SPLENIC COLONY FORMATION BY LIVER CELLS FROM MOUSE EMBRYOS OF DIFFERENT AGES IN THE PRESENCE OF THYMOCYTES

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A population of accessory cells, after interacting with which CFU-S form colonies in the spleens of irradiated mice, has been shown to exist in bone marrow [3]. A cell population with similar functions also is present in the thymus. On depression of the colony-forming activity of bone marrow from a donor due to damage to the accessory population, further injection of thymocytes into the recipient leads to more intensive growth of colonies. Bone marrow is the main source of stem cells of an adult animal. It takes over this function from the fetal liver at the end of intrauterine development. In embryogenesis not only is there a shift of the location of stem cells (yolk sac — embryonic and fetal liver — bone marrow), but also one of their natural characteristics [4]. The problem of the times of formation, during gestation, of the control mechanisms regulating hematopoietic stem cells in adult mice and, in particular, the appearance and beginning of functioning of the accessory population are problems of great interest.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 mice aged 2.5 months. Colony-forming activity of suspensions of embryonic liver cells was studied by the splenic colonies method [5]. Recipients were irradiated in a dose of 8.5 Gy by gamma-rays on a "Luch-1" radiotherapy apparatus. A cell suspension was prepared from the liver of embryos and fetuses at different times of gestation, obtained by crossing female CBA and male C57BL mice. A thymocyte suspension was prepared from the thymus of (CBA × C57BL)F_1 mice and injected intravenously, in a dose of 2×10^7 cells per mouse in 0.5 ml, 30 min before injection of the liver cells. The recipient mice were killed on the 9th day after transplantation of the cells, the spleens were removed and fixed, and the number of colonies was counted.

EXPERIMENTAL RESULTS

Activity of accessory bone marrow cells belonging to the T series cannot normally be found. Additional injection of a suspension of thymocytes, together with syngeneic bone marrow, into the recipient was not reflected in the number of colonies formed. Only in the case of transplantation of bone marrow suspensions, damaged by exposure to various injurious factors (SPGMN [3], enrichment of the suspension by means of a cell sorter [6], treatment with con A [2]), does injection of syngeneic thymocytes enable their role in the process of splenic colony formation to be revealed by restoration of colony formation.

A different picture was observed in the case of transplantation of embryonic liver cells at different times of gestation into irradiated recipients. Injection of syngeneic thymocytes parallel with intact liver cells from 12-16-day embryos led to stimulation of colony formation by 1.5-1.75 times. On transplantation of thymocytes together with liver cells from 18-20-day embryos, however, stimulation of colony formation was not observed (Table 1). These results indicate that T lymphocytes, which appear in the earliest stages of embryogenesis (7 days) [1], are not organized into an accessory cell population until the 18th day,

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TABLE 1. Effect of Thymocytes on Splenic Colony Formation from Embryonic Liver

Age of embryos	Number of colonies per 10^5 cells		Coefficient of stimulation
	embryonic liver cells	the same + thymocytes	
12—13	3,1±0,2 (42)	4,6±0,3 (3,2)*	1,48
15—16	1,8±0,2 (28)	3,15±0,2 (24)**	1,75
18—19	4,8±0,3 (40)	4,8±0,4 (40)	1,0
20—21	4,8±0,3 (10)	5,6±0,4 (11)***	1,1

Legend. *$p < 0.01$, **$p < 0.001$, ***Not significant; number of animals given in parentheses.

i.e., toward the end of fetal development and transfer of the function of principal hematopoietic organ from the liver to bone marrow. In the early stages of gestation, another control mechanism evidently acts, and CFU-S do not yet require accessory cells to regulate the rate of their proliferation. However, the fact that they can be stimulated at this time by thymocytes suggests that they are already capable of receiving a signal from the regulating cells.

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