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# EFFECT OF THE THYMUS ON PRECURSOR CELLS OF THE HEMATOPOIETIC STROMA

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It was shown by transplantation of a fragment of bone marrow beneath the capsule of the kidney that thymectomy reduces the number of cells constructing the hematopoietic microenvironment. Transplantation of the thymus abolishes the effect of thymectomy.

KEY WORDS: hematopoietic microenvironment; precursor cells; heterotopic focus of hematopoiesis; thymectomy; stroma of hematopoietic organ.

According to several recent reports the thymus influences not only differentiation of the T lymphocytes, but also earlier hematopoietic cells. Although data in the literature are contradictory, it can be concluded that thymectomy, whether neonatal or in the adult animal, modifies endocolonization and the number of hematopoietic stem cells (HSC) in the body [1-3, 11, 15], reduces the proliferative activity of HSC [8, 9], and disturbs the ability of the HSC to regenerate after sublethal radiation injury [4]. In all these cases it is not yet clear whether the results of thymectomy are due to its direct effect on hematopoietic cells or whether they can be partly attributed to its action on the hematopoietic microenvironment, controlling proliferation and differentiation of HSC [5].

It was therefore decided to study the effect of thymectomy and subsequent transplantation of the thymus on the function of cells transmitting the hematopoietic microenvironment.

## EXPERIMENTAL METHOD

Female CBA and (C57BL × CBA)$F_1$ mice were used.

Thymectomy was performed on mice at the age of 8-10 weeks under hexobarbital anesthesia [7]. For mock thymectomy the same operation was performed but without aspiration of the thymus.

The mice were irradiated with $^{137}Cs$ $\gamma$ rays (dose rate 21 rad/min) in a dose of 1300 rad. After irradiation the animals received an intravenous injection of syngeneic embryonic liver cells (age of the embryo 14.5 days) in a dose of $12 \cdot 10^6$ cells.

One lobe of neonatal mouse thymus was transplanted into thymectomized syngeneic recipients beneath the kidney capsule.

A focus of heterotopic hematopoiesis was obtained by transplanting a fragment of femoral marrow beneath the kidney capsule of a syngeneic recipient. Under these conditions the hematopoietic cells leave the implant and the stromal precursor cells form the hematopoietic microenvironment, which is secondarily colonized by the recipient's hematpoietic cells [10]. The size of the developing focus, proportional to the

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TABLE 1. Effect of the Thymus on Precursor Cells of Hematopoietic Stroma

| Experiment | Donors of bone marrow | Number of cells in focus of heterotopic hematopoiesis, · $10^6$ (M ± m) | | P |
|---|---|---|---|---|
| | | recipients | | |
| | | intact | thymectomized | |
| 1 | Mock-thymectomized | 10,5±1,08 | — | |
| | Thymectomized | 6,2±0,83 | — | <0,01 |
| | Thymectomized + transplantation of thymus | 8,2±0,95 | — | insignificant |
| 2 | Mock-thymectomized | 18,9±1,45 | 13,9±1,24 | |
| | Thymectomized | 14,5±1,27 | 10,7±1,09 | <0,05 |
| | Thymectomized + transplantation of thymus | 22,2±1,57 | 16,3±1,35 | insignificant |
| 3 | Not thymectomized, irradiated, and restored with embryonic liver cells | 14,7±1,28 | — | |
| | Thymectomized, irradiated, and restored with embryonic liver cells | 9,7±1,04 | — | <0,01 |

number of stromal precursors [6], was determined from the number of hematopoietic cells in it one month after implantation of bone marrow. Cells from 8-10 foci were used at each point.

The effect of thymectomy was studied 3-4 months after the operation (Table 1, experiments 1 and 2) or 8 months after thymectomy, irradiation, and transplantation of embryonic hematopoietic cells (Table 1, experiment 3). The time elapsing after transplantation of the thymus was 2 months, (Table 1, experiment 1) or 1 month (Table 1, experiment 2).

## EXPERIMENTAL RESULTS

The results are given in Table 1. They show that after thymectomy on the donors of bone marrow the size of the developing focus of heterotopic hematopoiesis was considerably reduced. The effect of thymectomy was abolished 2 months after transplantation of the thymus (Table 1, experiment 1).

Thymectomy on the recipients of the bone marrow did not potentiate the effect of removal of the thymus from the donors of bone marrow, although the size of the developing focus was smaller in thymectomized recipients of all groups than in the control mice (Table 1, experiment 2). In this case also, transplantation of the thymus abolished the effect of thymectomy of the donors after 1 month.

Lethal irradiation of the thymectomized mice, followed by injection of embryonic hematopoietic cells did not potentiate the effect of thymectomy on the ability of stromal precursors to build a focus of heterotopic hematopoiesis (Table 1, experiment 3).

Thymectomy thus affects the stromal precursor cells of hematopoietic tissue: a few months after thymectomy either their number or their ability to build the heterotopic hematopoietic stroma is reduced. The effect is specific, for mock thymectomy had no such action and transplantation of the thymus quickly abolishes it. It can tentatively be suggested that the thymus has a direct influence on the stroma and does not act on it indirectly, through the system of T lymphocytes for example. In fact, 3-4 months after thymectomy, before the functions of the system of T lymphocytes are substantially disturbed [12, 13, 15], the ability of the stromal precursors to transmit the hematopoietic microenvironment was already reduced. On the other hand, a marked reduction in the number of mature T cells in the body as a result of lethal irradiation with transplantation of hematopoietic cells not containing postthymic precursors of T lymphocytes (embryonic liver) did not potentiate the effect of thymectomy on stromal precursor cells.

The effect of thymic factors on the function of the immunologically incompetent cell system can significantly widen our ideas of the physiological role of the thymus, and it therefore requires further study.

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# PATHOLOGY OF MITOSIS AFTER RECOVERY OF CELLS FROM METAPHASE BLOCK

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Experiments on fibroblast-like Chinese hamster cells showed that agents inducing c-mitosis (colchicine, colcemid, low temperature) give rise to two distinct effects: stathmokinetic and radiomimetic. Toward the time of reversibility of the first of these effects, the second becomes clearly manifested as bridge formation. The appearance of this pathological form is evidently due to disturbance of cell nucleoprotein metabolism during c-mitosis.

KEY WORDS: pathology of mitosis; stathmokinetic effect; radiomimetic effect; chromosome bridges.

The appearance of colchicine-like mitosis, or c-mitosis, may be caused by several factors. The c-mitosis is connected with disturbance of several mechanisms of normal mitosis [1]. Our previous studies of colchicine-like mitosis were mainly concerned with the study of the character of injury to the microtubules of the division spindle and ways of its repair, depending on the factor causing the c-mitosis. We were also interested in the successive replacement of pathological forms of mitosis during the development of the stathmokinetic response and in the course of its reversibility [5, 6], due to differences in the degree of disorganization of the mitotic spindle [7]. The attainment of the control values of the mitotic index and of the relative proportions of the stages of mitosis demonstrated normalization of the spindle and the ability of the cells to recover from metaphase block and to complete the final stages of mitosis – anaphase and telophase. However, analysis of these last stages of mitosis was not studied at that time; the present investigation was devoted to this problem.

## EXPERIMENTAL METHOD

Just as in the previous investigations [2, 4, 5], experiments were carried out on a monolayer culture of Chinese hamster fibroblast-like cells (clone 237). Colchicine (exposure for 30 min, 1 $\mu$g/ml), colcemid (exposure for 2 h, 0.03 $\mu$g/ml), and cold shock (culture for 2 h at 21°C) served as the inducers of the stathmokinetic reaction. The doses and exposures used were such that, after removal of the alkaloid by washing or after heating (37°C) of the cooled cultures, the latter were able to recover from the metaphase block and to complete mitosis.

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