ROLE OF T- AND B-LYMPHOCYTES IN THE RESPONSE OF THE HEMATOPOIETIC SYSTEM TO STRESS

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UDC 612.119-06:612.112.94.017.1

KEY WORDS: T- and B-lymphocytes; stress.

The quantitative characteristics of responses of the blood system (peripheral blood, bone marrow, thymus, and spleen) of animals exposed to various types of stress have been described and the general principles governing them determined [2, 3, 5-7]. One of the most constant phenomena of the response of the bone marrow is a significant increase in the number of lymphocytes in the first 6-12 h after the beginning of stress. This phenomenon has been called the "lymphoid peak." Special experiments on (CBA × C57BL)F_1 hybrid mice showed previously that the bone marrow in this period contains T-lymphocytes, which are not found in intact mice of this genotype [8]. However, it is not yet clear what is the source of this rapid and considerable accumulation of lymphocytes in the bone marrow of animals exposed to stress: Is it due to regeneration or migration; likewise, the physiological significance of the increased number of lymphocytes in the bone marrow in the early phase of the response to stress has not been determined. Considering new data on the possible role of cooperation between the T-lymphocyte and the hematopoietic stem cell, it was deemed important to make a more detailed study of the role of T- and B-lymphocytes in responses of the hematopoietic system to stress.

Experiments were carried out for this purpose on (CBA × C57BL)F_1 hybrid mice with different degrees of T-lymphocyte deficiency. Altogether there were four groups of animals in the experiment.

Mice of group 1 were thymectomized 30 days before the beginning of the experiment to produce a deficiency of short-living nonrecirculating T_1-lymphocytes. Mice of group 2 underwent a mock thymectomy at the same time. Animals of group 3 received a single injection of antilymphocytic serum (ALS) 3 days before the beginning of the experiment, which led to a marked decrease in the circulating pool of T_2-lymphocytes. Group 4 consisted of so-called B-mice – animals in which T-lymphocytes were virtually completely absent. The thymus was removed from mice and, 1 month later, they were irradiated with γ-rays in a dose of 750 R, with protection by injection of syngeneic bone marrow from intact donors after irradiation. These mice were used in the experiment 30 days later.

All the animals were immobilized for 6 h in the supine position. They were decapitated 6, 9, 12, 24, and 48 h after the beginning of stress and, by methods described previously [1], the number of cells was counted in the spleen and femoral marrow. The number of cells of different series in the bone marrow also was counted. The leukocyte count and leukocyte formula were determined in the peripheral blood.

The data were obtained from repeated experiments.

Results obtained in all groups of mice before the beginning of immobilization were used as initial data. The results of experiments on mice undergoing mock thymectomy were used as the standard for comparison of all the other values obtained, for these animals gave the typical response of animals of this species to immobilization for 6 h.

A typical stress response appeared in the peripheral blood of the mice of all four groups in the first 12 h after the beginning of immobilization, namely marked neutrophilia and lymphocytopenia, returning to the initial level after 24 h. Only certain quantitative differences were found between the groups.

Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 4, pp. 415-417, April, 1980. Original article submitted July 10, 1979.

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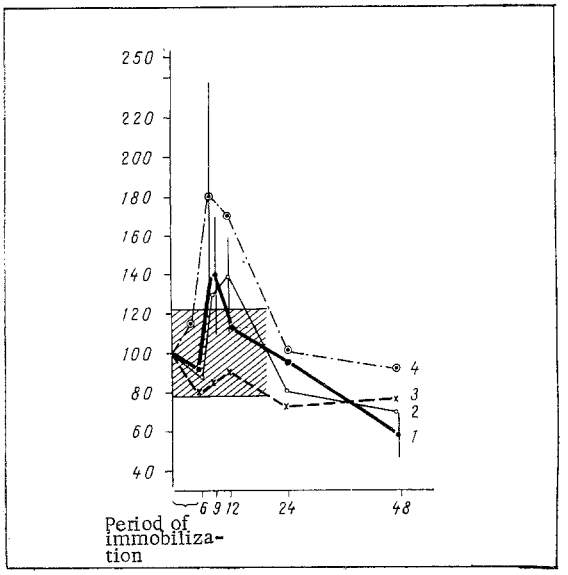

Fig. 1. Number of lymphocytes in femoral marrow of thymectomized mice (1), mice undergoing mock thymectomy (2), mice receiving ALS (3), and B-mice (4) after immobilization. Abscissa, time after beginning of stress (in h); ordinate, number of cells (in % of initial level). Vertical lines indicate confidence interval; shaded area shows confidence interval for intact animals; each point represents mean data for 10 animals.

The dynamics of the quantitative changes in the lymphocytes in the bone marrow of the different groups of mice is illustrated in Fig. 1. The graphs in Fig. 1 show that thymectomy did not affect the size of the lymphoid peak, which was about 140% of the initial level and was identical quantitatively to the analogous peak in the control group of mice undergoing mock thymectomy. The greatest increase in the number of lymphocytes occurred in the B-mice (to 180%). An artificial decrease in the pool of recirculating T-lymphocytes in the peripheral blood (injection of ALS) prevented the development of the lymphocyte peak during stress, for during the whole of this period of observation the number of lymphocytes remained within the lower level of confidence limits, i.e., about 20% below the initial level. These observations thus confirmed once again that both B- and T-lymphocytes, belonging to the pool of recirculating cells, participate in the formation of the lymphoid peak in the bone marrow of animals exposed to stress. Complete prevention of the increase in the number of lymphocytes in the group of animals receiving ALS indicates that the serum may affect not only T-lymphocytes, but also a certain proportion of the recirculating B-lymphocytes. It will be recalled that there is information in the literature to the effect that a certain proportion of T-cells surviving after irradiation and capable of recirculation is preserved in B-mice [13].

As regards granulocytes, during the first 12 h a decrease both in the number of mature neutrophils (stab cells and polymorphs) and in the number of young forms (myeloblasts–myelocytes) was observed in the bone marrow of all mice. Later, the number of these cells rose rapidly, to reach the initial level or even to exceed it. Signs of hyperregeneration were particularly marked in the group of blast cells of the neutrophil series in mice of the control group (Fig. 2). On the basis of previous investigations the writers postulated that the development of a lymphoid peak is essential for stimulation of granulocytopoiesis [3]. However, judging from the data given in Fig. 2, there was no increase in the number of young cells of the granulocyte series in the bone marrow of the B-mice during stress, despite the fact that these animals had the highest lymphoid peak. But this does not necessarily contradict the hypothesis. The absence of an effect of stimulation of medullary hematopoiesis in the B-mice during stress, in the writers' opinion, is evidence merely that T-lymphocytes are evidently necessary for normal hematopoiesis and for stimulation of granulocytopoiesis, for previous investigations showed that T-cells accelerate postradiation recovery of the cell composition of the bone marrow and spleen [9, 11]. The effect depends on the number of T-cells. Thymectomy removes the

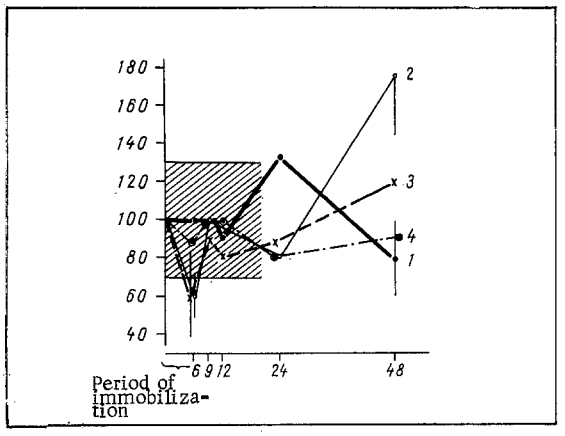

Fig. 2. Number of young cells of granulocyte series (myeloblasts–promyelocytes–myelocytes) in femoral marrow after immobilization. Legend as in Fig. 1.

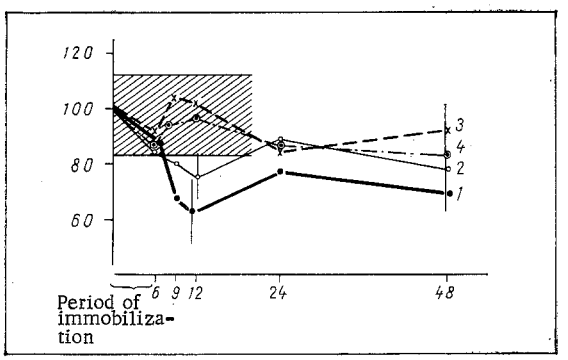

Fig. 3. Number of cells in spleen after immobilization. Legend as in Fig. 1.

subclass of T-cells whose interaction with stem cells is essential for granulocytopoiesis. In fact, hematopoietic stem cells from thymectomized mice lose their ability to differentiate along the granulocyte path, and predominantly erythroid colonies grow in the recipient's spleen [12].

Investigation of the number of cells in the spleen (Fig. 3) showed that the decrease in the number of cells in the organ, so characteristic during the first 6-9 h after exposure to stress, was observed in the control group and in the group of thymectomized animals. The number of cells in the spleen of the B-mice and also of mice receiving the preliminary injection of ALS remained unchanged during 2 days of observation. The decrease in the number of cells in the spleen during the first hours after stress is largely connected with contraction of the capsule of the organ, which is abolished by α-adrenoreceptor blockade [4], and it involves T-lymphocytes, for in B-mice, when T-lymphocytes were absent or their number was considerably reduced, the phenomenon of a decrease in the number of cells in the spleen during stress was completely absent. Confirmation that the disappearing cells are connected with the recirculating pool of T-lymphocytes may also be given by the fact that 3 days after injection of ALS, when there was a considerable decrease in the number of circulating lymphocytes, the response of the spleen to stress also was absent.

The experiments described above confirm the view that the increase in the number of lymphocytes in the bone marrow is due mainly to their migration, for only in the group of mice receiving ALS, in which the number of lymphocytes in the peripheral blood was reduced by 70%, was the number of lymphocytes in the bone marrow not only not increased after stress, but actually reduced to the lower limits of normal.

The results of these experiments thus showed that the lymphoid peak in the bone marrow during the first 12 h after stress evidently arises through migration of T- and B-lymphocytes into the bone marrow. The main source of the emergency increase in the number of T-lymphocytes in the bone marrow during stress is the spleen, a conclusion in good agreement with the results of previous experiments on spleenectomized animals [10].

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