## The Immunosuppressive Effects of Mouse Placental Steroids

One of the most intriguing biological questions is how the foetus, which inherits transplantation antigens from both parents avoids allograft rejection. Several explanations have been offered to explain this phenomenon[1], none of them being fully satisfactory. It is believed that either the uterus may be an immunologically privileged site[2], or the trophoblast is only feebly immunogenic[3], or even that it forms an immunologically inert barrier[4,5]. It has also been postulated that the prolonged survival time of skin allograft in pregnancy[6], as well as the inhibition of lymphocyte transformation by maternal plasma[7], are related to the excess of steroid hormones. Although cortisone and hydrocortisone have been known to suppress the immune response[8,9], the main adrenal cortex steroid in the mouse, rat and rabbit, corticosterone[10–12] has no such effect. Other steroids, such as estradiol, testosterone and progesterone, also do not directly influence the immune response[13–15]. Progesterone synthesized by mouse and rat placenta is metabolized to several 5α-reduced products and to androgens. These metabolites have been identified by Rembiesa et al.[16–18], but their biological role is unknown. We report here some effects of steroids naturally occurring in pregnant mouse on the immune reactivity. Contact sensitivity to oxazolone[19] was chosen as a cell-mediated reaction and the number of plaque-forming cells as the index of humoral immunity.

*Materials and methods.* The micronized test compounds were suspended at concentration 5 mg/ml in saline containing 0.5% carboxymethylcellulose. Swiss female mice, weighing 25-26 g, were given daily 1 or 2 mg the compound tested s.c. for 10 consecutive days. Control animals received the suspending medium only. At day +5 animals were sensitized to oxazolone. At day +11 a solution of 1% oxazolone in olive oil was smeared on both sides of the ear and the thickness of the ear was measured. For details see[19]. $10^8$ sheep red blood cells were injected i.v. to each mouse on day +8 and the number of antibody forming cells was recorded on day +12 by the JERNE technique[20]. As in subsequent experiments, the variability of ear swelling in both sensitized controls ($11.2 \pm 3.8 - 16.9 \pm 3.3$) and nonsensitized controls ($2.4 \pm 1.3 - 5.7 \pm 1.3$) was noted; for comparison of results indices of inhibition were calculated for each compound in each particular experiment, according to the equation

$$ii = \frac{A - C}{B - C}$$


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Table 1. Effects of Steroid Compounds on Cell-Mediated and Humoral Immune Responses

| Compound[a] | Contact sensitivity index of inhibition at daily dose | | Haemolytic plaques index of inhibition at daily dose | |
|---|---|---|---|---|
| | 1 mg | 2 mg | 1 mg | 2 mg |
| 1. Progesterone | 1.14[d] | 0.77[e] | 0.77[d] | 1.84[e] |
| 2. 6β-Hydroxyprogesterone | 0.69 | N.T. | 0.88 | N.T. |
| 3. 5α-Pregnane-3,6,20-trione | 0.64 | N.T. | 0.94 | N.T. |
| 4. 4-Pregnene-3,6,20-trione | 0.64 | N.T. | N.T. | N.T. |
| 5. 5α-Pregnane-3,20-dione | 0.97 | N.T. | 1.76 | N.T. |
| 6. 3β-OH-5α-pregnan-20-one | 1.0 | N.T. | N.T. | N.T. |
| 7. 5α-Pregnane-3β,20α-diol | 1.36 | N.T. | N.T. | N.T. |
| 8. Hydrocortisone[b,c] | −0.20[d] | N.T. | 0.04[e] | N.T. |
| 9. Testosterone[b] | 1.0[d] | 0.88[d] | 0.95[d] | 0.71[d] |
| 10. Dehydroepiandrosterone[b] | 0.74 | 0.70[d] | 0.94 | 0.68[e] |
| 11. 5α Androstane-3,17-dione | 0.33[f] | 0.21[f] | N.T. | 1.10 |
| 12. 3α-OH-5α-androstan-17-one | 0.73 | 0.26[f] | 1.0 | 1.0 |
| 13. 17β-OH-5α-androstan-3-one[b] | 0.71 | 0.50[d] | 1.0 | 0.57[d] |
| 14. 5β-Androstane-3,17-dione[b] | 0.83 | 0.77[d] | N.T. | 1.76[d] |
| 15. 4-Androstene-3,17-dione | 0.60 | 0.40[f] | 1.68 | 0.47 |

Each experimental group consisted of at least 6 animals. Control groups included 8–12 mice. Index = 1, no inhibition; index = 0, complete inhibition; index > 1, enhancement; N.T., not tested.

[a] Steroids No. 1 and 5 to 15 were purchased from Koch-Light Labs Ltd, Colnbrooke, Bucks, England. Steroids No.2–4 were a gift from Prof M. KOCÓR, Institute of Organic Chemistry, Warsaw. [b] Supposedly not present in mouse placenta. [c] Ear swelling in hydrocortisone-treated, sensitized mice was less than non-specific swelling in controls. [d] Mean of 2 independent experiments. [e] Mean of 3 independent experiments [f] Statistically significant $p < 0.01$.

Table II. Effects of Some Androstans on Lymphoid Tissues

| Compound | Thymus weight (mg ± S.D.) | Cells per thymus × $10^6$ | Spleen weight (mg ± S.D.) | Cells per spleen × $10^8$ |
|---|---|---|---|---|
| 5α-Androstane-3,17-dione | 25.4 ± 3.2 | 13.5 | 80.6 ± 27.1 | 0.38 |
| Testosterone | 27.4 ± 3.7 | 17.0 | 115.4 ± 50.7 | 1.78 |
| 17β-Hydroxy-5α-androstan-3-one | 20.6 ± 3.9 | 5.0 | 126.2 ± 31.9 | 1.10 |
| 4-Androstane-3,17-dione | 33.6 ± 4.2 | 27.0 | 98.2 ± 34.9 | 1.0 |
| 3α Hydroxy-5α-androstan-17-one | 31.8 ± 8.2 | 23.7 | 118.6 ± 46.3 | 1.69 |
| Controls | 52.1 ± 6.8 | 59.1 | 72.6 ± 22.5 | 0.42 |

Each experimental group consisted of 6 animals, the control group of 8 animals.

where *A* refers to ear swelling in sensitized animals receiving the tested compound; *B* refers to sensitized controls, receiving suspending medium only; *C* refers to non-specific swelling in normal animals tested with oxazolone. As the magnitude of response in JERNE test varied in control animals between 100–200 PFC/$10^6$ spleen cells similar indices were calculated for inhibition of haemolytic plaque formation. The Student *t*-test was used for evaluation of significance of inhibition. Results are given in Table I.

The influence of steroid compounds on the weight and cellularity of lymphoid tissue was tested in inbred CBA female mice weighing 20.0 ± 0.5 g. Each animal received s.c. injection of 1 mg of test compound daily for 7 days. Animals were bled out and the weight of spleen and thymus was recorded on day +8. The organs were teased apart and nucleated cells counted with haemocytometer. Results are shown in Table II.

*Results and discussion.* Our experiments were aimed to discover the relationship between the structure and immunosuppressive action of several androstane and pregnane derivatives. Some of these compounds are known to occur in mouse placenta, but their hormonal activity is very low. Whereas the immunosuppressive activity of testosterone, a compound which has not been identified in the placenta, was almost nil, some of its 5α-derivatives occurring in placenta decreased significantly the contact sensitivity and affected also, if slightly, the number of plaque-forming cells. In contrast, 5β-androstane-3,17-dione, a compound which is not present in the mouse placenta, was found to be inactive in inhibition of contact sensitivity, but enhanced significantly the PFC number. This suggests that steroid derivatives present in the mouse placenta exert the suppressive action. This suppressive activity depends on the steric configuration of the molecule.

As all androstane derivatives tested, regardless of their suppressive properties, promoted a significant decrease of thymus weight and cellularity, it cannot be excluded that the susceptibility of distinct subpopulations of thymocytes to particular androgens is different. It is known that testosterone stimulates erythropoiesis (for review see [21]). All androgens tested by us, except the most suppressive 5α-androstane-3,17-dione, caused a significant increase of spleen weight. Although we have no direct proof, it is possible that this is related to increased erythropoiesis. This effect of hormonally inert androgens deserves further attention.

The biological significance of our findings remains unknown, as we do not know the relationship between the doses of the compounds used and their natural output from the placenta. It should be noted, however, that the tested steroids were injected systematically to adult animals, whereas compounds synthetized in placenta may attain locally fairly high concentration and thus influence lymphocytes entering this area. This, in our opinion, may contribute to the other mechanism preventing the foetus rejection.

*Addendum.* When the manuscript of this paper was already completed, a paper of FABRIS [22] appeared in this Journal. He found that in the mouse the contact allergic reaction to picryl chloride is depressed during pregnancy. Our results might provide, at least partially, an explanation of this phenomenon.

*Zusammenfassung.* Der Einfluss der von uns in Mäuseplazenta gefundenen und identifizierten Progesteronmetaboliten auf humorale und zelluläre Immunität wurde untersucht. 5-α-Androstan-Derivate, vor allem aber 5-α-Androstan-3,17-dion setzen nur die Zellimmunität herab, während Pregnanderivate keinen Einfluss auf die Immunitätsvorgänge haben.

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