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ROLE OF THE STROMAL MICROENVIRONMENT IN REGULATION OF BONE-MARROW HEMATOPOIESIS AFTER ADMINISTRATION OF DIPYRIDAMOLE

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The construction of hematopoietic territories following heterotopic bone marrow transplantation is accompanied by transfer of the hematopoiesis-inducing microenvironment, which determines the type and volume of the developing hematopoietic tissue, and the cells transferring it are stromal cells of the hematopoietic organs [4-6]. A general feature of cells of connective-tissue nature is the presence of a receptor for platelet-secreted growth factor (PSGF), by means of which the stimulating action of PSGF on proliferative is realized [1, 10]. Blocking of platelet aggregation, leading to a decrease in the secretion of PSGF, causes inhibition of proliferative activity of connective-tissue cells.

The aim of the investigation was to study the role of the stromal microenvironment in the regulation of bone-marrow hematopoiesis following administration of dipyridamole, used as a platelet disaggregant, by the method of experimental heterotopic bone marrow transplantation.

EXPERIMENTAL METHOD

Experiments were carried out on 540 male (CBA × C57BL)F_1 mice weighing 18-20 g. A fragment of bone marrow isolated from the femoral medullary cavity was transplanted beneath the connective-tissue capsule of the kidney of syngeneic recipients under hexobarbital anesthesia [11]. Some animals received an intraperitoneal injection of dipyridamole (D) in a dose of 30 mg/kg body weight daily for 50 days after bone marrow transplantation. Control animals received physiological saline. From the rest of the experimental and control animals, the foci of heterotopic hematopoiesis (FHH), formed 7 days after transplantation, was retransplanted into a fresh group of intact recipients. The animals were killed by cervical dislocation on the 7th, 19th, 30th, and 50th days of administration of D. Animals with retransplanted foci were killed on the 30th day of the experiment. The dimensions of FHH were estimated from the weight of the bony capsule and of the cellular bone marrow in it, in both experiment and control. Part of the material was analyzed histologically. In preparations stained with hematoxylin-eosin the mitotic

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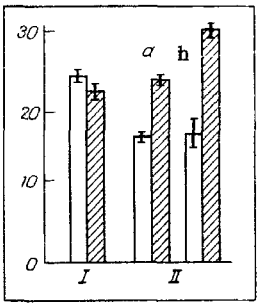

Fig. 1. Change in number of 8-day exogenous splenic colonies in irradiated recipients with transplanted intact bone marrow, after administration of D (I) and in irradiated recipients with transplanted bone marrow from FHH (a) and from mouse femur (b), and receiving D for 30 days (II). Ordinate, number of exogenous splenic colonies. Unshaded columns – control, shaded – injection of dipyridamole.

index (MI) of the myelokaryocytes and the number of megakaryocytes (MKC) in the femoral bone marrow and in FHH were determined. To determine the effect of D on the connective-tissue cells, millipore filters were implanted intramuscularly into mice, and D was injected into them daily for 7 days, whereas the control animals received physiological saline. The animals were given an intraperitoneal injection of ^{3}H-thymidine in a dose of 0.8 MBq (specific radioactivity 196 GBq/g) 1 h before sacrifice. Type "M" photographic emulsion was applied to dewaxed 5-μm sections of an area of tissue containing the encapsulated filter, and 3 weeks later the sections were developed and stained with hematoxylin and eosin. The intensity of proliferation of fibroblasts surrounding the filter was estimated from the value of ILN (in %). To estimate the effect of D on the population of hematopoietic stem cells (HSC) the method of exogenous splenic colony formation was used [12]. Intact animals were irradiated on the RUM-17 apparatus in a dose of 7.5 Gy, after which 2×10^5 bone marrow cells were transplanted into them intravenously in accordance with the following schedule: series I) intact bone marrow was transplanted together with intraperitoneal injection of D and physiological saline, series II) bone marrow from FHH and from the femur of mice which had received D and physiological saline for 30 days was transplanted. The survival control consisted of animals without bone marrow transplantation. The mice were killed after 8 days, their spleens fixed in Bouin's solution, and the number of exogenous splenic colonies was counted. The results were subjected to statistical analysis by the methods in [2].

EXPERIMENTAL RESULTS

Heterotopic transplantation of syngeneic bone marrow beneath the connective-tissue capsule of the kidney of intact animals led after 30 days to the formation of a medullary organ with developed osteogenic and hematopoietic tissues. Administration of D caused a sharp decrease in the number of bone marrow cells in FHH on the 19th and 30th days of the experiment, but in the femur only on the 19th day compared with animals of the control group. By the 50th day of administration of D no significant differences could be found in the number of bone marrow cells in FHH and in the femur of the experimental and control animals.

Administration of D for 50 days led to a sharp decrease in the osteogenic component in FHH compared with the control, which could indicate suppression of proliferation of osteogenic precursors by D and/or considerable activation of bone tissue resorption during the formation of FHH. Performance of retransplantation of the 7-day FHH showed that it is the stromal stem cells that are responsible for transfer of the hematopoiesis-inducing microenvironment, and which take part in the changes observed in the bone marrow of animals which have received D.

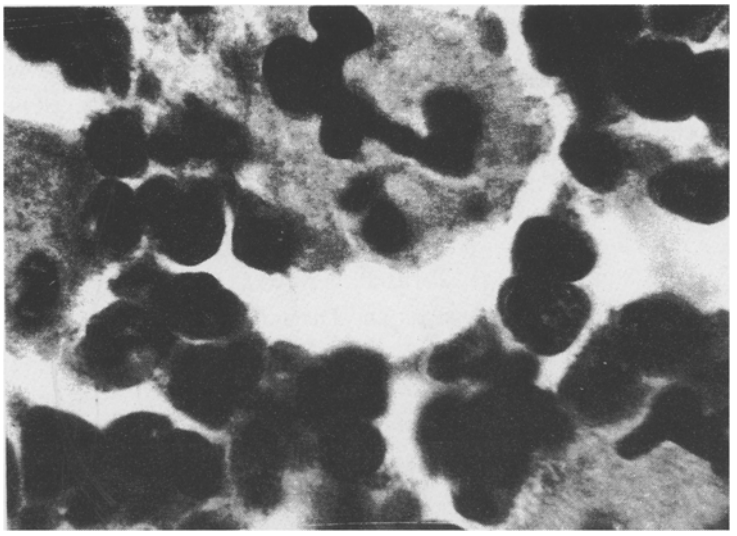

Fig. 2. Destruction of megakaryocytes in bone marrow of mice receiving D for 30 days. Hematoxylin-eosin. 700×.

To test the hypothesis that D, being a preparation inhibiting platelet aggregation, causes inhibition of proliferative activity of fibroblasts, the test with intramuscular implantation of a millipore filter into mice, accompanied by administration of D, was used for we know that encapsulation of a foreign body under these conditions is effected mainly by highly differentiated typical fibroblasts [3]. For instance, ILN of fibroblasts surrounding the filter amounted to 2.16 ± 0.46% after 7 days of its administration, compared with 4.70 ± 0.72% in the control, evidence that the effect of D is realized through blocking of PSGF secretion from the platelets.

Estimation of the effect of D on the HSC population in FHH and in the femur of the animals, using the method of exogenous splenic colony formation, showed that D, injected into irradiated mice, into which bone marrow of intact animals had been transplanted, reduced the number of exogenous colonies formed, but only by an insignificant degree. However, in bone marrow from FHH or from the femur of animals which had received D for 30 days was transplanted into irradiated animals, there was a sharp increase in splenic exogenous colony formation (Fig. 1). It follows from these data that D has the same action on the stromal component of splenic tissue as on the stroma of the bone marrow, although weaker, and this effect of the preparation on hematopoiesis, mediated through the bone marrow stroma, is realized only upon contact of the stromal and hematopoietic cells, probably with the aid of certain short-distance factors.

The experiments showed that D, at certain stages of the investigation, reduced the number of bone marrow cells in FHH and the femur, but the potential activity of the hematopoietic cells increased during this period. This is evidently reflected in a sharp increase in the number of HSC in th bone marrow, determined by the method of exogenous splenic colony formation. The effect of an increase in HSC may probably be realized both through the arrival of new HSC, repopulating the stromal territory of the ectopic organ, and through the introduction of HSC, which have already colonized the bone marrow stroma, into the cycle or shortening its duration.

Confirmation of the increased potential activity of the myelokaryocytes against the background of few cells in the bone marrow, after administration of D is given by the fact that their mitotic activity was higher than in the control animals. It can be tentatively suggested that the phenomenon of reduction of the osteogenic component of the growing hematopoietic organ during administration of D was connected not only with suppression of the proliferative activity of the osteogenic precursors, but also with activation of resorption processes through the system of mononuclear phagocytes.

In addition, accumulation of an excess of pathologically changed MKC was observed in the femoral marrow from the 7th day of administration of D, and in FHH from the 19th to the 30th days of the experiment, against the background of karyorrhexis and karyolysis of these cells (Fig. 2), possibly indicating ineffective megakaryocytopoiesis, when intramedullary destruction of MKC is intensified. A similar picture has been observed in patients with myeloproliferative diseases, and with chronic myelofibrosis [7, 8, 9]. This phenomenon is probably compensatory in nature, and the chain of reactions, arising under the influence of D and beginning with suppression of platelet aggregation, leads to activation of megakaryocytopoiesis and to intramedullary destruction of MKC, causing local release of PSGF. This evidently explains the time course of the changes in the number of

bone marrow cells in FHH, when a state of dynamic equilibrium is not established until the 50th day of the experiment and the degree of suppression of release of PSGF from granules of the platelets becomes comparable with the degree of intramedullary destruction of MKC. This state of equilibrium is reached faster in the stationary medullary cell population than in the regenerating bone marrow tissue of the ectopic hematopoietic organ.

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