EFFECT OF HEMATOPOIESIS ON BONE MARROW STROMAL PRECURSOR CELLS

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The number, proliferative activity, and degree of self-maintenance of hematopoietic stem cells are controlled in vivo by a hematopoietic microenvironment of stromal nature [5]. Dependence of the state of the microenvironment on the character of hematopoiesis taking place in it has not been studied. Meanwhile, in clinical practice diseases (myelofibrosis, myelosclerosis) have been found in which the predominant features are lesions of the hematopoietic stroma, whereas the primary lesions are located, not in that stroma, but in hematopoietic cells [3]. This suggests that the character of hematopoiesis plays an important role in the maintenance of its hematopoietic stroma.

The aim of the present investigation was an experimental study of the effect of hematopoiesis on precursor cells of the hematopoietic stroma, which are able to transfer the hematopoietic microenvironment in vivo with the formation of foci of ectopic hematopoiesis, and in vitro with the formation of a layer of adherent cells, maintaining hematopoiesis in long-term bone marrow cultures.

EXPERIMENTAL METHOD

Female CBA, CBAT6T6, (CBA × C57BL)F_1, and (CBAT6T6 × C57BL)F_1 mice aged 8-30 days were used. To obtain chimeras, the mice were irradiated in a dose of 12-13 Gy with ^{137}Cs γ-rays, in a dose rate of 0.25 Gy/min. The following types of chimeras were obtained: standard chimeras – syngeneic bone marrow from normal donors, differing in their chromosomal marker, was injected intravenously into irradiated mice in a dose of one-third of a femoral equivalent (this dose also was used to obtain all other groups of chimeras), one-passage chimeras – irradiated mice were restored with bone marrow of standard chimeras (obtained not earlier than 2 months after creation of the chimera), two-passage chimeras – irradiated mice were restored with bone marrow from one-passage chimeras, and double chimeras – standard chimeras were irradiated again in the same lethal dose and restored with bone marrow from normal donors. Experiments were carried out 3-9 months after creation of the chimeras. Self-maintenance of hematopoietic stem cells was determined from the number of daughter CFU-C in an 11-day splenic colony, produced by the stem cells under investigation in an irradiated (12.5 Gy) recipient (10 in a group). Long-term culture of bone marrow fragments was carried out as described previously [1]. The bone marrow or the removed sublayer of 3-4-week old cultures was implanted beneath the renal capsule of syngeneic normal or irradiated (standard chimeras) mice, and the size of the foci or ectopic hematopoiesis thus formed was determined 1 month later on the basis of their cell content. In some cases the ectopic focus was implanted entirely in reirradiated recipients. Karyological investigations were carried out [2].

EXPERIMENTAL RESULTS

All groups of chimeras used in metaphase analysis in the bone marrow contained only donors' hematopoietic cells (no fewer than 100 metaphases were analyzed from the pool of bone marrow cells of all chimeras of the group). The absence of revertants indicates that hematopoietic cells in the different groups actually passed through 1, 2, or 3 cycles of intensive proliferation in the course of restoration of hematopoiesis in the chimeras, and they were not the progenies of the irradiated recipient's cells. As regards the degree of direct

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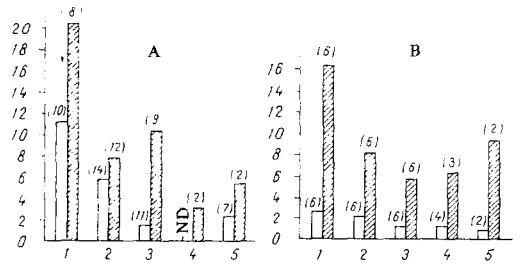

Fig. 1. Size of ectopic foci of hematopoiesis produced by normal bone marrow (A) or after culture (B) in syngeneic recipients. Abscissa, groups of donors: 1) normal mice, 2) standard chimeras, 3) one-passage chimeras, 4) two-passage chimeras, 5) double chimeras; ordinate, cell content of foci (1×10^{-6}). Unshaded columns – intact recipients. Numbers in parentheses give number of foci studied (A – mean of three experiments, B – mean of two experiments). ND) No data.

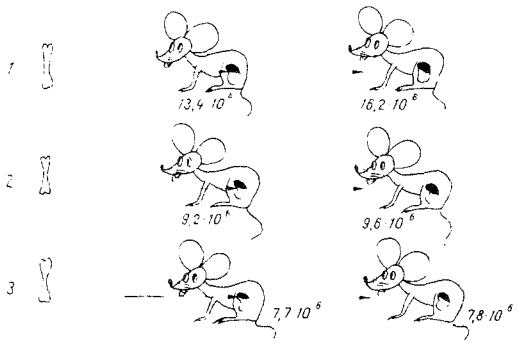

Fig. 2. Self-maintenance of precursors of hematopoietic stroma from ectopic foci. 1) Normal mice; 2) standard chimeras; 3) passage chimeras.

radiation injury, the stromas of the chimeras of groups 1, 2, and 3 were thus equivalent; differences between them were restricted to the proliferative history of the stem cells maintaining hematopoiesis in the chimeras.

Data on the ability of stromal precursors to transfer the hematopoietic microenvironment in vivo are given in Fig. 1A. Foci formed in standard recipients by bone marrow from standard chimeras were only half the size of foci produced by normal bone marrow. Foci produced by bone marrow from double chimeras were only half as large again, in good agreement both with the radiosensitivity of stromal precursors [4] and with their inability to recover completely from radiation injuries [7]. Ability of the passage chimeras to transfer the microenvironment was depressed just as strongly as that of the double chimeras, although the total dose of irradiation they received was only half as high. Differences similar in principle, but rather less in degree, also were found in the irradiated recipients.

On explantation into long-term culture the bone marrow of all chimeras maintained hematopoiesis about equally, but qualitatively worse than normal bone marrow. This was expressed morphologically as the smaller area of the bottom of the flask covered by regions of active hematopoiesis (region of "cobble stones"). The number of hematopoietic cells in the cultures of chimeras was 1×10^4 to 5×10^4/ml throughout the period of culture, whereas in cultures of normal bone marrow it was maintained at the level of 1×10^5/ml. The results of the study of the number of stromal precursors in the layer of adherent cells of the culture are given in Fig. 1B. On implantation of the sublayer into unirradiated recipients the size of the foci of ectopic hematopoiesis formed was reduced in the standard chimeras by 25% compared with the sublayer of normal bone marrow. A substantially greater decrease (by 80%) was observed in the case of double chimeras. One- and two-passage chimeras, in which the size of the foci was about 40% of the control, occupied an intermediate position. Conse-

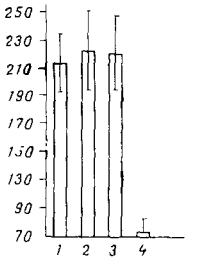

Fig. 3. Self-maintenance of hematopoietic stem cells of different origin. Abscissa, groups of donors: 1) normal mice, 2) standard chimeras, 3) double chimeras, 4) passage chimeras. Ordinate, number of CFU-C in 11-day-old splenic colony.

quently these results agree, in principle, with those obtained in vivo: Damage to the stroma was determined not only by the dose of irradiation, but also by the quality of the hematopoietic cells proliferating on it. After implantation of the layer of adherent cells into irradiated recipients the size of the foci was reduced about equally for cultures of all groups of chimeras.

The similarity in the behavior of stromal precursors in culture and on transfer from culture into an irradiated recipient, manifested as maintenance of hematopoiesis equally in all groups in the first case and the formation of foci of ectopic hematopoiesis of equal size in the second case, suggests that this result is not fortuitous. Among the many possible explanations, the simplest would seem to be the presence of a special population of stromal precursors, responding to external influences (interrogation by the irradiated organism in vivo or the creation of a sublayer on the whole area of the bottom of the flask in vitro) together with stromal stem cells not responding to interrogation, so that their number corresponds to the degree of injury and it differs in different chimeras.

After repeated transfer of foci produced both by normal bone marrow and by marrow obtained from standard or passage chimeras to reirradiated recipients, the size of the daughter foci of ectopic hematopoiesis was not reduced (Fig. 2). Hence it follows that, although the number of precursors transferring the microenvironment was reduced in the chimeras, their high self-maintaining ability, characteristic of stem cells, was fully preserved.

Hematopoiesis in all groups of chimeras was basically the same as regards both cell content of the femur and number of CFU-C per femur and per 1×10^5 nucleated cells (data not given). As regards self-maintenance, the hematopoietic stem cells after one passage were indistinguishable from normal; self-maintenance of stem cells of passage chimeras, which had gone through two cycles of intensive proliferation, was sharply reduced (Fig. 3). These results are in good agreement with the observed decrease in self-maintenance of stem cells which have gone through at least two passages [6].

The results provide the first experimental proof that cooperative interactions between stromal and hematopoietic cells, lying at the basis of the local regulation of hematopoiesis, are not one-way in character in the direction from stromal to hematopoietic cells. In particular, proliferation of stem cells with reduced self-maintenance in bone marrow leads to additional secondary damage to the stromal precursors of radiation chimeras. Mechanisms of this sort are not clear, although the results described above provide the opportunity for their further study.

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