The problem of the molecular mechanisms of interaction between the bacterial agents tested in this study and immune receptors of lymphocytes requires further investigation.

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ROLE OF THE THYMUS IN REGULATION OF STROMAL CELLS TRANSFERRING THE HEMATOPOIESIS-INDUCING MICROENVIRONMENT IN STRESS

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The thymus is a universal organ which takes part in the formation of several complex homeostatic reactions of the individual [1, 4]. The writers showed previously on a model of immobilization stress that the thymus exerts its regulatory influence on hematopoiesis through the T-cell system [6]. In the modern view, processes of proliferation and differentiation of committed cells (precursors of myelopoiesis) are largely determined by the functional state of the stromal cells involved in the formation of the hematopoiesis-inducing microenvironment (HIM) of the bone marrow [5, 7]. The thymus can evidently influence the activity of the cells composing HIM [5]. However, information on this problem is extremely insufficient, and the role of the thymus in the regulation of HIM during exposure of the individual to extremal factors not possessing a myeloinhibitory effect has not been studied. Accordingly, the aim of the present investigation was to determine the role of the thymus in the regulation of the functional state of cells responsible for the transfer of HIM and the precursor cells of myelopoiesis during stress.

EXPERIMENTAL METHOD

Experiments were carried out on 850 male (CBA × C57BL)F_1 mice weighing 18-21 g (from the "Rappolovo" nursery, Academy of Medical Sciences of the USSR). The animals were immobilized for 10 h in the supine position. At different times after immobilization the mice were killed by destruction of the spinal cord in the neck. The total number of myelokaryocytes (TNC) per femur was determined. The myelogram was counted in bone marrow films. The thymus of some of the mice was removed 1 month before immobilization or a corresponding mock operation was performed. The number of colony-forming units (CFU) was counted by the use of

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TABLE 1. Dynamics of TNC, Number of CFU, Cell Content, and Mass of "Primary" and "Secondary" Ectopic Foci of Hematopoiesis from Bone Marrow of (CBA × C57BL)F_1 Mice Subjected to Immobilization for 10 h and to Mock Thymectomy (I) or Thymectomized (II) (M, P_u)

Parameter	Time after immobilization, days						
	before immobilization, days	2	3	4	5	6	7
TNC, $\times 10^6$	18,5	18,4	17,6	18,7	23,9	25,8*	20,1
CFU, $\times 10^5$	22,3	20,5	23,7	38,5	76,9*	61,3*	24,5
erythroid, %	0	—	—	—	12,1*	7,8*	—
granulocytic, %	17,5	—	—	—	18,9	26,3*	15,0
granulocytic-macrophagal, %	35,5	—	—	—	26,3*	19,0*	25,3*
macrophagal, %	47,0	—	—	—	42,7	46,9	59,7
Ectopic focus:							
"primary" (cell content, $\times 10^6$; mass, mg)	14,1	—	20,8*	22,3*	23,4*	19,1	—
	1,58	—	2,2	2,0*	3,1*	2,1	—
"secondary" (cell content, $\times 10^6$; mass, mg)	7,5	—	8,6	11,6*	8,6	7,9	—
	1,0	—	0,8	1,8*	1,7*	1,2	—
TNC, $\times 10^6$	19,6	18,5	18,2	17,3	19,5	19,3	19,7
CFU, $\times 10^5$	24,5	21,7	22,8	25,3	21,9	23,7	22,5
Ectopic focus:							
"primary" (cell content, $\times 10^6$; mass, mg)	17,5	—	10,9*	17,5	20,3	11,6	—
	1,9	—	1,5*	1,5	2,3	1,9	—
"secondary" (cell content, $\times 10^6$; mass mg)	6,1	—	4,6	7,4	7,4	7,6	—
	0,9	—	0,6	1,0	1,9	1,0	—

*Indicates significance of differences: $P_u < 0.05$.

diffusion chambers, incubated in the peritoneal cavity of recipient mice (for 7 days), treated beforehand with cyclophosphamide, as described previously [6]. The optimal concentration of bone marrow cells (chosen as the result of a study of the number of cells transplanted into tissue culture and the number of colonies formed from them) was 0.4×10^9/liter of medium of the following composition: 10% embryonic calf serum, 40% of medium D-MEM, 20% intact rabbit plasma, 30% Alsever's solution, 290 mg/liter of L-glutamine, and 500 mg/liter of $CaCl_2$. The term colony is taken to mean a cellular aggregate containing more than 50 cells. The qualitative composition of the colonies was determined on thin-layer preparations of plasma clot after staining with azure II-eosin or after carrying out the benzidine test for hemoglobin [5, 6]. The functional activity of stromal cells responsible for transfer of HIM was studied by the method of heterotopic bone marrow transplantation [5, 7]. A column of bone marrow was grafted beneath the renal capsule of intact recipients. After 40 days the animals were killed, and the cell content and mass of the newly formed "primary" heterotopic focus of hematopoiesis were estimated. Another group of recipients was killed on the 7th day after implantation of bone marrow; the foci thus formed were retransplanted beneath the renal capsule of a new group of intact recipients and left in situ for 40 days, after which the cell content and mass of the "secondary" heterotopic focus of hematopoiesis were evaluated [5]. The results were subjected to statistical analysis by the nonparametric test with determination of the criterion of significance P_u [3].

EXPERIMENTAL RESULTS

Development of the general adaptation syndrome was accompanied by a number of successive functional changes in the hematopoietic tissue. Starting with the 3rd day activation of stromal cells responsible for transfer of HIM was observed in the bone marrow (Table 1). It must be emphasized that not only committed stromal cells (forming a "primary" heterotopic focus of hematopoiesis), but also stem cells (forming a "secondary" focus of hematopoiesis), were involved in this process. It is evident that later all this phenomenon comes to be realized as stimulation of proliferation and differentiation of the prescursor cells of erythro-granulomonocytopoiesis and development of a phenomenon of hyperplasia of bone-marrow hematopoiesis (Table 1). Thymectomy completely abolished the onset of these changes affecting the hematopoietic tissue in stress (Table 1). Moreover, on the 3rd day from the beginning of exposure a decrease in the ability of the stormal cells to form a "primary" ectopic focus beneath the renal capsule of the recipient animals was observed. No increase was found in the number of CFU and myelokaryocytes in the bone marrow and no colonies of erythroid type were formed. As a whole the results are evidence that the process of activation of medullary hematopoiesis is thymus-dependent. The interconnection of T-lymphocyte and stromal mechanisms of regulation of hematopoiesis is a matter of fundamental importance. On the one hand, as

already mentioned, no increase in production of hematopoietic cells took place in thymectomized mice subjected to immobolization. On the other hand, thymectomy abolished the development of stimulation of functional activity of the cells responsible for transfer of HIM during stress and the accumulation of T lymphocytes in the bone marrow tissue of intact animals, as the writers showed previously [2], coincides in time with activation of the stromal cells. These data were themselves evidence of at least the very close interconnection between thymus-dependent and stromal (HIM) mechanisms of regulation of hematopoiesis. The possibility of an indirect influence of the thymus on hematopoiesis (through factors of the stomal microenvironment) can be considered to be proven. It seems likely that we are dealing with activation of HIM by T lymphocytes migrating into the bone marrow. Whatever the case, the times of accumulation of the immature T-lymphocyte population (expressing antigens Lyt-1 and Lyt-2 on their surface) in the bone marrow and stimulation of HIM coincide, and injection of monoclonal antibodies leads to abolition of the phenomenon of stimulation of bone-marrow hematopoiesis in stress [2].

The experiments thus demonstrate the important role of the thymus in regulation of the functional activity of stromal cells that are responsible for transfer of HIM and of the processes of proliferation and differentiation of committed precursors of myelopoiesis during stress.

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