KINETICS OF THE MAIN SECTIONS OF THE HEMATOPOIETIC SYSTEM IN RADIATION CHIMERAS

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During the first 3 days after irradiation of mice and transplantation of syngeneic bone marrow into them the number of hematopoietic stem cells (CFUs) does not increase (about 0.5% of the number of injected cells) although induction of proliferation in these cells starts as early as 24 h after transplantation. It was shown by the "thymidine suicide" method that after 24 h all CFU were in a mitotic cycle. Conversely, the buffer section of the hematopoietic system – the section of committed cells or precursors of granulopoiesis (CFUc) immediately went into the logarithmic phase of growth. An exponential increase in the number of CFUs was observed after the 4th day, at the same times as increased proliferation of CFUc and the beginning of restoration of the total number of bone marrow cells. In late radiation chimeras (1 month after irradiation and transplantation) the number of stem cells was 50-70% of its initial value. The remaining hematopoietic parameters were within normal limits.

KEY WORDS: hematopoietic stem cells; bone marrow; proliferation; radiation chimeras.

Hematopoiesis is based on differentiation and proliferation of hematopoietic stem cells, the ancestors of all branches of blood formation: erythro-, myelo-, and lymphopoiesis. In addition, there are buffer sections of the precursor cells which are intermediate in position between stem cells and morphologically identifiable cells of the corresponding series.

To understand the mechanisms of regulation of hematopoiesis it is important to know how the various sections of the hematopoietic system interacts when its stable state is disturbed.

This paper gives data on changes in hematopoietic stem cells, committed precursors of granulocytopoiesis, and morphologically identifiable cells in the regenerating bone marrow of radiation chimeras.

EXPERIMENTAL METHOD

Female hybrid (CBA × C57BL)F_1 mice aged 2-3 months were used as both donors and recipients. Radiation chimeras were obtained as follows: Mice were irradiated with ^{137}Cs γ rays in a dose of 1300 rad, after which syngeneic hematopoietic cells were transplanted intravenously into them in a dose of 1×10^7 cells per mouse.

The number of stem cells (CFUs) was determined by the splenic colonies method [3].

To determine the number of committed cells (precursors of granulocytopoiesis – CFUc) a modified method of cloning hematopoietic cells in semisolid nutrient medium was used [2].

The fraction of proliferating CFUs and CFUc was determined by the "thymidine suicide" method in vitro; this method is based on the fact that if the isotope is present with high specific activity it will kill only those

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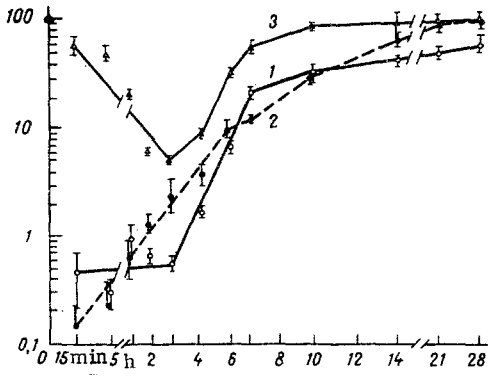

Fig. 1. Kinetics of stem (CFUs), committed (CFUc), and morphologically identifiable cells in regenerating bone marrow of radiation chimeras. Abscissa, time after transplantation (in days); ordinate, fraction of preserved cells (in % of initial level). 1) CFUs; 2) CFUc; 3) morphologically identifiable cells.

cells into whose DNA it has been incorporated, i.e., cells in the S period of the mitotic cycle. [^{3}H]Thymidine with a specific activity of 16 Ci/mmole was used in a concentration of 100 μCi/ml.

The cell suspension was obtained by flushing the bone marrow cells out of the femur with medium No. 199.

The results were subjected to statistical analysis and significance of differences was assessed by the Student–Fisher criterion.

EXPERIMENTAL RESULTS

The cellular kinetics of the three main sections of the hematopoietic system in the bone marrow of the radiation chimeras is shown in Fig. 1. During the first 3 days after transplantation the CFUs section did not grow but remained at a stable level. This indicates that a substantial proportion of them continues to differentiate; the stability of the CFUs section at these times shows that the probability that a stem cell (of the number counted per mitotic cycle) would remain a stem cell after division (P) and the value complementary to it (1 – P) – the probability that the stem cell would undergo differentiation, i.e., would move into the next section of committed precursors with loss of their polypotency and their capacity for prolonged self-maintenance, were equal, namely 0.5.

Logarithmic growth of the CFUs section, commencing from the 4th day (the number of CFUs was doubled after 18 h) was due primarily to an increase in the value of P, for increased proliferation of CFUs was observed much earlier: A sharp increase in the number of cells dying as a result of incorporation of the isotope was observed in the case of CFUs 24 h after transplantation (Table 1). This distinguishes the behavior of the hematopoietic cells of the radiation chimeras essentially from their behavior after whole-body sublethal irradiation (200 rad), when increased proliferation of CFUs was observed later; not until after 3-4 days [1]. Hence it follows that after higher doses of irradiation the hematopoietic microenvironment was modified so that it could immediately induce increased proliferative activity of CFUs. The increase in the number of stem cells slowed down in the 2nd week and by the 3rd week a plateau appeared at the level of about 50% of normal. It is unlikely that this was due to a decrease in their proliferative potential after intensive self-renewal (Table 1), for after transplantation of these cells into new irradiated recipients the hematopoietic stem cells of the chimeras were able to go through a new cycle of intensive proliferation [4]. The disturbance of proliferation in the radiation chimeras was evidently connected with a disturbance of the hematopoietic microenvironment, further confirmation of the role of the stroma of the hematopoietic organs in the regulation of stem cells.

A characteristic feature of the buffer section of committed cells – precursors of granulocytopoiesis (CFUc) – was their virtually complete absence in the bone marrow of the radiation chimeras 15 min after transplantation (Fig. 1). This shows that the cells of this section do not tolerate the transplantation procedures and their exponential growth is due to differentiation from hematopoietic stem cells. The resulting CFUc

TABLE 1. [^{3}H]Thymidine Suicide of CFUs and CFUc in Regenerating Bone Marrow of Radiation Chimeras

Time after transplantation, days	CFUs	CFUc
	%	
Intact mice	15*, 33*, 0, 15*	47%†, 52, 50, 31
1	50%†	—
2	69, 54	—
3	36, 70	47, 54
4	45	83, 84, 76, 70
5	89, 71	65
7	65, 64, 50	69
10	39, 70, 55, 37	—
14	38, 47, 33	58
21	19, 15, 9*	—
28	10*	

*Differences not significant.
†Differences significant at the level $P < 0.01$.

proliferate and, consequently, the number of these cells begins to increase immediately after transplantation. Despite the very high proliferative activity of the CFUc (Table 1), the rate of their proliferation was rather lower than that of CFUs: In the logarithmic phase of growth the number of CFUc doubled in about 30 h. This shows that cells of the buffer section differentiate intensively and cause rapid recovery of the number of cells in the bone marrow of the radiation chimeras. In fact, it was the section of morphologically identifiable cells that was restored fastest: By the 2nd week after transplantation its number was just below normal.

A particularly interesting fact was the coincidence of the time (4th day) of increased proliferative activity of CFUc, the beginning of logarithmic growth of the number of stem cells, and recovery of the number of morphologically identifiable cells. Whether this was accidental or evidence that hematopoietic stem cells "perceive" growth of the CFUc section (on account of their increased self-maintenance), so that the CFUs can reduce their differentiation $(1 - P)$ at that time, and so enable recovery of their own section to take place simultaneously with the increased differentiation of the committed precursor cells, is an important problem for the understanding of hematopoiesis. A further study of the state of the section of stem cells in the later stages after irradiation and transplantation could also provide fundamental information on the role of the microenvironment (see above) in the regulation of hematopoiesis.

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