# Effect of Dehydroepiandrosterone Sulfate on Maturation and Functional Properties of Interferon-α-Induced Dendritic Cells

**O. Yu. Leplina, M. A. Tikhonova, L. V. Sakchno, T. V. Tyrinova, A. A. Ostanin, and E. R. Chernykh**

Translated from *Byulleten' Eksperimental'oi Biologii i Meditsiny*, Vol. 148, No. 7, pp. 80-84, July, 2009
Original article submitted December 10, 2008

We studied the effect of adrenal cortex hormone dehydroepiandrosterone sulfate on maturation and functional activity of interferon-α-induced dendritic cells. Dehydroepiandrosterone sulfate stimulated differentiation and maturation of interferon-α-induced dendritic cell, which manifested in a decrease in the number of $CD14^+$ cells and increase in the ratio of mature $CD83^+$ dendritic cells expressing costimulatory molecules (CD80 and CD86). The induction of dendritic cell differentiation after treatment with dehydroepiandrosterone sulfate was accompanied by an increase in the production of interferon-γ. At the stage of dendritic cell maturation, the effect of dehydroepiandrosterone sulfate manifested in a 4-fold increase in tumor necrosis factor-α production. Dehydroepiandrosterone sulfate had little effect on the production of Th2/antiinflammatory cytokines at the stages of differentiation and maturation of interferon-α-induced dendritic cells. Dehydroepiandrosterone sulfate increased the ability of dendritic cells to stimulate Th1 cytokine production by T cells (interferon-γ). This hormone had no effect on the ability of interferon-α-induced dendritic cells to activate $CD3^+IL\text{-}4^+T$ cells in mixed lymphocyte culture.

**Key Words:** *dendritic cells; phenotype; cytokines; dehydroepiandrosterone sulfate*

Many functions of the immune system are regulated by hormones. Steroid hormones play a particular role in this respect. They are involved in the regulation of nearly all vital processes and play a role under pathological conditions. Glucocorticoids are produced by the adrenal cortex and exhibit antiinflammatory and immunosuppressive properties. Biologically, adrenal cortex hormone dehydroepiandrosterone (DHEA) and its sulfated form (dehydroepiandrosterone sulfate, DHEAS) are natural antagonists of glucocorticoids. DHEAS concentration in the peripheral blood is much higher than the content of other steroid hormones. It can be hypothesized that this hormone has a modulatory effect on the immune system. DHEAS can stimulate the cellular immune response. For example, DHEAS activates type 1 T helper cells (Th1) and natural killer cells (NK cells) [4,10]. Polarization of T helper cells by the Th1 or Th2 pathway and activation of NK cells are realized with the involvement of dendritic cells (DC) [5]. The role of DHEAS in DC regulation and involvement of DC in the immunostimulatory effect of this hormone remain unknown. Recent studies showed that DC express receptors for steroid hormones and are hormonally regulated by glucocorticoids, estrogens, and gestagens [7]. However, little is known about the effect of DHEAS on maturation and functions of DC.

DC are obtained by culturing of adherent mononuclear cells in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) and further treatment with a "cocktail" of ma-

Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** ct_lab@mail.ru. O. Yu. Leplina

turing cytokines (IL-1, IL-6, TNF-α, and prostaglandin $E_2$) [1,9]. However, recent experiments showed that partially mature DC are rapidly generated during culturing of monocytes with GM-CSF and interferon-α (IFN-α). This approach seems to be more physiological [11]. The so-called "interferon DC" (IFN-DC) have high migratory activity due to the expression of CC chemokine receptors R5 (CCR5) and R7 (CCR7) [8,12], activate the Th1 response, and can stimulate the Th2 response [3]. Here we studied the effect of DHEAS on differentiation, maturation, and functional activity of IFN-DC.

## MATERIALS AND METHODS

The study was performed with 23 healthy donors. Mononuclear cells were obtained by centrifugation of the heparinized venous blood in a Ficoll-Verografin density gradient. Monocytes were isolated on 33-mm Petri dishes (Nunclon) by adherence of mononuclear cells ($2\text{-}5\times10^6$ cells/ml) to plastic in the presence of 10% serum (AB(IV) group). DC were obtained by culturing of adherent mononuclear cells in 6-well plates (Nunc) with RPMI 1640 medium (Sigma-Aldrich) containing 0.3 mg/ml L-glutamine, 5 mM HEPES buffer, 100 μg/ml gentamicin, and 5% fetal bovine serum (BioloT) in the presence of GM-CSF (40 ng/ml, Sigma-Aldrich) and IFN-α (1000 U/ml, Roferon-A, Roche). Culturing was performed in a $CO_2$ incubator at 37°C for 5 days. DC maturation was induced by lipopolysaccharide (LPS, 10 μg/ml, *E. coli* 0114:B4, Sigma-Aldrich). LPS was added 24 h before the end of culturing. For evaluation of the effect of DHEAS (Sigma-Aldrich, catalogue number D5297) on DC phenotype, the hormone in a concentration of $10^{-6}$ M was added at the start of culturing or at the stage of maturation (24 h before the end of culturing). DC were phenotyped by means of single-color or two-color flow cytofluorometry (FACSCalibur, Becton Dickinson) with CellQuest software (Becton Dickinson). Surface markers were studied with phycoerythrin-labeled anti-CD1a, anti-CD123 mAB (BD PharMingen), anti-CD14, anti-CD16 mAB (Sorbent), allophycocyanin-labeled anti-CD11c mAB (BD PharMingen), FITC-labeled anti-CD25, anti-CD83 mAB (BD PharMingen) and anti-CD56 mAB (Sorbent).

Allostimulatory activity of DC was evaluated in a mixed culture of lymphocytes. Mononuclear cells from donors ($0.1\times10^6$ cells/well) were used as the responding cells. DC (mononuclear cell/DC ratio 10:1) served as stimulators. The proliferative response was assayed radiometrically by $^3$H-thymidine incorporation (1 μCi/well) on day 5 of the study. $^3$H-thymidine was added 18 h before the end of culturing.

The production of Th1/proinflammatory (IFN-γ, IL-2, IL-12(p70), and TNF-α) and Th2/antiinflammatory cytokines (IL-4, IL-5, IL-13, and IL-10) by DC was studied with 5-day-old supernatants of intact and DHEAS-modified DC. The measurements were performed by means of flow fluorometry on a dual-beam laser automated analyzer (Bio-Plex Protein Assay System, Bio-Rad) with commercial test systems according to manufacturer's recommendations.

The expression of intracellular cytokines in a population of T cells stimulated with allogeneic DC was studied by the method of three-color flow cytometry. Mononuclear cells (monocyte-free) were cultured in 96-well plates with complete culture medium that contained 10% fetal bovine serum in the absence or presence of DC (ratio 10:1) for 72 h. Brefeldin (10 μg/ml, ISN) was added to the culture 18 h before the end of incubation. The cells were washed and incubated with 5 μl allophycocyanin-labeled monoclonal anti-CD3 antibodies (Becton Dickinson) at room temperature for 15 min. Then the cells were permeabilized with 0.2% Tween 20 and incubated with FITC-conjugated anti-IFN-γ and phycoerythrin-labeled anti-IL-4 antibodies (Becton Dickinson). The samples were analyzed on a flow cytometer with CellQuest software.

The results were analyzed using Statistica 5.0 software.

## RESULTS

Addition of DHEAS on day 1 of culturing (Table 1) was accompanied by a significant decrease in the relative number of $CD14^+$ monocytes and increase in the percentage of cells expressing costimulatory molecules CD80 and CD86, which attests to the stimulatory effect of DHEAS on IFN-DC differentiation. Addition of DHEAS at the stage of maturation (in combination with LPS, 24 h before the end of culturing) was also followed by a decrease in the percentage of $CD14^+$ cells and significant increase in the number of $CD86^+$ cells. We revealed a significant increase in the number of cells that carried a marker of mature DC ($CD83^+$). The percentage of activated CD25-positive cells tended to increase under these conditions. At the stages of DC differentiation and maturation, the decrease in the relative number of $CD14^+$ cells in the presence of DHEAS was associated with a slight increase in the count of $CD1a^+$ cells. The observed changes reflect differentiation of additional amounts of monocytes into immature $CD1a^+$-DC. DHEAS had no effect on the expression of CD123, which is typical of plasmacytoid DC. Changes in the expression of stage-specific and costimulatory molecules on DC after treatment with DHEAS attest to the stimulatory effect of this hormone on differentiation and maturation of IFN-DC. Our findings are consistent with the results of previous experiments, where the effect of DHEAS on the production of DC

**TABLE 1.** Effect of DHEAS on Differentiation and Maturation of IFN-DC

| CD marker | In the absence of LPS | | In the presence of LPS | |
|---|---|---|---|---|
| | without DHEAS | in the presence of DHEAS | without DHEAS | in the presence of DHEAS |
| CD14 | 14.1±4.6 | 10.3±2.9* | 14.1±2.1 | 10.4±2.7 |
| CD1a | 9.9±6.3 | 42.7±6.5 | 39.1±5.4 | 44.1±5.5 |
| CD83 | 14.3±3.3 | 19.2±5.2 | 17.0±3.0 | 25.6±6.4* |
| CD123 | 40.4±9.1 | 43.1±9.3 | 60.6±6.0 | 61.9±6.4 |
| CD86 | 42.10±5.94 | 49.1±6.8* | 72.8±3.0 | 84.10±1.42* |
| CD80 | 59.4±10.0 | 75.6±7.2* | 64.4±6.6 | 73.2±9.4 |
| CD25 | 13.2±2.1 | 18.7±3.8 | 33.9±5.1 | 39.5±6.0 |
| CD11c | 8.9±3.1 | 19.9±3.0 | 18.6±2.8 | 19.9±3.0 |

**Note.** The data ($n$=13) are presented as a percentage of cells ($M\pm S.E.$) expressing the corresponding marker. Here and in Table 2: *$p$<0.05 compared to DC in the absence of DHEAS (paired Mann—Whitney $U$ test).

**TABLE 2.** Effect of DHEAS on Production of Th1/Proinflammatory Cytokines and Th2/Antiinflammatory Cytokines during Differentiation and Maturation of IDN-DC

| Cytokines, pg/ml | In the absence of LPS | | In the presence of LPS | |
|---|---|---|---|---|
| | without DHEAS | in the presence of DHEAS | without DHEAS | in the presence of DHEAS |
| Th1/proinflammatory | | | | |
| IFN-γ | 164±105 (38.8) | 316±177* (84.5) | 830±197 (836) | 910±137 (828) |
| IL-2 | 19.7±3.9 (15.2) | 18.8±4.9 (14.0) | 38.3±4.1 (36.8) | 40.0±3.9 (39.7) |
| IL-12(p70) | 0.5±0.3 (0.2) | 3.3±2.2 (0.8) | 10.2±3.8 (6.5) | 37.0±22.7 (8.0) |
| TNF-α | 123±87 (43.7) | 143±80 (49.3) | 627±218 (282) | 2311±657* (1669) |
| Th2/anti-inflammatory | | | | |
| IL-4 | 53.3±14.8 (41.5) | 65.1±14.6 (45.1) | 204.0±40.3 (210) | 214±29 (187) |
| IL-5 | 1.2±0.4 (0.7) | 1.2±0.3 (0.9) | 1.6±0.2 (1.4) | 1.8±0.5 (1.7) |
| IL-13 | 22.4±7.2 (41.5) | 20.0±5.5 (18.5) | 37.0±5.1 (39.6) | 34.5±4.7 (30.0) |
| IL-10 | 11.9±3.0 (11.5) | 13.5±4.0 (10.4) | 94.0±46.6 (57.3) | 113±55 (68) |

**Note.** The data ($n$=10) are presented as $M\pm S.E.$ (median value). DHEAS at a concentration of $10^{-6}$ M was added to the culture of DC at the initial stage (effect on differentiation) or in combination with LPS (effect on maturation).

induced in the presence of GM-CSF and IL-4 was evaluated [2]. The stimulatory effect of DHEAS on DC differentiation manifested in an increase in the number of CD1a-positive and CD80-positive cells. We showed that the stimulatory effect of DEHAS on IFN-DC manifested not only at the stage of differentiation, but also during DC maturation.

Differentiation and maturation of DC are accompanied by changes in the range and amount of secreted cytokines [9]. The concentration of cytokines was measured in culture supernatants of unstimulated DC (Table 2). IFN-DC were characterized by high production of IFN-γ and TNF-α and moderate secretion of IL-2. IL-12(p70) production was nearly undetected. DC maturation in the presence of LPS was accompanied by an increase in the production of IFN-γ, TNF-α, and IL-2 (by 5, 4, and 2 times, respectively). IL-12(p70) was found in supernatants. Studying the production of Th2/antiinflammatory cytokines showed that IFN-DC secrete a moderate amount of IL-4, IL-13, and IL-10, but not of IL-5. The concentration of these cytokines (particularly of IL-4 and IL-10) increased significantly during DC maturation in the presence of LPS. Addition of DHEAS at the stage of differentiation was followed by a significant increase in the production of IFN-γ. However, the concentration of IFN-γ under these conditions was lower than during LPS stimulation. The effect of DHEAS at the stage of DC maturation (combined treatment with the hormone and LPS 24 h before the end of culturing) was manifested in a 4-fold increase in TNF-α production. DHEAS had little effect on the production of Th2/

**TABLE 3.** Effect of Intact and DHEAS-Modified DC on Activation of T1 Cells and T2 Cells in a Mixed Culture of Lymphocytes

| % $CD3^+T$ cells | 0 | $+DC_{INT}$ | IE | 0 | $+DC_{DHEAS}$ | IE |
|---|---|---|---|---|---|---|
| IFN-$\gamma^+$ | 3.1±0.3 | 17.6±1.8* | 6.2±0.8 | 3.1±0.3 | 27.9±3.6*+ | 10.1±1.7 |
| IL-$4^+$ | 2.0±0.4 | 6.7±1.6* | 3.4±0.8 | 2.00±0.42 | 6.8±1.1* | 3.9±0.4 |

**Note.** Relative number of $CD3^+T$ cells with intracellular expression of IFN-γ and IL-4 in a mixed culture of lymphocytes in the presence of intact ($+DC_{INT}$) and DHEAS-modified DC ($+DC_{DHEAS}$). The hormone was added during differentiation of IFN-DC. IE, index of effect of DC on the number of T cells expressing IFN-γ and IL-4. $p<0.05$: *compared to cultures without DC; +compared to intact DC (Mann—Whitney *U* test).

antiinflammatory cytokines during differentiation and maturation of IFN-DC.

The effect of DHEAS on the ability of DC to stimulate the proliferative response in a mixed culture of lymphocytes was studied to evaluate the influence of this hormone on functional activity of IFN-DC. Addition of the hormone during differentiation and maturation of DC was followed by a moderate, but statistically insignificant increase in allostimulatory activity of DC (16,930±1190 *vs*. 14,290±1110 cpm; and 19,910±1870 *vs*. 18,780±1190 ppm, respectively; $n$=10).

The effect of DHEAS on functional activity of IFN-DC was also evaluated from the ability of intact and DHEAS-modified DC to stimulate T1 and T2 cells in a mixed culture of lymphocytes. We measured the relative number of $CD3^+T$ cells with intracellular expression of IFN-γ and IL-4. Intact DC induced an increase in the number of IFN-$\gamma^+T$ cells (effect index 6.2±0.8), but had less pronounced effect on $CD3^+$IL-$4^+T$ cells (effect index 3.4±0.8; Table 3). The increase in the number of IFN-$\gamma^+T$ cells in the presence of DHEAS-modified DC was more pronounced (up to 27.9±3.6%, effect index 10.1±1.7). The number of $CD3^+$IL-$4^+T$ cells in a mixed culture of lymphocytes induced by intact and DHEAS-modified DC increased similarly. Therefore, DHEAS significantly increased the ability of DC to stimulate Th1 cytokine production by T cells (IFN-γ). However, this hormone had little effect on the production of Th2 cytokines by T cells (IL-4).

We conclude that the immunostimulatory effect of DHEAS is mediated by DC. For example, the hormone in physiological concentrations has a stimulatory effect on differentiation and maturation of IFN-DC. The effect of DHEAS on DC maturation is probably related to the increased production of endogenous TNF-α. This cytokine serves as a potent maturation-inducing agent for DC [6]. The ability of DHEAS to increase the expression of costimulatory molecules and production of IFN-γ contributes to the stimulatory effect of this hormone on activation of type 1 T cells under the influence of DC. The increased production of IFN-γ by DC also mediates the stimulatory effect of DHEAS on NK cells [13]. DHEAS-modified DC cannot activate type 2 T cells, which is probably related to the absence of hormonal influence on the production of Th2/antiinflammatory cytokines by DC (IL-4, IL-5, IL-13, and IL-10).

This work was supported by the Russian Foundation for Basic Research (grants No. 07-04-00967 and No. 08-04-00700).

## REFERENCES

1. J. Banchereau and R. M. Steinman, *Nature*, **392**, 245-252 (1998).
2. M. O. Canning, K. Grotenhuis, H. J. de Wit, and H. A. Drexhage, *Eur. J. Endocrin*., **143**, No. 5, 687-695 (2000).
3. S. Della Bella, S. Nicola, A. Riva, *et al*., *J. Leuk. Biol*., **75**, No. 1, 106-116 (2004).
4. C. Dosiou and L. C. Giudice, *Endocr. Rev*., **26**, No. 1, 44-62 (2005).
5. H. Ida, P. J. Utz, P. Anderson, and K. Eguchi, *Mod. Rheumatol*., **15**, No. 5, 315-322 (2005).
6. H. Jonuleit, U. Kuhn, C. Muller, *et al*., *Eur. J. Immunol*., **27**, No. 12, 3135-3142 (1997).
7. T. Okabe, M. Haji, R. Takayanagi, *et al*., *J. Clin. Endocrinol. Metab*., **80**, No. 10, 2993-2996 (1995).
8. S. Parlato, S. M. Santini, C. Lapenta, *et al*., *Blood*, **98**, No. 10, 3022-3029 (2001).
9. W. F. Pickl, O. Majdic, P. Kohl, *et al*., *J. Immunol*., **157**, No. 9, 3850-3859 (1996).
10. R. Raghupathy, *Semin. Immunol*., **13**, No. 4, 219-227 (2001).
11. S. M. Santini, C. Lapenta, M. Logozzi, *et al*., *J. Exp. Med*., **191**, No. 10, 1777-1788 (2000).
12. S. M. Santini, T. Di Pucchio, C. Lapenta, *et al*., *Stem Cells*, **21**, No. 3, 357-362 (2003).
13. B. Solerte, S. Precerutti, C. Gazzaruso, *et al*., *Eur. J. Endocrin*., **152**, No. 5, 703-712 (2005).