | 11.6 ± 1.9 | 21.2 ± 3.3** | 14.0 ± 2.5 | 14.2 ± 2.1 | 14.7 ± 2.6 | 16.2 ± 2.9 | 17.9 ± 2.9** |
| CD3 (T cells) | 68.4 ± 8.8 | 66.7 ± 4.3 | 61.7 ± 3.8 | 65.4 ± 3.1 | 69.8 ± 2.7 | 68.7 ± 3.7 | 69.1 ± 4.0 |
| CD4 (T helper) | 44.2 ± 3.4 | 39.4 ± 4.7 | 39.8 ± 2.9 | 40.5 ± 3.1 | 44.6 ± 2.4 | 43.9 ± 3.6 | 41.3 ± 4.1 |
| CD8 (T suppressor) | 32.7 ± 3.7 | 33.0 ± 3.0 | 31.2 ± 3.1 | 35.4 ± 3.1 | 33.6 ± 2.9 | 36.2 ± 1.9 | 38.3 ± 3.8 |
| TCR α/β | 59.2 ± 2.5 | 55.9 ± 2.9 | 42.6 ± 5.8 | 57.0 ± 3.2 | 51.9 ± 3.1 | 61.7 ± 3.9 | 62.0 ± 5.8 |
| TCR γδ | 5.7 ± 1.1 | 6.2 ± 0.97 | 6.2 ± 1.2 | 7.4 ± 1.1 | 7.7 ± 1.2 | 10.9 ± 1.2 | 10.5 ± 1.1** |
| CD25 (IL-2 receptor) | 9.4 ± 1.2 | 6.7 ± 1.7 | 4.8 ± 0.83* | 10.6 ± 2.3 | 11.7 ± 2.7 | 15.1 ± 1.5* | 12.4 ± 2.4* |
| CD57 (NK) | 24.1 ± 3.5 | 23.3 ± 2.9 | 24.0 ± 3.3 | 26.0 ± 2.4 | 22.8 ± 3.6 | 30.9 ± 2.9** | 31.5 ± 3.2** |
| CD16 (NK) | 19.5 ± 1.9 | 17.9 ± 2.4 | 19.6 ± 1.9 | 24.0 ± 2.0 | 21.0 ± 2.7 | 31.0 ± 2.8** | 28.6 ± 3.1** |

*p < .05; **p < .01 vs placebo.

| Table 2. Serum Concentrations (mean ± SE) of DHEAS (μmol/L), Cortisol (nmol/L) and the Ratio of DHEAS/Cortisol at Baseline and After Treatment With Placebo and DHEA (50 mg/day) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                               | Weeks of Treatment |
|                               | Baseline | Placebo | 2 | 10 | 14 | 18 |
| DHEAS (μmol/L) | 3.15 ± 0.39 | 3.22 ± 0.39 | 11.8 ± 1.07 | 10.9 ± 1.12 | 10.7 ± 1.44 | 12.1 ± 0.81 | 12.9 ± 1.13 |
| Cortisol (nmol/L) | 273 ± 28.8 | 283 ± 25.1 | 282 ± 37.3 | 238 ± 26.3 | 246 ± 25.1 | 231 ± 31.3 | 245.0 ± 36.3 |
| DHEAS/cortisol | 13.0 ± 2.2 | 11.9 ± 1.7 | 48.2 ± 6.4 | 49.2 ± 7.6 | 44.4 ± 6.9 | 56.3 ± 6.7 | 53.0 ± 6.7 |
However, there was a doubling in the number of T cells expressing the T cell receptor γδ (TCR γδ) \( p < .01 \) but not the TCR αβ by 20 weeks of treatment (see Table 1). T cell function was activated as evidenced by an increased proliferative response to PHA (0.1 μg/ml) within 12 weeks (Figure 2). This was accompanied by an increase in T cells expressing the IL-2R (CD25) by 18 weeks \( p < .05 \) (Table 1 and Figure 4), and enhanced PHA-induced IL-2 secretion by 20 weeks (Figure 2). Serum sIL2-R levels also increased significantly \( p < .01 \) by 12 weeks (Figure 4). Moreover, DHEA treatment led to a significant \( p < .01 \) increase in NK cell number (CD16, CD57) by 18–20 weeks (Table 1). This was accompanied by a parallel increase in their cytotoxicity at both high and low E:T ratios \( p < .01 \) (Figure 5).

DHEA was well tolerated without significant effects on blood pressure, body weight, blood count, or chemistry profile.

**DISCUSSION**

Our study demonstrates the stimulatory effects of DHEA on the immune function of age-advanced men. These effects of DHEA appear to require a latent phase of several weeks. This delay in the immune response to DHEA is to be expected as changes in immune function occur at a slower pace in aging populations (34). The ex vivo evidence presented here confirms and extends animal-derived data concerning the effects of DHEA on T cell function, and provides novel B cell findings. DHEA treatment rejuvenated the immune system by increasing the secretion of IL-2, a potent T cell growth factor, increasing the number of cells expressing the IL-2R (CD25), inducing a rise in serum sIL2R, and enhancing T cell responsiveness to mitogen stimulation, all of which decline during physiologic aging (34). These findings suggest that activation of T cells expressing IL-2R may permit the growth-promoting effects of IL-2 on T cells via an autocrine mode of action. In addition to serving as a growth factor to T lymphocytes, IL-2 may also stimulate the prolif-
IMMUNE ACTIVATION BY DHEA

Figure 4. Top panel depicts the % lymphocytes expressing the IL-2R (CD25) as determined by flow cytometry. Middle panel shows circulating sIL-2-R as determined by ELISA. Lower panel shows the concentration of IL-2 as determined by ELISA in media from cultured PHA stimulated (20 µg/ml) lymphocytes following placebo and DHEA treatment. **p < .01.

The significant increase in NK cell cytotoxicity in DHEA treated subjects was potentially related to the increased number of NK cells, both events being mediated by IL-2 stimulation. In postmenopausal women (25), an increase in NK cell cytotoxicity was also observed, but at an earlier time course of DHEA treatment, i.e., 3 weeks versus 18 weeks in men. Moreover, the inhibition of T-cell mitogenic response and the decreased number of cells expressing the CD4 antigen observed in postmenopausal women treated with DHEA (25) were not found in the present study. Although the reasons for these disparities are unknown, they suggest that a gender difference in the immune response to DHEA may exist and that sex steroids may modulate the effects of DHEA, a proposition that requires further study. The question of fluctuations in immune cell function over time also needs to be addressed. Limited data suggest a lack of variation in NK cell cytotoxicity measured over a 20-month interval (41). Because of the presence of circadian rhythm of most of the cytokines in circulation (44), such variations can clearly contribute to heterogeneity in clinical studies. It was emphasized that proper timing with uniformity of sampling would improve the usefulness of cytokine data (45).

The mechanism to account for the immune-enhancing properties of DHEA is currently unknown. The steroid may exert a direct action through binding to a specific cytosolic DHEA-binding complex (46), or by a non-genomic, direct action on immune cells. Alternatively, the effects of DHEA may be mediated by the elevation of IGF-I levels, given the observed temporal increase in bioavailable IGF-I levels and immune activation, and the demonstration of unambiguous
stimulatory effects, both in vivo and in vitro, of IGF-I on immune cells (47–49). In all probability, the activation of immune system by DHEA is not related to its androgenic potential, since androgens have a negative impact on the CD4+/CD8+ ratio (50). Nonetheless, our findings add support to an emerging body of evidence suggesting that the relative levels of DHEA (immune activation) and cortisol (immune suppressor) may determine, in part, immune/metabolic function (9,51). Since ACTH and cortisol secretion remain relatively constant throughout life (52), increases and decreases in the secretion of DHEA and DHEAS, as seen during adolescence/puberty (53) and with aging, may respectively determine, among other factors, immunocompetence. The 4-fold increase in DHEAS/cortisol ratio in response to a 50 mg dose of DHEA as seen in our age-advanced male cohort may thus be viewed as a favorable adrenal hormonal milieu for upregulating immune function, albeit at a slow pace.

In summary, in this single blind placebo-controlled trial in age-advanced men, we present preliminary evidence demonstrating that oral DHEA in a dose of 50 mg/day can significantly and safely activate the immune system by increasing the number of monocytes and B cells, stimulate T and B cell mitogenic response, increase the number of T cells expressing the IL-2R, TCR 7/8 and stimulate IL-2 secretion, autocrine/paracrine production of IL-2, and thus augmenting the number and cytotoxicity of NK cells expressing the IL-2R, TCR 7/8 and stimulate IL-2 secretion, cytokine production, NK cell mitogenic response, increase the number of T cells expressing the IL-2R, TCR 7/8 and stimulate IL-2 secretion, increase the number of T cells expressing the IL-2R, TCR 7/8 and stimulate IL-2 secretion, and increase the number of T cells expressing the IL-2R, TCR 7/8 and stimulate IL-2 secretion.


Received February 12, 1996
Accepted May 7, 1996

Geriatrician — Seattle, WA

Board-certified or eligible geriatrician to join a team of five other geriatricians who are part of a 70-physician multiple-specialty group practice. For the right superbly trained, dynamic, and energetic individual who can help expand the department, this is an excellent opportunity.

Please mail or Fax your CV to:

Martin Greene, M.D., Medical Director
Minor & James Medical, P.L.L.C.
Suite 200, 515 Minor Avenue, Seattle, WA 98104
Fax: (206) 386-9605