TRANSFER OF THE BONE MARROW MICROENVIRONMENT BY STROMAL CELLS GROWN IN CULTURE AND TRANSPLANTED INTO GELATIN SPONGES

A. G. Grosheva, Yu. F. Gorskaya, and A. Ya. Fridenshtein

UDC 612.419:612.6.02]-085.23

KEY WORDS: adhesive cells of bone marrow cultures; transfer of the bone marrow microenvironment by cells grown in cultures.

The bone marrow microenvironment can be transferred by transplantation of pure strains of rabbit medullary fibroblasts [4] and of single clones of medullary fibroblasts of guinea pigs and mice [5] in vivo. The effectiveness of transfer of the microenvironment by cells grown beforehand in culture largely depends on the method of regrafting into the animal. For instance, if mouse bone marrow stromal cells, grown in culture, were injected beneath the kidney capsule neither osteogenesis nor transfer of the hematopoietic microenvironment was obtained [6]. In the preceding paper [2] the writers described a highly effective method of transferring the hematopoietic microenvironment by transplanting suspensions of bone marrow cells into sponges. In the investigation now described this method was used to transplant mouse bone marrow cells grown beforehand in culture. In this way the bone marrow microenvironment could be transferred by stromal cells grown in culture, with a high degree of reproducibility.

EXPERIMENTAL METHOD

Bone marrow cells from CBA mice were used for culture. Cell suspensions were prepared by treatment with trypsin or by mechanical mincing [2]. Before explantation the cells were filtered through Kapron to produce a suspension of isolated cells. To determine the efficiency of cloning of the fibroblasts [1] they were cultured in 100-ml Roux flasks, but stromal cells were grown for subsequent transplantation in 1000-ml flasks. The number of cells in the cultures for cloning was the same as described in [1], and between 4×10^7 and 5×10^7 cells of trypsinized bone marrow were explanted into each 1000-ml flasks. Fisher's medium with 20% embryonic calf serum was used for culture. The medium was changed every 4 days. Cloning efficiency was counted on the 12th-14th day of culture [1]. Cells growing on the surface of the glass in 15-30-day cultures and removed by treatment with 0.25% trypsin solution were used for transplantation. The contents of the medullary cavity of one femur also were explanted by Dexter's method [7], in the form of unfiltered tissue fragments, into plastic 25-ml flasks. Cells of the adhesive layer of these cultures were removed by trypsin after 20-25 days. Cells removed from the cultures were introduced into gelatin sponges by the method described in [2].

Sponges with cells were grafted beneath the kidney capsule of syngeneic or semisyngeneic recipients. In the control, empty sponges or sponges containing bone marrow cells irradiated in a dose of 6000 rads were used. Cell films were stained by Giemsa's method and for nonspecific esterase. The grafts were fixed on the 24th-60th day with alcohol–formol and processed by histological methods [2].

EXPERIMENTAL RESULTS

The cloning efficiency of the fibroblasts was on average 10 times higher for cells of trypsinized bone marrow than of untrypsinized. The layer of adhesive cells on the surface of 15-30-day cultures was formed mainly by growth of colonies of fibroblasts, and by the end of the 2nd week it was almost confluent. The centers of many colonies under these circumstances were composed of several layers of cells. Macrophages also were present among the adhesive cells. Hematopoiesis virtually ceased in the cultures after the 7th day. The cells

Laboratory of Immunomorphology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 608-610, May, 1981. Original article submitted December 12, 1980.

0007-4888/81/9105-0677$07.50

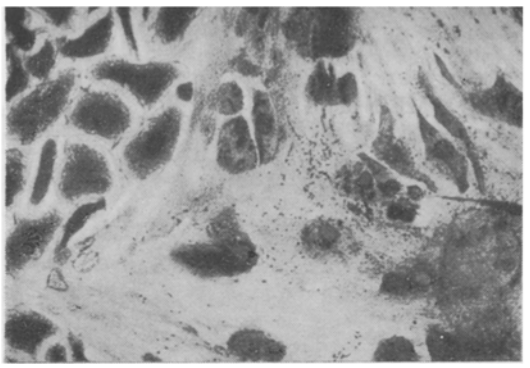

Fig. 1

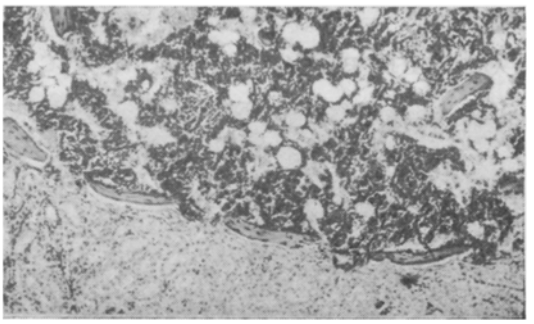

Fig. 2

Fig. 1. Culture of bone marrow cells, 16 days. Reaction for nonspecific esterase in macrophages (left) and fibroblasts (right). Objective 24×.

Fig. 2. Graft of 10^6 cells from 22-day culture after 60 days, objective 4×.

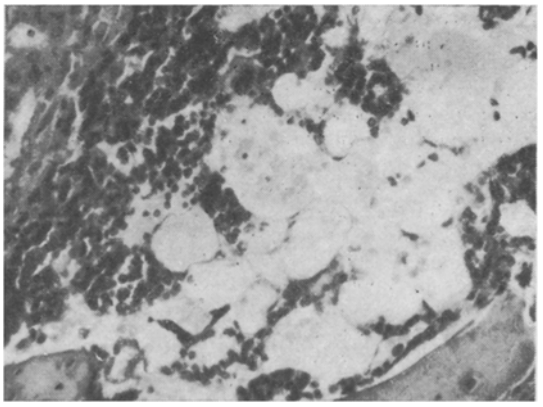

Fig. 3. The same preparation, objective 24×.

removed from the cultures included fibroblasts (from 75 to 85%) and macrophages. The distinguishing features of these two types of cells when grown in monolayer cultures of bone marrow were described previously [3]. In the present investigation, identification of the cells was based on morphological features and on nonspecific esterase activity (positive in macrophages and negative in fibroblasts; Fig. 1).

Empty sponges and sponges with irradiated bone marrow cells were fixed 47-60 days after implantation. In all 26 cases the result was negative for bone or hematopoietic tissue in the sponges.

In four experiments with CBA cells, 10^7 cells were taken from three flasks on the 15th day, 5×10^6 cells from one flask on the 15th day, 13.5×10^6 cells from three flasks on the 21st day, and 1.4×10^7 cells from three flasks on the 33rd day. The cells were introduced into 42 sponges in doses of between 5×10^3 and 2×10^6 cells per sponge and were transplanted beneath the kidney capsule. In each of these experiments the bone marrow microenvironment was transferred, i.e., the formation of a bone marrow organ was achieved within the transplanted sponges (altogether in 31 or 35 sponges containing from 2×10^6 to 8×10^5 cells at the time of transplantation). In the cultures in plastic flasks by Dexter's method the layer of adhesive cells was the same as that described in [7]. On average 10^6 cells were taken from each flask and 3×10^5 cells were introduced into each of 10 sponges. On the 45th day a bone marrow organ was found in each sponge. In sponges containing at least 8×10^5 cells, proliferation of reticular tissue with many capillaries and the formation of discrete foci of hematopoietic cells on the side facing the surface of the kidney and large bony trabeculae in the center of the sponge were observed on the 24th day. By the 34th day, a bone marrow organ with bony capsule and medullary cavity with bridges of bone, foci of hematopoietic cells, and vessels of sinusoidal type, was organized in the sponges (the tissue of which had largely been absorbed (Figs 2 and 3). The ratio between the amounts of bone and hematopoietic tissue varied in different sponges. In some cases the same sponge contained bone and hematopoietic tissue (as a rule topographically interconnected) and also areas of connective tissue or zones with few cells, which were characteristic features of sponges with irradiated bone marrow cells.

In previous investigations the writers obtained data indicating that transfer of the bone marrow microenvironment takes place as a result of survival of stromal mechanocytes with osteogenic potential, and not macrophages [3]. It has so far proved impossible to grow mouse bone marrow fibroblasts in culture without admixture of macrophages. There is reason to suppose that in cultures of mouse bone marrow cells the microenvironment is transferred by adhesive osteogenic fibroblasts, which have multiplied in culture. The use of sponges for transplanting cells grown in culture enabled successful transfer of the microenvironment to take place by stromal cells from primary mouse cultures, which has hitherto been impossible. In the present investigation transfer took place more effectively and constantly than by transplantation of strains of rabbit bone marrow fibroblasts (when sponges were not used). It must also be pointed out that adhesive cells from bone marrow cultures used to transfer the bone marrow microenvironment during transplantation were from a line of mice (CBA) whose adhesive cells are known not to maintain its hematopoietic microenvironment in culture [7]. This may indicate a difference between the cells responsible for each of these two processes. It is interesting to note that, as the large number of osteogenic cells revealed, more of them accumulate in the layer of adhesive cells of Dexter cultures, evidently, than in bone marrow used for explantation.

LITERATURE CITED

1. A. Ya. Fridenshtein, Yu. F. Gorskaya, et al., Exp. Hemat., 4, 267 (1976).
2. A. Ya. Fridenshtein, A. G. Grosheva, et al., Byull. Éksp. Biol. Med., No. 5, 606 (1981).
3. A. Ya. Fridenshtein and E. A. Luriya, Cellular Basis of the Hematopoietic Microenvironment [in Russian], Moscow (1980).
4. A. Ya. Fridenshtein, R. K. Chailakhyan, et al., Transplantation, 17, 331 (1974).
5. R. K. Chailakhyan, Yu. V. Gerasimov, et al., Byull. Éksp. Biol. Med., No. 12, 705 (1978).
6. R. W. Anderson, H. L. Lipscomb, et al., Exp. Hemat., 8, Suppl. 7, 93 (1980).
7. T. M. Dexter, M. A. S. Moore, et al., J. Exp. Med., 145, 1612 (1977).

FORMATION OF FOCI OF MYELOID CELLS ON COLONIES OF THYMUS AND BONE MARROW FIBROBLASTS IN MONOLAYER CULTURE

S. A. Kuznetsov, E. A. Luriya, and E. N. Genkina

UDC 612.419-612.438].014.2:612.6]-085.23

KEY WORDS: colonies of stromal thymus and bone marrow mechanocytes; foci of myeloid cells.

Stromal mechanocytes (fibroblasts) of hematopoietic and lymphoid organs play the leading role in the local regulation of hematopoiesis [3]. It has recently been shown that stromal mechanocytes of different hematopoietic organs bind differentiated granuloid cells in vitro to an equal degree [2].

In the present investigation the ability of stromal fibroblasts of bone marrow and thymus to interact in culture with hematopoietic precursor cells forming colonies of myeloid cells was studied.

EXPERIMENTAL METHOD

Noninbred guinea pigs weighing 180-250 g and (CBA × C57BL)F_1 mice weighing 18-22 g were killed with chloroform. Filtered suspensions of mouse and guinea pig femoral marrow cells and mouse thymus cells were prepared by the standard method [6] in Fisher's medium. Between 1×10^6 and 2×10^6 bone marrow cells or 6×10^6 and 12×10^6 mouse thymus cells were explanted into plastic flasks and Petri dishes (the area of the

Laboratory of Immunomorphology, N. F. Gamalaya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Department of Medical Biophysics, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 610-613, May, 1981. Original article submitted August 12, 1980.