Contribution of Dehydroepiandrosterone Sulfate and Progesterone to *In Vitro* Regulation of Tolerogenic Activity of IFN-α-Induced Dendritic Cells

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Dehydroepiandrosterone sulfate and progesterone exhibited an immunomodulatory effect on the tolerogenic characteristics of IFN-α-induced dendritic cells. The hormone effects depended on the initial level of allostimulatory activity of dendritic cells in mixed lymphocyte culture. However, dehydroepiandrosterone sulfate significantly more often stimulated allostimulatory activity by attenuating the tolerogenic properties of dendritic cells, while progesterone potentiated their tolerogenic potential. The capacity of the hormones (dehydroepiandrosterone sulfate and progesterone) to attenuate tolerogenic activity of dendritic cells was associated with reduction of FasL expression on these cells, while the increase in tolerogenic activity was associated with the increase in the percentage of $CD123^+$ dendritic cells, and under conditions of modification with dehydroepiandrosterone sulfate it was associated with increased B7-H1 expression. Possible contribution of indolamine-2,3-dioxygenase and prostaglandin E2 to stimulation of tolerogenic characteristics of dendritic cells modified with dehydroepiandrosterone sulfate and progesterone, respectively, was demonstrated.

Key Words: *dendritic cells; tolerogenic activity; dehydroepiandrosterone sulfate; progesterone*

Hormone modulation of the immune system is a well-known fact. The effects of different hormone can be opposite. Dehydroepiandrosterone and its sulfated form (DHEAS) stimulate, while progesterone (PG) inhibits activities of Th1 and NK cells [8,9]. Hence, changes in the hormone levels may be essential for the immune response. For example, reduction of PG level in pregnant women with the lutein phase insufficiency and/or elevation of DHEAS in women with adrenal hyperandrogenia are paralleled by disorders in the "suppressor" rearrangement of the immune system and development of gestosis [2]. Recent studies showed that dendritic cells (DC) acquiring tolerogenic characteristics in normal pregnancy play the key role in the reorganization of the immune system during gestation [10,14]. On the other hand, DHEAS and PG modulate DC differentiation and maturation [1,3,9,15]. These facts suggest that DHEAS and PG play an important role in the regulation of tolerogenic/regulatory activity of DC and prompted this study. DC are usually generated by culturing adherent fraction of mononuclear cells (MNC) with GM-CGF and IL-4 with subsequent maturation of DC (IL4-DC) in the presence of the cytokine "cocktail" or LPS. In addition, partially mature DC could be generated from monocytes after replacement of IL-4 for IFN-α [11];

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the resultant IFN-DC population was characterized by high individual heterogeneity, as it included cells with the mature, intermediate, and immature DC phenotypes. According to modern concepts, tolerogenic/regulatory activity of IL4-DC was associated with immature DC phenotype and manifested *in vitro* by low capacity of DC to induce proliferation of T cells in mixed lymphocyte culture, while mature IL4-DC were characterized by high allostimulatory activity. We analyzed the effects of DHEAS and PG on the tolerogenic characteristics of IFN-DC and studied the role of coinhibitory/proapoptotic molecules and of indolamine-2,3-dioxygenase and prostaglandin E2 in hormone-mediated regulation of DC.

MATERIALS AND METHODS

The study was carried out on blood specimens from 36 donors. Mononuclear cells were isolated by centrifugation of heparin-treated venous blood in Ficoll-verografin density gradient. Monocytes were isolated in Petri dishes (33 mm^2; Nunclon) by adhesion of MNC ($2\text{-}5\times10^6$/ml) to plastic in the presence of 10% AB(IV) group serum. Dendritic cells were generated by culturing the adherent MNC fraction in 6-well plates (Nunc) for 5 days in RPMI-1640 (Sigma-Aldrich) with 0.3 mg/ml L-glutamine, 5 mM HEPES buffer, 100 μg/ml gentamicin, and 5% fetal calf serum (BioloT) in the presence of GM-CSF (40 ng/ml, Sigma-Aldrich) and IFN-α (1000 U/ml, Roferon-A, Roche) at 37°C in a CO_2 incubator. The DC maturation was induced by adding LPS (10 μg/ml, *E. coli* 0114:B4 LPS, Sigma-Aldrich) 24 h before the end of culturing. The effects of DHEAS (10^{-6}, M, Sigma-Aldrich) or PG (100 ng/ml, Sigma-Aldrich) on DC was evaluated by adding the hormones during the maturation stage 24 h before the end of culturing. Phenotyping of DC was carried out by flow cytofluorometry (FACSCalibur, Becton Dickinson) using Cell Quest software (Becton Dickinson). Surface markers were evaluated using monoclonal antibodies labeled with phycoerythrin (anti-CD123, B7-H1, and TRAIL) and FITC-labeled anti-FasL antibodies (BD PharMingen). Allostimulatory activities of intact and hormone-modified DC (DC_{int}, DC_{DHEAS}, and DC_{PG}) were evaluated in a mixed lymphocyte culture. Donor MNC served as the respondent cells (0.1×10^6/well). Dendritic cells in 10:1 MNC:DC proportion served as the stimulants. The proliferative response was evaluated on day 5 by radiometry (by incorporation of ^{3}H-thymidine, 1 μCi/well; added 18 h before the end of culturing).

In order to evaluate possible involvement of prostaglandin E2 in the realization of IFN-DC tolerogenic characteristics, hormone-modified DC were additionally incubated (45 min, 37°C) with indomethacin (Sigma-Aldrich) in a dose of 50 μg/ml and after washout allostimulatory activity was compared with intact and hormone-modified DC without indomethacin treatment. In order to evaluate possible contribution of indolamine-2,3-dioxygenase, allostimulatory activities of intact and hormone-modified DC were evaluated without and with the indolamine-2,3-dioxygenase substrate and inhibitor analog 1-methyltryptophane (95%, Sigma-Aldrich) in a dose of 500 μmol/ml.

The results were mathematically processed using Statistica 6.0 software.

RESULTS

Functional activity of *in vitro* generated IFN-DC was evaluated in mixed lymphocyte culture with MNC as respondent cells and allogenic donor DC as stimulatory cells (1:10). The intensity of lymphocyte proliferative response in the presence of intact DC was 15,210±1560 cpm (n=36). The individual values of allostiomulatory activity of donor DC varied from 4240 to 44,680 cpm. These data indicate functional heterogeneity of donor IFN-DC, which were generated *in vitro* under identical culturing conditions, but differed from each other by the levels of allostimulatory activity in mixed lymphocyte cultures, presumably because of different proportions of immature, semimature, and mature DC in the total population.

TABLE 1. Distribution of IFN-DC and Patterns of Their Response to Hormone Treatment

Hormone treatment effects on functional activity of DC in mixed lymphocyte culture	DC_{DHEAS}		DC_{PG}		P_{EFT}
	%	abs.	%	abs.	
Stimulatory	53	19	25	9	0.0287
Neutral	25	9	25	9	
Inhibitory	22	8	50	18	0.0266

Note. EFT: exact Fisher test.

Hormone treatment of DC was associated with modulatory effects depending on the initial level of allostimulatory activity of DC. That was confirmed by inverse correlation between the indexes of intact and DHEAS- and PG-modified DC effects (r=-0.33 and r=-0.35, respectively; p<0.05). As a result, DHEAS and PG increased allostimulatory activity (and hence, reduced the tolerogenic potential) of DC with low allostimulatory activity. On the other hand, hormone treatment of DC with high initial allostimulatory activity led to inhibition of this function because of potentiation of the DC tolerogenicity. Comparative analysis of the direction of effects of the studied hormones (Table 1) showed that on the whole, DHEAS significantly more often increased allostimulatory activity by suppressing the tolerogenic characteristics of DC, while PG more often amplified the tolerogenic potential of IFN-DC.

Evaluation of the hormone effects on the expression of coinhibitory/proapoptotic molecules (Table 2) showed that the increase in allostimulatory activities of DHEAS- or PG-modified DC was associated with significant reduction of the level of FasL-positive DC, while the inhibitory/tolerogenic effect of the hormones was associated with an increase in the percentage of cells expressing CD123, and for cells modified with DHEAS also of those expressing the coinhibitory B7-H1 molecule.

In order to evaluate possible involvement of prostaglandin E2 and indolamine-2,3-dioxygenase in induction of IFN-DC tolerogenic properties, allostimulatory activities of intact and hormone-modified DC generated without and with indomethacin or 1-methyltryptophan were compared in a group of 14 donors. The inhibitory/tolerogenic effects of DHEAS and PG on allostimulatory activity of IFN-DC in this experimental series was detected in 35% (5/14) and 64% (9/14) cases, respectively. Treatment of hormone-modified DC with indomethacin and 1-methyltryptophan (Table 3) caused no appreciable shifts in allostimulatory activity, but leveled the inhibitory effects of PG and DHEAS on allostimulatory activity of DC after indomethacin and 1-methyltryptophan treatment, which became statistically negligible (p_W=0.11 and p_W=0.08, respectively). This suggested the involvement of prostaglandin E2 and indolamine-2,3-dioxygenase in the realization of suppressor activities of hormone-modified DC.

The data indicated immunomodulatory effects of DHEAS and PG on the IFN-DC tolerogenic characteristics at DC maturation stage. The direction of the hormone effects was determined by the initial level of DC allostimulatory activity. However, analysis of the hormone effect vectors and their comparison clearly indicated that DHEAS significantly more often

TABLE 2. Allostimulatory Activities and Phenotypes of Intact, DHEAS- and PG-Modified DC Differing by Their Response to Hormone Modification ($M \pm SE$)

Parameter	Stimulatory effect of DHEAS		Inhibitory effect of DHEAS		Stimulatory effect of PG		Inhibitory effect of PG	
	DC_{int}	DC_{DHEAS}	DC_{int}	DC_{DHEAS}	DC_{int}	DC_{PG}	DC_{int}	DC_{PG}
Response in mixed lymphocyte culture, cpm	13,170±1920 *n*=19	17,320±2250** *n*=19	17,190±3160 *n*=8	10,080±3180** *n*=8	10,680±2320 *n*=9	16,400±3130** *n*=9	16,900±2580 *n*=18	13,170±2440** *n*=18
B7-H1, %	43±4 *n*=7	50.4±7.5 *n*=7	48.8±10.8 *n*=7	69.7±5.2* *n*=7	43.8±5.7 *n*=9	52.2±5.1 *n*=9	53.7±7.6 *n*=12	53.6±6.8 *n*=12
CD123, %	55.7±6.5 *n*=8	60.5±8.6 *n*=8	51.3±11.0 *n*=8	59.0±9.8* *n*=8	57.9±6.4 *n*=9	52.4±8.4 *n*=9	47.3±11.8 *n*=6	66.8±9.5* *n*=6
TRAIL, %	14.4±2.5 *n*=13	13.7±3.0 *n*=13	7.6±1.1 *n*=8	7.1±1.6 *n*=8	13.0±3.2 *n*=7	14.0±4.7 *n*=7	9.2±1.6 *n*=8	11.0±2.1 *n*=8
FasL, %	21.0±4.4 *n*=7	16.8±3.2* *n*=7	18.9±2.6 *n*=8	16.4±2.9 *n*=8	21.3±3.5 *n*=9	15.4±2.4* *n*=9	18.6±2.7 *n*=12	21.8±4.9 *n*=12

Note. Here and in Table 3: *P_W<0.05, **P_W<0.01 compared to intact DC in the corresponding subgroups (W: Wilcoxon's test for conjugated pairs).

TABLE 3. Effects of Indomethacin and 1-Methyltryptophan on Functional Activities of DHEAS- and PG-Modified DC in Mixed Lymphocyte Culture

Culture variant	Inhibitor preparations	*n*	*M*±*SE*	P_w
MNC+DC_{int}	—	5	17,170±2830	
MNC+DC_{DHEAS}	—	5	8550±2880*	0.043
MNC+DC_{DHEAS}	+indomethacin	5	9530±3230*	0.043
MNC+DC_{DHEAS}	+1-methyltryptophan	5	7920±2700	0.08
MNC+DC_{int}	—	9	14,500±1880	
MNC+DC_{PG}	—	9	9730±1780**	0.007
MNC+DC_{PG}	+indomethacin	9	10,310±1970	0.11
MNC+DC_{PG}	+1-methyltryptophan	9	10,170±2690*	0.038

reduced the tolerogenic properties of DC, while PG more often stimulated them.

We found only one report about the stimulatory effect of DHEAS on DC differentiation and maturation [6]. Our previous studies revealed a similar effect of DHEAS on donor IFN-DC. It was found that induction of DC maturation was paralleled by an increase in allostimulatory activity of DC. This effect manifested in donor DC [1], but was particularly pronounced in women with normal gestation, their DC exhibiting signs of non-maturity. The increase in allostimulatory activity of DC in the presence of DHEAS in this latter case was also associated with reduction of DC capacity to stimulate the Th2 response and indicated a reduction of the tolerogenic potential of immature IFN-DC in pregnant women [3,4]. The effects of PG on DC were also contradictory, judging from published reports. Some authors demonstrated PG stimulation of DC maturation [15], while others observed its inhibitory effect on DC generation and capacity to stimulate apoptosis and production of IL-10 in cultured DC [10]. These facts suggested that hormone sensitivity of DC was largely determined by the initial status of DC, which was confirmed in our study.

No data on the mechanisms of DHEAS and PG effects on regulatory activity of DC are in fact available. The only study [10] showed that the increase in tolerogenic activity of DC in the presence of PG could be due to stimulation of IL-10 production. Our data indicated that attenuation of tolerogenic activity of DC under the effects of DHEAS and PG was associated with reduced expression of FasL, while the inhibitory/tolerogenic effect of the hormones was associated with the increase in CD123 and also B1-H1 expression (after DHEAS treatment of DC). The involvement of FasL in the realization of the DC cytostatic effect on tumor cells was described [7]. Since stimulated T-cells expressed Fas, they could also undergo apoptosis. Hence, reduction of FasL expression on DC under the effect of hormones would presumably reduce suppressor activity of DC associated with their proapoptogenic effect. As for the role of CD123 molecule, IFN-DC (in contrast to DC generated in the presence of IL-4) contained many $CD123^+$DC, and these very cells in the IFN-DC population according to previous data [14] were characterized by higher cytostatic activity towards tumor cells. The association of the tolerogenic effect of DHEAS with increased expression of B7-H1 coinhibitory molecule seemed also logical, because the interactions of that molecule with its Pd1 ligand on T-cells led to triggering T-cell anergy or apoptosis [12]. As for indolamine-2,3-dioxygenase and prostaglandin E2 as possible molecular targets of the hormone effects, their role in the realization of DC tolerogenic activity was discussed [5,13]. However, according to our findings, the specific share of those mechanisms in the realization of tolerogenic characteristics of LPS-stimulated IFN-DC was hardly great.

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REFERENCES

1. O. Yu. Leplina, M. A. Tikhonova, L. V. Sakhno, *et al.*, *Byull. Eksp. Biol. Med.*, **148**, No. 7, 80-85 (2009).
2. N. V. Seledtsova, N. A. Khonina, A. V. Dudareva, *et al.*, *Byull. Sibirsk. Otdelen. Rossiisk. Akad. Med. Nauk*, No. 1, 35-40 (2006).
3. N. V. Seledtsova, N. A. Khonina, M. A. Tikhonova, *et al.*, *Med. Immunol.*, **9**, 589-596 (2007).
4. E. R. Chernykh, N. V. Seledtsova, O. Yu. Leplina, *et al.*, *Ibid.*, 11**,** 541-548 (2009).
5. S. M. Blois, U. Kammerer, C. Alba Soto, *et al.*, *Biol. Reprod.*, **77**, No. 4, 590-598 (2007).
6. M. O. Canning, K. Grotenhuis, H. J. de Wit, and H. A. Drexhage, *Eur. J. Endocrin.*, **143**, No. 5, 687-695 (2000).

7. C. Chauvin and R. Josien, *J. Immunol.*, **118**, No. 1, 11-16 (2008).
8. C. Dosiou and L. C. Giudice, *Endocr. Rev.*, **26**, No. 1, 44-62 (2005).
9. R. Druckmann and M. A. Druckmann, *J. Steroid Biochem. Mol. Biol.*, **97**, No. 5, 389-396 (2005).
10. D. Kyurkchiev, E. Ivanova-Todorova, and I. Altankova, *Biotechnol. Biotechnol. Eq.*, **21**, 468-470 (2007).
11. S. M. Santini, C. Lapenta, M. Logozzi, *et al.*, *J. Exp. Med.*, **191**, No. 10, 1777-1788 (2000).
12. N. Selenko-Gebauer, O. Majdic, A. Szekeres, *et al.*, *J. Immunol.*, **170**, No. 7, 3637-3644 (2003).
13. R. M. Steinman, D. Hawiger, and M. C. Nussenzweig, *Annu. Rev. Immunol.*, **21**, 685-711 (2003).
14. M. Wang, J. Shi, Y. Wan, *et al.*, *In Vitro Cell. Dev. Biol. Anim.*, **45**, No. 7, 398-404 (2009).
15. L. Yang, X. Li, J. Zhao, and Y. Hou, *Steroids*, **71**, No. 10, 922-929 (2006).