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QUANTITATIVE CHARACTERISTICS OF HEMATOPOIETIC MICROENVIRONMENT TRANSFER

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During heterotopic bone marrow transplantation a new bone marrow organ with bony capsule and medullary cavity is formed and is colonized by hematopoietic cells. In the heterotopic bone marrow organ (HBMO) the donor's microenvironment acts [8] although the hematopoietic cells in it are recipient's cells [1]. Heterotopic transplantation is essentially transfer of the hematopoietic microenvironment [2, 4]. The transfer is effected by clonogenic bone-marrow mechanocytes (CBMM), which preserve their donor origin in HBMO [1, 3]. The size of the HBMO is a characteristic which can be used to judge the factors regulating morphogenesis of the bone marrow stroma. It has been shown that the size of HBMO depends on the quantity of transplanted bone marrow tissue, but this relationship is multifactorial [9, 10]. In the present investigation parameters of transplants determining the size of HBMO are analyzed.

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 mice weighing 20-22 g were used. Fragments of bone marrow flushed out of the medullary cavity of the femur were transplanted beneath the renal capsule of syngeneic recipients by the method in [1]. There were three series of experiments: I) marrow from one femur of each of three donors was transplanted into three separate recipients, and the marrow of the remaining three femora was transplanted together into the 4th recipient (six experiments), II) marrow from one femur was transplanted in its entirety into one recipient, marrow from the 2nd femur was divided into four parts: 1/2, 1/4, and 2 parts each of 1/8 were grafted each into a separate recipient (nine experiments). III) 10 Min before removal of the marrow the donors were irradiated in doses of 1.5-7.0 Gy on a cobalt source with dose rate of 28.2 rads/min; fragments of 1/4 or 1/8 of the marrow of the irradiated femora were transplanted, and in the control, fragments of the same size of unirradiated bone marrow were grafted (five experiments, 111 irradiated and 21 control grafts). After 2.5 months each HBMO was removed, a suspension was prepared from the cells of its medullary cavity, and the number of cells was counted in a Goryaev's chamber. The results were subjected to statistical analysis.

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TABLE 1. Size of Heterotopic Medullary Organs

Size of original transplant	No. of transplants	No. of cells in heterotopic bone marrow organs (10^7)
3	6	3,0±0,5
1	27	1,96±0,27
1/2	7	1,78±0,44
1/4	8	1,21±0,22
1/8	16	0,72±0,11
1/2+1/4+2/8 of one donor	5	3,82±0,78

*In parts relative to contents of medullary cavity of one femur.

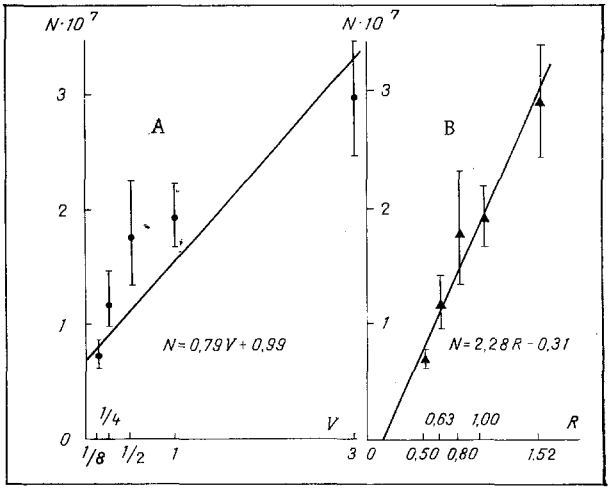

Fig. 1. Linear regression lines for dependence of size N of HBMO on volume (V) and radius (R) or original transplant. A: abscissa, volumes of original transplants relative to volume of transplant of one femoral marrow; ordinate, size of HBMO (number of cells in medullary cavity); B: abscissa, radii of original transplants relative to radius of transplant of one femoral marrow; ordinate, size of HBMO (number of cells in medullary cavity).

EXPERIMENTAL RESULTS

Data on the number of cells in the medullary cavity of HBMO are given in Table 1. They show the following relationship between the size of the original transplant (measured in fractions of one femoral marrow) and the size of the HBMO (measured as the number of cells in its medullary cavity): with an increase in size of the original transplant and, consequently, of its volume and the number of transplanted cells, the size of the HBMO increased. The coefficient of correlation between these values was 0.50. The same result was obtained by dispersion analysis: with a level of significance of over 0.999, this increase really took place. Meanwhile simple comparison of the mean numbers of cells in HBMO gave the impression that the size of HBMO was not directly proportional to the size of the original grafts. In fact, after transplantation of one femoral narrow, the HBMO was not 3 times less, but only 1.5 times less than HBMO after transplantation of three femoral marrows, and after transplantation of 1/2, 1/4, and 1/8 of a femoral marrow HBMO was only 1.1, 1.6, and 2.7 times less than HBMO after transplantation of a whole femoral marrow. This was independent of the unavoidable inaccuracy of division of the femoral contents into parts, for the total size of HBMO produced after transplantation of 1/2, 1/4, and 1/8 of one femoral marrow was significantly larger than HBMO after transplantations of one whole femoral marrow. The results likewise cannot be explained by unequal trauma to the tissue, or in the case of the

contents of one femur and three femora, no additional trauma was inflicted due to division into parts. The hypothesis that the size of HBMO is a linear function of the size of the original transplant [6] thus appears doubtful. To test it, dispersion analysis was undertaken. It showed that, with a degree of significance of over 0.999, a linear relationship does not exist between these values.

This means that cells which are carriers of the medullary microenvironment do not behave additively on heterotopic transplantation. The question arises of the parameters of the original transplant which affect interaction between the cells transferring the microenvironment. Bone marrow fragments transplanted beneath the renal capsule assume a near-spherical or hemispherical shape. HBMO formed at the site of transplantation also has the same shape. On transplantation of bone marrow fragments of different sizes not only the volume of the original transplants changes, but also their surface and radius. Bone tissue, which evidently is an important component of the hematopoietic microenvironment, is located on the surface of HBMO. Hence the size of the surface may affect the quantity of bone, whereas the radius may affect the intensity with which influences arriving from the surface of HBMO act on the cells filling its cavity. We attempted to determine how the size of HBMO depends on the radius (R) and surface area (S) of spheres with the volume (V) of the original transplant (bearing in mind that $R \sim \sqrt[3]{V}$, a $S \sim \sqrt[3]{V^2}$.

Linear regression equations for dependence of the number of cells in HBMO, on the one hand, and on the parameters R, S, and V of the original transplants, on the other hand, were calculated (Fig. 1). It will be clear from Fig. 1 that better approximation of the point to a straight line occurred if R was taken as the independent variable, and worse if the parameter V was taken. Hypotheses on a linear relationship between the number of cells in HBMO and the parameters R and S were tested by dispersion analysis. It was found that both hypothesis were confirmed if a 5% level of significance was used. Agreement with the hypothesis of a linear function of the parameter R was found to be very good (about 50%). Using the chosen criterion, the hypothesis of linear dependence on radius R was thus more plausible. This problem requires further study.

Under all conditions the results showed that no hypothetical linear relationship exists between the size of HBMO and the number of transplanted medullary cells. This invalidates attempts to judge the population of transplanted cells transferring the microenvironment from the size of HBMO or to determine their radiosensitivity by this method [6, 7]. In fact, in the experiments of series III the dose–response curve for the size of HBMO after transplantation of 1/4 of a femoral marrow had D_0 = 1.7 Gy and n = 1.9, whereas after transplantation of 1/8 of a femoral marrow D_0 = 1.7 and n = 1.7. Not only do these values differ from each other, but they also differ sharply from those obtained after transplantation of a whole femoral marrow (D_0 = 350 Gy, n =5) [7]. This proves directly that assessment of the radiosensitivity of cells carrying the hematopoietic microenvironment according to dose–response curves for the size of HBMO [7] is meaningless.

It has been suggested [5] that during heterotopic bone marrow transplantation a fraction of "surplus" or reserve carriers of the microenvironment arises. Judging from the results of the present investigation, the fraction of these cells increases with an increase in volume of the original transplants. This fact may be important for analysis of the mechanisms regulating the concentration of stromal precursor cells in hematopoietic organs.

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STRUCTURAL CHANGES IN THE CYTOSKELETON IN REGENERATING MOUSE LIVER CELLS

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KEY WORDS: intermediate filaments; actin cytoskeleton; regeneration of the liver.

Synthesis of the embryo-specific serum protein a-fetoprotein (AFP) is observed during regeneration of the liver induced, in particular, by the action of CCl_4 vapor [1, 2]. In most lines of mice AFP synthesis is carried out after exposure to CCl_4 by only a small proportion of hepatocytes (not more than 10%), which are characterized by a mainly perinecrotic distribution [5]. Redistribution of membrane antigens is observed under these circumstances on the surface of perinecrotic hepatocytes [4]. This redistribution, which correlates well with expression of AFP synthesis, has led the "embryonalization" of the perinecrotic cells to be linked with local structural changes in junctions between hepatocytes. The system of prekeratin intermediate filaments (tonogilaments) is, as we know [6, 7], in close connection with interhepatocytic junction structures, and evidently plays a role in their creation and stability. Accordingly the reorganization of intercellular interactions arising in regenerating parts of the liver must somehow or other be reflected in the tonofilament system and also, probably, in other structures of the hepatocyte cytoskeleton.

The aim of this investigation was to study the connection between the above-mentioned phenomena by indirect immunofluorescence with specific antibodies.

EXPERIMENTAL METHOD

AKR and SWR mice of both sexes, aged 3-4 months, were used. Liver damage was induced by poisoning with CCl_4 vapor [2]. The mice were killed 1-4 days after poisoning. Pieces of liver either were frozen in 7% gelatin at the temperature of liquid nitrogen, after which sections 5 μ thick were cut in a cryostat and then fixed for 5 min in 4% formaldehyde in 0.1M phosphate buffer, pH 7.4, or were fixed by perfusion with 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4, with the addition of 0.05% of saponin, and were then embedded in paraffin wax. Frozen sections revealed only water-insoluble antigens, including cytokeratins and actin. Both water-soluble AFP antigen and cytokeratins could be demonstrated in paraffin sections. To demonstrate the latter it was necessary to incubate the dewaxed sections, before treatment with antibodies, for 30-60 min at room temperature in 0.05% trypsin solution in physiological saline. The distribution of AFP, cytokeratin, actin, and biliary capillary antigen (AGI) were investigated by the indirect immunofluorescence method. Monospecific rabbit antibodies against AFT were provided by A. I. Gusev and V. S. Poltoranina, and antibodies against action by A. D. Bershadskii. Rabbit antibodies against Poltoranina, and antibodies against actin by A. D. Bershadskii. Rabbit antibodies against AGI were provided by N. I. Kuprina and T. D. Rudinskaya [4]. Preliminary experiments showed that monoclonal antibodies against rat liver cytokeratin interact equally effectively with mouse cytokeratin. FITC-Labeled pig antirabbit antiserum (from "Daco," Denmark) and FITC-labeled rabbit antimouse serum (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) were used as the second antibodies.

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