# TWO POPULATIONS OF BONE MARROW CELLS CREATING AN ECTOPIC HEMATOPOIETIC FOCUS

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A fragment of bone marrow transplanted beneath the capsule of the kidney creates a focus of ectopic hematopoiesis the size of which, measured according to the number of hematopoietic cells, is proportional to the size of the implant. In irradiated recipients the focus was 1.5-2.5 times larger than in intact recipients. Cells creating a focus of heterotopic hematopoiesis in intact and irradiated recipients differed in their radiosensitivity: their $D_0$ values were about 160 and 350 rad, respectively. In this connection it is postulated that two populations of precursor cells participate in the creation of the focus. Their possible relations with predetermined and inducible osteogenic precursor cells are discussed.

KEY WORDS: *bone marrow*; *heterotopic transplantation*; *osteogenic precursor cells*; *radiosensitivity*.

In heterotopic transplantation of a bone marrow fragment, beneath the capsule of the kidney for example, osteogenic precursor cells build bone which is repopulated by hematopoietic cells. As a result a chimeric focus of ectopic hematopoiesis is formed, the stroma of which consists of the donor's cells whereas the hematopoietic elements belong mainly to the recipient [2]. The size of the heterotopic focus can be most conveniently estimated from the size of the hematopoietic microenvironment suitable for settlement by hematopoietic cells, i.e., by the number of nucleated cells and of hematopoietic stem cells (CFUs) in the graft. The size of the focus depends on the size of the transplanted fragments of bone marrow and also on the state of the recipients. In particular, in an irradiated animal the size of the developing focus of ectopic hematopoiesis is substantially larger than if the fragment of bone marrow of the same size is transplanted into an intact recipient [1].

The question accordingly arises: Do the same cells form the focus in intact and irradiated recipients? In the investigation now described this problem was studied by determining the radiosensitivity of cells creating the focus in intact and irradiated recipients.

## EXPERIMENTAL METHOD

CBA × C57BL/F1 hybrid mice aged 8-12 weeks were used. To obtain a focus of ectopic hematopoiesis, a fragment of bone marrow expelled from the femur by air or by a stilet was transplanted beneath the capsule of the kidney of animals anesthetized with hexobarbital. The size of the developing focus was estimated 1-1.5 months later from the number of hematopoietic cells (flushed out of the bone with medium No. 199) and hematopoietic stem cells (CFUs) contained in it. Cells from 8 to 10 foci of ectopic hematopoiesis were mixed at each time. The number of CFUs was determined by cloning in the spleen of irradiated mice [4]. The animals were irradiated with $^{137}Cs$ $\gamma$-rays in a dose of 1300 rad. For 2 h after irradiation, hematopoietic cells were injected into the mice (10-12 animals in the group) in the course of 2 h after irradiation. The spleens were fixed after 8 days in Bouin's fluid and the number of colonies counted under a magnifying glass. The number of endogenous colonies did not exceed 0.2 per spleen. To determine the radiosensitivity of the cells creating the focus, femora removed from the donors were irradiated by a cesium source in doses of between 170 and 1700 rad at a dose rate of 530 rad/min. The bones were kept on ice until transplantation (not more than 3 h). Control tests showed that the size of the heterotopic focus was not reduced by keeping the grafts under these conditions for at least 4 h.

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TABLE 1. Size of Focus of Heterotopic Hematopoiesis in Relation to Size of Graft

| Experiment | Size of bone marrow graft | No. of cells per focus | No. of CFUs per focus |
|---|---|---|---|
| 1 | 1/4 of femur | $2,5\times10^6$ | 344±37 |
| | 1 femur | $4,7\times10^6$ | 971±129 |
| 2 | 1/4 of femur | $2,6\times10^6$ | 591±52 |
| | 1/2 of femur | $3,4\times10^6$ | 586±59 |
| | 1 femur | $10,0\times10^6$ | 2000±200 |
| 3 | 1/4 of femur | $3,8\times10^6$ | |
| | 1 femur | $8,1\times10^6$ | |

TABLE 2. Effect of Irradiation of Recipient on Size of Focus of Heterotopic Hematopoiesis

| Experiment | Recipient | Number of cells per focus | Weight of bone per focus (mg) |
|---|---|---|---|
| 1 | Intact | $8,1\times10^6$ | |
| | Irradiated 800 rad | $19,3\times10^6$ | |
| 2 | Intact | $11,4\times10^6$ | 1,4 |
| | Irradiated 250 rad | $21,1\times10^6$ | 1,8 |
| | " 500 " | $13,4\times10^6$ | 1,5 |
| | " 750 " | $24,0\times10^6$ | 1,9 |
| | " 1000 " | $18,9\times10^6$ | 2,0 |
| 3 | Intact | $9,7\times10^6$ | |
| | Intact * | $5,6\times10^6$ | |
| | Irradiated 600 rad | $34,7\times10^6$ | |
| | " 600 rad* | $15,3\times10^6$ | |
| 4 | Intact | $14,6\times10^6$ | 1,8 |
| | Chimera (6 months after irradiation) | $36,0\times10^6$ | 2,2 |

*Donor's bone marrow irradiated in vitro in a dose of 600 rad before transplantation.

TABLE 3. Radiosensitivity of Cells Transferring Hematopoietic Microenvironment

| Dose of irradiation of graft (in rad) | Recipient | Number of cells per focus | Number of CFUs per focus | Weight of bone per focus |
|---|---|---|---|---|
| 0 | Intact | $6,8\times10^6$ | 1006±307 | 0,9 |
| 500 | " | $6,3\times10^6$ | 929±126 | 1,0 |
| 800 | " | $0,9\times10^6$ | 177±16 | 0,7 |
| 1100 | " | $0,7\times10^6$ | 33±9 | 0,7 |
| 1400 | " | $0,7\times10^6$ | 91±11 | 0,8 |
| 1700 | " | $0,2\times10^6$ | 4±2 | 0,2 |
| 0 | Irradiated (750 rad) | $17,0\times10^6$ | 2800±339 | 1,1 |
| 500 | The same | $12,0\times10^6$ | 2460±480 | 1,0 |
| 800 | " | $14,0\times10^6$ | 3290±560 | 0,9 |
| 1100 | " | $12,2\times10^6$ | 1616±335 | 1,3 |
| 1400 | " | $6,1\times10^6$ | 997±151 | 0,6 |
| 1700 | " | $2,3\times10^6$ | 207±92 | 0,4 |

## EXPERIMENTAL RESULTS

The size of the developing focus of ectopic hematopoiesis, determined from the number of nucleated cells and hematopoietic stem cells in it, was proportional to the size of the original transplanted bone marrow fragments (Table 1), i.e., ultimately to the number of transplanted osteogenic precursor cells. Hence it follows that assessment of the size of the focus from the number of cells can be used as a means of determining the radiosensitivity of cells transferring the hematopoietic microenvironment.

In the irradiated animal the developing focus was 1.5-3 times larger than in the intact recipient (Table 2). The effect of preliminary irradiation in this case evidently followed the "all or nothing" law: Doses of whole-body irradiation of between 250 and 1000 rad gave a virtually identical increase in size of the focus (Table 2, experiment 2). In the radiation chimeras the state intensifying growth of the heterotopic focus was maintained for an indefinite time. Six months after creation of the chimera, when stable hematopoiesis was well restored, the heterotopic bone marrow graft formed a focus more than twice as large as that formed in the intact recipients (Table 2, experiment 4). As Table 2 shows, the weight of newly formed bone reflected the size of the heterotopic hematopoietic focus significantly less well, for usually with an increase in the size of the focus and in the number of hematopoietic cells in it the thickness of the bone decreased.

The results of determination of the radiosensitivity of the cells carrying the hematopoietic microenvironment are shown in Table 3. The values of $D_0$ and of the extrapolation number are shown graphically in Fig. 1. Clearly the radiosensitivity of the cells forming the hematopoietic focus in the irradiated (750 rad) and intact recipients differed sharply. In both cases the cells were distinguished by very high ability to repair postradiation injuries, as reflected in the high value (about 20) of the extrapolation number. The radioresistance of cells forming the hematopoietic focus in the irradiated recipients was very high: $D_0$ was 350 rad and the exponential decrease in their number began only after a very large dose of irradiation (1400 rad). The radiosensitivity of the cells forming the focus in the unirradiated recipients was significantly higher: $D_0$ was 160 rad and the exponential part of the curve began with a dose of irradiation of 800 rad. The radiosensitivity curve of the cells creating the focus in the unirradiated recipent had a second plateau and area of exponential decline which were observed at higher doses of irradiation. This region was similar in its characteristics to the process of focus formation in the irradiated recipients.

The results are evidence that a focus of ectopic hematopoiesis is formed in intact and irradiated recipients by two different categories of stromal precursors. The histogenetic relations between them are not clear. It is possibly a question of two different stages of differentiation of the precursor cells of the stroma of hematopoietic organs: for example, of stem and committed precursors, or of two subpopulations of cells of the same line: for example, cells at different stages of the cell cycle, although this is less likely. Finally, another possible explanation is that two independent cell lines participate in the formation of the focus. In all cases, as Fig. 1 shows, both cell populations participate in the process of formation of the focus but in the intact recipients the focus is formed mainly through the functioning of the radiosensitive line of cells, and in the irradiated recipients through that of the radioresistant line.

It has been shown that there are two categories of osteogenic precursor cells [3]. One of them is found in bone marrow and is called the determined osteogenic precursor cell (DOPC). These cells are self-maintaining during repeated transplantation and are capable of osteogenesis without special addition of an external inducer. The second category of precursor cells is found in other lymphoid organs, such as in the spleen, and is called the inducible osteogenic precursor cell (IOPC). It is not self-supporting and is capable of osteogenesis only in the presence of a special inducer, such as that secreted by the cells of transitional epithelium. The hypothesis that both DOPC and IOPC are present in bone marrow is very attractive. In the unirradiated recipient the formation of a focus could be mainly due to DOPC and in the irradiated recipient to IOPC. The presence of these two cell populations could be of great biological importance. The maintenance of a stable hematopoietic microenvironment in this case would be due to self-supporting DOPC. Activation of the hematopoietic microenvironment taking place after exposure to various disturbing factors could take place on account of non-self-supporting IOPC, responding to the action of an inducer. After disappearance of the inducer, the system would automatically revert to the stable state as a result of exhaustion of the IOPC. Some of the results given above support this hypothesis. In fact, as is clear from the radiosensitivity curve, many of the radiosensitive precursors (hypothetical DOPCs) die in radiation chimeras irradiated in a dose of 1300 rad. Meanwhile the size of hematopoietic microenvironment in the chimeras, judging from the number of cells of their bone marrow, was not significantly reduced. Hence it follows that the stroma of the hematopoietic organs of chimeras may be composed of radioresistant precursors (hypothetical IOPCs). Since, according to this hypothesis, these cells are not self-supporting, an inducer activating the radioresistant precursors, even after the stabilization of hematopoiesis, must be constantly present in the chimeras. This is what was in fact found in the chimeras 6 months after irradiation.

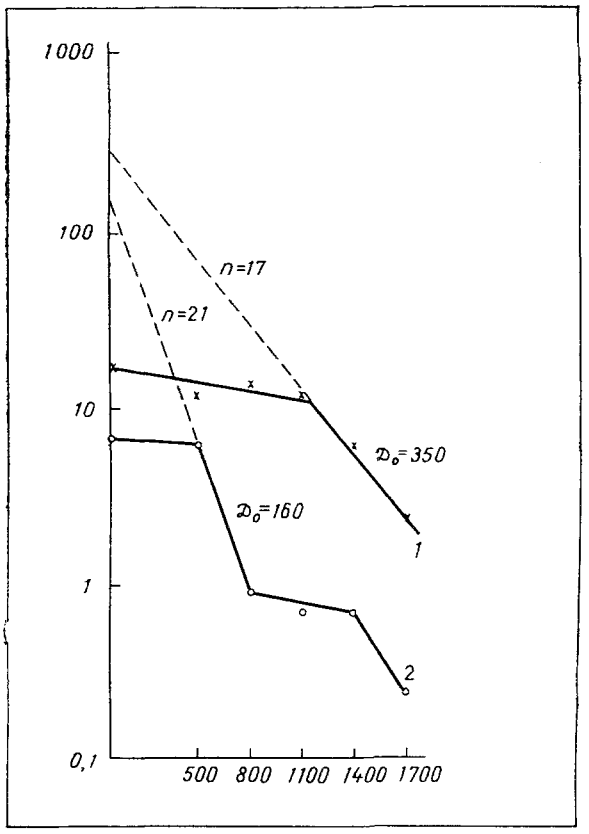


Fig. 1. Radiosensitivity curves of cells forming hematopoietic microenvironment in intact and irradiated recipients. Abscissa, dose of irradiation (in rad); ordinate, number of hematopoietic cells in focus ($\times 10^6$). 1) Unirradiated recipients; 2) irradiated recipients (750 rad).

It is too early as yet to judge the validity of these arguments. Data reflecting the ability of radiosensitive and radioresistant stromal precursor cells to maintain themselves would be of decisive importance in this respect and investigations to obtain them are in progress.

LITERATURE CITED

1. W. Chamberlin, J. Barone, A. Kedo, et al., Blood, 44, 385 (1974).
2. A. J. Friedenstein (A. Ya. Fridenshtein) et al., Transplantation, 6, 230 (1968).
3. A. J. Friedenstein (A. Ya. Fridenshtein), in: Symposium on Hard Tissue Growth, Repair and Remineralization, Amsterdam (1973), pp. 169-182.
4. J. E. Till and E. A. McCulloch, Radiat. Res. 14, 213 (1961).