MIGRATION OF T-LYMPHOCYTES INTO BONE MARROW AS AN INITIAL RESPONSE TO STRESS

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From 6 to 9 h after the beginning of the response to stress caused by immobilization for 6 h, cells with antigenic (θ-antigen) and functional (helper cells) characteristics of T-lymphocytes appeared in the bone marrow of CBA mice. Migration of T-lymphocytes into the bone marrow is regarded as a mechanism for increasing the nonspecific reactivity of the body.

KEY WORDS: bone marrow; stress; migration of T-lymphocytes.

A decrease in the number of lymphocytes in lymphoid tissues and in the circulating blood is a characteristic feature of the state of stress. Several factors are involved in this response, the most important of which are mobilization of lymphocytes and their redistribution within the lymphomyeloid system, with migration into the bone marrow [2-5]. Methods recently developed for identifying T- and B-lymphocytes make it possible to investigate the principles governing their migration in the intact organism [6].

The object of this investigation was to discover the pathways of migration and the origin of lymphocytes migrating into the bone marrow during stress and to assess the possible physiological consequences.

EXPERIMENTAL METHOD

Experiments were carried out on mice of strains AKR, CBA, and F_1(CBA × C57BL) obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR and from the laboratory of Experimental Models in the village of Svetlye Gory. The number of nucleated cells in the femur was determined by the method described previously [3]. The absolute number of lymphocytes in the femur was determined from the myelogram counted in squash preparations stained by Pappenheim's method.

To obtain anti-θ-serum the method described in [12, 14] was used. Immunization was carried out by six weekly intraperitoneal implantations of 10 million thymocytes from CBA mice into AKR mice.

The cytotoxic test with Trypan Blue was carried out by the method of Boyse et al. [7] in the modification of Schlesinger [15] and the writers. Living cells numbering $0.5 \cdot 10^6$-$1 \cdot 10^6$/ml were incubated in antiserum (dilution 1:4) at room temperature for 30-45 min. After centrifugation the antiserum was removed, complement was added in a final concentration of 1:4, and the samples were incubated for 30 min at 37°C. Guinea pig serum, twice adsorbed in the cold with spleen cells of CBA mice and with cells of NK/Ly mouse ascites tumor, in the proportion of one-tenth by volume, were the source of complement. After treatment with complement the cells were sedimented, the supernatant was removed, and the sedimented cells were resuspended in Trypan Blue solution in a concentration of 1:2000. The number of living cells was counted in a Goryaev's chamber. The cytotoxic index obtained was corrected by subtracting the number of dying cells in the control series (the same manipulations but

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TABLE 1. Morphological and Functional Changes in Mouse Femoral Marrow During Stress Response

Index	Control	Time after immobilization (in h)	
		6–9	24
Number of cells in femur × 10^6	33,9±1,81 (13)	32,8±1,45 (12)	35,4±2,74 (5)
Number of lymphocytes in femur × 10^6	7,9±0,54 (13)	10,1±0,53 (12) $P<0,05$	9,5±0,38 (5)
Cytotoxic index (anti-θ) (in %)	2,4±0,89 (12)	12,5±3,85 (6) $P<0,05$	2,8±2,48 (6)
Number of AFCs in 10^8 spleen cells	9,0±2,40 (20)	25,0±3,00 (9) $P<0,05$	—
Number of CFUs in femur × 10^3	4,8±0,30 (9)	7,0±0,38 (10) $P<0,05$	5,3±0,51 (6)

Legend. Number of animals shown in parentheses.

without antiserum) from the result [16].

Cytotoxic C3H anti-θ-serum in a concentration of 1:8-1:16 "killed" 100% of thymocytes.

The number of antibody-forming cells (AFCs) was determined in the spleen of recipient mice (culture *in vivo*) by the local hemolysis test [9] on the eighth day after transfer of the bone marrow cells and sheep's red cells.

Colony-forming stem cells (CFUs) in the bone marrow were determined by the method of Till and McCulloch [18].

All the results were subjected to statistical analysis by means of Student's parametric criterion.

EXPERIMENTAL RESULTS

During the first 24 h of the stress response evoked by immobilization for 6 h no significant change took place in the number of nucleated cells in the femoral marrow (Table 1). Morphological investigation after 6 h showed a stereotyped nonspecific response consisting of an increase in the number of lymphocytes similar to that described previously [2, 3]. The problem is whether the lymphocytes which migrate into the bone marrow during stress are T (thymus-dependent) or B (thymus-independent). By means of certain tests the origin of the migrating cells could be determined. As a result of cooperative intercellular interaction some T-lymphocytes (helper cells) can become involved in the immune response of bone marrow cells (B-lymphocytes).

One of the markers of T-lymphocytes also is the θ-antigen, located on the cell membrane. θ-Positive cells can be detected by the cytotoxic test with anti-θ-serum [8, 12, 13].

Under physiologically normal conditions no T-lymphocytes are present in the bone marrow of CBA mice [8, 13]. This was confirmed by the present experiments. However, 6 h after the beginning of stress cells with characteristics of T-lymphocytes appeared in the marrow of the CBA mice. As Table 1 shows, the number of θ-positive cells increased at this period in the marrow. There was also a marked increase in the number of AFCs in the culture of bone marrow cells. After transplantation of 10^7 bone marrow cells from intact mice and $2 \cdot 10^6$ sheep's red cells into lethally irradiated recipients (culture *in vivo*), for instance, 9 ± 2.4 AFCs were formed in the recipients' spleens. Immobilization for 6 h led to a significant increase in the number of AFCs in the spleen of recipients receiving the same dose of marrow cells and antigen (Table 1). This increase in the number of AFCs could most likely have been the result of interaction between B-cells and T-cells migrating into the bone marrow.

In the same period there was an increase in the number of stem (colony-forming) cells in the marrow of the experimental mice.

There is thus every reason to conclude that during exposure to an unfavorable factor (stress) T-lymphoyctes migrate into the bone marrow. Migration of T-lymphocytes into the marrow has also been observed in a number of artificially created experimental situations: after intravenous injection of large numbers of thymocytes [11, 17] and after injection of a large dose of hydrocortisone [8].

What is the physiological importance of this redistribution of the T-lymphocytes? Ultimately an increase in the immune competence of the bone marrow and an increase in the number of stem cells could be aimed at increasing the resistance of the body to the harmful factor and could probably be the basis for development of the next stage of the adaptation syndrome, i.e., resistance. A similar situation can arise evidently in response to stressors of antigenic nature.

A special role in this response belongs to T-lymphocytes, the regulatory role of which in the immune response is at present under intensive study. Their action extends not only to precursors of immunocompetent cells, but also evidently to hematopoietic stem cells. Experimental evidence in support of this last conclusion already exists [1, 10].

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