# HEMATOPOIETIC STEM CELLS IN THE PERIPHERAL BLOOD OF MICE

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The properties of the hematopoietic stem cells of the peripheral blood were studied by the method of cloning hematopoietic cells in the spleen of irradiated (850 R) mice. With respect to all the parameters studied the hematopoietic stem cells of the blood were indistinguishable from those of the bone marrow. It is concluded that the stem (colony-forming) cells from both these sources are identical in principle. Hematopoietic stem cells circulating in the blood are a random fraction, and not a specially determined fraction of a single population of hematopoietic stem cells.

Proliferation and differentiation of the hematopoietic stem cells lie at the basis of the processes of hematopoiesis. In man and many species of animals, these cells are found not only in the bone marrow but also in the peripheral blood. The discharge of hematopoietic stem cells into the blood stream can be brought about only by the need for their transportation from the bone marrow into the thymus in order to make good the quantity of lymphoid tissue [3]. At the same time it can be accepted that a mechanism exists for regulating the exchange of hematopoietic stem cells between different zones of hematopoiesis and which may be connected, for example, with the selective discharge only of proliferating hematopoietic stem cells into the blood stream [6]. Finally, the migration of these cells should be a purely random process.

This raises the question whether the hematopoietic stem cells of the peripheral blood and bone marrow are identical or whether they differ and the first are not a random fraction but a subpopulation of the bone marrow hematopoietic stem cells specially "selected" on the basis of certain characteristics. In the investigation described below an attempt was made to answer this question by studying the proliferative activity, the directions of differentiation, and the affinity for the spleen of hematopoietic stem cells derived from both sources.

## EXPERIMENTAL METHOD

CBA mice and (C57BL × CBA) $F_1$ hybrids aged 6-10 weeks were used. To determine the number of stem (colony-forming) cells (CFU) the number of macroscopically visible colonies was counted in the spleen of lethally irradiated (850 R) mice 8 days after transplantation of syngeneic hematopoietic cells [10]. For the histological study of the colonies serial sections (6 $\mu$) were cut through the spleens stained with hematoxylin and eosin. The methods of obtaining cell suspensions of bone marrow, spleen, and peripheral blood leukocytes were described previously [1]. The proliferative activity of the CFU in systems in vivo and in vitro was studied by vinblastin treatment [5] and by the "thymidine suicide" method [4]. During the action of vinblastin on the CFU only those cells which have begun mitosis die [11]. For this reason, by using vinblastin it is possible to determine the proportion of CFU which passes through mitosis during the time of action of this metaphase poison. Vinblastin was injected intraperitoneally twice a day in a total dose of 0.2 mg per mouse. These conditions give an effect lasting not less than 24 h [8]. The "thymidine suicide" method is based on the fact that, if the specific activity of the isotope is high, it kills only those cells into whose DNA it has been incorporated. Cells in the S-period of the cell cycle are thus selectively killed.

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TABLE 1. Effect of Vinblastin Treatment in Vivo on CFU of Bone Marrow and Peripheral Blood of (C57BL × CBA) $F_1$ Mice

| Source of cells | Vinblastin | Number of cells injected | Number of mice | Number of macrocolonies (M ± m) | Number of CFU per femur or per ml blood | Portion of dying CFU (in percent) | P | Number of microcolonies analyzed | Proportion of colonies of each type (in percent) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | E | G | Me | Mi |
| Bone marrow | — | $4{,}0 \cdot 10^4$ | 9 | 16,6±1,3 | 5104 | 95 | <0,001 | 167 | 62 | 24 | 3 | 11 |
| | + | $8{,}6 \cdot 10^4$ | 10 | 9,2±1,0 | 256 | | | 187 | 65 | 20 | 7 | 8 |
| Leukocytes | — | $1{,}3 \cdot 10^6$ | 11 | 5,3±0,6 | 12,3 | 55 | <0,001 | 46 | 71 | 16 | 4 | 9 |
| | + | $0{,}94 \cdot 10^6$ | 11 | 1,3±0,3 | 5,6 | | | 36 | 75 | 17 | 3 | 5 |

Legend: E) erythroid colonies; G) granulocytic; Me) megakaryocytic; Mi) mixed; +) vinblastin given, −) not given.

TABLE 2. "Thymidine Suicide" in vitro of CFU of Bone Marrow and Peripheral Blood of CBA Mice

| Source of cells | Thymidine-$H^3$ | Number of cells injected | Number of mice | Number of colonies (M ± m) | Proportion of CFU dying (in %) | P |
|---|---|---|---|---|---|---|
| Spleen of early radiation chimeras | — | $1{,}25 \cdot 10^6$ | 5 | 8,4±1,7 | 64 | <0,05 |
| | + | $1{,}25 \cdot 10^6$ | 5 | 3,0±1,1 | | |
| Bone marrow of intact mice | — | $4 \cdot 10^4$ | 10 | 1,8±0,9 | 6 | NS |
| | + | $4 \cdot 10^4$ | 6 | 1,7±0,4 | | |
| Blood leukocytes of intact mice | — | $0{,}6 \cdot 10^6$ | 9 | 3,1±0,7 | 0 | NS |
| | + | $0{,}6 \cdot 10^6$ | 13 | 4,0±0,8 | | |

Legend: NS) difference not significant; +) thymidine-$H^3$ given, −) not given.

In this investigation the proportion of CFU synthesizing DNA was determined in vitro by using thymidine-$H^3$ with high specific activity (13.2 Ci/mmole) in a concentration of 100 $\mu$Ci/ml with an exposure of 25 min. Full details of the method were given previously [2]. To determine the fraction of CFU colonizing the spleen of the irradiated recipient [9], the spleen cells were regrafted into other irradiated animals.

## EXPERIMENTAL RESULTS

With the dose of irradiation used there was virtually no endocolonization: in 27 spleens of control mice three spontaneous colonies were found, i.e., the rate of endocolonization was 0.1 colony per spleen. The CFU of the bone marrow and blood formed four types of differentiated colonies in the spleen of the irradiated recipients: erythroid, granulocytic, megakaryocytic, and mixed. The relative distribution of the types of colonies is shown in Table 1. It will be clear from this table that the results were identical for CFU from both sources and confirmed previous findings [7].

Prolonged (28 h) treatment with vinblastin led to death of the overwhelming majority (95%) of CFU of the bone marrow (Table 1). During this time of action of the alkaloid the relative number of CFU in the blood fell by only one-half, and the destruction of the proliferating CFU by vinblastin did not change the character of distribution of the types of colonies produced by the hematopoietic cells from the two sources (Table 1), as would have been expected had these cells been two different lines of CFU. The relatively smaller decrease in the number of CFU in the blood (compared with that in the bone marrow) after treatment with the cytostatic for 24 h may be due not to their lower sensitivity, but to their increased liberation into the blood stream under the influence of vinblastin. To test this hypothesis the proliferative activity of the CFU was studied in a system in vitro, where it was impossible for the CFU to be made good at the expense of the bone marrow. Using pulse-labeling with thymidine-$H^3$ the fraction of the CFU synthesizing DNA was measured. The preliminary results of the experiments studying "thymidine suicide" of the CFU from the regenerating spleen of irradiated mice 6 days after transplantation with $1 \cdot 10^6$ syngeneic bond marrow cells confirmed the suitability of the thymidine-$H^3$ used; as will be clear from Table 2, under the influence of thymidine 64% of the splenic CFU of the early radiation chimeras died, in good agreement with

TABLE 3. Determination of Splenic Fraction (f) of CFU from Peripheral Blood of CBA Mice

| Time after transplantation | Intermediate recipients | | Final recipients | | Number of colonies (M±m) | f* |
|---|---|---|---|---|---|---|
| | number of cells injected | number of mice | number of cells injected | number of mice | | |
| Control | — | — | $0{,}63 \cdot 10^6$ | 5 | 5,0±1,3 | — |
| 2 h | $6{,}3 \cdot 10^6$ | 5 | 1/2 spleen | 8 | 5,5±1,0 | 0,22 |
| 24 » | $6{,}3 \cdot 10^6$ | 5 | 1/2 spleen | 6 | 1,7±0,5 | 0,07 |

*Value of f for CFU from bone marrow determined 2 h after injection was 0.12-0.25 and 24 h after injection it was 0.07-0.10 [1].

the published observations [4]. Meanwhile CFU both from the peripheral blood and from the bone marrow of animals with stable hematopoiesis were virtually insensitive to thymidine-$H^3$.

If CFU already destined to replenish the lymphoid tissue are discharged into the blood stream, the distribution of the CFU of the peripheral blood in the irradiated animals ought to differ from that in the case of transplantation of bone marrow: in the first case these cells would have increased affinity for the thymus, and so on. Accordingly the character of migration into the spleen and also the ability to be retained in it should differ for CFU of the bone marrow and of the peripheral blood. On these grounds, the fraction of CFU colonizing the spleen of the irradiated recipient and also the fraction of CFU abandoning it during the 1st day after primary migration into it were determined for both sources. After injection of peripheral blood leukocytes, about 20% of the injected CFU settled in the spleen. During the first 24 h two-thirds of these CFU colonizing the spleen to begin with later abandoned it (Table 3). Changes in the affinity of CFU from the peripheral blood for the spleen compared with that of CFU from the bone marrow thus were not found.

These investigations suggest that the characteristics of the CFU of the bone marrow and blood were identical in principle in all the experimental situations studied. The hypothesis that CFU circulating in the blood are a random fraction and not a specially determined fraction of a single population of hematopoietic stem cells thus seems most probably correct.

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