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Low serum levels of dehydroepiandrosterone may cause deficient IL-2 production by lymphocytes in patients with systemic lupus erythematosus (SLE)

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SUMMARY

The principal cause of IL-2 deficiency, a common feature of both murine lupus and human SLE, remains obscure. Recent studies of our own as well as others have shown that dehydroepiandrosterone (DHEA), an intermediate compound in testosterone synthesis, significantly up-regulates IL-2 production of T cells, and that administration of exogenous DHEA or IL-2 via a vaccinia construct to murine lupus dramatically reverses their clinical autoimmune diseases. Thus, we have examined serum levels of DHEA in patients with SLE to test whether abnormal DHEA activity is associated with IL-2 deficiency of the patients. We found that nearly all of the patients examined have very low levels of serum DHEA. The decreased DHEA levels were not simply a reflection of a long term corticosteroid treatment which may cause adrenal atrophy, since serum samples drawn at the onset of disease, which are devoid of corticosteroid treatment, also contained low levels of DHEA. In addition, exogenous DHEA restored impaired IL-2 production of T cells from patients with SLE *in vitro*. These results indicate that defects of IL-2 synthesis of patients with SLE are at least in part due to the low DHEA activity in the serum.

Keywords systemic lupus erythematosus dehydroepiandrosterone IL-2

INTRODUCTION

SLE is a classical organ non-specific autoimmune disorder, and is characterized by an aberrant immunoregulatory T cell function and B cell hyperactivity, both of which are associated with pathogenic autoantibody production [1–3]. Deficient IL-2 activity is a well characterized abnormality of T lymphocytes from patients with SLE as well as murine models of lupus [4–6]. The deficient IL-2 activity could result in disruption of T cell differentiation into effector cells which prevent the autoimmune reactions leading to disease manifestations [4–6]. Participation of IL-2 deficiency in the pathogenesis and development of SLE is clearly shown by a murine study where deficient IL-2 activity is corrected by infection of recombinant vaccinia virus harboring human IL-2 gene [7]. However, the principal cause of IL-2 deficiency in patients with SLE is not yet elucidated. Family studies of patients with SLE showed that IL-2 deficiency could result from genetic predisposition, and precede rather than follow the onset of clinical symptoms [8].

Dehydroepiandrosterone (DHEA) is the most abundant adrenal steroid hormone in humans. Although DHEA is known to serve as an intermediate in sex hormone synthesis, the physiologic role of circulating DHEA has been elusive. Serum levels of DHEA and its precursor, DHEA-sulphate, are lower in early life but rise to a maximum at about 25 years of age and then decline gradually thereafter, reaching 15–20% of the maximum in individuals over the age of 70 [9]. The decline of DHEA levels with ageing correlates with a general decline of cell-mediated immunity and increased incidence of malignancies, suggesting immunomodulatory effects of DHEA.

We have recently found that treatment of normal human T cells with DHEA *in vitro* can enhance IL-2 production by the T cells; in the presence of DHEA, normal T cells produce more IL-2 upon mitogenic activation and the DHEA effects may be due to the enhanced transcription of IL-2 gene [10,11]. In addition, administration of exogenous DHEA to murine lupus dramatically reversed their IL-2-deficient states [12] as well as their clinical autoimmune disease [13]. Taken together, these data suggest that deficient IL-2 activity of T lymphocytes

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observed in patients with SLE could result, at least in part, from DHEA deficiency of the patients.

In the present study we have addressed this issue. Our results indicate that serum level of DHEA is in fact low in patients with SLE, and supplementation of exogenous DHEA to the *in vitro* culture of T lymphocytes can restore defects of IL-2 production in patients with SLE. Thus, deficient IL-2 production by T lymphocytes in patients with SLE can be, at least in part, attributed to the low serum level of DHEA.

PATIENTS AND METHODS

Patients

All of the 12 patients that were studied satisfied the 1982 revised criteria of the American Rheumatism Association for the classification of SLE [14]. The mean age of the patients was 33 years, and all were female. Patients receiving immunosuppressive drugs were excluded. Two patients at disease onset had received no medication at all. Two patients who had been treated with corticosteroids successfully were having no corticosteroids at the time of the study. The remaining eight patients were receiving low doses of corticosteroids ($\leqslant$ 10 mg of prednisolone/day). Eleven healthy blood donors served as control subjects; their mean age was 29 years and all were female. Blood sampling was done early in the morning with fasting.

Determination of serum DHEA and DHEA-sulphate level

Serum DHEA and DHEA-sulphate were estimated by the commercial radioimmunoassay (RIA) kits using a 'coat-a-count' procedure (Diagnostic Products Corp., Los Angeles, CA) [15–18]. In brief, coat-a-count procedure is a solid-phase RIA based on an anti-DHEA or DHEA-sulphate antibody being immobilized to the wall of a polypropylene tube. ^{125}I-labelled DHEA or DHEA-sulphate competes for a fixed time with the hormone in the patient's sample for antibody sites. The sensitivity of the DHEA RIA kit and DHEA-sulphate RIA kit is 0·01 ng/ml and 0·6 μg/ml, respectively. Both RIA kits are highly specific for a relevant hormone. The DHEA RIA kit does not cross-react with irrelevant hormones including DHEA-sulphate, testosterone, dihydrotestosterone, androsterone. Similarly, the DHEA-sulphate RIA kit does not cross-react with irrelevant hormones including DHEA, DHEA-glucuronide, androsterone-sulphate, estrone-3-sulphate, and testosterone.

Reagents

DHEA was purchased from Sigma Chemical Co. (St Louis, MO). Human rIL-2 was obtained from Ajinomoto Corp. (Kawasaki, Japan). Phytohaemagglutinin (PHA) was purchased from Wellcome Research Laboratories Diagnostic (Dartford, UK). OKT4A and OKT8 MoAbs were obtained from Ortho Pharmaceutical Corp. (Raritan, NJ). Anti-Leu-11c MoAb was purchased from Becton Dickinson Monoclonal Centre Inc. (Mountain View, CA).

Cell separation and cell culture

Peripheral blood lymphocytes (PBL) were separated into T cells and non-T cells by a sheep erythrocyte-rosetting technique [19,20]. $CD4^+$ and $CD8^+$ T cell subsets were negatively enriched from T cells by the panning method [19,20]. Monocytes were purified from non-T cells by plastic dish adherence. T cells or T cell subsets with 3% irradiated (25 Gy) autologous monocytes were stimulated at 37°C with PHA (1 μg/ml) in the presence of DHEA or solvent (ethanol) diluted in medium as control. After 48 h incubation, the supernatants were harvested and stored at −70°C until assay. Cell culture media were RPMI 1640 supplemented with 1% Nutridoma-HU (Boehringer-Mannheim, Germany).

Lymphokine assays

The lymphokine activity in the culture supernatants was determined by cytokine-ELISA. Human IL-2 ELISA was obtained from Otsuka Assay Inc. (Tokushima, Japan). Human IL-4 and IL-6 ELISA were both from Research and Diagnotic System (Minneapolis, MN).

Statistical analysis

Statistical significance was ascertained by Student's *t*-test.

RESULTS

Serum concentrations of DHEA

IL-2 production by T lymphocytes has been shown to be deficient in patients with SLE regardless of their disease activity and treatment with medication [5,6]. Indeed, we confirmed that IL-2 activity in culture supernatants derived from PHA-stimulated T cells of SLE patients examined in the present study was significantly lower than that in the control females (data not shown).

There are huge numbers of reports which describe abnormal sex hormone activity in patients with SLE [21–26]. Since DHEA is an intermediate of sex hormone synthesis, and DHEA did increase IL-2 production by normal T cells [10,11], it is possible that DHEA activity may be low in

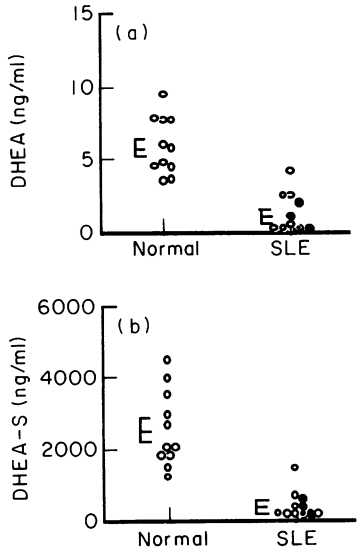

Fig. 1. Serum dehydroepiandrosterone (DHEA) (a) and DHEA-sulphate (b) concentrations of patients with SLE. Serum samples drawn in the morning were tested for their DHEA and DHEA-sulphate concentrations by radioimmunoassay (RIA) methods as described in Patients and Methods. ●, Patients without corticosteroid treatment at the time of study. The lines show mean ± s.e.m.

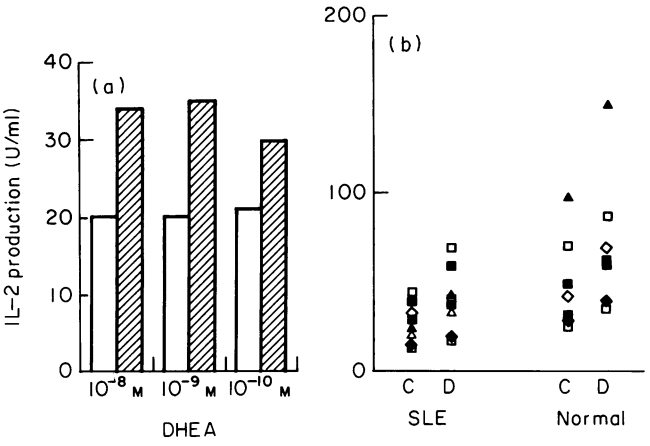

Fig. 2. Treatment of SLE T cells with dehydroepiandrosterone (DHEA) restores defective IL-2 production upon phytohaemagglutinin (PHA) stimulation. (a) Effects of DHEA on IL-2 production by PHA-stimulated SLE T cells. Note that physiological concentration of DHEA is approximately 10^{-8}–10^{-9} M in humans. □, Control; ▨, DHEA. (b) Improvements of defective IL-2 production by SLE T cells. T cells were stimulated with PHA (1 μg/ml) in the presence of either DHEA 10^{-9} M or solvent (ethanol) as a control. After termination of cell culture for 48 h, supernatants were harvested and IL-2 activity determined. Each symbol represents one subject. C, Control; D, DHEA.

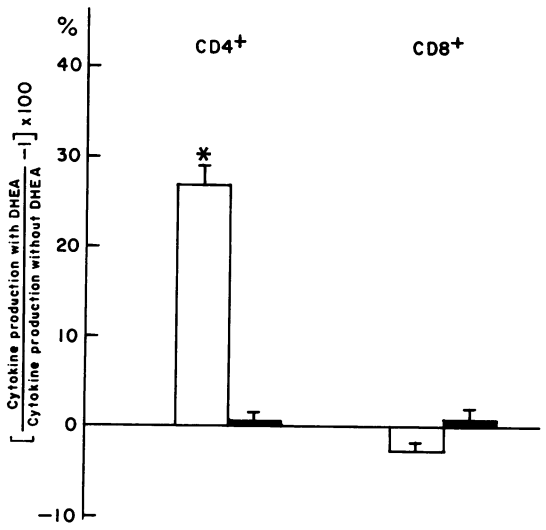

Fig. 3. Dehydroepiandrosterone (DHEA) restores IL-2 (□), but not IL-6 (■), secretion by $CD4^+$, but not $CD8^+$, T cells from patients with SLE. $CD4^+$ and $CD8^+$ T cells were purified with negative panning, and these T cell subsets, supplemented with irradiated monocytes, were tested for their ability to produce IL-2 and IL-6 in the presence of DHEA, as described in Patients and Methods. The results are expressed as mean per cent increase ± s.e.m. in three patients with SLE. * Significantly different from per cent increase of corresponding cytokine production by $CD8^+$ T cells ($P < 0.01$).

patients with SLE, resulting in decreased IL-2 production by SLE T cells. To test this hypothesis, we measured serum DHEA concentrations in patients with SLE by RIA methods. As shown in Fig. 1a, serum DHEA levels of patients with SLE were lower than those of age- and sex-matched normal females ($P < 0.01$). Even those without corticosteroid treatment (two fresh cases and two cases in complete remission) showed decreased serum DHEA levels (Fig. 1a, closed circles). We also examined concentrations of serum DHEA-sulphate in patients with SLE. DHEA-sulphate is an inactive precursor of DHEA, which is secreted from adrenal glands. As shown in Fig. 1b, serum DHEA-sulphate concentrations were also lower in patients with SLE than in normal controls ($P < 0.01$).

Effects of DHEA on lymphocytes from patients with SLE

It is possible that low serum concentrations of DHEA down-regulate the ability of SLE T cells to secrete IL-2. Thus we next examined whether supplementation of exogenous DHEA at physiological concentrations (approximately 10^{-8}–10^{-9} M) restores defective IL-2 production by T cells from patients with SLE *in vitro*. A representative result of such experiments is shown in Fig. 2a. Supplementation of DHEA at concentrations of 10^{-8} M and 10^{-9} M restored the responses. As shown in Fig. 2b, PHA-stimulated T lymphocytes from all the patients with SLE examined secreted more IL-2 in the presence of 10^{-9} M DHEA than in the absence of DHEA. The degree of per cent enhancement by DHEA in SLE patients was as great as in normal females. This result suggests that the signal transduction pathway of DHEA is functional even in T lymphocytes of patients with SLE. It also indicates that low concentrations of serum DHEA may be, in part, responsible for the deficient IL-2 activity of patients with SLE.

T cell subsets involved in the correction of deficient IL-2 synthesis by DHEA

We have previously found that DHEA treatment augments IL-2 production of $CD4^+$, but not $CD8^+$, T cells in normal subjects [11]. In addition, we have reported that many T cell defects, including deficient IL-2 secretion, observed in patients with SLE reside mainly within the $CD4^+$ T cell subset [5,6]. It is intriguing to know whether DHEA corrects impaired IL-2 secretion by $CD4^+$, but not $CD8^+$, T cells of patients with

Table 1. Effects of dehydroepiandrosterone (DHEA) upon IL-4 and IL-6 production by phytohaemagglutinin (PHA)-stimulated T cells

	Per cent increase	
Source of lymphocytes	IL-4	IL-6
SLE	105·1 + 6·6 ($n = 5$)	99·7 + 0·8 ($n = 3$)
Normal	105·7 + 1·9 ($n = 3$)	100·1 + 3·7 ($n = 3$)

$$\text{Per cent increase} = \frac{\text{IL-4 or IL-6 production in the presence of DHEA}}{\text{IL-4 or IL-6 production in the presence of solvent}} \times 100$$

Fresh T cells supplemented with irradiated monocytes were stimulated with PHA (1 μg/ml) in the presence of DHEA. After termination of cell culture, IL-4 or IL-6 activity of the supernatants was determined by cytokine-ELISA.

SLE. We have therefore studied effects of DHEA on the T lymphocyte subsets of SLE patients. As shown in Fig. 3, DHEA acted mainly on the $CD4^+$ T cell subset of the patients to augment IL-2 production. This finding observed in patients with SLE suggests that $CD4^+$ T cell defects could be overcome by DHEA treatment, as has been observed in the normal $CD4^+$ T cell subset [11].

Effects of DHEA on B cell-tropic lymphokines

We turned our attention to the DHEA effects on the other lymphokines synthesized by SLE lymphocytes, which modulate B cell growth and maturation. Recently, we found that pathogenic autoantibody production of B cells is largely dependent on IL-6, rather than IL-2 in SLE patients [27]. As shown in Table 1, DHEA affected the ability of SLE T lymphocytes to secrete neither IL-4 nor IL-6. In addition, IL-6 production by both $CD4^+$ and $CD8^+$ T cell subsets was not affected by the supplementation of exogenous DHEA to the cell cultures (Fig. 3). The results suggest that DHEA would not act effectively on T cells to secrete IL-4 and IL-6, so that DHEA would neither induce nor exacerbate B cell hyperfunction of SLE patients via increased production of IL-4 and IL-6.

DISCUSSION

Defective IL-2 production is a common feature in both murine lupus and human SLE, and is independent of age or overall disease activity [3–6]. Deficient IL-2 activity may thus initiate or perpetuate the abnormality of the immunoregulatory T cell function in patients with SLE. In fact, Gutierrez-Ramos *et al.* examined the effects on disease progression of MRL/lpr mice using live vaccinia recombinant virus expressing human IL-2 gene, and found that vaccinated mice show neither clinical symptoms nor immunological abnormalities [7].

Although defects of IL-2 production can be observed invariably in murine lupus and human SLE, the principal cause of impaired IL-2 production remains to be studied. Previously, we found that 15 out of 29 healthy, consanguinous relatives of SLE patients had impaired IL-2 production, in contrast to non-consanguinous household members who produced normal amounts of IL-2 [8]. This result suggests that IL-2 defects in SLE may be genetically determined and precede rather than follow the onset of the clinical disease [8].

It has been reported that exogenous administration of DHEA is effective for treating murine lupus, where defective IL-2 production has been improved [12,13]. Our results showed that serum concentrations of both DHEA and DHEA-sulphate in patients with SLE are lower than those of normal controls. A similar decline of serum concentrations of the hormones in patients with SLE has been reported [28].

It is possible that the adrenal cortex function of patients with SLE, who have been given long-term prednisolone as a therapeutic formula, is suppressed entirely. However, those who are not treated with corticosteroids showed low serum DHEA concentrations, suggesting that low serum DHEA is not due solely to long-term corticosteroid treatment. Rather, some intrinsic factors may have some influence on the serum levels of the hormones.

IL-2 deficiency of T cells has also been reported in patients with rheumatoid arthritis (RA). It is intriguing that low serum DHEA-sulphate concentration is also noted in patients with RA [29,30]. Recently, this finding was confirmed by a study where a large number of women with RA were included [31]. We have also confirmed this finding in patients with RA (data not shown). Thus, low serum level of DHEA is not specific for patients with SLE, but rather is shared with patients with RA. In addition, it has been reported that DHEA activity is decreased in male SLE patients compared with male controls [32]. Thus, abnormal DHEA activity is common between male SLE patients and female SLE patients. This indicates that low DHEA activity may be a rather common feature and an underlying substratum of certain autoimmune diseases, in particular systemic autoimmune diseases in humans.

Since DHEA is an intermediate compound of androgen synthesis, a low serum DHEA concentration may result in low androgen synthesis, which agrees with previous observations [24–26]. Our preliminary study showed, however, that (i) IL-2 mRNA is increased when T cells are stimulated by PHA in the presence of DHEA [10]; (ii) this increase of IL-2 mRNA is mediated by the enhanced transcription of IL-2 gene, since IL-2 promoter/enhancer + chloramphenicol acethyltransferase gene transfectants produce more chloramphenicol acethyltransferase activity in the presence of DHEA than in its absence; and (iii) for the purpose of clarifying the evidence for direct interaction between the DHEA and DHEA-receptor complexes and IL-2 promoter elements, we have tried South-Western blotting and gel retardation assay, and confirmed the binding between IL-2 promoter/enhancer elements and protein factors present in DHEA-treated T cells (unpublished observations). Our previous study showed that phorbol myristate acetate provided signals necessary for correction of deficient IL-2 activity of SLE T cells [33]. The present study showed that exogenous DHEA restores IL-2 defects *in vitro*. Of note is the fact that the degree of enhancement of IL-2 production by DHEA is modest, since excessive production of IL-2 should cause various side effects [34]. Taken together, these data suggest that DHEA exerts its beneficial effects by correcting immunoregulatory T cell abnormalities of SLE, rather than by correcting hormonal abnormalities of SLE. The results further suggest that defects of SLE T cells could locate within signal transduction systems, and that machinery for protein synthesis of SLE T cells has been kept intact, and once appropriate signals are provided, SLE T cells would produce normal amounts of lymphokines.

In accordance with this finding, it has been shown that IL-2/vaccinia recombinant virus infection improves clinical and serological abnormalities of MRL/lpr mice via continuous IL-2 production [7]. Oral administration of DHEA resulted in prevention of autoantibody formation and prolonged survival in NZB/NZW F_1 mice [13]. Treatment of human SLE T cells with DHEA restored their defective IL-2 production upon PHA stimulation; enhancement of IL-2 secretion was moderate but significant: 120–174% (mean + s.e.m. of eight SLE patients is 151·5 + 7·0), which may be sufficient for immune potentiation. We believe that our present study gives a rationale for the clinical trial of DHEA in SLE [35], and may contribute to the design of new forms of therapeutic intervention in this disease.

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