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Dehydroepiandrosterone Protects Mice from Endotoxin Toxicity and Reduces Tumor Necrosis Factor Production

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Recent reports have demonstrated an immunomodulating activity of dehydroepiandrosterone (DHEA) different from that described for glucocorticoids. The present study was designed to test DHEA's activity in endotoxic shock and to investigate its effect on endotoxin-induced production of tumor necrosis factor (TNF). Mortality of CD-1 mice exposed to a lethal dose of lipopolysaccharide (LPS; 800 μg per mouse) was reduced from 95 to 24% by treatment with a single dose of DHEA, given 5 min before LPS. LPS administration resulted in high levels of TNF, a response that was significantly blocked by DHEA, both in vivo and in vitro. DHEA treatment also reduced LPS-induced increments in serum corticosterone levels, a parameter considered not to be mediated by TNF. In another experimental model, mice sensitized with D-galactosamine, followed by administration of recombinant human TNF, were subjected to 89% mortality rate, which was reduced to 55% in DHEA-treated mice. These data show that DHEA protects mice from endotoxin lethality. The protective effect is probably mediated by reduction of TNF production as well as by effecting both TNF-induced and non-TNF-induced phenomena.

Dehydroepiandrosterone (DHEA) is an abundantly secreted, weak androgenic, adrenocortical steroid hormone that is an intermediate in the biosynthesis of other hormones including testosterone and estradiol-17β. Concentrations of DHEA in plasma gradually decline after the third decade of life, reaching 10 to 20% of the peak level in the elderly (20, 24).

The precise biological functions of DHEA are uncertain. A growing body of evidence, both experimental and epidemiological, suggests an inverse relationship between low levels of DHEA in serum and morbidity from atherosclerotic cardiovascular disease (1, 9), cancer (10, 11, 26) and human immunodeficiency virus (HIV) infection (31). Moreover, low levels of DHEA were found to be independently predictive of death from any cause (1).

Recent reports proposed an immunomodulating activity of DHEA. It was found to prevent dexamethasone-induced thymic involution in mice (19), increase interleukin-2 production both in vitro and in vivo (6), and prevent the development of systemic lupus erythematosus in a mouse model (18). Lymphopoiesis, but not myelopoiesis or erythropoiesis, was inhibited in irradiated mice fed with DHEA (28, 29). Although lacking an in vitro antiviral activity, DHEA was effective in protecting mice from a variety of lethal viral infections (2, 17).

During the course of gram-negative infections, bacterial cell wall products, such as endotoxin (lipopolysaccharide [LPS]), are released, inducing intense pathophysiologic alterations (22). It is not LPS alone that causes the damage, but rather the host response, which may be described as an "overshoot" of the immune system. One of the major responses to LPS in vivo is the rapid production and secretion of cytokines, the soluble mediators of inflammation, like tumor necrosis factor (TNF) (3, 5).

LPS toxicity can be reduced by administration of potent immunosuppresive glucocorticoids (GC) (12) which inhibit the production of TNF and other cytokines if given prior to the LPS challenge (4, 33). Steroid hormones other than GC such as sex steroids (17-β estradiol and 5-α dihydrotestosterone) and mineralocorticoids (aldosterone and 11-deoxycorticosterone) were found ineffective in suppressing TNF production after LPS challenge (34).

This study examined the effect of DHEA on LPS toxicity. DHEA protected mice challenged with a lethal dose of LPS and was effective in reducing TNF response to LPS and protecting mice from TNF toxicity.

MATERIALS AND METHODS

Mice. CD-1 female mice (Charles River, London, United Kingdom) were used at 4 to 6 weeks of age. In all experiments mice of the same batch and age were compared.

High-dose endotoxin shock model. A lethal dose (800 μg per mouse) of LPS (lipopolysaccharide W, *Escherichia coli* 055:B5; Difco Laboratories, Detroit, Mich. was diluted in pyrogen-free saline and given intraperitoneally (i.p.). DHEA (Sigma) was diluted in 2.5% alcohol in 10% rabbit serum in saline containing penicillin to designated concentrations, and 0.5 ml was injected i.p. Control animals received only the diluent at the same times. Mice were observed daily for mortality for 10 days.

Sensitization with D-Gal. Mice were sensitized by i.p. injection of 18 mg of D-galactosamine (D-Gal; Sigma) diluted in pyrogen-free saline. Immediately after, mice were given 1.5 μg of recombinant human TNF (Genentech). DHEA (2 mg per mouse) was injected 1 h before challenge with TNF–D-Gal.

Peritoneal cells. Cells were obtained by peritoneal lavage of mice given 1 ml of 4% thioglycolate i.p. 4 days previously, by using cold pyrogen-free saline containing 1 U of heparin per ml and 5 μg of polymyxin B (Sigma) per ml. Cells were suspended in 10% fetal calf serum in endotoxin-free RPMI 1640 containing 5 μg of polymyxin B per ml and counted. Cell suspensions were adjusted to give 2×10^6 per ml in the same medium and 0.5-ml volumes were dispensed into wells

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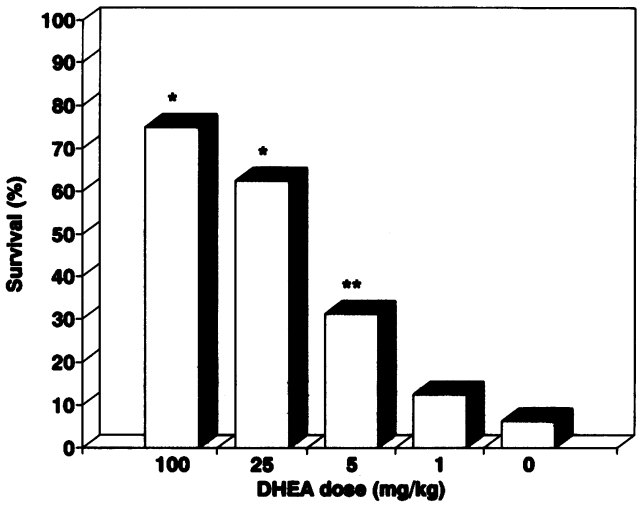

FIG. 1. Protection from LPS lethality by different DHEA doses. Mice (16 per group) were challenged with LPS (800 μg). DHEA at indicated doses was given 5 min before LPS. Survival was evaluated at 72 h after LPS challenge; no mortality was observed at 10 days postchallenge. *, $P < 0.01$ compared with control.

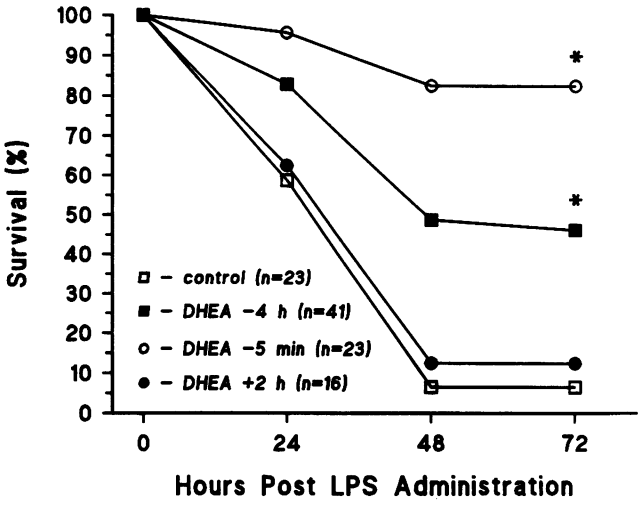

FIG. 2. Effects of DHEA administration schedules on LPS lethality. CD-1 mice were treated with LPS (800 μg). DHEA (2 mg per mouse, approximately 100 mg/kg) was given 4 h or 5 min before or 2 h after LPS. Control groups were injected with diluent at the same time. Survival was assessed daily for 10 days on groups of 6 to 10 mice. No mortality was observed later than day 3. The results presented are the accumulated data from several experiments; in all experiments mice of the same age and batch were used. *, $P < 0.01$ compared with LPS-diluent.

of 24-well plates. The cells were incubated for 2 h at 37°C in an atmosphere of 5% CO_2. Nonadherent cells were removed by washing with the same medium, and 0.5 ml of RPMI with and without DHEA was added. An hour later, 0.5 ml of medium containing LPS was added and the cells were incubated for 24 h. Supernatants were then collected and assayed for TNF.

TNF and CS assays. For quantitation of TNF and corticosterone (CS), mice were injected with LPS, 5 min after DHEA or diluent, and then bled at 90 and 300 min (CS only). Blood was collected into microtainers (Beckton-Dickinson). Serum was separated and frozen immediately at −70°C until use. Serum CS levels were quantitated by radioimmunoassay (ICN Biomedicals INc., Irvine, Calif.).

TNF-α bioactivity in serum was determined by using A-9 cells (ATCC, Rockville, Md.). Briefly, 3×10^4 A-9 cells were seeded in wells of microtiter plates and allowed to adhere overnight. Wells were treated with serial dilutions of the sera and then by addition of cycloheximide (25 μg/ml). After 8 to 10 h of incubation, the wells were washed and the number of surviving cells was determined by uptake of neutral red. Standard curves of human recombinant TNF were run on each plate, and results are weight equivalents of human TNF activity.

Data analysis. Data are reported as means ± standard errors. The two-tailed Fisher's exact probability test was used for proportion survival analysis. Statistical analysis of TNF and CS values was performed by using the Student's *t* test.

RESULTS

The set of experiments summarized in Fig. 1 was designed to investigate DHEA's effect on LPS lethality. High doses of LPS were needed to induce mortality in CD-1 mice as noted by others (8), and a dose of 800 μg per mouse, which caused 95% mortality, was selected for further studies. DHEA was found effective in preventing mortality. Figure 1 presents the dose-response of the effect of DHEA, given 5 min prior to challenge, on LPS lethality. It can be seen that a dose of 100 mg/kg of body weight was found most protective, reducing mortality to 25%. A significant protective effect was still evident in animals treated with 25 and 5 mg/kg but not in those treated with a 1-mg/kg dose.

The efficacies of different schedules for administration of DHEA on protection from high-dose LPS were then compared. Pretreatment with DHEA (2 mg per mouse, approximately 100 mg/kg) given 4 h before challenge reduced mortality to 50% compared with 24% mortality if treatment was administered 5 min before LPS. No significant protection was observed when DHEA was given 2 h after the LPS (Fig. 2).

To evaluate DHEA effect on TNF production, TNF levels were measured in the serum of mice given various doses of LPS (1, 10, or 100 μg per mouse). No TNF activity was detected in mice treated only with saline or with DHEA. LPS induced high TNF levels that were markedly reduced in DHEA-treated mice (2 mg per mouse, 5 min before LPS) in all three different dose groups. TNF levels in the serum 90 min after LPS administration in DHEA-treated and nontreated groups are shown in Fig. 3. To examine whether DHEA would block TNF production in vitro, peritoneal cells were stimulated by LPS. Pretreatment with DHEA significantly suppressed TNF production (Table 1).

To determine whether DHEA can protect animals from TNF-mediated toxicity, we employed the TNF/D-Gal septic shock model. In this model mice were sensitized with D-Gal and challenged with exogenous TNF. DHEA given 1 h before challenge significantly delayed the time of death and reduced the overall mortality rate from 89 to 55% (Fig. 4).

To investigate whether DHEA would intervene only in LPS-induced phenomena that are mediated by TNF, we measured levels of CS in serum after LPS challenge. CS levels after LPS were sharply raised and were still high at 5 h postchallenge. DHEA treatment moderated the increase in

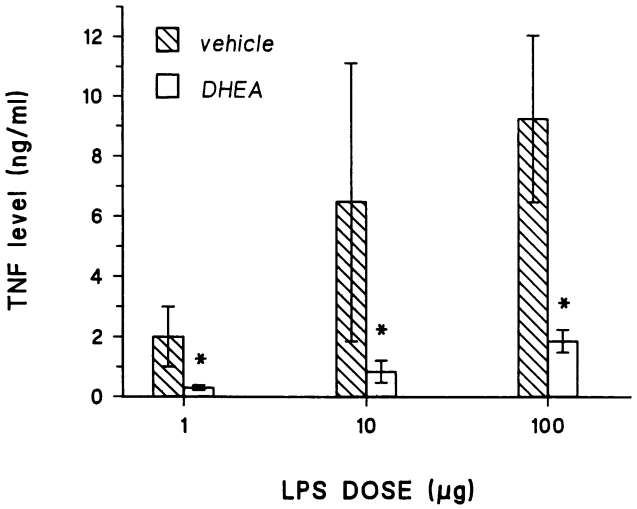

FIG. 3. Effects of DHEA on LPS-induced TNF levels in serum. Mice (8 per group) were treated with DHEA (2 mg per mouse) or diluent 5 min before challenge with LPS at the indicated doses. TNF levels were measured 90 min after challenge. Data are means ± standard errors. *, $P \leq 0.01$ compared with control.

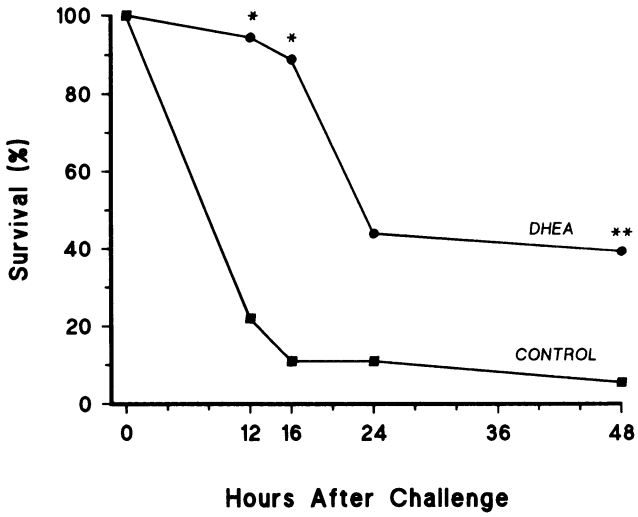

FIG. 4. DHEA protection from D-Gal-TNF lethality. Mice (18 per group) were treated with DHEA (100 mg/kg) or diluent 5 min before challenge with TNF (1.5 μg) and D-Gal (18 mg). Survival was assessed at indicated intervals. No additional mortality was observed 10 days later. *, $P \leq 0.01$ compared with control. **, $P = 0.019$ compared with control.

CS levels at 90 min after LPS, but no significant difference was observed at 5 h after challenge (Fig. 5).

DISCUSSION

The present experiments indicate that DHEA protects mice from death in various endotoxic shock models. We suggest three different possible sites of action for the involvement of DHEA in endotoxic shock.

TNF levels in serum following LPS challenge were significantly reduced in DHEA-treated mice. Reduced TNF response to LPS following DHEA treatment was also observed in vitro by using murine peritoneal macrophages. TNF is considered to be a major proximal mediator of septic shock, a claim substantiated by the finding that passive immunization against TNF protects mice from the lethal effects of LPS (5). TNF is not a sole mediator of LPS-induced phenomena (32) but rather acts in conjunction with other cytokines, augmenting their activities (35). Thus, our results may indicate that the protective effect of DHEA is accomplished by lowering TNF levels in the blood and preventing its deleterious cascade of events. The lack of positive effect in late administration of DHEA, after the release of TNF has occurred, further supports the view of suppression of TNF production as a mechanism of the protection offered by DHEA.

Beneficial effect was observed not only in LPS-challenged mice but also in those sensitized with D-Gal and challenged with a TNF, in a dose that is not lethal to unsensitized mice. In this model TNF toxicity is probably enhanced by the inhibition of hepatic enzymes and induction of hepatotoxic effect, similar to that observed following ischemia (16, 36). Since in this model exogenous TNF is given, reduction of TNF production cannot by itself explain the protection obtained by DHEA, and we may assume that DHEA exerts

TABLE 1. Production of TNF by peritoneal macrophages after stimulation with LPS, with and without DHEA treatment

Treatment[a]		TNF level (pg/ml)[b]
−1 h	0 h	
M	M	<35
M	LPS	2408 ± 509
DHEA	M	<35
DHEA	LPS	312 ± 77[c]

[a] Peritoneal macrophages in 24-well plates were treated with medium (M) containing LPS (100 ng/ml) with and without pretreatment with DHEA (10^6 M/ml).

[b] Following a 24-h incubation, supernatant were assayed for TNF. Data are means ± standard errors of the mean for x experiments.

[c] $P < 0.01$ compared with medium-LPS.

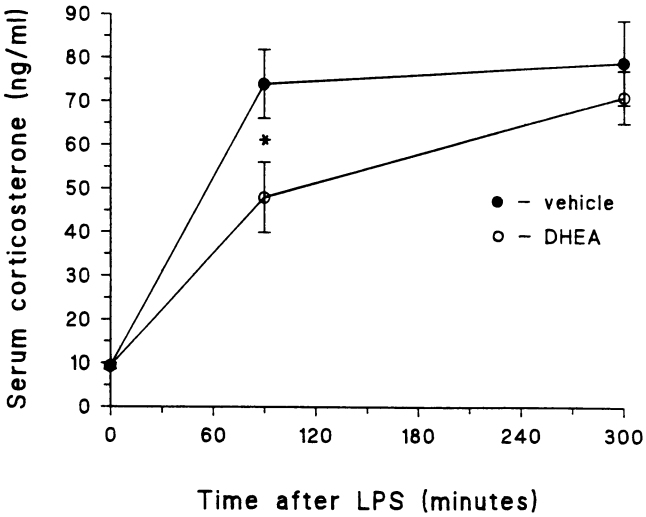

FIG. 5. Effects of DHEA on LPS-induced serum CS production. Mice (5 per group) were treated with DHEA (2 mg per mouse) 5 min before challenge with LPS (100 μg). Mice were bled at 90 min and 5 h, and sera were assayed for CS. Data are means ± standard errors. *, $P \leq 0.01$ compared with control.

its protective effect by abrogating effects in the endotoxic shock cascade which are mediated by TNF.

As mentioned already, endotoxic shock is mediated not only by TNF. In a recent work that studied inhibition of LPS-induced phenomena by using TNF antibodies, it was suggested that LPS-induced CS production is mediated by a mechanism that does not involve TNF (32). DHEA treatment partly suppressed the production of CS following LPS challenge. A significant difference in CS levels in the serum was observed at 90 min postchallenge but was abolished at 5 h. The difference probably indicates that DHEA action in endotoxemia is mediated not only by reducing TNF production or blocking TNF effects but also by modulating non-TNF-mediated LPS effects. Further studies are needed to explore DHEA effect on the production of other cytokines involved in septic shock, including interleukin-1 and interleukin-6.

The beneficial effect of DHEA in mice subjected to the TNF–D-Gal shock model was more prominent in the first 16 h after challenge. As mentioned above, a short-term DHEA effect was also observed on CS production. It seems that there is more than one pathway for LPS toxicity and more than one site for DHEA to act. TNF release after LPS is of short duration and can be blocked by a single dose of DHEA. A multiple-dose regimen of DHEA may block other effects, which are probably of longer duration.

DHEA is a natural adrenocortical androgenic steroid which has effects similar to that of GC on protection from LPS toxicity and on TNF production. On the basis of these, one could assume that DHEA immunomodulating activity is similar to that of GC or that it acts by inducing the production of GC. However, multiple data have shown that DHEA actually reverses the effects of GC on various components of the immune system with inhibition of GC immunosuppressive effects (2, 6, 17, 19, 23). Furthermore, DHEA probably does not act by inducing the production of GC, since reduced levels of CS, the most abundant natural GC in mice, were observed in DHEA-treated mice. The major metabolites of DHEA (androstenedione, testosterone, and estradiol-17β) do not hold the immunomodulating properties of either GC or DHEA (19, 34) and cannot be held responsible for the protective effects observed. Thus, DHEA seems to hold both immunosuppressive and immunoenhancing properties. Considering previous studies and its abundance in the plasma, the present study just adds to the confusion regarding DHEA's role in physiologic and pathologic states. Regelson et al. have reviewed DHEA's profound effects on a wide variety of physiologic and pathophysiologic events and suggested that it acts as a "buffer hormone," a hormone that demonstrates its presence during physiologic change in response to stress or injury to ensure homeostasis (27).

A property which is common to TNF and DHEA is the inhibition of differentiation of 3T3-L1 fibroblast clones to adipocytes (14, 30). An effect opposite to that is induced by dexamethasone (14). The metabolic significance of this is not yet clear, but it seems that the findings that DHEA reduce TNF production and block its effect are only one side of a more complex relation between DHEA and the host response.

Low levels of DHEA were found in AIDS patients (31). Recent studies have shown that low DHEA levels can independently predict disease progression of HIV infection to AIDS, and it was suggested that DHEA may have a therapeutic value in HIV patients (13). On the basis of our data we suggest a possible mechanism for DHEA involvement in HIV patients and a rationale for treatment: expression of HIV can be activated by TNF (7, 25). Elevated levels of serum TNF have been observed in AIDS patients and were presumed to worsen prognosis (15, 21). If, indeed, TNF is involved in the pathogenesis of HIV infection, DHEA may have a beneficial role in HIV patients that is mediated by suppression of TNF production and toxicity.

DHEA was administered to humans and mammals in high doses with only minor adverse effects, and it is devoid of the harmful immunosuppressive effects of GC (37). Its efficacy in the protection from endotoxemic shock as well as in protection from lethal viral infections warrants further investigations on its mechanisms of action, its activities on the immune system, and on the possibility of treating various infections and other pathologic states of the immune system with DHEA.

REFERENCES

1. **Barrett-Connor, E., K. T. Khaw, and S. S. C. Yen.** 1986. A prospective study of dehydroepiandrosterone sulfate, mortality and cardiovascular disease. N. Engl. J. Med. **315**:1519–1524.
2. **Ben-Nathan, D., B. Lachmi, S. Lustig, and G. Feurstein.** 1991. Protection of dehydroepiandrosterone in mice infected with viral encephalitis. Arch. Virol. **120**:263–271.
3. **Beutler, B., and A. C. Cerami.** 1989. The biology of cachectin/TNF, a primary mediator of the host response. Annu. Rev. Immunol. **6**:625–655.
4. **Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami.** 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanism of endotoxin resistance. Science **232**:977–980.
5. **Beutler, B., I. W. Milsark, and A. C. Cerami.** 1985. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science **229**:869–871.
6. **Daynes, R. D., D. J. Dudley, and B. A. Araneo.** 1990. Regulation of murine lymphokine production in vivo. II. Dehydroepiandrosterone is a natural enhancer of interleukin 2 synthesis by helper T cells. Eur. J. Immunol. **20**:793–802.
7. **Duh, E. J., W. J. Maury, T. M. Folks, A. S. Faucy, and A. B. Rabson.** 1989. Tumor necrosis factor α activates human immunodeficiency virus type through induction of nuclear factor binding to the NF-κB sites in the long terminal repeat. Proc. Natl. Acad. Sci. USA **86**:5974–5978.
8. **Gadina, M., R. Bertini, M. Mengozzi, M. Zandalasini, A. Mantovani, and P. Ghezzi.** 1991. Protective effect of chlorpromazine on endotoxin toxicity and TNF production in glucocorticoid-sensitive and glucocorticoid-resistant models of endotoxic shock. J. Exp. Med. **173**:1305–1310.
9. **Gordon, G. B., D. F. Bush, and H. F. Weismann.** 1988. Reduction of atherosclerosis by administration of dehydroepiandrosterone. A study in hypercholesterolemic New Zealand white rabbits with aortic intimal injury J. Clin. Invest. **82**:712–720.
10. **Gordon, G. B., K. J. Helzlsouer, and G. W. Komstock.** 1991. Serum levels of dehydroepiandrosterone and its sulfate and the risk of developing bladder cancer. Cancer Res. **51**:1366–1369.
11. **Gordon, G. B., L. M. Shantz, and P. Talalay.** 1987. Modulation of growth, differentiation and carcinogenesis by dehydroepiandrosterone. Adv. Enzyme Regul. **26**:355–382.
12. **Hinshaw, L. B., B. K. Beller-Todd, and L. T. Archer.** 1982. Review update. Current management of septic shock patient: experimental basis for treatment. Circ. Shock **9**:543–553.
13. **Jacobson, M. A., R. E. Fusaro, M. Galmarini, and W. Lang.** 1991. Decreased serum dehydroepiandrosterone is associated with increased progression of human immunodeficiency virus infection in men with CD4 cell counts of 200–499. J. Infect. Dis. **164**:864–868.
14. **Kawakami, M., P. H. Pekala, M. D. Lane, and A. Cerami.** 1982. Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induced mediator from exudate cells. Proc. Natl. Acad. Sci. USA **79**:912–916.
15. **Lahdervirta, J., C. P. J. Maury, A. M. Teppo, and H. Repo.** 1988. Elevated levels of circulating cachectin/tumor necrosis

factor in patients with acquired immunodeficiency syndrome. Am. J. Med. **85:**289–291.
16. **Lehmann, V., M. A. Freudenberg, and C. Galanos.** 1987. Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and D-galactosamine-treated mice. J. Exp. Med. **165:** 657–663.
17. **Loria, R. M., T. H. Inge, S. S. Cook, A. K. Szakal, and W. Regelson.** 1988. Protection against acute lethal viral infections with the native steroid dehydroepiandrosterone (DHEA). J. Med. Virol. **26:**301–314.
18. **Lucas, J. A., S. A. Ahmed, M. L. Casey, and P. C. MacDonald.** 1985. Prevention formation and prolonged survival in New Zealand black/New Zealand white F1 mice fed dehydroisoandrosterone. J. Clin. Invest. **75:**2091–2093.
19. **May, M., E. Holmes, W. Rogers, and M. Poth.** 1990. Protection from glucocorticoid induced involution by dehydroepiandrosterone. Life Sci. **46:**1627–1631.
20. **Migeon, C. J., A. R. Keller, B. Lawrence, and T. H. Shepard.** 1957. Dehydroepiandrosterone and androsterone levels in human plasma effect of age, sex, day and diurnal variations. J. Clin. Endocrinol. Metab. **17:**1051–1061.
21. **Mintz, M., R. Rapaport, J. M. Oleske, E. M. Connor, M. R. Koenigsberger, T. Denny, and L. G. Epstein.** 1989. Elevated serum levels of tumor necrosis factor are associated with progressive encephalopathy in children with acquired immunodeficiency syndrome. Am. J. Dis. Child. **143:**771–774.
22. **Morrison, D. C.** 1983. Bacterial endotoxins and pathogenesis. Rev. Infect. Dis. **5:**733s–747s.
23. **Munck, A., P. M. Guyre, and N. J. Holbrook.** 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Rev. **5:**25–44.
24. **Orenstreich, N., J. L. Brind, R. L. Rizer, and J. H. Vogelman.** 1984. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J. Clin. Endocrinol. Metab. **59:**551–555.
25. **Osborn, L., S. Kunkel, and G. J. Nabel.** 1989. Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κB. Proc. Natl. Acad. Sci. USA **86:**2336–2340.
26. **Ratko, T. A., C. J. Detrisac, G. M. Rajendra, G. J. Kellof, and R. C. Moon.** 1991. Inhibition of rat mammary gland chemical carcinogenesis by dietary dehydroepiandrosterone or a fluorinated analogue of dehydroepiandrosterone. Cancer Res. **51:** 481–486.
27. **Regelson, W., R. Loria, and M. Kalimi.** 1988. Hormonal intervention: "buffer hormones" or "state dependency." The role of dehydroepiandrosterone (DHEA), thyroid hormone, estrogen and hypophysectomy in aging. Ann. N.Y. Acad. Sci. **521:**260–273.
28. **Risdon, G., V. Kumar, and M. Bennett.** 1990. Mechanism of chemoprevention by dietary dehydroisoandrosterone. Inhibition of lymphopoiesis. Am. J. Pathol. **136:**759–769.
29. **Risdon, G., V. Kumar, and M. Bennett.** 1991. Differential effects of dehydroepiandrosterone (DHEA) on murine lymphopoiesis and myelopoiesis. Exp. Hematol. **19:**128–131.
30. **Rubin, C. S., A. Hirsch, C. Fung, and O. M. Rosen.** 1978. Development of hormone receptors and hormonal responsiveness in vitro. J. Biol. Chem. **253:**7570–7578.
31. **Villette, J. M., P. Bourin, C. Doinel, I. Mansour, J. Fiet, P. Boudou, C. Dreux, R. Roue, M. Debord, and F. Levi.** 1990. Circadian variations in plasma levels of hypophyseal, adrenocortical and testicular hormones in men infected with human immunodeficiency virus. J. Clin. Endocrinol. Metab. **70:**572–577.
32. **Vogel, S. N., and E. A. Havell.** 1990. Differential inhibition of lipopolysaccharide-induced phenomena by anti-tumor necrosis factor alpha antibody. Infect. Immun. **58:**2397–2400.
33. **Waage, A.** 1987. Production and clearance of tumor necrosis factor in rats exposed to endotoxin and dexamethasone. Clin. Immun. Immunopath. **45:**348–355.
34. **Waage, A., and O. Bakke.** 1988. Glucocorticoids suppress the production of tumor necrosis factor by lipopolysaccharide-stimulated human monocytes. Immunology **63:**299–302.
35. **Waage, A., and T. Espevik.** 1988. Interleukin 1 potentiates the lethal effect of tumor necrosis factor α/cachectin in mice. J. Exp. Med. **167:**1987–1992.
36. **Wallach, D., H. Holtmann, H. Engelmann, and Y. Nophar.** 1988. Sensitization and desensitization to lethal effects of tumor necrosis factor and IL-1. J. Immunol. **140:**2994–2999.
37. **Welle, S., R. Jozefowicz, and M. Statt.** 1990. Failure of dehydroepiandrosterone to influence energy and protein metabolism in humans. J. Clin. Endocrinol. Metab. **71:**1259–1264.