EFFECT OF THYMALIN ON SOME IMMUNOLOGIC PARAMETERS AND ON UTERINE STRUCTURE AND FUNCTION IN GUINEA PIGS WITH ENDOMETRIAL HYPERPLASIA

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A distinguishing feature of the pathogenesis of hyperplastic changes in the endometrium is that as a rule they arise and develop against the background of pre-existing neuroendocrine-metabolic disturbances, with inhibition of the immunity system [2, 5, 6].

The aim of this investigation was to study the efficacy and mechanisms of action of an immunomodulator (thymalin) in grandular hyperplasia of the endometrium.

EXPERIMENTAL METHOD

Experiments were carried out on 125 mature female guinea pigs weighing 350-420 g, of which 60 constituted the experimental and 65 the control (intact animals) group. Glandular hyperplasia of the endometrium was induced in the experimental animals by subcutaneous injection of an oily solution of dihydrostilbestrol (DHS) in a weakly dose of 1 mg for 19 weeks. Control guinea pigs received solvent (olive oil) only by the same schedule. On the 20th week 10 guinea pigs of each group were killed for histological confirmation of the presence of hyperplasia. In the experiments of series I, parameters of the immune system of the experimental animals were studied during the development of endometrial hyperplasia and administration of estrogens, whereas in series II the therapeutic effect of thymalin, its effect on morphology and structure of the endometrium, and the same immunologic parameters were studied. For the experiments of series I 40 experimental and 40 control animals were used. The immunologic investigations in the experimental group were carried out after 2, 5, 8, 10, 15, and 19 injections of DHS, and in the control group, after 5, 8, 10, 15, and 19 injections of oil. In the experiments of series II, of 30 animals with endometrial hyperplasia, 15 were given daily intramuscular injections of thymalin (0.4 mg in 0.2 ml physiological saline) for 5 days, and 15 animals received 0.2 ml of physiological saline at the same times. The immunologic investigations in this series of experiments were carried out before and 24 h after the last injection of DSH. After the end of the experiment all the animals were killed under ether anesthesia and the uterus was removed and weighed.

Material for subsequent histologic investigation was fixed in 10% formalin solution. Histologic sections were stained with hematoxylin and eosin.

For the immunologic investigations blood was taken from the hearts of the guinea pigs under ether anesthesia in a volume of 5 ml, mixed with 0.25 ml heparin (5000 U/ml), diluted with Hanks' solution in the ratio of 1:2, and layered on a density gradient of 1.107 g/cm^3 Ficoll (Pharmacia Fine Chemicals, Sweden) and Verografin (Spofa, Czechoslovakia). Mononuclear cells were isolated by the method of Belotskii and Snastina (1982) [1]. In the experiments with bovine erythrocytes loaded with IgG, the total population of B lymphocytes (B_{tot}), the total number of T lymphocytes, using rabbit lymphocytes [9], the number of active T lymphocytes (T_{act}) [10], the subpopulation of T lymphocytes with receptors for the Fc fragment of immunoglobulin M (IgM) and G (IgG) (T_μ and T_γ) [12]; and the number of B lymphocytes with receptors for mouse erythrocytes (B_m) [11], were studied. The relative percentages of lymphocytes (blood formula) were determined and the total number of leukocytes counted. The results were subjected to statistical analysis by Student's test.

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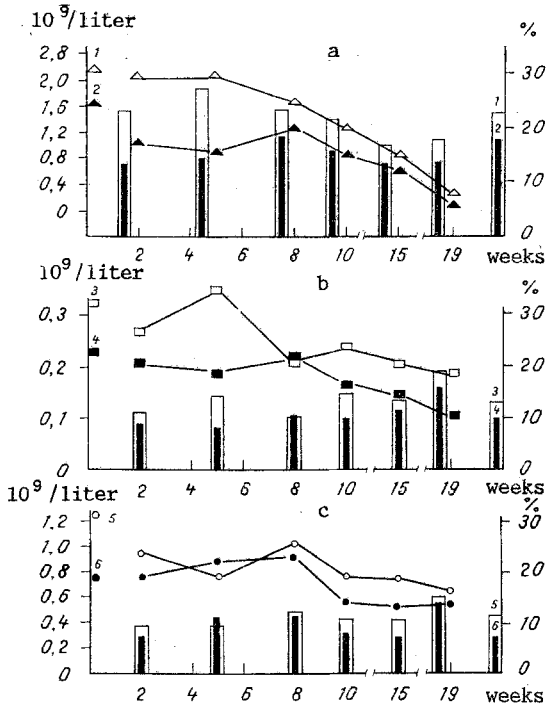

Fig. 1. Changes in parameters of immunity in female guinea pigs (n = 25) 2, 5, 8, 10, 15, and 19 weeks after injection of DHS (M ± m). a, b, c) Rosette-forming lymphocytes of guinea pigs. Changes in number of: 1) total T lymphocytes, 2) active T lymphocytes, 3) T_γ lymphocytes, 4) T_μ lymphocytes, 5) B lymphocytes, 6) B lymphocytes forming rosettes with mouse erythrocytes. Abscissa, time, in weeks; ordinate: on left, absolute number of rosette-forming cells (in 10^9/liter), on right, their relative percentages.

EXPERIMENTAL RESULTS

Injection of DHS led to marked changes in the epithelium of the endometrium of paired and unpaired parts of the uterus of the experimental animals. For instance, changes arising after injection of the estrogens took the form of the development of marked glandular hyperplasia of the endometrium. The uterus was cyanotic and congested, on section the folds were thickened, and the mucous membrane of the uterus showed marked hyperplasia. The relative volume of the glandular crypts was greatly increased. This increase took place both due to proliferation of the epitheliocytes lining the crypts and due to widening of the lumen of the glands, to more than twice their normal size. Glandular crypts were arranged irregularly in the stroma. Epitheliocytes lining the glandular crypts contained many mitoses, and in some places they were packed into epithelial sheets, forming outgrowths. The stroma of the endometrium was edematous. The lumen of many blood vessels was dilated and some of them had thickened walls and showed signs of thrombosis. The cyst-like expansion of the lumen of the glandular crypts was observed with areas of squamous-cell metaplasia of the prismatic epithelium. As a result of injection of DHS, especially in a dose of 19 mg, marked hyperplasia of the uterine mucosa thus developed. Incidentally, injection of DHS led to an increase in weight of the animals' uterus. In intact guinea pigs, for instance, the uterus weighed 2.0 ± 0.21 g, compared with 3.2 ± 0.04 g after administration of DHS ($p < 0.01$). As a result of injection of thymalin there was a tendency for the weight of the uterus to fall (to 2.6 ± 0.17 g). Histological investigation of the uterus showed that besides endometrial hyperplasia, regions of the uterine mucosa with the normal morphological structure were also observed, and the glands became shorter and tubular. Meanwhile the relative volume occupied by the glands decreased to 18.6 ± 3.2% whereas the volume of the stroma increased to 81.4 ± 5.3%. The lumen of the glandular crypts in this case was reduced by more than half (8.1 ± 0.8%), and they had the appearance of narrow slit-like spaces, lined with epitheliocytes, the relative volume of which was reduced to 10.5 ± 1.2%. In animals with endometrial hyperplasia and receiving physiological saline, no statistically significant changes were found in the morphometric parameters of the endometrium.

The study of the effect of DHS on the parameters of immunity in the guinea pigs demonstrated the inhibitory effect of estrogens on T and B lymphocytes. A significant change in the parameters was found to appear after 15 and 19 injections of DHS, coinciding with the development of glandular cystic hyperplasia of the endometrium in the experimental animals (Fig. 1). It was found, for instance, that the relative percentages and absolute numbers of T_{tot} and T_{act} were significantly reduced compared with intact animals ($p < 0.05$). Changes in T_μ (predominantly helper cells) and T_γ (predominantly suppressor cells) were not consistent. For instance, after 19 injections of DHS a tendency was observed for the relative

percentage of T_μ cells to increase, whereas the absolute number of these cells was reduced after only 10 injections of DHS, and this decrease continued until the end of the experiment. The relative percentage of T_μ also increased after 19 injections of DHS, but the absolute number of cells of the same type was unchanged. After 19 injections of DHS a twofold increase in the number of B_m cells was observed compared with intact animals (7.2 ± 0.7 and 14.23 ± 1.8%, respectively, $p < 0.05$). Estimation of the absolute number of these cells showed a tendency to decrease after the 10th injection of DHS (Fig. 1). Injection of estrogens into the guinea pigs also caused increased formation of circulating immune complexes.

Injection of thymalin led to restoration of the altered parameters of the immunity system. In animals with endometrial hyperplasia, besides the parameters already mentioned, there was also a significant decrease in the total number of leukocytes (from 15.37 ± 1.21 10^9/liter in intact animals to 6.3 ± 0.65 10^9/liter in the experimental animals; $p < 0.001$) and in the relative percentage of lymphocytes (from 78.4 ± 1.88 to 57.1 ± 4.9%, respectively; $p < 0.001$). Meanwhile there was a decrease in the total number of T lymphocytes, their active fraction T_{act}, and also T_μ and T_γ. Thymalin led to an increase in the number of leukocytes back to the normal values (15.37 ± 1.21 10^9/liter normally, 11.7 ± 0.9 10^9/liter in animals with hyperplasia and receiving thymalin). After administration of thymalin the number of T_{tot} and T_{act} increased, although it still remained low. Normalization of the numbers of T_μ, T_γ, and B_m also was observed, and the number of A-RFC was actually increased.

The experiments showed that the development of endometrial hyerplasia under these conditions is accompanied by an immunodeficiency state. Equilibrium is disturbed in various systems of the body, especially the endocrine and immune systems. Changes found in the T and B lymphocyte systems reflect processes taking place under the influence of the synthetic estrogen analog DHS in the central and peripheral organs of immunogenesis. In particular, it was shown experimentally that under the influence of estrogens involution of the thymus takes place, with a decrease in the number of lymphocytes in the thymus, spleen, and lymph nodes [6]. Administration of thymalin against this background to animals with endometrial hyperplasia induced by DHS led not only to normalization of the parameters of the immune system, but also to partial normalization of the morphological structure of the pathologically changes uterine mucosa. This effect suggests that there are several mechanisms of action of this preparation, not only on the immune, but also on the endocrine system. On the one hand, it may have a regulatory action on intracellular biochemical processes and on expression of differential antigens on the lymphocyte surface [8], a stimulating effect on interferon production when administered [4], and on the other hand, an inhibitory action on the glucocorticoid function of the adrenals [8]. It can also be postulated that thymalin, like other thymic hormones, influences pituitary function and, in particular, stimulates the production of luteinizing hormone and its releasing factor [13].

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