Kinetics of Hemopoietic Clones in Sublethally Irradiated Mice

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Hemopoiesis in sublethally irradiated mice is provided by a succession of clones of hemopoietic cells containing unique radioactive markers. Dozens of clones with short life-span (not more than 4 months) are functioning during mouse life. Ten percent of these clones are long-lived and can be revealed during 20 months. These clones are formed exclusively by bone marrow stem cells in the G_0-period of the cell cycle. A new structure of hemopoietic stem cell population is discussed.

Key words: *hemopoietic stem cell; radioactive markers; colony-forming spleen units; G_0-period of the cell cycle*

It is generally recognized that huge production of blood cells is provided by specific precursors — hemopoietic stem cells (HSC) capable of differentiation and self-support. It was previously shown that self-supporting cells are absent in the hemopoietic system [6]. Even the most early precursors — primitive HSC (pHSC) have high but limited proliferative potential. Hemopoiesis is provided by successive differentiation of HSC forming a chain of short-term hemopoietic clones [1-3]. Functioning of individual HSC was studied using foreign gene markers [1-3]. This technique has a number of disadvantages. First of all, the studies are conducted with lethally irradiated animals reconstituted by bone marrow transplantation, but not on intact animals. To reconstitute hemopoiesis, donor cells dissociated from the stroma are injected to recipient as a single cell suspension. This is accompanied by about 100-fold expansion of HSC *in vivo*. The main disadvantage of the method is that retrovirus can be incorporated only into proliferating cells. During stable hemopoiesis, HSC are in resting state and, therefore, pharmacological concentrations of the growth factors or irradiation of the stroma are required to mobilize HSC into the cell cycle. HSC which started proliferating and incorporated the marker may differ from the initial cells. Besides, HSC remaining in the G_0-phase during gene transportation cannot be marked and analyzed.

In the present study we used the method of radioactive markers based on the registration of chromosome changes caused by sublethal irradiation. Karyotypic changes are seldom associated with preservation of cell viability. That is why the cells with similar markers can be regarded as posterity of a common precursor. Thus, radioactive markers allow to identify clones of individual HSC with the same reliability as the method of gene transfer. Besides, the applied method allows to avoid the disadvantages of gene marking because sublethal irradiation does not require bone marrow transplantation and the examined cells are not isolated from their microenvironment. Since radiation marks the cells independently on the cell cycle phase, all HSC, including those in deep G_0 period, were examined for the first time in the present study.

MATERIALS AND METHODS

The study was carried out on 12-14-week-old female CBF_1 (CBA/Lac×C57Bl/6) F_1 mice. The experimental

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animals were irradiated on a ^{137}Cs-device made for Institute of Hemotransfusion. Each mouse received 5.5 Gy at dose power 0.187 Gy/min. Four, 9, 12, 16 and 20 months postirradiation bone marrow was taken from left or right femur in turn under light ether anesthesia. Part of marrow cells was used for karyotypic analysis. The mitoses were blocked with 10 μg/ml colcemide (10 μl/ml suspension) for 1 h. The rest of the cells were injected to irradiated (10 Gy) recipients, which received 2 fractions with 3 h interval. After 10 days the recipients were injected intraperitoneally with 0.2 ml colcemide (10 mg/ml) for mitosis inhibition, after 1 h individual colonies were isolated from the spleen and suspended in 0.5 ml phosphate buffer supplemented with 1% fetal calf serum for karyotypic analysis. Chromosome preparations were processed according to standard technique. G-differential chromosome staining was carried out with Wright dye as described previously [11]. Chromosome analysis was carried out as described elsewhere [10]. The colonies originating from individual precursor cells were detected by unique chromosome rearrangement. The changes were regarded as clonal if 2 or more mitoses with identical chromosome rearrangements were found. A total of 933 spleen colonies were examined. Statistical analysis was performed using Student's *t* test.

RESULTS

The applied sublethal irradiation dose proved to be effective for marking of a sufficient number of HSC. The number of marked colonies varied from 27 to 64% at all stages (Table 1). Eight mice had 136 marked clones, 16±1.1 clones on average were revealed in each animal. On the whole, clones kinetics confirms previous data. Hemopoiesis during mouse life is normally maintained by a number of short-lived small clones functioning simultaneously. Similarly to spleen colonies, femoral bone marrow preparations contained up to 17 individual clones at each examined stage. Hemopoiesis was not oligomonoclonal. Most of the examined clones were revealed once, seldom twice, which means that their lifespan did not exceed 4 months. These data correspond to the earlier suggested model of clonal succession in hemopoietic system based on genetic HSC marking [1,2,6], which was confirmed by other authors on the basis of gene polymorphism [3, 5,7]. Nevertheless, some differences should be noted. Thus, about 10% clones (15 of 136) proved to be long-lived and function during the whole life. No long-lived clones were found among 659 ones studied by the method of retroviral gene transfer for HSC marking. This result shows that long-lived clones originate from primitive HSC remaining in the resting state at the moment of irradiation. These cells could not be marked by gene transfer and were not analyzed.

These results allow to propose the following scheme of organization of the hemopoietic stem system: about 10% pHSC remaining in the deep resting state possess the highest proliferative potential and produce long-lived clones functioning throughout the animal life. Most pHSC (90%) are derived from resting pHSC which underwent 3-4 mitoses and returned to a less deep rest than the initial one. This organization of the stem system appears to be biologically expedient. Urgent mobilization into hemopoiesis is provided by a major population of superficially resting pHSC which can be easier mobilized into the G_1 period than mature pHSC. This organization of pHSC population is confirmed by the presence of cells which never proliferate during postembryonic period as well as cells which underwent 1-4 mitoses and returned to the resting

TABLE 1. Percent of Marked Colonies in Irradiated Mice

Mouse number	Number of marked colonies/number of examined colonies (% of marked colonies)					Mean number (%) of marked colonies per mouse
	Time postirradiation, months					
	4	9	12	16	20	
1	N.d.	N.d.	5/6 (83)	34/73 (47)	46/78 (60)	63.3±8.5
2 (1N)	N.d.	24/27 (89)	13/14 (92)	N.d.	N.d.	90.5
3	1/4 (25)	18/58 (31)	N.d.	21/57 (37)	18/31 (58)	37.8±6.2
4 (2N)	N.d.	27/35 (77)	13/14 (92)	N.d.	N.d.	84.5
5	5/13 (38)	11/19 (58)	14/47 (30)	27/50 (54)	N.d.	45.0±5.7
9	20/55 (36)	2/18 (11)	9/31 (29)	1/6 (17)	N.d.	23.3±4.9
10	N.d.	13/14 (92)	34/36 (94)	7/18 (39)	N.d.	75.0±8.5
13	5/48 (10)	10/35 (29)	12/27 (44)	16/55 (29)	22/43 (51)	32.6±6.3
Mean...	27.3±5.6	55.3±11.3	63.5±12.0	37.2±4.9	56.3±2.2	

Note. N.d. — not determined.

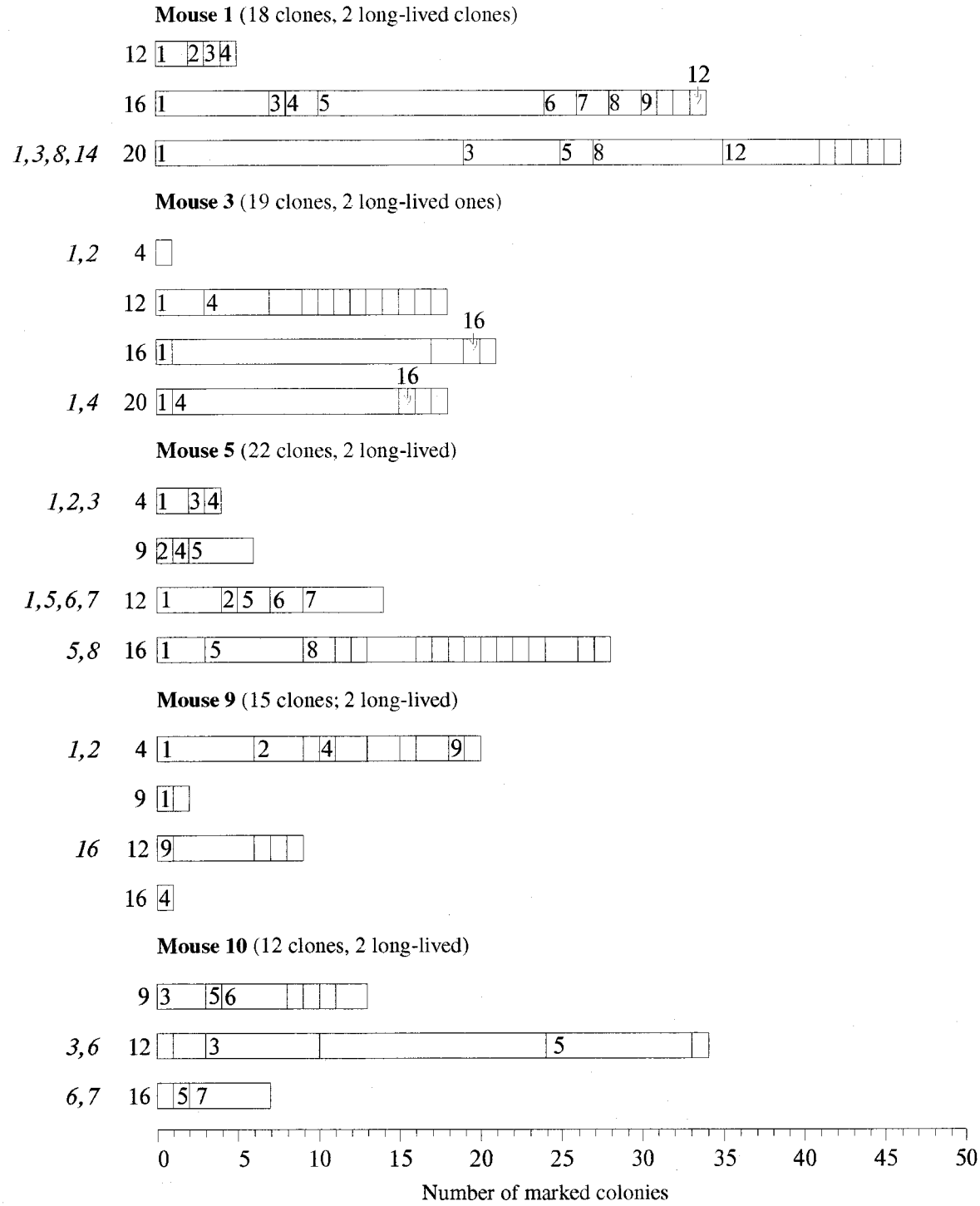

Fig. 1. Kinetics of hemopoietic clones with unique radioactive markers in 5 sublethally irradiated mice. Numbers in column: time afterirradiation (months). Figures in italics on the left: numbers of marked cells clones revealed in the bone marrow. Each rectangle indicates colonies with radioactive markers, figures in rectangles indicate individual clones numbers.

state. Non-proliferating cells were in the deep G_0 period and their *ex vivo* mobilization into proliferation with pharmacological cytokine concentrations took about 2 weeks. At the same time, HSC which underwent proliferation were in a less deep G_0 period and could be mobilized by the same factors into the cell cycle within 1 day [4,8]. Thus, these data showed for the first time the involvement of HSC in quantitative hemopoiesis regulation, which was earlier revealed only for committed precursors [9].

On the whole, our findings change the accepted concept of HSC. The idea that the initial cell elements

of this system are not immortal and self-supporting, but produce successive clones of blood cells makes senseless the notion that hemopoiesis is provided by polypotent and self-supporting HSC. All cells of the hemopoietic system can be regarded as transitory cell populations aging constantly in the process of hemopoiesis. Transitory cells are changing in the course of transformation in hemopoietic hierarchy, which explains the heterogeneity of pHSC similarly to all other hemopoietic cell populations.

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