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REDISTRIBUTION OF T AND B LYMPHOCYTE POPULATIONS AMONG LYMPHOID ORGANS DUE TO HYDROCORTISONE

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KEY WORDS: hydrocortisone; lymphoid organs; T and B lymphocytes.

The mechanism of action of corticosteroid hormones on lymphoid tissue has been studied by no means completely. However, it is already clear that corticosteroids have a varied influence, depending on many factors: the type of corticosteroid, the dose and duration of its administration, and the species of the test animal. Reports have recently been published indicating that glucocorticoids affect predominantly migration and recirculation of lymphocytes *in vivo*. The most marked changes are those in recirculation of the T lymphocyte pool [1, 3, 5, 6]. However, the possibility of an effect of corticosteroid hormones on redistribution of the B cells also cannot be ruled out, although the data on this question are contradictory [7-10].

The object of this investigation was to study the redistribution reactions of T and B lymphocytes after injection of hydrocortisone into guinea pigs which, like man, are a cortisol-resistant species.

EXPERIMENTAL METHOD

Experiments were carried out on 105 guinea pigs weighing 300-400 g. Hydrocortisone acetate (from Gedeon Richter, Hungary) was used in the investigations. Hydrocortisone was given as a single intramuscular injection in doses of 30 and 100 mg/kg body weight. The relative and absolute numbers of T and B lymphocytes in the thymus, bone marrow, paratracheal lymph nodes, spleen, and peripheral blood were determined 4, 12, and 24 h after injection of the hormone. T lymphocytes were identified by their ability to form spontaneous rosettes with rabbits' erythrocytes [9]. B lymphocytes were identified by their possession of receptors for the 3rd component of complement [4].

Rosette-forming cells were counted after staining with acridine orange in a mixture of ordinary and UV light. Structural changes in the lymphoid organs were analyzed in histological preparations stained with hematoxylin and eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

The results indicated early changes in the ratio between T and B lymphocyte populations after administration of hydrocortisone (Fig. 1).

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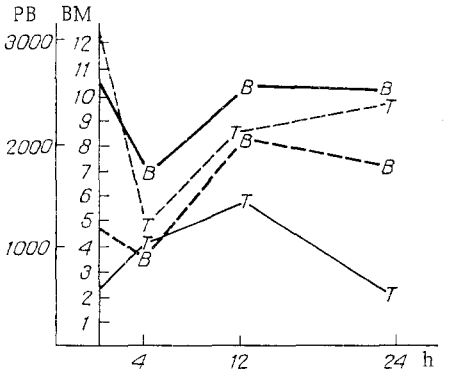

Fig. 1. Changes in absolute number of T and B lymphocyte populations in peripheral blood (broken lines) and bone marrow (continuous lines) at various times after injection of hydrocortisone (100 mg/kg). Abscissa, time of investigation (in h); ordinate: PB) number of cells in 1 μl blood, BM) number of bone marrow cells per tibia ($\times 10^6$).

In the peripheral blood of animals of the control group T cells accounted for 44% of the total number of lymphocytes. Lymphocytes with surface receptors for the 3rd component of complement accounted for 16%. Injection of hydrocortisone led to a similar reaction to both doses, in the form of lymphocytopenia, which reached its lowest level by the 4th hour after injection. The lymphocyte count fell from 6400 ± 600 to 3100 ± 400/μl with a dose of 100 mg/kg and to 3900 ± 600 with a dose of 30 mg/kg. The decrease in the percentage of T lymphocytes was accompanied by a marked decrease in their absolute number — by more than half (Fig. 1). Conversely, the number of B lymphocytes showed no marked changes by this time: The increase in their number to 28-30% against the background of lymphocytopenia reflected only very small fluctuations in their absolute number. At later stages gradual normalization of the total lymphocyte count was observed in the peripheral blood. There was a simultaneous and sharp rise in the number of B lymphocytes, and by 24 h their number was 50-100% higher than the normal level.

In the bone marrow the original content of T cells was relatively high, namely $(2.5 \pm 0.4) \cdot 10^6$ cells per tibia. Whereas in response to injection of small doses of hydrocortisone the changes in the number of T lymphocytes in the bone marrow were slight, doses of 100 mg/kg caused a marked increase in their number, reaching a maximum — $(5.8 \pm 1.4) \cdot 10^6$ — after 12 h. The number of B cells in the bone marrow fell in the early stages, both in relative and in absolute terms, without any significant changes in the total number of lymphocytes.

No characteristic changes were found in the lymphocyte populations in the thymus, spleen, and lymph nodes after injection of the various doses of hormones.

Histological analysis revealed no sign of massive death of the lymphocytes in any of the organs tested.

Injection of hydrocortisone was thus accompanied by marked redistribution of the lymphocyte populations between the central lymphoid organs and peripheral blood. In particular, under the influence of hydrocorticoids, an early transient T lymphocytopenia developed in the peripheral blood. The mechanism of the corticosteroid T-lymphocytopenia may be connected in part with the transfer of T cells from the recirculating pool to the bone marrow. The increase in the number of B lymphocytes in the blood, however, coincided with their release from the bone marrow. Transfer of T lymphocytes into the bone marrow may have a regulating effect on medullary hematopoiesis [1]. The changes found in the populations of T and B lymphocytes after injection of hydrocortisone must be regarded as predominantly redistributive.

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EFFECT OF TRYPSINIZATION OF BONE MARROW ON EFFICIENCY OF FIBROBLAST COLONY FORMATION IN MONOLAYER CULTURES

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Stromal mechanocytes are responsible for the transfer of the hematopoietic microenvironment during heterotopic bone marrow transplantation [4]. These cells, together with hematopoietic cells, are readily liberated from tissue structures during preparation of cell suspensions by the ordinary mechanical method, such as by passing fragments of bone marrow through a needle from a syringe. On explantation of bone marrow cells into monolayer cultures stromal precursor cells give rise to colonies, which are clones of fibroblasts [3]. Cells forming fibroblast colonies (CFFC) are present in a suspension of bone marrow cells in a very low concentration — not more than one CFFC/10^4 hematopoietic cells [2]. However, in bone marrow sections and films stromal cells — reticular cells of all types — account for up to 3% of the total number of nucleated cells [5], i.e., fewer than 1% of stromal cells exhibit clonogenic properties. It can be tentatively suggested that the remaining stromal cells either have no clonogenic properties or, since they are firmly bound in bone marrow structures, they are deprived of their colony-forming ability because of partial injury in the process of suspension of the tissue.

The possibility of increasing the number of clonogenic stromal cells in a bone marrow suspension by preliminary treatment of fragments of hematopoietic tissue with trypsin was studied in the investigation described below.

EXPERIMENTAL METHOD

The bone marrow donors were guinea pigs weighing 180-350 g and (CBA × C57BL)F_1 mice weighing 18-20 g. Bone marrow from the femoral or tibial diaphysis was flushed out as fragments into 0.25% trypsin solution, in which it was incubated for 60-90 min at room temperature, with periodic gentle shaking. During incubation some of the cells were washed from the fragments into the trypsin, and for that reason every 20-30 min the cell suspension was drawn off and transferred to centrifuge tubes, with the addition of 10% embryonic bovine serum. The remaining fragments of bone marrow were covered with a fresh portion of trypsin solution. At the end of trypsinization the bone marrow cells were sedimented by centrifugation, resuspended by forcing through a needle from a syringe into medium No. 199, filtered

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