DETERMINATION OF "SETTLING FACTOR" (F) BY CLONING OF HEMATOPOIETIC STEM CELLS IN THE MOUSE SPLEEN AND BONE MARROW

V. N. Shvets* and V. V. Portugalov

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The effects of distribution of injected colony-forming units (CFU) in the recipient were studied by the use of the "settling factor" (F) as the criterion. This factor was found from the number of colonies discovered in histological sections of the spleen and femoral bone marrow compared with the number of colonies distinguishable on the surface of the spleen. It was shown that the value of F both for the whole volume of the spleen and for the femoral marrow can be estimated by the method of macroscopic counting of colonies on the spleen. The value of F in this case was independent of the character of differentiation of the CFU. It was shown that the value of F can vary depending on the physiological state of the CFU population. After irradiation of bone marrow CFU in doses of 200, 400 and 600 R the value of F in the spleen was reduced, whereas in the bone marrow it was unchanged compared with the control.
KEY WORDS: *"F" factor; colony-forming units; spleen; bone marrow.*

After transplantation of hematopoietic cells into lethally irradiated mice only a certain fraction of the colony-forming units (CFU) settles in the spleen. The "settling factor" (F) can be found by retransplantation of CFU which have settled in the spleen into other lethally irradiated recipients [8]. Colonies formed in the spleen of these recipients, but their number was several times smaller than in the spleen of the first recipient. The ratio between the number of colonies growing in the spleens of the second and first recipients (F) gives the number of CFU settling the spleen. The value of F in the spleen has been shown to be 0.11-0.25, and in the femoral marrow 0.01-0.02 [1, 2, 5, 7, 8, 10]. If physical and chemical agents of different nature act on the CFU population the value of F in the spleen may vary [3, 4].

Investigations to determine the value of F in the spleen or bone marrow have used the method of counting macroscopically distinguishable colonies in the spleen [9]. Only 40-45% of the total number of colonies in the whole volume of the spleen can be detected macroscopically. It has also been shown that mainly colonies of erythroid type are counted macroscopically [6].

For the above reasons, in the investigation described below an attempt was made to discover whether the method of counting colonies on the surface of the spleen gives a reliable estimate of the value of F for CFU settling and forming colonies throughout the volume of the spleen and the femoral marrow.

EXPERIMENTAL METHOD

The value of F was determined by the method of Siminovitch et al. [8]. Female (CBA × C57BL)F_1 mice weighing 20-22 g were used. The recipients were irradiated in a dose of 950-990 R (^{137}CS γ rays, dose rate 33-37 R/min). The value of F was determined for CFU in the bone marrow and spleen of intact mice and also for CFU in the bone marrow of mice irradiated in doses of 200, 400, and 600 R. The value of F was estimated by counting colonies in histological sections of the spleen and bone marrow by the scheme shown in Fig. 1, and compared with the value of F estimated from counting macroscopically visible colonies on the spleen [9]. This paper is based on an analysis of the results of experiments on 240 recipient mice.

*Corresponding Member of the Academy of Medical Sciences of the USSR.

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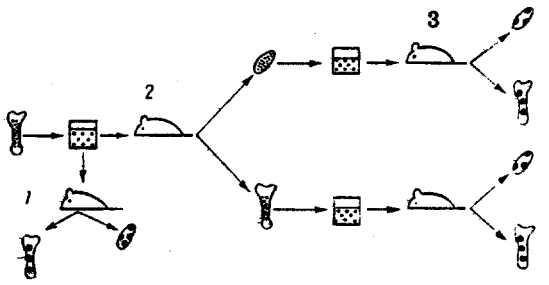

Fig. 1. Scheme of method of determining the value of F. 1) Group of mice receiving injection of 1×10^5 bone marrow or 1×10^6 spleen cells from intact or irradiated animals; on the ninth day after transplantation of cells colonies were counted in the spleen and femoral bone marrow; 2) group of mice receiving injection of an excess of cells (2.5×10^6-6.5×10^6 bone marrow or 3×10^7-10×10^7 spleen cells from intact mice or 2×10^7-4×10^7 bone marrow cells from mice irradiated in a dose of 200 - 600 R); the animals were killed 2 h after transplantation of the cells, the spleen and femur removed, and a suspension of spleen or femoral marrow cells was injected into the next recipient (group 3); 3) group of mice receiving transplanted cells from mice of group 2; on ninth day after injection of cells colonies were counted in spleen and femoral marrow. Value of F calculated by equation: $F = N_1/N_0$, where N_1 is the number of CFU found by counting colonies in spleen or bone marrow of mice of group 3 and N_0 the number of CFU found by counting colonies in spleen or femoral marrow of mice of group 1. Correct value of F can be obtained only when $F_1 = F_2 = F_3$, number of settling CFU as a proportion of number of injected cells is the same in the spleen or bone marrow of the first, second, and third recipients.

TABLE 1. Determination of Fraction of Injected CFU Settling in Recipient's Spleen

Transplant (source of CFU)	Value of F in spleen, found by counting colonies								
	in spleen					in femoral marrow			
	macroscopically	microscopically							
		E	M	MK	total number	E	M	MK	total number
Bone marrow	0,2	0,18	0,21	0,25	0,2	0,014	0,08	0,09	0,04
	0,2	0,15	0,28	0,18	0,16	0,016	0,07	0,08	0,037
	0,17	—	—	—	—	0,02	0,083	0,09	0,044
	0,19	—	—	—	—	—	—	—	—
	0,24	—	—	—	—	—	—	—	—
Spleen	0,21	0,18	0,17	0,14	0,18	0,04	0,058	0,097	0,056
	0,17	0,17	0,17	0,18	0,2	0,045	0,05	0,078	0,047
	0,18	—	—	—	—	—	—	—	—

Legend. Here and in Table 2 E) Erythroid M) myeloid, MK) megakaryocytic colonies.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the value of F in the spleen, determined from the number of macroscopically distinguishable colonies, was 0.17-0.24 for CFU of bone marrow and

TABLE 2. Determination of Fraction of Injected CFU Settling in Femoral Marrow of Recipients

Transplant (source of CFU)	Value of F in bone marrow found by counting colonies								
	in spleen					in femoral marrow			
	macroscopically	microscopically							
		E	M	MK	total number	E	M	MK	total number
Bone marrow	0,018	0,051	0,014	0,014	0,025	0,018	0,02	0,013	0,018
	0,015	0,053	0,017	0,02	0,028	0,021	0,016	0,023	0,019
	0,016	—	—	—	—	0,02	0,02	0,02	0,021
	0,017	—	—	—	—	—	—	—	—
	0,018	—	—	—	—	—	—	—	—
Spleen	0,036	0,047	0,043	0,03	0,048	0,041	0,042	0,06	0,041
	0,047	0,05	0,044	0,06	0,052	0,043	0,042	0,055	0,048
	0,044	—	—	—	—	—	—	—	—

TABLE 3. Value of F for Irradiated CFU Settling in Spleen and Bone Marrow of Recipients (from counting macroscopically distinguishable splenic colonies)

Dose of irradiation of bone marrow cells, R	Value of F	
	in spleen	in bone marrow
0	0,2	0,018
	0,22	0,017
200	0,084	0,018
	0,1	0,015
400	0,14	—
	0,15	0,02
600	0,12	0,015
	0,15	—

0.17-0.21 for CFU of the spleen. Analysis of the number of different types of colonies in histological sections through the spleen gave the same values of F. However, when its value was determined in the spleen by the bone-marrow colonies test, significant variations depending on the character of differentiation of the CFU were found. The value of F in the spleen for CFU of bone marrow and spleen was thus the same regardless of the method used to count the colonies: macrosopic or microscopic. Meanwhile determination of the value of F in the spleen by the bone-marrow colony test was not always reliable.

Table 2 shows that the value of F in the femoral marrow found from the number of macroscopically distinguishable colonies on the surface of the spleen, was 0.015-0.018 for CFU of bone marrow and 0.036-0.047 for CFU of the spleen. Counting colonies in histological sections of the spleen likewise revealed no significant differences in the value of F. The same value of F was found in the bone marrow in the test of bone marrow colonies as in the cloning test in the spleen.

The results of these experiments indicate that the method of counting colonies distinguishable on the surface of the spleen is suitable for estimating the value of F in the whole volume of the spleen and bone marrow.

The results of an investigation of the value of F for different physiological states of the CFU population of bone marrow are given in Table 3. The value of F in the spleen was reduced by the greatest degree after irradiation of the CFU in a dose of 200 R and remained below normal after irradiation in doses of 400 and 600 R. Meanwhile the value of F in bone marrow showed no change compared with the control.

Counting colonies distinguishable on the surface of the spleen is thus a suitable method of estimating the value of F both in the spleen and in the femoral marrow. The value of F in this case is independent of the character of differentiation of CFU in the bone marrow and

spleen. Meanwhile the value of F can vary in the spleen depending on the physiological state of the CFU population, whereas in the bone marrow it is stable. Differences in the value of F for CFU obtained from different sources were demonstrated.

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FUNCTIONAL MORPHOLOGY OF THE ACCESSORY NEUROSECRETORY CELLS OF THE CAT HYPOTHALAMUS

E. A. Borisova

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Accessory groups of neurosecretory cells were studied by staining serial paraffin sections of the hypothalamus by Gomori's method after stimulation of the supraoptic (SON) and postoptic (PON) nuclei, the preoptic region of the hypothalamus, the cervical sympathetic nerve (CSN) and afferent fibers of the vagus nerve in acute experiments on cats. Four paired accessory groups in the rostral hypothalamus were discovered (before the division of the chiasma into tracts); periventricular (along the walls of the third ventricle), preoptic (above the preoptic recess), parafornical (on both sides of the columns of the fornix), and fusiform (see: Byull. Éksp. Biol. Med., 1977, No. 2, p. 236). The fusiform group was found constantly in both control and experimental animals in all series of experiments. Stimulation induced an increase in synthesis of neurosecretory substance by its cells. In response to stimulation of SON and PON directly, and also of CSN and the vagus nerve, the direction of its reaction coincided with that in SON and PON, whereas to stimulation of the preoptic region of the hypothalamus activation of synthesis was observed against the background of an unchanged state of the neurosecretory nuclei by comparison with the control. Three other groups were found only during stimulation of the preoptic region. Accessory groups of cells can react in the same direction as the neurosecretory nuclei (mainly SON) or independently of them.

KEY WORDS: *neurosecretion; hypothalamic-hypophyseal neurosecretory system; stimulation of hypothalamus*

Steadily increasing attention has been paid in the recent literature to the neurosecretory centers. The study of serial sections through the hypothalamus has revealed accessory cells and groups in rats [7, 11], mice [3, 4], susliks [6], cats, dogs, and man [5]. They have been investigated in most detail in rats [11]. The workers cited describe the results of light- and electron-microscopic investigations of the morphological similarity between the accessory NSC and the NSC of the supraoptic nucleus (SON). However, their role in the neurosecretory process has not yet been completely explained.

Laboratory of Physiology of the Cerebral Circulation, A. L. Polenov Leningrad Neurosurgical Research Institute.

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