Administration of Dehydroepiandrosterone Suppresses Experimental Allergic Encephalomyelitis in SJL/J Mice

Caigan Du, M. Wahid Khalil and Subramaniam Sriram

*J Immunol* 2001; 167:7094-7101;
doi: 10.4049/jimmunol.167.12.7094
http://www.jimmunol.org/content/167/12/7094

**References**
This article cites 51 articles, 12 of which you can access for free at:
http://www.jimmunol.org/content/167/12/7094.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Administration of Dehydroepiandrosterone Suppresses Experimental Allergic Encephalomyelitis in SJL/J Mice

Caigan Du, 1* M. Wahid Khalil, † and Subramaniam Sriram*

Experimental allergic encephalomyelitis (EAE) is a Th1-mediated inflammatory demyelinating disease in the CNS, an animal model of multiple sclerosis. We have examined the effect of dehydroepiandrosterone (DHEA) on the development of EAE in mice. The addition of DHEA to cultures of myelin basic protein-primed splenocytes resulted in a significant decrease in T cell proliferation and secretion of (pro)inflammatory cytokines (IFN-γ, IL-12 p40, and TNF-α) and NO in response to myelin basic protein. These effects were associated with a decrease in activation and translocation of NF-κB. In vivo administration of DHEA significantly reduced the severity and incidence of acute EAE, along with a decrease in demyelination/inflammation and expressions of (pro)inflammatory cytokines in the CNS. These studies suggest that DHEA has potent anti-inflammatory properties, which at least are in part mediated by its inhibition of NF-κB activation. The Journal of Immunology, 2001, 167: 7094–7101.

E xperimental allergic encephalomyelitis (EAE)2 is an inflammatory demyelinating disease in the CNS, and it shows many pathological and clinical similarities with multiple sclerosis (MS; Refs. 1 and 2). The disease is induced in susceptible strains of rodents or primates by either immunization with neural Ags or adoptive transfer of neural Ag-specific T cells. Ags well known to induce EAE are myelin basic protein (MBP), proteolipoprotein, and myelin oligodendrocyte protein. EAE is an archetypal CD4+ Th1 cell-mediated autoimmune disease in which Th1 cells, infiltrating macrophages, proinflammatory cytokines (e.g., IL-12, IFN-γ, and TNF-α), and NO (free radical NO) play a critical role in the pathogenesis of the disease (1–3).

NF-κB, a nuclear transcriptional factor, is activated in response to inflammatory factors such as microbial products (e.g., LPS, dsRNA, and DNA) and cytokines (TNF-α, IL-1, lymphotoxin, and IL-2; Refs. 4 and 5). At least five members of NF-κB family have been identified: p50/p105 (NF-κB1), p52/p100 (NF-κB2), p65 (RelA), RelB, and c-Rel, which form homo- and/or heterodimers. Of them, the p50/p65 dimer is the most abundant complex (4, 6). Activation of NF-κB plays a critical role in innate immune responses by up-regulating expression of cytokines and chemokines (6, 7). Some of these cytokines (IL-1, TNF-α, lymphotoxin, IL-2, IL-12, and IFN-γ) also directly contribute to the development of Th1-mediated immune response (8).

Dehydroepiandrosterone (DHEA), a C19 adrenal steroid, and its sulfated form (DHEAS) are synthesized by the adrenal glands, gonadal tissue, and CNS cells (9–11). In young adult humans, the levels of DHEAS (5–6 μg/ml) and DHEA (2–4 ng/ml) in plasma are considerably higher than any other steroids (9). DHEAS is converted to the bioactive form DHEA by intracellular sulfatases, which are present in a number of cell types, including monocytes and macrophages (12, 13). Although DHEA is an abundant steroid hormone in serum, its biological functions are still unclear.

Although DHEA is widely considered to be a precursor of androgens and/or estrogens (14), increasing evidence indicates that DHEA has, in addition, potent immunoregulatory functions. In animal model systems, administration of DHEA enhances the ability of mice to resist experimental viral and bacterial diseases (15–20). In humans, many chronic inflammatory diseases are associated with lower serum levels of DHEA or DHEAS (21). Therapy with DHEA has shown benefit in some patients with systemic lupus erythematosus (22) and HIV infection (23). Our previous study has shown that the addition of DHEA to in vitro cultures inhibits the development of Th1 cells (24). In the present study, we examined the effect of DHEA on EAE. We predicted that administration of exogenous DHEA would inhibit Th1-mediated inflammatory responses and would consequently prevent the development of EAE.

Materials and Methods

Animals and reagents

Female SJL/J mice (6–8 wk old) were purchased from the National Institutes of Health (Bethesda, MD) and were maintained in the animal care facility at Vanderbilt University (Nashville, TN). DHEA was obtained from Sigma-Aldrich (St. Louis, MO). The DHEA stock solution (10 mM) was prepared by dissolving it in DMSO (Sigma-Aldrich), and it was diluted to the experimental concentrations (2–20 μM) in the culture medium. The monoclonal anti-mIL-12 Abs (C17.5 and C15.6), gifts from Dr. G. Trinchieri (Wistar Institute, Boston, MA), were purified by QAE Sephadex A-50 and Sephadex G-25 M column (Pharmacia Biotech, Uppsala, Sweden) from hyperidoma ascetic fluid. C15.6 was biotinylated according to manufacturer’s protocol and was used as a detecting Ab, whereas C17.5 was used as a capture Ab for IL-12 p40 ELISA. Abs against p50 and p65 of NF-κB were brought from Santa Cruz Biotechnology (Santa Cruz, CA). rMIL-12 was kindly provided by Genetics Institute (Cambridge, MA). MBP was purified from guinea pig spinal cord (Rockland, Gibertsville, PA) by the methods described previously (25).

ELISA of cytokines

Levels of cytokines (IL-12 p40, IFN-γ, IL-4, and TNF-α) were measured by ELISA as described previously (26). The matched Ab pairs and standards for IL-4, IFN-γ, and TNF-α were purchased from Endogen (Woburn, Massachusetts), and all other commercial reagents were from Sigma-Aldrich (St. Louis, MO), unless otherwise indicated.
The Journal of Immunology

MA), and the Abs and standards used for IL-12 p40 ELISA were described above.

**Measurement of NO**

NO secreted from cells is rapidly oxidized to nitrite in the culture medium, therefore, determination of nitrite concentrations was used as a measure of NO production. Fifty microliters of culture supernatant was mixed with 50 µl of Greiss reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride, and 2.5% phosphoric acid) per well in a 96-well plate in triplicate. After incubation at 25°C for 10 min, the absorbance was read at 550 nm. The levels of NO in the culture medium were calculated based on sodium nitrite standards.

**Proliferation assay**

T cell cultures were grown in a 96-well microtiter plate in triplicate. MBP-primed lymphocytes (2 × 10^5) per well were cultured in the presence of MBP for 72 h in RPMI 1640 complete medium (RPMI 1640 medium, 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% FBS; Life Technologies, Rockville, MD) under the atmosphere of 5% CO₂ and 95% air at 37°C. The cultures were harvested using a Harvester96 (Tomtec, Orange, CA) after incubation with 0.5 µCi/well [³H]thymidine (DuPont Pharmaceuticals, Boston, MA) for the last 18 h. The radioisotope incorporation as index of T cell proliferation was determined using a betaplate liquid scintillation counter (Wallac, Turku, Finland).

**Nuclear extraction and EMSA**

After treatment, MBP-primed or naive splenocytes (2.5 × 10^6 cells/ml) were added to 1 mM Na3VO4 at a final concentration and were pelleted by centrifugation at 1,000 × g for 5 min at 4°C. The pellets were washed with 0.5 ml of buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1 mM Na3VO4, and 5 µg/ml each of aprotinin, leupeptin, pepstatin, chymostatin, and antipain) and were centrifuged again at 1,000 × g for 5 min at 4°C. The cells were lysed in 100 µl of buffer A containing 0.5% Igepal CA-630 (Sigma-Aldrich) for 10 min at 4°C. The resultant nuclei were harvested by centrifugation at 1,500 × g for 15 min at 4°C, resuspended in 30 µl of buffer B (20 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 0.42 M NaCl, 1 mM EDTA, 1 mM EGTA, 0.5 mM DTT, 25% glycerol, 0.5 mM PMSF, and 5 µg/ml each of aprotinin, leupeptin, pepstatin, chymostatin, and antipain), and extracted by vigorous whirling for 15 min at 4°C. The nuclear extracts were cleared by centrifugation at 16,000 × g for 10 min at 4°C. Two microliters of nuclear extract from each sample was taken for measurement of protein content by Bio-Rad Protein Assay (Bio-Rad, Hercules, CA), and the final nuclear extracts were equalized among samples using buffer B, frozen in liquid nitrogen, and kept at −75°C.

The activation or nuclear translocation of p50NF-κB was performed by using EMSAs. NF-κB consensus oligonucleotide (5’-AGTTGAGGG GACCTTTCCAGGC-3’) was purchased from Promega (Madison, WI), and nuclear factor-Y box binding (NF-Y) oligonucleotide (5’-GATCT GAGAAATCTTCTGATGGTCTGCGGATGGTTG-3’) (27) was synthesized at IDT (Coralville, IA) and purified by polyacrylamide gel. Double-stranded oligonucleotide probe was labeled by [³²P]ATP (3000 Ci/mmol; Amersham, Arlington Heights, IL), 1 µl of T4 polynucleotide kinase (5–10 U; New England Biolabs, Beverly, MA), and 5 µl of nuclelease-free water for 10 min at 37°C. The reaction was stopped by the addition of 1 µl of 0.5 M EDTA (pH 8.0). The labeled probe was purified with a Micro Bio-Spin 30 Chromatography Column (Bio-Rad). DNA-nuclear protein binding reactions were done in a final volume of 20 µl for 30 min at 25°C. NF-κB-binding reactions contained 5 µg of nuclear protein, 4 µl of binding buffer (5×) (20 mM HEPES, pH 7.9, 50 mM KCl, 1 mM MgCl₂, 0.5 mM EDTA, 0.5 mM DTT, 20% glycerol, and 0.25 mg/ml fish sperm DNA) and 0.5–1 × 10⁶ cpm ³²P-labeled probe; NF-Y-binding reactions contained 5 µg of nuclear protein, 4 µl of binding buffer (5×) (20 mM HEPES, pH 7.9, 50 mM KCl, 1 mM MgCl₂, 0.5 mM EDTA, 0.5 mM DTT, 20% glycerol, and 0.25 mg/ml poly(dI-dC)), and 2–5 × 10⁵ cpm ³²P-labeled probe. For supershift assays, 2 µl of anti-NF-κB (p50 or p65) Abs was added to NF-κB-binding reactions after binding reaction completed, and they were then incubated for 1 h. The complex of probe and binding proteins was electrophoresed in a 4% polyacrylamide gel (16 × 20 cm) in 1× Tris-borate-EDTA at 100 V, and it was autoradiographed at −75°C.

**Induction of EAE**

Active EAE was induced by s.c. immunization with 800 µg of mouse spinal cord homogenate (MSCH) in CFA (Sigma-Aldrich) on days 0 and 7, along with i.p. injection of pertussis toxin (200 ng in 0.5 ml of PBS per animal; Sigma-Aldrich) on days 0 and 2. Passive EAE was induced by adoptive transfer of MBP-sensitized T lymphocytes (28). Briefly, SJL J mice were s.c. immunized with 400 µg of MBP in CFA on days 0 and 7. On day 14, the lymph nodes and spleen cells from the immunized mice were harvested and purified by gradient centrifugation (Histopaque-1077; Sigma-Aldrich), and then incubated in RPMI 1640 complete medium in the presence of 50–100 µg/ml MBP. A proliferation assay of MBP Ag-reactive T cells was performed at the time the lymph nodes and spleen cells were harvested. Cells (2 × 10⁵ per well) were incubated in RPMI 1640 complete medium in the presence of various concentrations of MBP Ag, and the Ag-reactive T cell proliferation assay was conducted as described above. To induce EAE, viable T cell blasts were harvested from MBP-stimulated cultures (a 96-h culture), washed twice with PBS, and injected i.p. into recipient mice (1.0 × 10⁶ cells in 0.5 ml of PBS per animal).

**Treatment of animals, and clinical and pathologic evaluation of disease**

Mice were treated with DHEA (2 mg/dose) or its carrier, DMSO (Sigma-Aldrich), daily by s.c. injection in a volume of 50 µl. The treatment began from the time of the first immunization (active EAE) or T cell transfer (passive EAE; day 0) until disease recovery. Paralysis was graded as follows: 0, normal; 0.5, mild; 1, limp; 1.5, limp tail with inability to right; 2, paralysis of one limb; 2.5, paralysis of one limb and weakness of one other limb; 3, complete paralysis of both hind limbs; 4, moribund state; and 5, dead.

To assess the degree of inflammation or demyelination, EAE mice with were euthanized on day 25 and were perfused by intracardiac injection of 0.9% paraformaldehyde and 1% glutaraldehyde in PBS. Transverse sections of the cervical, upper thoracic, lower thoracic, and lumbar regions of the
spinal cord were stained with Luxol Fast Blue or H&E. Each spinal cord section was further subdivided into an anterior, posterior, and two lateral columns. Each of them that displayed either lymphocyte infiltration or demyelination was assigned a score of one, thus, each animal had a potential maximum score of 16.

Isolation of total RNA and semiquantitative RT-PCR

Mice with EAE were randomly selected by cage numbers for mRNA isolation and analysis on day 20. Following perfusion with PBS, total RNA from the spinal cord tissue was extracted using TRI Reagent (Sigma-Aldrich) in accordance with the manufacturer’s protocol. If necessary, RNA was further purified by digestion using DNase I. Four micrograms of total RNA was reverse transcribed (RT) to cDNA using a GeneAmp RNA PCR kit with oligo(dT)16 primers (PerkinElmer, Branchburg, NJ; RT reaction). PCR amplification of each cDNA target (CD3ε, IFN-γ, IL-4, IL-12 p40, TNF-α, and inducible NO synthase (iNOS)) was performed from the same RT reaction as an internal control GAPDH. Each PCR contained 5 μl of cDNA, 2 μl of 10× PCR buffer (PerkinElmer), 1 μl of 25 mM MgCl₂, 0.5 μl of each dNTP (10 mM), 0.5 μl of sense and antisense gene-specific primers (50 pmol/μl), 0.25 μl of AmpliTaq DNA polymerase (5 units/μl; PerkinElmer), and 13.75 μl of nuclease-free H₂O, and was conducted in PTC-200 Peltier Thermal Cycler (MJ Research, Watertown, MA). The specific sense and antisense oligonucleotide PCR primers for each cDNA target are listed in Table I. Amplification conditions including annealing temperatures, number of cycles, and extension times were optimized for each target. PCR products were run on a 1.5% agarose gel containing 0.5 μg/ml ethidium bromide, and were visualized under UV light. The density of the band was quantitated using a Digital Imaging System (IS-1000 Version 2.0; Alpha Innotech, San Leandro, CA).

Results

DHEA inhibits lymphocyte proliferation in response to MBP

The addition of DHEA to the MBP-primed lymphocyte cultures resulted in a decrease of MBP-stimulated T cell proliferation, which was dependent on the doses of both DHEA and MBP (Fig. 1). Following stimulation with 100 μg/ml MBP, the proliferative response of T cells was suppressed at all concentrations of DHEA. When 50 μg/ml MBP was added, no inhibition was seen in the cultures with the addition of 5 μM of DHEA, but significant reduction was observed at 10 or 20 μM of DHEA. These results are similar to those observed in our earlier studies with keyhole limpet hemocyanin-primed lymphocytes (24), indicating that the inhibitory effect of DHEA on the Ag-stimulated T proliferation depends upon stimulation of Ag. In addition, although the background of [³H]thymidine incorporation was low in the cultures without stimulation of MBP, it was reduced by the presence of DHEA as well.

DHEA inhibits secretion of Th1-associated cytokines (IFN-γ, IL-12, and TNF-α) and NO in MBP-primed splenocyte cultures

We examined whether the inhibition of lymphocytic proliferation by DHEA was accompanied by a reduction in the levels of Th1-associated cytokines and NO. As shown in Fig. 2, the addition of DHEA reduced production of IFN-γ, IL-12 p40, TNF-α, and NO in MBP-stimulated culture supernatants in a dose-dependent manner. In cultures treated with 20 μM DHEA, inhibition of IFN-γ was complete (100% reduction). IL-12 p40 levels decreased from 3.5 ± 0.12 ng/ml to 1 ± 0.008 ng/ml (72% reduction), TNF-α decreased from 101.4 ± 9.7 pg/ml to 32.3 ± 3.3 pg/ml (68% reduction), and NO levels were reduced from 9.5 ± 0.5 μM to 2.4 ± 0.002 μM (75% reduction). These observations indicate that DHEA reduces Th1-mediated inflammatory responses in MBP-primed T cells following stimulation with Ag. In addition, Th2

FIGURE 1. DHEA inhibits Ag MBP-stimulated T cell proliferation. MBP-primed splenocytes were harvested from mice after 14 days of immunization with 400 μg of MBP in CFA on days 0 and 7. The splenocytes (2 × 10⁶ cells/well in a 96-well plate) were stimulated with indicated MBP in the presence or absence of DHEA. The data represent the mean values (cpm) and SD of triplicates at each point from a representative of three separate experiments. *, Significantly reduced from the controls (0 μM DHEA: addition of DMSO only), p < 0.001 (n = 9).

FIGURE 2. DHEA inhibits secretion of (pro)inflammatory cytokines/mediator in the cultures of MBP-primed splenocytes in response to the Ag. The MBP-primed splenocytes (2.5 × 10⁶ cells/ml) were stimulated with 75 μg/ml MBP in the presence or absence of DHEA for 72 h. The levels of cytokine (IFN-γ, IL-12 p40, and TNF-α) and NO in the supernatant of the cultures were determined by ELISA. The data represent the mean and SD of triplicates at each point from a representative of three separate experiments. *, Significantly reduced from the controls (0 μM DHEA: addition of DMSO only), p < 0.001 (n = 9).
cytokine IL-4 production in these cultures following stimulation of MBP was very low (64 ± 10 pg/ml), and was reduced to undetectable levels by the presence of DHEA.

**DHEA inhibits Ag-stimulated nuclear translocation of NF-κB**

The activation of NF-κB is a prerequisite for the expression of a number of proinflammatory cytokines and mediators, including TNF-α, IL-12 p40, IL-2, and iNOS (4, 29). To determine whether the molecular mechanism of DHEA action occurred at a site proximal or distal to NF-κB, we examined the effect of DHEA on the induction of NF-κB activation by EMSA in MBP-primed splenocytes. As shown in Fig. 3, nuclear translocation of NF-κB was seen in response to stimulation with MBP in the MBP-primed splenocytes. Supershift studies showed that the migration of NF-κB was retarded completely by anti-p50 Ab and to a lesser degree by anti-p65 Ab, indicating that the NF-κB complex included, at least in part, a heterodimer of p50/p65. Because the entire panel of anti-NF-κB Abs was not examined, the presence of other Rel proteins and homodimers of p50/p50 could not be excluded. The addition of DHEA to the cultures of MBP-primed splenocytes reduced the activation of NF-κB in a dose-dependent manner (Fig. 4). Using a digital image system to quantify the levels of NF-κB, the translocation of NF-κB to the nucleus was inhibited by 43% following the addition of 20 μM DHEA (Fig. 4B). Furthermore, kinetic studies showed that in the cultures treated with 10 μM DHEA, inhibition occurred at as early as 1 h and reached it maximum at 8 h (Fig. 5). These data suggest that DHEA inhibits T cell proliferation and proinflammatory cytokine/mediator production at least in part by inhibiting translocation of NF-κB. Because mixed cultures were used in these investigations, our studies do not identify the phenotype of the cell in which the effects of DHEA on NF-κB activation are maximal.

**DHEA prevents development of EAE**

The development of EAE is closely regulated by the expression of proinflammatory cytokines/mediators (30). Our data has shown that DHEA inhibited immune responses of MBP-specific T cells against MBP by decreasing production of (pro)inflammatory cytokines and NO and/or proliferation. Therefore, we predicted that parental administration of DHEA in vivo would alter the development of autoantigen specific T cells and thereby the incidence and severity of EAE.

When EAE was induced by active immunization in the absence of pertussis toxin (Fig. 6A), only one of six mice treated with DHEA was paralyzed (mean maximal clinical score: 0.17), whereas in the vehicle-treated group, five of six mice were paralyzed (mean maximal clinical score: 1.42; p < 0.01). In the second experiment, EAE was induced with the addition of pertussis toxin (Fig. 6B). Seven of nine DHEA-treated mice developed clinical paralysis of EAE. All nine mice treated with vehicle were paralyzed with high severity, and one died from disease attack. The severity of EAE was reduced by DHEA treatment from a mean maximal clinical score of 2.82 in the control group to 1.25 (p < 0.01). In the adoptive transfer model of EAE (Fig. 6C), none of the nine mice that received DHEA developed clinical paralysis of

![FIGURE 3](http://www.jimmunol.org/) Activation of NF-κB in MBP-primed splenocytes following stimulation with MBP. The MBP-primed or naive splenocytes (2.5 × 10⁶ cells/ml) were stimulated with 75 μg/ml MBP for 3 h. NF-κB in the nuclear extracts (5 μg of total protein) was identified by EMSA with 32P-labeled NF-κB oligonucleotide probe (pointed by a big arrow), which was further confirmed by supershift assay using anti-p50 and anti-p65 Abs (pointed by small arrows; representative of three experiments).

![FIGURE 4](http://www.jimmunol.org/) DHEA inhibits activation of NF-κB in MBP-primed splenocytes in a dose-dependent manner. The MBP-primed splenocytes (2.5 × 10⁶ cells/ml) were stimulated with 75 μg/ml MBP in the presence or absence of DHEA for 3 h. The levels of NF-κB and internal control NF-Y in the nuclear extracts (5 μg of total protein) were determined by EMSA with their own specific binding probes. A, Autoradiographs of NF-κB and NF-Y in the nuclear extracts; B, The levels of NF-κB and NF-Y on the autoradiograph were quantitated using a digital imaging system. In each sample, the activation of NF-κB was normalized with internal control NF-Y, and the inhibition (%) of NF-κB was calculated by the decrease from the DHEA-untreated sample (representative of three experiments).
EAE, whereas seven of nine mice in the control group developed clinical signs of EAE with a mean maximal clinical score of 1.2 (p < 0.001). These data indicate that administration of DHEA protects animals from the development of active and adoptively transferred EAE.

To confirm whether the beneficial actions of DHEA against clinical paralysis were due to a decrease in demyelination and inflammation in the CNS, the spinal cords of EAE mice were examined histologically (Fig. 7). In the mice receiving DMSO (mean clinical score: 1.33), the mean pathologic scores were 3.5 for demyelination and 4.5 for lymphocyte infiltration. However, in DHEA-treated mice (mean clinical score: 0), the score of demyelination or lymphocytes infiltration was reduced to 0.4 or 0.7, respectively (Fig. 7B). These data indicate that the reduction of EAE severity in the DHEA-treated mice closely correlates with a decrease of demyelination and/or lymphocyte infiltration in the CNS.

**DHEA reduced Th1 immune responses in the CNS of mice with EAE**

To examine whether the reduction of demyelination/lymphocyte infiltration following DHEA treatment was accompanied by a decrease in inflammatory mediators in the CNS, we analyzed the expression of inflammation/Th1-related genes (CD3γ, IFN-γ, IL-12 p40, IL-4, TNF-α, and iNOS) from the spinal cord of mice with EAE. As shown in Fig. 8, four mice randomly selected from the DHEA-treated group showed a decrease in levels of all targeted mRNA (CD3γ, IFN-γ, IL-12 p40, TNF-α, and iNOS) as compared with four mice from the vehicle-treated group. The level of each mRNA in each mouse closely correlated with its clinical score. The digital image analysis indicated that the levels of expression of CD3γ, IFN-γ, IL-12 p40, TNF-α, and iNOS were higher in the vehicle-treated animals (clinical scores: 3, 2, 3, and 3) than those in the DHEA-treated mice (clinical scores: 0, 2, 0, and 0; p < 0.05; Fig. 8B). Among the DHEA-treated mice, one animal with paralysis showed comparable levels of the presence of T cells (marked by CD3γ) and (pro)inflammatory cytokines/mediator in the CNS, but in the remaining animals in which no clinical paralysis was observed, the levels of all targeted mRNA were either lower (one mouse) or the same as seen in the naive animal. No expression of IL-4 was seen, and this would argue against a switch in the immune response from Th1 to Th2 following treatment with DHEA. These data indicate that in vivo administration of DHEA reduces Th1-mediated autoimmune responses in the CNS via inhibition of T cell proliferation and/or production of Th1-related cytokines and inflammatory mediator; as a result, it prevents EAE.
Discussion

Our studies show a profound anti-inflammatory effect of DHEA on the immune response to MBP and on the development of EAE. We have shown three major effects of DHEA on immune function: DHEA inhibits T cell proliferation and secretion of (pro)inflammatory cytokines/mediators in Ag-activated cultures of MBP-primed splenocytes; DHEA inhibits activation and translocation of NF-κB in splenocytes following stimulation with Ag; and in vivo administration of DHEA inhibits Th1-mediated inflammatory responses in the CNS, and ameliorates the development of EAE.

DHEA is the most abundant steroid in the circulation of humans and many other warm-blooded animals (9, 31). Plasma levels of DHEA peak in early adulthood (reaching levels of 2–4 ng/ml) and then decline through adult life (9). In adult mice, DHEA levels are ~1 ng/ml and they dramatically increase following parental administration of DHEA (32). In the present investigation, the
DHEA doses (2–20 μM) we tested in vitro are undoubtedly high when compared with its levels in sera, but the levels of local and intracellular DHEA in the steroid target tissue are uncertain. The average serum DHEAS level in healthy “younger” adults is ~7.5 μM, and increases to 20–70 μM following administration of pharmacological dosages of DHEA without any sign of systemic toxicity (33). These high levels of recirculating DHEA can be converted to DHEA by ubiquitous steroid sulfatase. Therefore, DHEA levels in plasma may not reflect the real concentrations of DHEA in the target tissue, which could be much higher than that observed in the circulation.

The ability of DHEA to regulate proliferation and cytokine production suggests its potential role in mediating inflammatory response. Prior studies of DHEA on T cell function have shown conflicting results. It has been reported that DHEA enhances IL-2 production and, consequently, T cell proliferation in murine and human T cells (34, 35), whereas others have noted an inhibition of lymphocyte proliferation and IL-2 production in the presence of DHEA (36–38). Our previous work also has demonstrated that the addition of DHEA in vitro inhibits T lymphocyte proliferation stimulated by ligation of TCR complex and favors the induction of a Th2 immune response to Ag (24). In LPS-stimulated macrophages, DHEA reduces the production of IL-12 (24), TNF-α, IL-1 (39), and NO (data not shown). In this study, the most dramatic effect of DHEA was in the suppression of the T cell proliferative response and the development of EAE.

EAE is a Th1 cell-mediated inflammatory autoimmune disease of the central nervous system that serves as a prototypic animal model for MS. The suppression of this disease by DHEA (Fig. 6) disagrees with the data presented by Kipper-Galperin et al. (40). This conflicting result could be due to the differences in the dosage and the route of administration of DHEA. In this study, we used 2 mg/mouse every day by s.c. injection, by which way the highest bioavailability of DHEA in vivo is obtained (41). Although Kipper-Galperin et al. gave 0.5 mg/mouse every other day by i.p. injection (40), that may not be enough to suppress the autoimmune responses in the EAE animals.

The mechanism by which DHEA mediates its effect on T cells in vitro and in vivo is not known. So far, all known steroid receptors belong to a group of nuclear (orphan) receptors, including those for glucocorticoid and progesterone. They undergo structural alternation upon binding to the steroid ligands, conferring upon it the ability to bind DNA and regulate gene transcription (42). The receptors for DHEA/DHEAS are not identified yet, but DHEA binding activity has been detected in murine and human T cells, suggesting that receptors for DHEA may be present in T cells (43, 44). The action of DHEA in vivo is also not fully understood. One of the important questions is whether exogenous DHEA acts directly on T cells and/or other immune cells in vivo as it does in vitro. In many peripheral steroid tissues such as the prostate and mammary glands, DHEA can be converted into androgens and/or estrogens (45), which also have immunomodulatory functions. However, a role independent of its metabolic functions and, in particular, on immune regulation has been previously observed (31, 46). For example, DHEA was shown to have immunomodulatory properties in androgen-unresponsive mice (47). Further studies using an inhibitor of DHEA metabolic conversion or androgen/estrogen receptors double knockout mice are needed to clarify this point.

NF-κB is clearly one of the most important transcriptional factors regulating expression of many proinflammatory genes, including IL-2, IL-12 p40, TNF-α, IL-1, and iNOS (4, 29). Our gel shift assay showed that activation of NF-κB was reduced in the presence of DHEA in Ag-primed splenocytes (Figs. 4 and 5), indicating that the inhibitory action of DHEA on nuclear translocation of NF-κB is a key mechanism mediating the reduction of the proliferative response and (pro)inflammatory cytokine production. GRs, structurally similar to DHEA, are known to inhibit the activation of NF-κB. Activated GR directly inhibits activation of NF-κB subunits and/or up-regulates transcriptional activity of IkBα, resulting in inactivation of NF-κB (48). It is possible that activated DHEA receptor inhibits NF-κB activation in Ag-primed splenocytes in our study through one of these mechanisms.

It has been demonstrated that the development of the autoimmune disease EAE requires the activation of inflammation-related genes such as IL-12, TNF-α, and iNOS (2, 49). Virtually all of these genes have shown the presence of binding sites for NF-κB in their promoter regions (4, 29). In rats with EAE, the activation of NF-κB (p50/p65) is seen in the spinal cord and persists throughout the disease (50, 51). Disruption of the NF-κB gene or treatment with pyrroline dihidrocarbomate, an inhibitor of NF-κB activation, prevents the development of EAE (51–53). We believe that the immunosuppressive effect of DHEA on EAE is closely associated with the inhibition of activation of NF-κB. Because glial cells synthesize DHEA (10), our findings imply that the presence of the neurosteroid DHEA may protect the CNS against autoimmune injury. Additionally, it is possible that genetic differences in the levels of DHEA in the brain and/or plasma may predict susceptibility to autoimmune diseases including EAE.

In conclusion, a number of strategies aimed at interrupting the proinflammatory cascade are currently being applied to the treatment of human diseases. Invariably, they are initially tested in experimental model systems with the hope that they will be successful in human disease. Our data show that DHEA has potent anti-inflammatory properties in vitro and in preventing the development of EAE. Furthermore, because the inhibition of EAE was seen in adoptive transfer model system, it is likely that the effect of DHEA is present even after the generation of an immune response. Because DHEA does not possess the undesirable side effects of glucocorticoids, it has the potential to be applied to the treatment of chronic inflammatory diseases in the CNS such as MS.

Acknowledgments

We thank Sandy Watkins for her administrative assistance and Åsa Ljunggren-Rose for her technical assistance.

References


