EFFECT OF DOSE OF INJECTED HEMATOPOIETIC CELLS ON NUMBER OF DAUGHTER CFU-S IN SPLENIC COLONIES

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One of the most important problems in the regulation of differentiation of hematopoietic stem cells (HSC) is that of their self-maintenance, their ability to divide without further differentiation. The method of splenic colonies is often used to determine self-maintenance of HSC, i.e., the number of splenic daughter colony-forming cells (CFU-S) is determined in an 11-12-day primary colony [3, 5].

The investigation described below confirmed data in [6] showing that production of daughter CFU-S depends not only on self-maintenance of HSC, but also on the cell doses used, and the reasons for this phenomenon are analyzed. It may substantially distort the results and must be taken into account when such experiments are planned.

EXPERIMENTAL METHOD

Experiments were carried out on female (CBA × C57B1)F_1 mice aged 10-16 weeks. The animals were irradiated with ^{137}Cs γ-rays (IPK source) in a dose of 12 Gy, with a dose rate of 25 cGy/min [1]. With the dose of irradiation used the number of endogenous colonies was under 0.2 per spleen. To determine CFU-S, bone marrow cells were injected into irradiated recipients (10 animals in a group) and the number of colonies in their spleens, fixed with Bouin's solution, was counted 8-11 days later. The number of daughter CFU-S in 11-day colonies also was determined. For this purpose individual 11-day colonies were excised from the spleen of mice which had received bone marrow in low doses (10^4-3×10^4), homogenized, and injected in a dose of 0.1-0.2 colony into secondary irradiated recipients. The number of splenic colonies in the latter was determined 8 days later. Individual colonies were not isolated from mice receiving high doses of cells, but the whole spleen was homogenized and cells equivalent to 0.2 colony were injected. The number of colonies in intermediate recipients of this kind was counted on the basis of the number of CFU-S in the suspensions of hematopoietic cells studied, for the fraction of CFU-S settling in the spleen did not change with an increase in the dose of bone marrow cells injected within very side limits — from

TABLE 1. Dependence of Number of Daughter CFU-S in 11-Day Splenic Colonies on Number of CFU-S Injected

No. of experiment	Number of hematopoietic cells injected	Number of CFU-S injected	Number of CFU-S per 11-day splenic colony
1	10^4	3,0±0,6	53±5
	$3 \cdot 10^4$	9,8±1,4	80±12
	$9 \cdot 10^4$	28,4±2,3	19±4
	10^7	3100±465	7±1
2	$3 \cdot 10^4$	5,1±1,0	94±19
	$1,2 \cdot 10^7$	2040±400	7±1
3	$5 \cdot 10^4$	29,0±4,5	122±11
	10^7	5800±900	2±0,2

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TABLE 2. Dependence of Number of Daughter CFU-S in 7-Day Splenic Colonies on Number of CFU-S Injected

Number of hemato-poietic cells injected	Number of CFU-S injected	Number of CFU-S per spleen	Number of CFU-S per 7-day splenic colony	P
10^4	3,3	12,4±2,1	3,7±0,6	
10^5	33,3	143±27,1	4,3±1,3	ND
10^6	333	1465±102	4,4±0,7	ND
10^7	3330	8125±406	2,4±0,3	<0,02

Legend. Number of CFU-S injected was determined by injecting bone marrow cells into irradiated mice in doses of 10^4 and 3×10^4 and counting the number of splenic colonies 11 days later. ND) Not determined.

10^6 to 100×10^6 cells [2]. This same principle of counting also was used when the number of daughter CFU-S was determined in 7-day splenic colonies.

EXPERIMENTAL RESULTS

With an increase in the number of hematopoietic cells injected the number of daughter CFU-S in 11-day colonies (regarded as an indicator of self-maintenance of CFU-S) diminished (Table 1). The effect was observed after a threefold increase in the dose (from 3×10^4 to 9×10^4) and a further increase in the number of cells injected up to 10^7 reduced self-maintenance of the CFU-S (i.e., the number of daughter CFU-S in the colonies) by 10-15 times. The apparent decrease in self-maintenance of CFU-S depends on the time of recording the colonies (Table 2). If the recording was done 7 days after injection a 100-fold increase in the number of bone marrow cells injected (from 10^4 to 10^6) did not affect self-maintenance of CFU-S. Only after a further increase in the dose of cells to 10^7 was self-maintenance of CFU-S significantly reduced.

The results show that the number of daughter CFU-S discovered in splenic colonies depends not only on self-maintenance of CFU-S, but also on their kinetics. Exponential growth of the number of CFU-S in the spleen begins 4 days after injection and continues only for a few days, after which it falls to normal immediately after the overshoot phase [4]. The time of slowing of the increase in number of CFU-S is determined by the dose of cells injected: the larger the dose the more rapidly the plateau is reached. When colonies are recorded after 11 days, the exponential phase thus is maintained only after injection of the smallest doses used in the investigation (10^4-3×10^4). With a further increase in the dose of the cells to only 10^5, exponential growth is already retarded by the 11th day and the results are distorted. The sooner the recording is done, the larger the dose of cells needed for the plateau to be reached and for introduction of an artefact into the results.

For the reasons described above, when self-maintenance of HSC is determined from the number of daughter CFU-S produced by them, the kinetics of CFU-S for the chosen dose of injected cells and the chosen time of recording must be monitored. It is essential to make sure that the CFU-S population at this time is in the phase of exponential growth.

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