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Protection Against Acute Lethal Viral Infections With the Native Steroid Dehydroepiandrosterone (DHEA)

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A significant protective effect of a native adrenal steroid, dehydroepiandrosterone (DHEA), was demonstrated in studies of two lethal viral infection models in mice: systemic coxsackievirus B4 and herpes simplex type 2 encephalitis. The steroid was active either by long-term feeding or by a single subcutaneous injection. A closely related steroid, etiocholanolone, was not protective in these models. Histopathological analysis, leukocyte counts, and numbers of spleen antibody forming cells in the coxsackievirus B4 model suggests that DHEA functions by maintaining or potentiating the immune competence of mice otherwise depressed by viral infection. DHEA was not effective in genetically immunodeficient HRS/ J hr/hr mice and did not demonstrate antiviral activity in vitro. While the molecular basis for DHEA's effect on the immune system is not known, studies by others suggest that it may counteract the stress related immunosuppressive effects of glucocorticoids stimulated by viral infection. Because DHEA is a native steroid that has been used clinically with minimal side effects, the utility of DHEA in the therapeutic modulation of acute and chronic viral infections including the acquired immune deficiency syndrome deserves intensive study.

Key words: virus-infection, immune up-regulation, coxsackievirus, herpesvirus, DHEA

INTRODUCTION

Dehydroepiandrosterone, 3-beta-hydroxyandrost-5-en-17-one or dehydroiso-androsterone (DHEA), is quantitatively one of the major adrenal cortical steroid hormones in humans and other mammals [Windholz, 1976; Diem, 1975]. DHEA is sulfated by an adrenal sulfokinase to DHEA sulfate (DHEAS) in humans, but to a lesser extent in rodents [Tyrell, 1983]. DHEAS is quantitatively the major secretory

89 4 06 089

product of the human adrenal gland [Migeon, 1957] and the levels of this hormone begin to decline in the second decade of life reaching 5% of the original level in the elderly [Barrett-Connor, 1986]. Although DHEA appears to serve as an intermediary in gonadal steroid synthesis the primary physiological function of DHEA is unclear.

Our previous studies [Loria, 1984, 1986; Montgomery, 1986] have shown that the diabetic mutation db+/db+ is also associated with an impaired immune response in the inbred C57BL/KsJ mouse, and this host is markedly more susceptible to coxsackievirus B4 (CVB4) infection. It is now recognized that diabetes mellitus in humans may be a virus mediated autoimmune reaction, which may result in the destruction of the islets of Langerhans [Markhost, 1987; Bottazzo, 1986]. Since dietary DHEA was reported to have an anti-diabetic effect [Coleman, 1982, 1984, 1984b, 1985] in the diabetic mutant mouse, we examined whether the anti-diabetic effect of DHEA could be mediated in part by an effect on the immune response and/or on the pathogenicity of the enterovirus CVB4. Two acute virus infection models, with distinct replicative and pathogenic mechanisms were examined to determine the effects of DHEA on virus-mediated pathophysiology. The results show that peroral (p.o.) and subcutaneous (s.c.) administration of DHEA up-regulates the host immune system and reduces the virulence of an RNA and DNA virus that are lethal by widely different mechanisms.

MATERIALS AND METHODS
Viruses and Tissue Culture Procedures

Two different human virus isolates were used to challenge C57BL/6J inbred mice; one was the CVB4 Edwards strain and the second was herpes simplex type 2 (HSV2). Details on the passage history of CVB4 and tissue culture procedures have been published previously [Loria, 1976, 1984, 1986]. The HSV2 strain MS was obtained from the American Type Culture Collection (ATCC VR-540). This virus was grown and plaqued on Vero cells. For staining HSV2 plaques a 1% crystal violet was used for 20 minutes and then rinsed.

Animals

Male mice have been shown to be more susceptible than female mice to enterovirus infection [Berkovitch, 1965, 1967] and the reverse is true for HSV2 susceptibility [Mogensen, 1977; Baker, 1978; Yirrell, 1987]. Therefore male inbred C57BL/6J mice 6 to 8 weeks old (Jackson Laboratories, Bar Harbor, ME) were infected with CVB4, while female inbred mice of the same age and strain were used for HSV2 infections. The genetically immunodeficient hairless female HRS/J hr/hr inbred mice (Jackson Laboratories) at 6–8 weeks of age were used to test the effect of a functional immune system [Heiniger, 1974; Johnson, 198?] on the response to DHEA.

Diet

All animals were maintained on normal laboratory mouse chow Agway RMH-3000 (Agway, Syracuse, NY). In experiments where animals were maintained on a semipurified diet high in animal fat the diet contained 20% casein, 52.5% sucrose, 18% animal fat (lard), 5% cellulose, 4% salts, 0.2% choline chloride, 0.1% inositol,

and 0.1% vitamin mix. This semipurified diet has been used extensively [Loria, 1976a,b; Campbell,1978, 1982].

Route, Vehicle and Dose

Several routes of DHEA administration were examined. These included feeding as 0.4% of the diet (p.o.), subcutaneous injection (s.c.), or intraperitoneal injection (i.p.). For injection, DHEA (Searle, Chicago IL) was suspended in 0.2 ml dimethyl sulfoxide (DMSO). In CVB4 experiments, animals were infected with virus 1 hour after DHEA injection, and each group was challenged (i.p.) with a dose of CVB4 ranging from 10^2 pfu to 10^5 pfu/animal. In HSV2 infection experiments, young mice were challenged by intracranial (i.c.) injection with a dose of HSV2 ranging from 10^5 to 10^8 PFU/animal. Four hours prior to viral infection animals were injected s.c. with 1 g/kg of DHEA. Control animals were injected with virus and 0.2 ml of DMSO.

The optimal dose of DHEA-mediating antiviral activity was determined by injecting animals with DHEA doses of 2 g, 1 g, 500 mg, and 250 mg/kg, respectively. Protection from lethal CVB4 and HSV2 infection was observed when DHEA was injected s.c. at a dose of 1 g/kg. Also, feeding DHEA at 0.4% concentration protected from lethal virus infection. No significant protection from lethal virus infection was evident with any other s.c. dose of DHEA, or with any dose of etiocholanolone, 3 α-hydroxy-5β-androstan-17-one (Sigma Chemical Co., St Louis, MO). In all subsequent experiments a dose of 1 g/kg DHEA s.c. (25 mg/mouse) was used.

Enumeration of Spleen Antibody Forming Cells

As previously described [Montgomery, 1986] 10 days after CB4 infection test animals were subjected to an i.p. injection with 5×10^8 sheep red blood cells (SRBC), while control animals were immunized only with SRBC. Four days after SRBC immunization, animals were killed with an overdose of ether and the spleen removed. The procedure of Moller et al. [1973], for the enumeration of spleen cells secreting IgM-antibody, was used in these experiments.

Peripheral White Blood Cell Counts

Peripheral white blood cells were counted following Diff-Quik (American Scientific Products, McGaw Park, IL) staining of blood smear. No differentiation of lymphocytes or monocytes by special stains or cell marker was done.

Histopathological Examinations

For histopathological studies animals were sacrificed by an overdose of methoxyflurane (Metofane, Pitman-Moore, Inc., Washington Crossing, NJ), tissues were removed and fixed in phosphate-buffered formaldehyde at room temperature. Specimens were embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Statistical Analysis

The General Linear Models Procedure (SAS) was used to determine the significance of the particular changes in a given cell type. Whether there was a significant difference between the various groups was determined using Tukey's studentized range test for each variable at a $P \leqslant 0.05$ level. A confirmation of these results was obtained from the non parametric Wilcoxon Rank Sums test.

RESULTS

Protection Against Lethal Virus Infection

The effects of 25 mg DHEA injected s.c. on the percent survival following CVB4 and HSV-2 infection are presented in Figures 1a and 1b, respectively. The results show that the percent cumulative survival of animals following CVB4 infection was close to 90% in DHEA-treated mice as compared to about 58% in control animals ($P \leqslant 0.03$), (Fig. 1a). An almost identical increase in the percent cumulative survival was evident in HSV2-infected and DHEA-treated mice 92% versus 58% in control HSV2-infected animals, (Fig. 1b). The effects of s.c. injected DHEA on virus-dose-dependent mortality following infection with CVB4 and HSV2 are presented in Figure 2a,b. The findings show that animals infected with CVB4 LD_{100} dose (10^5 PFU/animal) had mortality reduced from 90% to 37.5% with DHEA treatment, while HSV2 induced mortality was reduced from 88% to 0 at a dose of 10^7 pfu/animal. This protective effect of DHEA against i.p. CVB4 and intracranial HSV2 infections was statistically significant, $P \leqslant 0.03$. These results confirmed and extended our earlier observations, which showed that inbred C57BL/6J mice fed 0.4% DHEA in a semipurified diet high in animal fat for 16 weeks prior to challenge was also associated with a significant resistance to CVB4 infection (Fig. 3, $P \geqslant 0.05$).

In these experiments, we also tested the effects of the sulfated metabolite, DHEAS, by s.c. or i.p. injection as well as the effects of the related steroid, etiocholanolone at the above mentioned doses. There was no evidence of protection against virus lethality with either DHEAS or etiocholanolone.

Number of Spleen Antibody Forming Cells

The effect of DHEA on the number of spleen antibody forming cells (AFC) in virus-infected and uninfected animals was determined by sheep red cell immunization
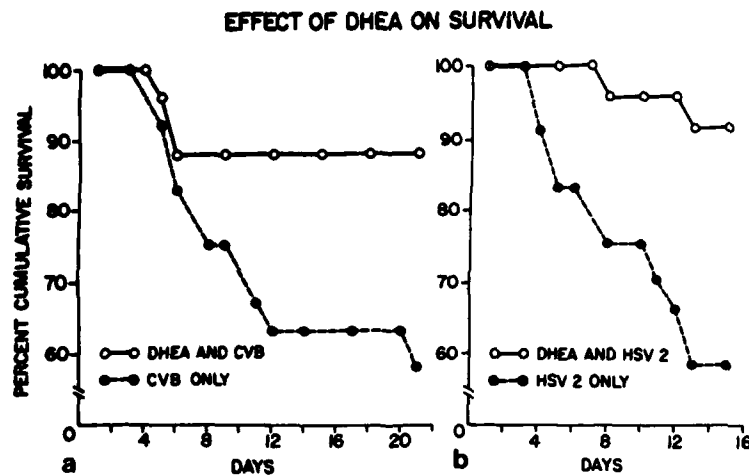
Fig. 1. The effects of DHEA injection on the percent cumulative survival of C57BL/6J mice following virus infection. a) Male mice were injected i.p. with CVB4 or with virus and DHEA. b) Female mice were injected i.c. with HSV2 or with virus and DHEA.
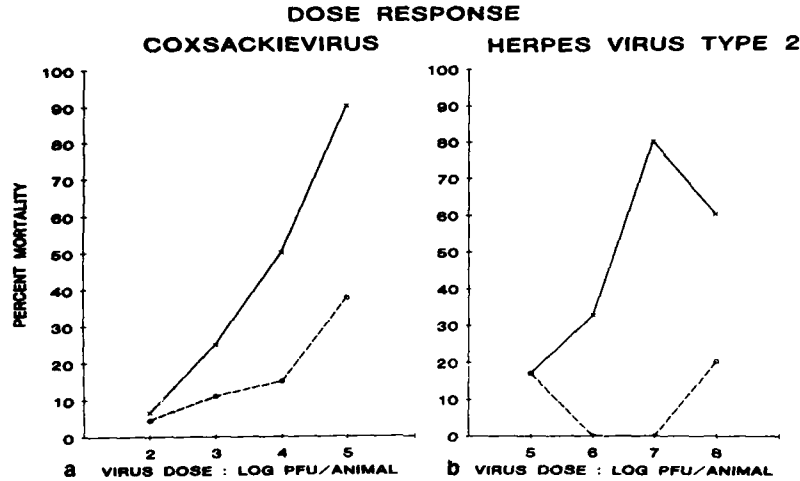
DOSE RESPONSE

COXSACKIEVIRUS   HERPES VIRUS TYPE 2

Fig. 2. The effects of DHEA injection on virus dose response of C57BL/6J mice. X—X virus only;
O----O virus and DHEA.

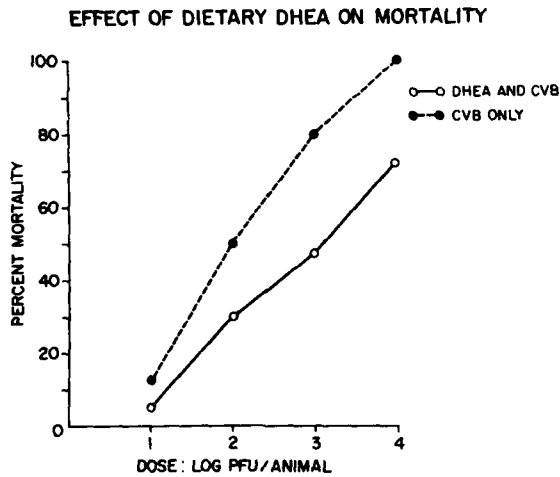EFFECT OF DIETARY DHEA ON MORTALITY

Fig. 3. The effects of feeding 0.4% DHEA on CVB4 dose response of C57BL/6J mice.

as described previously. The number of IgM AFC per 10^6 spleen cells in uninfected
and CVB4-infected mice with and without DHEA treatment are presented in Figure
4a. As can be seen, the number of IgM AFC per 10^6 spleen cells was 35% higher in
uninfected DHEA-treated mice as compared to the uninfected control mice. This
increase was not statistically significant. However, in CVB4-infected DHEA-treated
mice the number of spleen IgM AFC was 80% higher than the number of IgM AFC
in CVB4-infected control mice, $P \leqslant 0.025$. The number of spleen IgG AFC were
also enumerated (Fig. 4b); in DHEA-treated/CVB4-infected mice a 70% increase in
the number of IgG AFC was observed as compared to virus-infected mice not treated
with DHEA. This increase however was not statistically significant.

NUMBER OF SPLEEN ANTIBODY FORMING CELLS
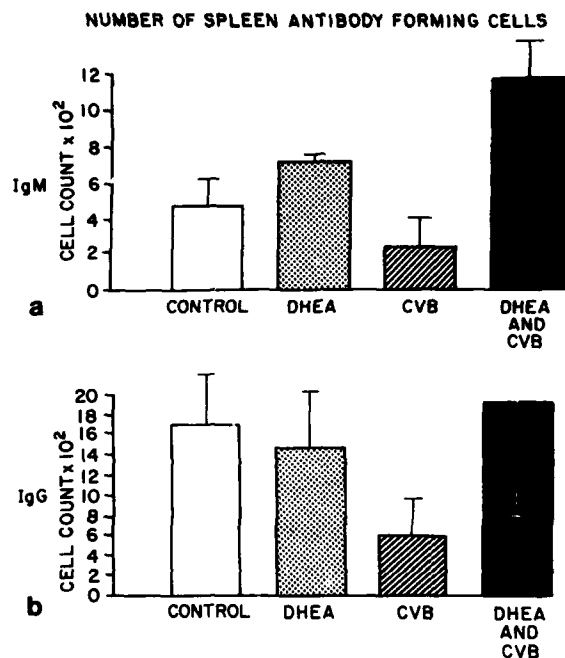
Fig. 4. The effect of DHEA on the number of spleen antibody forming cells (APF) in virus infected and uninfected C57BL/6J mice. a) the number of spleen IgM AFC. b) the number of spleen IgG AFC.

Histopathological Examination

Histopathological studies of hematoxylin and eosin stained spleen sections revealed that the spleen peri-arteriolar sheath of lymphocytes (PALS), which is composed largely of T lymphocytes that are primarily Thy 1.2 cells, were well developed in both DHEA-treated and in CVB4-infected animals. However, infection with CVB4 was associated with reduction in the number and size of spleen germinal centers. In contrast, in DHEA-treated animals, there was a marked increase in the number and size of splenic germinal center, suggesting B lymphocyte hyperplasia and a marked increase in the hematopoietic activity in the spleen red pulp areas. Furthermore, in untreated CVB4-infected animals, the spleenic white pulp was characterized by a prominent "starry sky" pattern [Sorger, 1987; Sinkovics, 1969] indicative of phagocytosis of large number of dead lymphocytes. In DHEA-treated/CVB4-infected animals, the "starry sky" pathological picture was reduced. These histological observations are suggestive of DHEA-mediated changes in the splenic lymphocyte and the hematopoietic cell populations.

Peripheral Leukocyte Counts

We also evaluated the effect of DHEA on peripheral leukocyte concentrations in the following four groups: 1) control, 2) 1 g/kg DHEA s.c., 3) CVB4-infected, and 4) 1 g/kg DHEA s.c./ CVB4-infected. All data were analyzed for statistical significance and the results are presented in Table I.

TABLE I. Effects of DHEA and Virus Infection on Peripheral Leukocytes Counts*

Treatment	Day	Total leukocytes	Monocytes	Neutrophiles	N
None	−1	11.20 ± 0.74	0.55 ± 0.13	1.04 ± 0.17	10
Control	3	11.30^a ± 1.87	0.84 ± 0.27	0.64 ± 0.16	3
DHEA	3	8.37 ± 1.04	0.58 ± 0.07	0.74 ± 0.12	6
CVB4	3	7.98 ± 0.53	0.42^c ± 0.08	3.94^c ± 1.00	4
DHEA + CVB4	3	3.94^b ± 0.88	0.13^{c,d} ± 0.02	2.60^b ± 0.30	12
Control	7	10.30 ± 1.84	0.58 ± 0.22	0.94 ± 0.33	3
DHEA	7	12.30 ± 1.30	0.94 ± 0.11	1.40 ± 0.25	6
CVB4	7	5.08 ± 1.32	0.44 ± 0.16	1.44 ± 0.46	6
DHEA + CVB4	7	8.13 ± 0.61	1.38^e ± 0.29	2.16^d ± 0.29	12
Control	14	9.73 ± 0.49	0.31 ± 0.14	0.73 ± 0.21	3
DHEA	14	14.20 ± 1.92	0.50 ± 0.22	1.84 ± 0.17	5
CVB4	14	11.10 ± 2.35	0.67 ± 0.41	4.95 ± 2.43	2
DHEA + CVB4	14	14.00 ± 1.50	0.87 ± 0.15	4.73 ± 1.12	7

*All values are mean cells count × 10^3/mm^3 blood ± S.E.
^{a}Control animals were injected with both vehicle and medium at the respective sites. Based on analysis of variance (ANOVA) the overall change on the third day were statistically significantly different for the total leukocyte, monocyte, and neutrophils counts at $P \leqslant 0.002$, $P \leqslant 0.0001$ and 0.0003, respectively. On day 7, the change in monocytes count was statistically significant, $P \leqslant 0.02$. Tukey's studentized range test for multiple comparison at a level of $P \leqslant 0.05$ was used to determine whether the difference between the particular groups was significant.
^{b}Different from uninfected control and DHEA-treated groups.
^{c}Different from uninfected control.
^{d}Different from DHEA-treated uninfected group.
^{e}Different from CVB4-infected group.

There was no significant effect of DHEA alone on the total leukocyte count as compared to untreated control animals. However, the total leukocyte count 3 days after infection was significantly lower in the DHEA-treated/CVB4-infected animals, as compared to uninfected control or DHEA injected controls, $P \leqslant 0.05$. There were no significant differences in the total leukocyte counts between any of the experimental groups at subsequent sampling. Three days after CVB4 infection only, or in the DHEA-treated/CVB4-infected group, the monocyte counts were 50% and 84.5% lower than the control group, respectively, $P \leqslant 0.05$. In contrast, 7 days after infection the monocyte counts of the DHEA-treated CVB4-infected group were 214% higher than the monocyte counts in the group infected with CVB4 that did not receive DHEA, $P \leqslant 0.05$. DHEA injection alone without CVB4 infection resulted in a 62% elevation of monocyte counts over control animals. There was no significant difference in the monocyte counts between the CVB4-infected animals not receiving DHEA and uninfected controls.

A biphasic response in peripherally sampled polymorphonuclear leukocyte (PMN) numbers was evident in CVB4-infected animals. Three days after infection the PMN counts reached 3.94 × 10^3 cells/mm^3 which was 515% higher than the PMN count in the control group of 0.64 × 10^3 cell/mm^3, $P \leqslant 0.05$. A second elevation in PMN counts was seen at 14 days in CVB4-infected or DHEA-treated/CVB4-infected animals only. This elevation was not quite as accentuated and not statistically significant. In noninfected DHEA-treated animals no real change was noted in the number of PMNs.

Host Immunogenetics

The mutation hairless hr/hr in the inbred HRS/J mouse is associated with hereditary immunodeficiency and leukemogenesis [Heiniger, 1974; Johnson, 1982; Holmes,1982]. Experiments were done to test whether s.c. DHEA injection could affect the resistance of this strain to CVB4 infection. Inbred HRS/J hr/hr mice were injected s.c. with 1 g/kg DHEA and challenged i.p. with 10^5 pfu/animal of CVB4 1 hour later. In contrast to the immunologically normal inbred C57BL/6J mice, DHEA did not protect this immunodeficient mutant from CVB4 lethality.

Mode of DHEA Administration

In contrast to protective s.c. DHEA injection our initial results show that DHEA given by the i.p. route was not associated with host protection from virus induced mortality or up-regulation of the immune response. We observed that s.c. injection of DHEA is associated with the formation of a local deposit leading to a prolonged DHEA interaction with the lymphoid system. Prolonged feeding of 0.4% DHEA was also protective in the CVB4 model (Fig. 3). However, it is of particular interest to note that the magnitude and the range of protection against lethal virus infection associated with s.c. injection of DHEA was considerably greater than when DHEA was fed in the diet.

In Vitro Effect of DHEA

In vitro experiments were done to determine whether DHEA had any direct effect on CVB4 infectivity and replication. HeLa cells in culture were incubated with either 2 μM or 20 μM DHEA and inoculated with 100 pfu of CVB4. No evidence of a reduction in the number of CVB4 plaque forming units could be detected at these concentrations of DHEA.

DISCUSSION

In general, steroid hormones of adrenocortical origin when administered at pharmacological doses have been regarded as immunosuppressive [Cupps, 1982; Claman, 1984; Grosmann, 1984; Goldien, 1987; Parillo, 1979], particularly in viral infections [Kilbourne, 1951; Gaitmaitan, 1970; Rytel, 1969]. In viral infections the administration of glucocorticoids results in higher viral tissue titers and increased symptomatology [Lynden, 1984; Meek, 1972; Hollinger, 1985; Johnson, 1985; Yir-rell, 1987].

In contrast, the results of this study demonstrate that DHEA, a native adrenal steroid hormone, which has been thought to be primarily an intermediary in testosterone and estradiol metabolism [Tyrell, 1983], can prevent mortality normally seen with two distinct classes of viruses.

We are inclined to attribute the protection against viral lethality seen with a single s.c. injection of DHEA (but not DHEAS) to an effect upon the host resistance and/or the immune system rather than upon the viruses per se. This supposition is supported by the observations that 1) DHEA failed to influence CVB4 replication in vitro, where immune mechanisms are not present; 2) DHEA was ineffective in the inbred HRS/J hr/hr mouse which is genetically immunodeficient; 3) up-regulation of the immune response by DHEA was seen in CVB4-infected mice with regard to the number of spleen IgM and IgG AFC (Fig. 4); 4) administration of DHEA alone was

also associated with enlargement of the spleen germinal centers which suggests stimulation of the B lymphocyte dependent areas; 5) DHEA treatment of CVB4-infected animals resulted in a reduction of the "starry sky pattern", an indicator of cell killing, which was prominent in the spleens of CVB4-infected mice not treated with DHEA, and 6) finally, an increase in circulating mononuclear cells was observed in DHEA-treated/CVB4-infected mice which is consistent with the role of these cells in host defense against CVB4 infection [Woodruff, 1979], as does the DHEA mediated decline in the splenic "starry sky" pattern.

While our studies do not reveal the specific effect(s) of DHEA on the immune system, there are suggestions from the work of Riley [1983] that DHEA may interfere with the immunosuppressive action of glucocorticoids such as corticosterone. In Riley's studies, mice subjected to "rotation stress" experienced increased serum corticosteroid levels and developed thymic involution and reduced resistance to transplantable tumors. These involutional effects of stress were antagonized by the s.c. injection of 1 mg/animal of DHEA [Riley, 1982]. In addition, DHEA also antagonized the effects of corticosterone injections on thymus involution.

Viral infections have been shown to cause an increase in glucocorticoid responses [Smith, 1982; Dunn, 1987; Blalock, 1987; Hammond, 1972; Spackman, 1974; Santisteban, 1972] and thymic involution as well as a generalized immunosuppression [Escobar, 1983; Woodruff, 1975; Rager-Zisman, 1973; Thong, 1975]. Thus it is reasonable to speculate that DHEA or its metabolites could act to protect the immune system from the stressful effects of the infection, i.e., glucocorticoid-mediated immune suppression, and thus enhance the ability of the host to control virus-mediated cytotoxicity, and possibly virus replication through various immune mechanisms. In this regard, a potent blocker of glucocorticoid synthesis, metyrapone, protects chickens against the lethal effects of Marek's disease, a herpesvirus-mediated lymphoproliferative disorder and also protects mice against murine sarcoma virus [Thompson, 1980; Rettura, 1973; Spangelo,1987]. Presently, the effects of exogenous DHEA on glucocorticoid synthesis and action are unknown. Similarly, it is not known whether DHEA can antagonize glucocorticoid action on T lymphocytes or other lymphoid cells. Both of these potential mechanisms of DHEA regulation of the immune system need to be investigated.

Since DHEA is considered to be a weak androgen, its host protective antiviral effect must be examined in the context of known sex hormone effect on the immune system. In particular, estradiol and progesterone have been reported to have effects on the natural killer cells [Grossman, 1985; Mohammad, 1985; Berci, 1986]. Thus DHEA like other gonadal and pituitary hormones [Davila, 1987; Russell 1985] could have independent regulatory effects on the immune response.

An alternative explanation for the sparing effect of DHEA in these acute viral infection models is that this steroid hormone may reduce virus-mediated T lymphocyte killing and reduce the number of anti-viral cytotoxic T cells, leading to a reduced tissue pathology. Indeed, cytotoxic T lymphocytes have a major role in the pathogenesis of CVB4 infection while humoral immunity is protective [Woodruff, 1975, 1979; Escobar, 1983; Rager-Zisman, 1973; Thong, 1975]. The opposite is seen in primary HSV infections [Lopez, 1984; Rouse, 1984] resistance is primarily mediated by T lymphocytes, while antibody protection is not as significant. Our observation of an increased proliferation of the spleen germinal centers in DHEA-treated animals and the reduction in the viral killing of lymphocytes in DHEA-treated/CVB4-infected

animals supports this hypothesis. Furthermore, the alteration of circulating leukocytes and the elevation in monocytes seen in DHEA-treated/CVB4-infected mice suggest a modulatory effect of DHEA on monocytes at various stages of the infection process. Monocytes have been reported to serve as effector cells in CVB4 infections [Woodruff, 1979] and it is possible that the changes in circulating monocyte levels reflect on the action of DHEA on tissue distribution or generation of these cells.

In our studies, anti-viral effects were observed only when DHEA was given s.c. or p.o., indicating that the route of DHEA administration may be a critical factor in the up-regulation of the host immune response. As is evident from Figures 2 and 3, the magnitude and the range of protection against lethal virus infection associated with s.c. injection of DHEA were considerably greater than when DHEA was fed in the diet. Recent reports show that the skin may have unique immune functions [Romerdahl, 1986; Streilein, 1983]. Indeed the skin is known to contain a population of cutaneous immune cells, which includes the epidermal Langerhans cells and keratinocytes that produce an epidermal thymocyte-activating factor, similar to IL-1 [Sauder, 1984]; in the murine system the Thy-1$^+$ dendritic epidermal cell. It has been suggested that the Thy-1$^+$ cell has a role in immune surveillance [Bergstresser, 1983; Tschachler, 1983] or in the presentation of antigen [Sullivan, 1985]. Consequently, it is possible that the increase in resistance following s.c. DHEA injection is associated with activation of the skin's particular immune functions.

The ability of DHEA to influence the immune system is also supported by the reports that DHEA and its bromo derivative have inhibited lymphoblastic transformation in human lymphocytes in vitro. In addition, DHEA has prevented the autoimmune lupus like syndrome in the NZB mouse that is thought by some to be caused by a slow virus [Henderson, 1981; Schwartz, 1985].

Whatever the mechanism of DHEA's action in the acute viral models, our studies suggest that prolonged exposure to DHEA is also an important factor for obtaining the protective effect. Either prolonged feeding for 16 weeks or s.c. deposition of the hormone appeared to be required for achieving antiviral protection (Fig. 3), while i.p. bolus administration did not protect the host against CVB4 infection. Furthermore, injection of DHEA sulfate in the mouse, either s.c. or i.p. showed no antiviral action, suggesting that the protective action of DHEA is through a pathway independent of sulfation.

An unexpected finding in these studies was that the protection seen with DHEA was enhanced by increasing the virus dose in both infection models (Fig. 2a,b). These results suggest that a certain critical virus load is required to activate the protective mechanism(s) induced by the hormone. This phenomenon could be mediated by the need for a certain amount of viral antigen to trigger the pertinent DHEA-modulated immunological process. Another possibility is that a threshold amount of virus might be required to activate the adrenal cortex if the protective DHEA effect should prove to be mediated through antagonism of glucocorticoids or other steroid effects.

The protective effect of s.c. DHEA injection against intracranial HSV2 (Figs. 1 and 2), was obtained by injection of the hormone 4 hours prior to infection. However, dose timing is critical; if injection of HSV2 i.c. and DHEA s.c. was 1 hour apart, no antiviral effect was produced. This observation suggests that either DHEA has to penetrate the blood-brain barrier to achieve its effect or the lag is necessary for DHEA to achieve up-regulation of the host immune system.

Clinically, DHEA has been used systemically and/or topically for psoriasis and has been used in the treatment of gout, hyperlipemia, and in post-coronary patients

[Regelson, 1988]. In animal models [Yen, 1977] and humans it has anti-obesity effects [Nestler, 1988] and anti-carcinogenic action in animals [Lopez, 1984; Rouse, 1984; Henderson, 1981; Schwartz, 1985]. It is still used clinically in Europe, in conjunction with estrogen as an agent to reverse menopausal systems and has also been used in the treatment of manic depression, schizophrenia, and Alzheimer's disease. Our group has studied DHEA clinically at 40 mg/kg/day in the treatment of advanced cancer and we are involved in an ongoing study of its role in multiple sclerosis [Regelson, 1988]. Mild androgenic effects, hirsutism, and increased libido were the side effects observed.

Our results show that dietary and s.c. administration of DHEA provides a new, effective approach to the treatment of both RNA and DNA viral infection; it may have broad clinical value where immunosuppression is a manifestation of infectious pathology or aging. DHEA, in contrast to clinical corticosteroids, is not diabetogenic nor anti-inflammatory. Its benign clinical side effects [Regelson, 1988; Nestler, 1988] suggest that it may have a place in the clinical treatment of viral infections where immunosuppression is an important concomitant of the infectious process.

ACKNOWLEDGMENTS

REFERENCES

Baker DA, Plotkin SA (1978): Enhancement of vaginal infection in mice by herpes simplex virus type I with progesterone. Proceedings Society of Experimental Biology and Medicine 158:131–134.

Barrett-Connor E, Khaw KT, Yen SS (1986): A Prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. New England Journal of Medicine 315:1519–1524.

Berci I (1986): Immunoregulation by pituitary hormones. In Berci I (ed): "Pituitary function and Immunity." Boca Raton, FL: CRC Press, pp 227–240.

Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW (1983): Thy-1 antigen bearing dendritic cells populate murine epidermis. Journal Investigative Dermatology 81:286–288.

Berkenbosh F, Van Oers J, Del Rey A, Tilders F, Besedovsky H (1988): Corticotropin-releasing factor producing neurons in the rat activated by interleukin-1. Science 238:524–526.

Berkovich S, Ressel M (1965): Effect of gonadectomy on susceptibility of the adult mouse to coxsackie B1 virus infection. Proceedings Society of Experimental Biology and Medicine 119:690–694.

Berkovich S, Ressel M (1967): Effect of sex on susceptibility of adult mice to coxsackie B1 virus infection. Archiv Fur die Gesamte Virusforschung 22:246–251.

Blalock JE (1987): Virus-induced increase in plasma corticosterone. Science 238:1424–1425.

Bottazzo GF, Pujol-Borrell R, Gale EM (1986): Autoimmunity and diabetes progress consolidation and controversy. In Alberti KGMM, Krall LP (eds): "The Diabetes Annual, 2." Amsterdam: Elsevier, pp 13–29.

Campbell AE, Loria RM, Madge GE (1978): Coxsackievirus B cardiopathy and angiopathy in the hypercholesteremic host. Atherosclerosis 31:295–306.

Campbell AE, Loria RM, Madge GE, Kaplan AM (1982): Dietary hepatic cholesterol elevation: Effect on coxsackievirus B infection and inflammation. Infection and Immunity 37:307–317.

Claman HN (1984): Anti-inflammatory effects of corticosteroids. Clinical Immunology and Allergy 4:317–329.

Coleman DL (1985): Antiobesity effects of Etiocholanolones in Diabetes (db), viable yellow (A^y), and normal mice. Endocrinologia 117:2279–2283.

Coleman DL, Leiter EH, Applezweig N (1984b): Therapeutic effects of dehydroepiandrosterone metabolites in diabetes mutant mice (C57BL/KsJ db/db). Endocrinologia 115:238–248.

Coleman DL, Leiter EA, Schizer RW (1982): Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. Diabetologia 31:830–833.

Coleman DL, Schizer RW, Leiter EA (1984): Effect of genetic background on the therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic-obesity mutants and in aged normal mice. Diabetes 33:26–32.

Cupps TR, Fauci AS (1982): Corticosteroid mediated immunoregulation in Man. Immunological Reviews 65:133–155.

Davila DR, Brief S, Simon J, Hammer RE, Brinster RL, Kelley KW (1987): Role of growth hormone in regulating T-dependent immune events in aged nude and transgenic rodents. Journal Neuroscience Research 18:108–116.

Diem K, Lentner C (1975): "Geigy Scientific Tables." Geigy Pharmaceuticals, Ciba-Geigy Co., Ardley, NY.

Dunn AJ, Powell ML, Gaskin JM (1987): Virus-induced increase in plasma corticosterone. Science 238:1423–1424.

Escobar MR, Swenson PD (1983): Mechanisms of viral immunopathology. In Rose NR, Siegel BV (eds): "The Reticuloendothelial system. Vol 4. Immunopathology." New York: Plenum Press, pp 201–253.

Gatmaitan BG, Chason JL, Lerner AM (1970): Augmentation of virulence of murine coxsackievirus B3 myocardiopathy by exercise. Journal Experimental Medicine 131:121–1136.

Goldien A (1987): Adrenocorticosteroids and adrenocortical antagonists. In Katzung BG (ed): "Basic and Clinical Pharmacology." Third edition. Norwalk, Connecticut: Appleton and Lange, pp. 449–460.

Grossman CJ (1984): Regulation of the immune system by sex steroids. Endocrine Reviews 5:435–455.

Grossman CJ (1985): Interactions between the gonadal steroids and the immune system. Science 227:257–260.

Hammond JB, Rosenberg JL (1972): Stimulation of small intestinal mucosal enzyme during coxsackievirus infection in neonatal mice. Journal of Laboratory Clinical Medicine 79:814–823.

Heiniger HJ, Meier H, Kaliss N, Cherry M, Chen HW, Stoner RD (1974): Hereditary Immunodeficiency and Leukemogenesis in HRS/J mice. Cancer Research 34:201–211.

Henderson E, Schwartz A, Pashko L, Abou-Gharbia M, Swern D (1981): Dehydroepiandrosterone and 16 α-bromoepiandrosterone: Inhibitors or Epstein-Barr virus-induced transformation of human lymphocytes. Carcinogenesis 2:683–686.

Hollinger BI (1985): Features of Viral Hepatitis. In Fields BN, Knipe DM, Chanock RM, Melnick JL, Roizman B, Shope RR (eds): "Virology." New York: Raven Press, p 1424.

Holmes K, Phibbs R, Shultz L, Tew J (1982): Antigen trapping of follicular dendritic cells in normal and hairless mice. Federation American Societies for Experimental Biology 82:3333.

Johnson DA, Schultz LD, Bedigian HG (1982): Immunodeficiency and reticulum cell sarcoma in mice segregating for HRS/J and SJL/J genes. Leukemia Research 6:711–720.

Johnson KM (1985): Arenaviruses. In Fields BN, Knipe DM, Chanock RM, Melnick JL, Roizman B, Shope RR (eds): "Virology." New York: Raven Press, p 1046.

Kilbourne ED, Horsfall FL (1951): Lethal Infection with Coxsackievirus of Adult Mice given Cortisone. Proceedings Society of Experimental Biology and Medicine 77:135–138.

Loria RM, Kibrick S, Downing D, Madge G, Fillios LC (1976a): Effects of prolonged hypercholesteremia in the mouse. Nutrition Reports International 12:509–518.

Loria RM, Kibrick S, Madge G (1976b): Infection in hypercholesteremic mice with coxsackievirus B. Journal Infectious Diseases 133:655–662.

Loria RM, Montgomery LB, Corey LA, Chinchilli V (1984): Coxsackie virus B4 infection in animals with diabetes-mellitus genotype. Arch Virology 81:251–262.

Loria RM, Montgomery LB, Tutle-Fuller N, Gregg HM, Chinchilli VM (1986): Genetic predisposition to diabetes mellitus is associated with impaired humoral immunity to coxsackievirus B4. Diabetes Research & Clinical Practice 2:91–96.

Lopez C (1984): Natural resistance mechanisms against herpesvirus in health and disease. In Rouse BT, Lopez C (eds): "Immunobiology of herpes simplex virus infection." Boca Raton FL: CRC Press, pp 45–69.

Lynden DL, Huber SA (1984): Aggravation of coxsackievirus, group B, type 3 induced myocarditis increase in cellular immunity to myocyte antigens in pregnant Balb/C mice and animals treated with progesterone. Cellular Immunology 87:462–472.

Markholst H, Lernmark A (1987): Autoimmunity in Diabetes. In Becker Y (ed): "Virus Infections and Diabetes Mellitus." Boston: Martinus Nijhoff, pp 111–124.

Meek ES, Golden B (1972): Infections of the eyelids and periorbital cellulitis. In Hoeprich PD (ed): "Infectious Diseases." New York: Harper and Row, pp 1241–1242.

Migeon CJ, Keller AR, Lawrence B, Shepard TH (1957): Dehydroepiandrosterone and androsterone levels in human plasma. Effect of age sex, day and diurnal variations. Journal Clinical Endocrinology and Metabolism 17:1051–1062.

Mogensen SC (1977): Genetics of macrophage-controlled resistance to hepatitis induced by herpes simplex type 2 in mice. Infection and Immunity 17:268–273.

Mohammad M, Couderc B, Chateaureynaud P, Mayer G (1985): Preliminary study in the female rat of the immunoregulatory effects of estradiol progesterone and hypophyseal prolactin on natural killing-type cell-mediated cytotoxic activity. Annals of Endocrinology 46:415–419.

Moller E, Bullock W, Makela O (1973): Affinity of T and B lymphocytes receptors for hapten determinants. European Journal of Immunology 3:172–179.

Montgomery LB, Loria RM (1986): Humoral immune response in hereditary and overt diabetes mellitus. Journal Medical Virology 19:255–268.

Nestler JE, Barlascini CO, Clore JN, Blackard WG (1988): Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men. Journal Clinical Endocrinology and Metabolism 66:57–61.

Oels HC, Harrison EG, Kiely JM (1968): Lymphoblastic lymphoma with histiocytic phagocytosis ("starry sky" appearance) in adults. Guide to prognosis. Cancer 21:368–375.

Parillo JE, Fauci AS (1979): Mechanisms of glucocorticoid action on immune processes. Annual Reviews of Pharmacology and Toxicology 19:179–201.

Rager-Zisman B, Allison AC (1973): The role of antibodies and host cells in the resistance of mice against infection by coxsackie B3 virus. Journal General Virology 19:329–338.

Regelson W, Loria R, Kalimi M (1988): Hormonal Intervention: "Buffer Hormones" or "State dependency." The role of Dehydroepiandrosterone (DHEA); Thyroid Hormone, Estrogen and Hypophysectomy in Aging. New York Academy of Sciences 518:260–273.

Rettura G, Seifter J, Zisblatt M, Levenson S, Levine N, Seifter E (1973): Metyrapone-inhibited oncogenesis in mice inoculated with murine sarcoma virus. Journal National Cancer Institute 51:1983–1985.

Riley V (1982): Basic and applied studies on the physiology and pathology of stress: The anti-stress action of dehydroepiandrosterone. In Murdoch MJ Foundation Report. Pacific Northwest Research Foundation Publication.

Riley V (1983): Psychoneuroendocrine influence on immune competence and neoplasia. Science 212:1100–1109.

Romerdahl CA, Kripke ML (1986): Advances in the immunology of the skin: Implication for cutaneous malignancies. Cancer and Metastasis Review 5:167–178.

Rouse BT (1984): Cell mediated Immune mechanisms. In Rouse BT, Lopez C (eds): "Immunobiology of Herpes Simplex Virus Infection." Boca Raton FL: CRC Press, pp 107–120.

Rytel MW (1969): Interferon Response During Coxsackie B-3 Infection in Mice. I. The effect of cortisone. J. Infect. Dis. 120:379–382.

Russell DH, Kibler R, Matrisian L, Larson DF, Poulos B, Magun BE (1985): Prolactin receptors on human T and B lymphocytes antagonism of prolactin binding by cyclosporine. Journal Immunology 134:3027–3031.

Santisteban GA, Riley V, Fitzmaurice MA (1972): Thymolytic and adrenal cortical responses to lactate dehydrogenase elevating virus. Proceedings Society of Experimental Biology and Medicine 139:202–206.

Sauder DN (1984): Epidermal cytokines: properties of epidermal cell thymocyte-activating factor (ETAF). Lymphokine Research 3:145–151.

Schwartz A (1985): The effects of dehydroepiandrosterone on the rate of development of cancer and autoimmune processes in laboratory rodents. In Woodhead AD (ed): "Molecular biology of aging Vol 35." New York: Plenum, pp 181–191.

Sinkovics JG, Pienta RJ, Trujillo JM, Ahearn MJ (1969): An immunological explanation for the starry sky histological pattern of a malignant lymphoma. Journal Infectious Diseases 120:250–254.

Smith EM, Meyer WJ, Blalock JE (1982): Virus-induced corticosterone in hypophysectomized mice: A possible lymphoid adrenal axis. Science 218:1311–1312.

Spackman D, Riley V (1974): Increase corticosterone a factor in lactic dehydrogenase virus induced alterations of immunological response in mice. Proceedings American Association for Cancer Research 15:143.

Sorger K, Gessler M, Hubner FK, Kohler H, Olbing H, Schulz W, Thoenes GH, Thoenes W (1987): Follow-up studies of three subtypes of acute postinfectious glomerulonephritis ascertained by renal biopsy. Clinical Nephrology 27:111–24.

Spangelo BL, Hall NRS, Ross PC, Goldstein AL (1987): Stimulation of in-vivo antibody production and concanavalin A-induced mouse spleen cell mitogenesis by prolactin. Immunopharmacology 14:11–20.

Streilein JW, Tigelaar RE (1983): SALT: skin-associated lymphoid tissues. In Parrish JA, Kripke ML, Morison WL (eds): Photoimmunology. New York: Plenum pp 95–130.

Sullivan S, Bergstresser PR, Tigelaar RE, Dees JH, Streilein JW (1985): FACS purification of bone marrow derived epidermal populations in mice: Langerhans cells and Thy-1+ dendritic cells. Journal Investigative Dermatology 84:491–495.

Thompson L, Elgert KD, Gross WB, Siegel PB (1980): Cell-mediated immunity in Mare's disease virus-infected chicken genetically selected for high and low concentration of plasma corticosterone. American Journal Veterinary Research 41:91–96.

Thong HY, Vincent MM, Hensen SA, Fuccillo DA, Rola-Plesczynski M, Bellanti J (1975): Depressed specific cell mediated immunity to herpes simplex virus type 1 in patients with recurrent herpes labialis. Infection and Immunity 12:76–80.

Tschachler E, Schuler G, Hutterer J, Leibl H, Wolff K, Stingl G (1983): Expression of Thy-1 antigen by murine epidermal cells. Journal Investigative Dermatology 81:282–285.

Tyrell JB, Forsham PH (1983): Glucocorticoids and adrenal androgens. In Greespan FS, Forsham PH (eds): "Basic and Clinical Endocrinology." Los Altos California: Lange Medical Publications, pp 258–294.

Windholz ME (1976): "The Merck Index," Ninth Edition. New Jersey: Merck & Co., Inc.

Woodruff JF, Woodruff JJ (1975): The effects of viral infection on the function of the immune system. In Notkins AL (ed): "Viral Immunology and Immunopathology." New York: Academic Press, pp 393–418.

Woodruff JP (1979): Lack of correlation between neutralizing antibody production and suppression of coxsackievirus B3 replication in target organs: Evidence for involvement of mononuclear inflammatory cells in host defense. Journal of Immunology 123:31–36.

Yen TT, Allan JA, Pearson DV, Acton JM (1977): Prevention of obesity in mice by dehydroepiandrosterone. Lipids 12:409.

Yirrell DL, Blyth WA, Hill TJ (1987): The influence of androgens paralysis in mice following intravenous inoculation of herpes simplex virus. Journal of General Virology 68:2461–2464.