SELF-SUPPORT OF EMBRYONIC MIGRATING HEMATOPOIETIC STEM CELLS

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KEY WORDS: embryonic colony-forming units; migration; self-support.

In their ability to support themselves hematopoietic stem cells (HSC) detectable in the blood of adult mice are significantly inferior to the resident HSC of bone marrow [1, 2]. The lowering of the proliferative potential of migrating HSC can be attributed either to inadequacy of the environmental provided for them by the blood stream, increasing the likelihood of their differentiation, or to the presence of mechanisms in the bone marrow aimed at expelling "aged" HSC, i.e., cells which have gone through more mitoses, into the blood.

Which of these hypotheses is correct can be settled by studying the ability of HSC of embryonic blood to support themselves. In embryogenesis all HSC migrate (yolk sac — liver — bone marrow); consequently, if embryonic migrating HSC have reduced ability for self support, this would confirm that the first hypothesis (inadequate microenvironment) is correct, whereas reduced ability for self support would be in favor of the second hypothesis — the presence of a mechanism increasing the likelihood of expulsion of "aged" HSC into the blood stream in the adult, but not in the embryo.

In the investigation described below the self-supporting ability of HSC from the liver and blood of 15-17-day mouse embryos was compared.

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 mouse embryos were used. HSC were determined by the splenic colonies method [3] in the same hybrid mice irradiated in a dose of 1300 rad (in groups of ten recipients). Self-support of HSC from different sources was determined (5 experiments) by counting colony-forming units (CFU_S) in primary splenic colonies. Embryonic liver cells in a dose of $4 \cdot 10^4$ or embryonic blood escaping on decapitation of the embryo into Hanks' solution containing 50 units/ml of heparin were injected into irradiated intermediate recipients (in groups of 8); blood from one embryo was injected into one recipient (mean, $7 \cdot 10^4$ leukocytes). Separate colonies were excised from the spleen of the intermediate recipients 12 days later and cells of the colonies were injected into the final recipient (in groups of 10) in two doses: $^1/_5$ and $^1/_{50}$ colony. Altogether four experiments were carried out with embryos aged 15-17 days (day 0 corresponded to the day of discovery of a vaginal plug). Since the results were identical they are described together.

EXPERIMENTAL RESULTS

The number (cell content) of the colonies in the intermediate recipients is given in Table 1. On average each spleen contained one to two colonies, so that separate colonies could easily be isolated without overlapping. The colonies produced by HSC from blood and liver were of the same size.

Self-support of CFU_S from different sources is shown in Table 2. In their ability to support themselves, the migrating embryonic HSC were not inferior to resident HSC in the liver. This shows that the stay of the HSC in the blood stream does not reduce their ability to support themselves. The hypothesis that the bone marrow of adult animals contains a special structural organization, leading to the expulsion of the more intensively proliferating

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TABLE 1. Number and Cell Content of Colonies in Spleens of Intermediate Recipients

Source of CFU_s	Number of colonies per spleen	Number of nucleated cells per colony
Embryonic blood	1,4±0,5	$12,6 \cdot 10^6$
Embryonic liver	0,8±0,3	$14,5 \cdot 10^6$

TABLE 2. Self Support of Embryonic CFU_s from Peripheral Blood and Liver

Source of CFU_s	No. of cells injected (in colony equivalents)	Number of colonies per spleen	Number of CFU_s per colony	
			in each group	$M \pm m$
Embryonic blood	1/5	23,5±3,5	118	169±26
	1/50	4,4±0,7	220	
				$P>0,05$
Embryonic liver	1/5	19,3±5,6	96	
	1/50	3,2±0,5	160	128±26

CFU_s with reduced ability for self support into the blood stream preferentially, thus seems more likely to be correct.

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IMMUNOREACTIVE ACTH LEVEL IN THE HUMAN PITUITARY AND BLOOD SERUM DURING PRENATAL DEVELOPMENT

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From the published evidence there is no doubt that the hypophyseo-adrenocortical system in man becomes functionally active before birth [6, 8, 9, 11]. The first evidence of the presence of ACTH in the pituitary of human fetuses at 21 and 27 weeks was published in 1953 by Taylor et al. [12]. Skebel'skaya [4] showed that adrenocorticotrophic activity is present in acetone extracts of pituitary glands of human fetuses after the 9th-10th week of intrauterine life. The indicator of ACTH activity was a fall in the ascorbic acid concentration in the adrenal cortex of male rats previously treated with DOCA.

So far, however, no direct systematic study has been made of the adrenocorticotrophic function of the human fetal adenohypophysis. This is largely explained by the small size of

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