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ANAPSOS, AN ANTIPSORIATIC DRUG WHICH INCREASES THE PROPORTION OF SUPPRESSOR CELLS IN HUMAN PERIPHERAL BLOOD

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SUMMARY

Anapsos, an antisporiatic drug, was administered to normal volunteers in order to study its possible activity on the immune system. After three days of treatment, the drug decreased the lymphoblastic response to PWM and very slightly reduced the serum levels of immunoglobulins. After five days of treatment, however, both values were normal. The response to ConA decreased, and there was an increase in the suppressor index and in the proportion of OKT 8^+. The drug did not vary the proportion of OKT 4^+ and OKT 3^+ cells. These results suggest that anapsos acts by increasing the number of suppressor cells. Such anapsos-induced suppressor cells are probably responsable for the diminished response to ConA, but did not seem to significantly affect response to PWM and serum levels of Ig after five days of treatment.

KEY-WORDS: Psoriasis, Immunosuppression, Anapsos; Lymphoblastic response, Suppressor index, OKT antibodies, Suppressor cells.

INTRODUCTION

The administration of anapsos — an infusion of rhizomes of the fern *Polipodium leucotomos* — to psoriatic patients appears to be of great therapeutic interest because of the clinical improvement it produces and the lack of secondary effects [5]. The active principle of this fern is a saponine formed by a ketosteroid and a deoxyhexose [6]. Metabolic studies

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suggest that the antisporiatic action of anapsos is not cytostatic and that its effects on normal tissues are anabolic, both *in vitro* and *in vivo* [6, 14].

The etiology and pathogenesis of psoriasis remain unknown, but in recent years a series of reports suggest that autoimmunity may play an important role in this disease [3, 4, 7]. Atopic dermatitis — another immune disease [11, 12] — also improves when treated with anapsos [1]. These clinical findings impelled us to study the possible activity of anapsos on the immune system.

MATERIALS AND METHODS

Treatment with anapsos. — Twelve healthy volunteers with ages ranging between 18 and 30 were chosen, taking special care that they had not received any medication in the three previous months. Anapsos (360 mg/day; Psoriacen, Centrum Pharmaceutical International, Spain) was administered orally to each volunteer for a period of five days, three times per day before meals.

Lymphoblastic response to mitogens. — Ten millilitres of blood from each volunteer were obtained by venous puncture before treatment with anapsos and after 1, 3 and 5 days of treatment. The heparinized blood was mixed with an equal volume of Ca^{++}- and Mg^{++}-free PBS, layered on a Ficoll-Hypaque gradient (Lymphopred, Nyegaard) and centrifuged at 400 *g* for 30 min at room temperature. The peripheral blood mononuclear cell (PBMC) layer at the interface was carefully removed and washed three times in PBS. Cells were then resuspended to a concentration of 5×10^5/ml of RPMI-1640 (Gibco) supplemented with 10 % heat-inactivated foetal calf serum (Gibco), 50 μg/ml gentamicin, 100 IU/ml penicillin and 2 mM l-glutamine (Gibco). PBMC were cultured in flat-bottomed microtest plates, 200 μl per well, and stimulated with 5 μg/ml ConA (Sigma) or 5 μl/ml PWM (Gibco). Cultures were incubated at 37° C in a humidified atmosphere with 5 % CO_2. One μCi of H^3-thymidine was added to each well after 72 h, and 24 h later, cultures were harvested by a « Titertek Cell Harvester » and counted for radioactivity in a beta-scintillation counter.

Spontaneous suppressor cell assay. — This was carried out according to the method described by Bresnihan and Jasin [2]. PBMC were cultured separately in two microplates (10^5 cells/well). In one plate, 5 μg/ml of ConA was immediately added to these fresh cells. The second plate containing PBMC in culture medium alone was incubated for 24 h at 37° C before addition of the same mitogen concentration. The two plates were incubated for 72 h after the addition of mitogens. The H^3-thymidine uptake was determined as before. The suppressor index (SI) was calculated using the following formula:

$$SI = \frac{\text{DPM}/10^5 \text{ lymphocytes preincubated for 24 h}}{\text{DPM}/10^5 \text{ lymphocytes without preincubation}}$$

Baselines of unstimulated cultures were subtracted from the disintegrations of stimulated cultures.

AISC = anapsos-induced suppressor cell.
ConA = concanavaline A.
Ig = immunoglobulin.
IU = international unit.
PBMC = peripheral blood mononuclear saline.
PBS = phosphate-buffered saline.
PWM = pokeweed mitogen.
SEM = standard error of mean.
SI = suppressor index.

T-cell subpopulations. — T-lymphocytic subpopulations were determined by monoclonal anti-T human cell antibodies (OKT3, OKT4 and OKT8) (Ortho Pharmaceutical Co.) in a complement-dependent cytotoxic assay [13]. Ten µl of monoclonal antibody (1 µg of protein) and 80 µl of cells (2.5×10^6/ml) were placed in 75 × 11 mm round-bottomed plastic tubes and incubated for five min at 37° C. Ten µl of rabbit serum were added and the tubes incubated at 37° C for 45 min. Then, 100 µl of a 2 % trypan-blue solution were added and, 10 min later, the number of dead and live cells was counted in a « Neubauer » haemocytometer. All counts were made in triplicate and at least 300 cells were counted.

Serum concentration of Ig. — The serum concentration of IgG, IgA and IgM were measured by an immunonephelometric system (Auto ICS; Beckman).

Statistical analysis. — Group comparisons to determine significance were performed using Student's test and Dunnett's t test. All values are expressed as arithmetical mean ± standard error of mean (SEM).

RESULTS

Mitogen stimulation.

The response to PWM remained unaltered after one day of treatment. It decreased after 3 days ($p < 0.01$) and returned to the level of day zero after 5 days (fig. 1).

The response to ConA decreased after one day of administration of the drug ($p < 0.05$), reaching its lowest value after 3 days ($p < 0.01$). After 5 days of treatment, the response to ConA increased slightly, but remained significantly lower than the level at day zero ($p < 0.01$) (fig. 1).

Suppressor index.

The SI increased after 3 days of treatment, but decreased slightly after 5 days (fig. 1). Both values were significantly higher than those obtained before treatment ($p < 0.01$).

Serum concentration of Ig.

As shown in table I, the serum levels of IgG, IgA and IgM decreased slightly only after 3 days of treatment, but returned to the levels of day zero after five days.

TABLE I. — **Concentration of serum Ig (mg/100 ml, mean ± SEM) before and during treatment with anapsos.**

Days of treatment	IgG	IgA	IgM
—	1 011 ± 106	189 ± 37	133 ± 10
1 day	1,017 ± 89	211 ± 34	142 ± 14
3 days	858 ± 41	172 ± 36	120 ± 8
5 days	1,016 ± 47	222 ± 34	149 ± 13

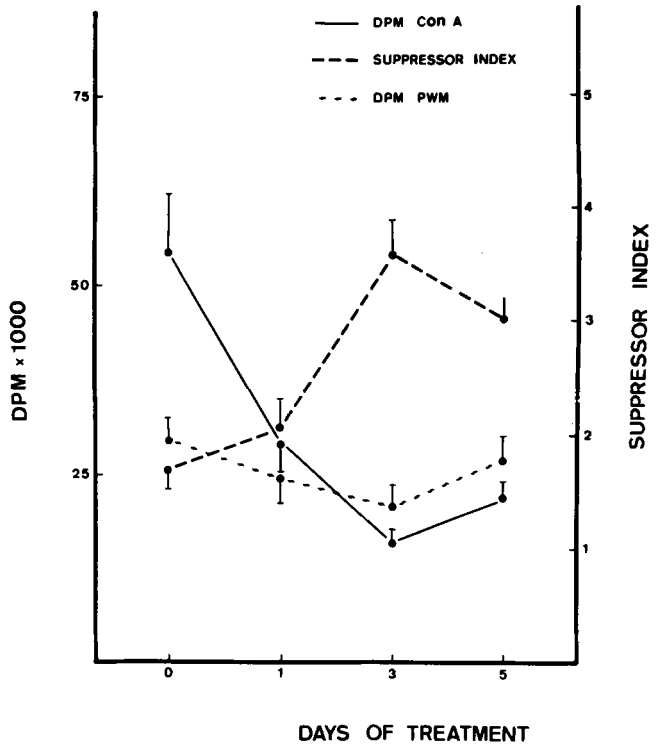

FIG. 1. — *Evolution of different immunological parameters in 12 anapsos-treated volunteers.*

Lymphoblastic response to ConA, lymphoblastic response to PWM and SI. All values are expressed as arithmatical mean ± standard error.

T cell subpopulation.

Reactivity of the different OKT monoclonal antibodies with PBMC of untreated and five-day-treated volunteers are given in table II. There was no difference in the reactivity of OKT 3^+ and OKT 4^+ antibodies before and after treatment. The proportion of OKT 8^+ cells, however, increased significantly after five days of treatment with anapsos ($p < 0.01$) compared to that of untreated volunteers.

TABLE II. — **T-cell subsets (mean % ± SEM) determined by OKT monoclonal antibodies before and after five days of treatment with anapsos.**

Monoclonal antibody	Before treatment with anapsos	After 5 days of treatment with anapsos
OKT 3	56.5 ± 4.00	56.6 ± 5.5
OKT 4	41.4 ± 3.7	43.1 ± 3.9
OKT 8	16.2 ± 3.8	30.6 ± 2.2 ($P < 0.01$)

DISCUSSION

This study shows that anapsos is a drug which acts on the immune system. The inhibition of the response to ConA (fig. 1) indicates that anapsos acts directly or indirectly on T cells, since ConA is a mitogen specific for T cells. The activity of the drug on B cells is not clear, however. Although, after three days of treatment, there was a reduction in the response to PWM (fig. 1) and also a slight reduction in the serum levels of Ig (table I), both values increased to those of day zero after five days of treatment with anapsos (fig. 1 and table I).

The SI rose progressively, reaching a peak after three days of treatment. This peak coincides with the lowest response to mitogens and with a slight reduction in the serum levels of Ig (fig. 1), suggesting that the increment in the SI caused these alterations. Similarly, the slight increment observed in the response to mitogens and serum levels of Ig after 5 days of treatment could be due to a mild decrease in SI (fig. 1 and table I).

Although the experiments shown here were performed for only five days, some of the volunteers continued taking anapsos for one month and were studied on different days. In these cases, SI, lymphocyte response to mitogen and serum concentrations of Ig were similar to those recorded after 5 days of treatment (unpublished results). In the light of these results, we consider that the variations in SI between days 3 and 5 of treatment could be interpreted as an initial accommodation of the immune system to the effects of the drug.

Table II shows that anapsos induced a strong increment in the proportion of OKT 8^+ cells (suppressor/cytotoxic T cells), but did not alter the percentage of OKT 3^+ cells (peripheral T lymphocytes), nor that of OKT 4^+ cells (inducer/helper T cells) [8]. The addition of the proportion of OKT 4^+ cells and the proportion of OKT 8^+ cells exceeds the percentage of OKT 3^+ cells. This suggests that the anapsos-treated volunteers have OKT 4^+ OKT 8^+ cells in their blood. This phenomenon occurs in some immunological diseases and is explained as an abnormal release of OKT 4^+ OKT 8^+ immature T cells in peripheral blood [9, 10].

If we accept this hypothesis, we should expect to find in our results not only an increase in OKT 8^+ cells, but also in OKT 4^+ cells. The proportion of OKT 4^+ cells, however, did not vary after treatment with anapsos (table II). A possible explanation for this is that the increase in OKT 4^+ cells resulting from release of OKT 4^+ OKT 8^+ immature T cells by the thymus is compensated by a diminished release of OKT 3^+ OKT 4^+ cells. In other words, anapsos might block the differentiation of OKT 4^+ OKT 8^+ cells to OKT 3^+ OKT 8^+ cells (fig. 2), in which case, the OKT 4^+ OKT 8^+ cells have two possibilities: 1) either they leave the thymus directly without further differentiation, or 2) their development into OKT 3^+ OKT 8^+ cells is enhanced. In summary, the final balance could be a decreased proportion of OKT 3^+ OKT 4^+ cells and an increased proportion of OKT 4^+ OKT 8^+ and OKT 3^+ OKT 8^+ cells in the blood (fig. 2).

Further experiments are necessary to confirm this hypothesis; according to our results however, a higher SI after treatment with anapsos seems to be directly related to the increase in OKT 8^+ suppressor cells. These AISC could act directly or indirectly on B cells, since the highest value of SI coincides with the lowest response to PWM and with the slight decrease observed in Ig serum levels (fig. 1). After the accommodation of the immune system referred to above, however, both parameters increase to normal levels and the action of AISC on B cells cases. On the other hand, the principle targets of AISC appear to be ConA responder cells, since the response to ConA remained low throughout the experiment.

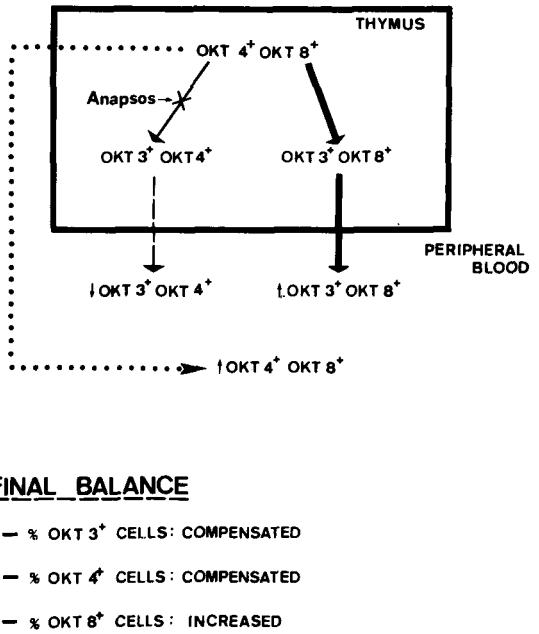

FIG. 2. — *Possible action mechanism of anapsos on thymus differentiation of T-cell subsets.*

None of the experiments carried out to clarify the action of anapsos *in vitro* produced a significant result. This suggests that the drug must be metabolized in order to act. The stimulation of suppressor cells *in vivo* could be the mechanism by which anapsos ameliorates psoriasis [5] and atopic dermatitis [1], since there is an impairment of the activity of suppressor cells in both of these skin diseases [7, 11, 12].

Experiments are now being carried out different immunological parameters in psoriatic patients treated or non-treated with anapsos in order to further determine the therapeutic action mechanism of anapsos.

RÉSUMÉ

ANAPSOS, UNE DROGUE ANTIPSORIASIQUE QUI AUGMENTE LA PROPORTION DES CELLULES SUPPRESSIVES DANS LE SANG PÉRIPHÉRIQUE HUMAIN

Anapsos, une drogue antipsoriasique, a été administrée à des volontaires en parfaite santé pour une étude des effets sur le système immunitaire. Après trois jours de traitement, la drogue réduit la réponse lymphoblastique au PWM et légèrement les taux d'immunoglobulines sériques. Cependant, après cinq jours, les deux valeurs sont normales. La transformation lymphoblastique par la ConA est diminuée, montrant une élévation de l'index suppresseur et aussi de la proportion de cellules OKT 8^+. La drogue n'a pas altéré la proportion de cellules OKT 3^+ et OKT 4^+. Tous ces résultats suggèrent que l'anapsos produit une augmentation du nombre des cellules suppressives dans le sang périphérique. Ces cellules sont probablement responsables de la réponse réduite à la ConA, mais il ne semble pas qu'elles affectent d'une façon significative la réponse au PWM ni les taux sériques des Ig après cinq jours de traitement.

MOTS-CLÉS : Psoriasis, Immunosuppression, Anapsos ; Réponse lymphoblastique, Index suppresseur, Anticorps OKT, Cellules suppressives.

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