KINETICS OF THE PRINCIPAL PARTS OF THE HEMATOPOIETIC SYSTEM DURING POSTRADIATION REGENERATION

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During the first 24 h after sublethal (200 rad) irradiation of mice there was a sharp decrease in the number of stem cells (to 4%), of committed precursors of granulocytes and macrophages (to 20%), and of morphologically identifiable cells (to 50%) in the bone marrow. Complete restoration of hematopoiesis ended two weeks after irradiation and was sufficient to maintain exponential growth in the number of stem cells and their intensive proliferation. The increase in the number of commited precursors was delayed until recovery of the total number of cells in the bone marrow.

KEY WORDS: hematopoietic stem cells; commited cells; bone marrow; sublethal irradiation.

Hematopoietic stem cells are the ancestors of all branches of hematopoiesis: erythro-, myelo-, and lymphopoiesis. There are also groups of precursor cells that occupy an intermediate position between stem cells and morphologically identifiable cells of the corresponding series.

This paper presents data on the kinetics of groups of stem cells which are committed precursors of granulocytes and macrophages, as well as of morphologically identifiable cells in the process of regeneration after sublethal irradiation of mice.

EXPERIMENTAL METHOD

CBA mice of both sexes aged 2-3 months were used as donors and recipients. Whole-body irradiation of the mice was carried out in revolving cells by Cs^{137} γ rays in a dose rate of 26.5 rad/min.

The number of stem cells (CFUs) was determined by the splenic colony method [4].

To determine the committed precursor cells of granulocytes and macrophages (CFUc) a modified method of cloning of hematopoietic cells in semisolid nutrient gel 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 specific activity of the isotope is high, it will kill the cells in whose DNA it was incorporated [1]. Thymidine-H^3 with a specific activity of 11 Ci/mmole was used in the experiments in a concentration of 100 μCi/ml.

A cell suspension was obtained by flushing out the bone-marrow cells from the femur into medium No. 199 (3 mice in each group). The results were subjected to statistical analysis, the significance of differences being determined by the Student–Fisher criterion.

EXPERIMENTAL RESULTS

The cellular kinetics of the three main divisions of the hematopoietic system in the bone marrow of mice after exposure to a relatively low dose – whole-body sublethal irradiation in a dose of 200 rad – is

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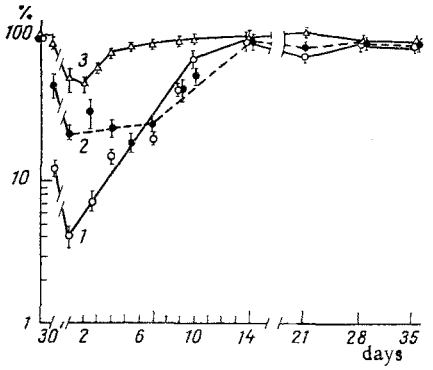

Fig. 1. Kinetics of CFUs, CFUc, and morphologically identifiable cells after sublethal irradiation (200 rad) of mice: 1) CFUs; 2) CFUc; 3) morphologically identifiable cells. Abscissa, time after irradiation (in days); ordinate, fraction of cells remaining intact (in % of original level).

TABLE 1. "Thymidine Suicide" (in %) of CFUs and CFUc in Mouse Bone Marrow Regenerating after Irradiation (200 rad)

Time after irradiation (in days)	CFUs	CFUc
Intact mice	8*	44
1—2	9*	42
3	17	—
4	36	46
7—8	26	45
9—10	39	49
12	36	51
14	33	49
18—21	42	45
28—35	1*	—

* Differences not significant; in all other cases differences significant ($P < 0.001$).

shown in Fig. 1. The CFUs division was clearly the most severely damaged. Only 4% of CFUs remained 24 h after irradiation. This value is much greater than the true radiosensitivity of these cells, for 30 min after irradiation more than 10% of CFUs remained intact.

It thus follows that in the first 24 h after whole-body irradiation the CFUs continued to differentiate without any significant self-renewal. Not until 24 h later did exponential growth of the CFUs begin, and it then continued for 10 days. The increased self-renewal of the CFUs was induced later under these circumstances, for a considerable increase in the rate of proliferation, judging from the degree of "thymidine suicide," was not found until 3-4 days later (Table 1). It thus follows that the entry of the CFUs into the logarithmic phase of growth was brought about primarily by an increase in the value of P. This symbol denotes the probability that a hematopoietic stem cell (calculated per single mitotic cycle) will remain a stem cell after division; its complementary value (1−P) is the probability that the hematopoietic stem cell will start to differentiate, i.e., will change into the next section of committed precursors, with loss of its pluripotency and its powers of prolonged self-maintenance. In the case of stable hematopoiesis, the value of P is 0.5 [3]. Further maintenance of the logarithmic phase of growth of CFUs is associated with their increased proliferation (Table 1).

By the second week the number of CFUs was fully restored and stabilized at the level reached. This was due primarily to a chage in the value of P, which fell to 0.5, for when the equilibrium state of the CFUs was reached, the latter still continued to proliferate very intensively for a further week, and not until one week after irradiation did their proliferative activity decline to its initial level (Table 1). The evident biological significance of this kinetics could be that the system is maintained in a state of increased preparedness for a second response: Against the background of continuing intensive proliferation of stem cells, repeated irradiation, causing an increase in the value of P, leads to more rapid recovery of hematopoiesis.

As Fig. 1 shows, the time for the number of CFUs to double itself in the logarithmic phase of growth was about 60 h. Meanwhile the duration of the mitotic cycle of the proliferating CFUs was 12-18 h [3]. Hence it follows that their differentiation into more mature cells did not cease in this period but the departure of CFUs for the CFUc division continued at a constant rate.

The kinetics of the CFUc division was quite different (Fig. 1). During the first day after irradiation a decrease in their number was observed; this decrease was greater than could be attributed to their direct damage by the radiation, i.e., here also differentiation of the granulocytic precursors into mature cells continued during the first 24 h. In the course of the first week after irradiation the size of the CFUc division did not increase (Fig. 1) but was kept at the minimal level reached, despite the fact that precursors from the CFUs division were being received all the time (see above). Meanwhile the number of morphologically identifiable cells in the bone marrow was restored during the first week after irradiation. Not until this had been done did the logarithmic phase of increase of the CFUc population begin, and their number was completely restored toward the end of the second week after irradiation. It must be emphasized that CFUc are unable to support themselves for a long time and the rate of their proliferation after this relatively weak hematopoietic stress was unchanged: The degree of "thymidine suicide" remained stable (Table 1).

The results show that restoration of hematopoiesis after sublethal irradiation (200 rad) of mice takes place primarily on account of CFUs that persist intact in the bone marrow. An increase in the value of P (the probability of self-maintenance) and induction of proliferation in resting CFUs quickly bring this division into a state of exponential growth, as a result of which the total number of CFUs is restored to normal within a short time (2 weeks). The number of mature cells is restored much sooner, thanks to the presence of the buffer division of CFUc. Its intensified differentiation into mature cells is compensated by subsequent restoration of the division as a result of the arrival of precursors from CFUs. The presence of the buffer division thus facilitates quantitative regulation of hematopoiesis and permits the number of functioning cells to be restored rapidly, without any increased utilization of CFUs for differentiation in the critical period at the beginning of regeneration, when the total number of CFUs is minimal.

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