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TRANSFER OF THE HEMATOPOIETIC MICROENVIRONMENT DURING HETEROTOPIC TRANSPLANTATION OF A BONE MARROW CELL SUSPENSION

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Heterotopic (i.e., extraskeletal) bone marrow transplantation has been widely used in recent years to study the hematopoietic microenvironment and, in particular, the cells responsible for its transfer [1-3, 6]. Meanwhile the cellular mechanisms of formation of the new hematopoietic organ during transplantation, including the formation of the hematopoietic microenvironment, have not yet been explained. We do not know the minimal tissue structure capable of the transplantation effect. Heterotopic transplantation of bone marrow with the formation of a new hematopoietic organ by transfer of a suspension of hematopoietic cells has not yet proved successful. Conversely, many workers have specially stated that this would be impossible, i.e., that self-assembly of stromal structures from dissociated cells cannot take place [5] and that transfer of certain undisturbed stromal structures is essential for the formation of new hematopoietic territories.

The investigation described below showed that successful heterotopic transplantation of bone marrow may be achieved by the transfer of initially isolated bone marrow cells, and the conditions necessary for such transplantation and the subsequent stages of self-assembly of the hematopoietic tissue also are described.

EXPERIMENTAL METHOD

CBA and (CBA × C57BL)F_1 mice aged 8 weeks were used as donors of bone marrow. The bone marrow was flushed out of the femora and tibiae by means of a syringe with a needle, and was suspended by means of a syringe and needles of diminishing diameter in medium 199 or an MEM spinner and filtered through two to four layers of Kapron gauze. The filtered suspension was centrifuged so that (4-6) × 10^6 bone marrow cells were deposited on a millipore filter (of HA, AUFS, or RA type) with an area of 20-25 mm^2. The filter was then folded in half with the cells inside and transplanted syngeneically beneath the kidney capsule. Filters without bone marrow cells and filters with a suspension of bone marrow cells irradiated in a dose of 5000 R served as the control.

After 1, 2, 3, 4, and 8 weeks the grafts were fixed in alcohol-formol, decalcified, and embedded in paraffin wax; the PAS reaction was carried out on sections, which were counterstained with hematoxylin.

To determine whether the bone marrow cells responsible for transfer of the hematopoietic microenvironment are phagocytes, phagocytic cells were removed beforehand from the cell sus-

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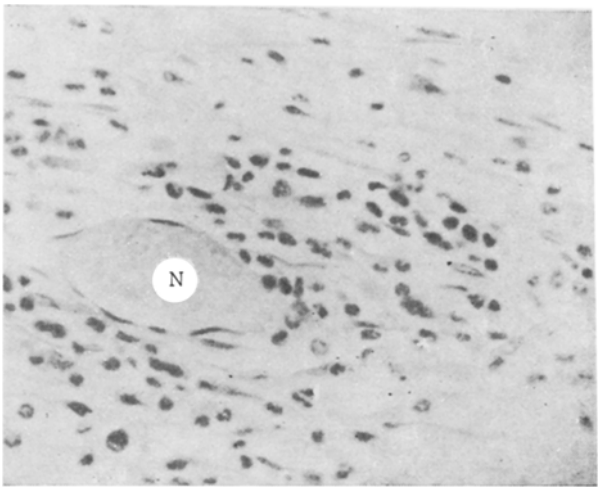


Fig. 1. Graft of bone marrow suspension after 2 weeks. Microvascular network (N) and bands of reticular cells, with myeloid cells distributed anong them; 400 ×.

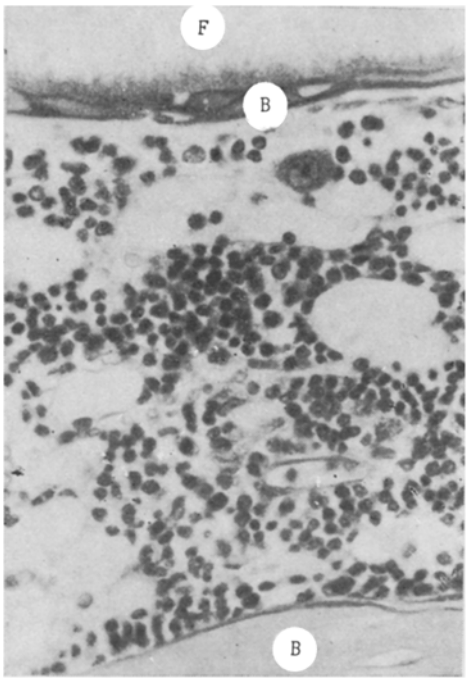
pension [4]. Into a silicone-treated flat-bottomed vessel 10 ml of a bone marrow cell suspension in a concentration of (1-3) × 10^7 cells/ml was poured, with the addition of 0.4 g of sterile iron carbonyl powder. The mixture was incubated for 30 min at 37°C, with gentle mixing every 5 min. The suspension was then carefully resuspended and the iron particles and cells which had phagocytosed them were removed by means of a powerful magnet. The remaining suspension was filtered and the residue resuspended in fresh medium. A cell suspension treated in the same way but without the addition of iron carbonyl served as the control. Cells from the experimental and control suspensions were applied to HA millipore filters by the method described above and transplanted into mice. Two months later nine experimental and 15 control grafts were fixed.

EXPERIMENTAL RESULTS

By the end of the first week, many dilated capillaries were found in the grafts between the HA filters, together with large reticular cells arranged in bands, between which hematopoietic cells could be seen, mainly of the myeloid series. After 2 weeks the number of hematopoietic cells was greatly reduced (Fig. 1). After 3 weeks the well-developed microvascular network was still present and bone tissue was formed between the filters. By the end of the first month the graft had acquired the shape of a hematopoietic organ. The surfaces of the filters were covered with a layer of bone tissue. Between these two laminae of bone a medullary cavity was present, with many vessels of sinusoid type, between which the densely packed myeloid cells were arranged in bands, among which megakaryocytes and a few scattered areas of adipose tissue also were present (Fig. 2). The ground substance of the bone was deposited in a layer 20-50 μ thick in the substance of the millipore filters. In grafts aged 2 months the structure of the bone-marrow organ still remained, but compared with at the previous time the quantity of fatty marrow was increased (Fig. 3). On the whole, grafts also developed similarly when AUFS filters, permeable for cells, were used, the only difference being that the bone tissue in such grafts not only was formed on the surface, but also was deposited within the filters. When 5 × 10^6 cells were introduced initially between the filters, (1-3) × 10^6 cells could be counted in the graft 2 months later.

In the experiment in which all cells with high phagocytic activity were removed from the suspension with the aid of iron powder and placing the cells in a magnetic field, all 15 control grafts contained bone with bone marrow. Seven of nine grafts contained neither bone nor hematopoietic tissue; one graft contained a fragment of dead bone, and one a small focus of hematopoiesis and a fragment of living bone.

The results show that a suspension of bone marrow cells, grafted heterotopically, can form hematopoietic territories which are colonized with hematopoietic cells. A microvascular network forms very quickly (in the course of 1 week) in such grafts, by contrast with the control filters, and it persists for a long time. The bone-marrow organ thus formed is



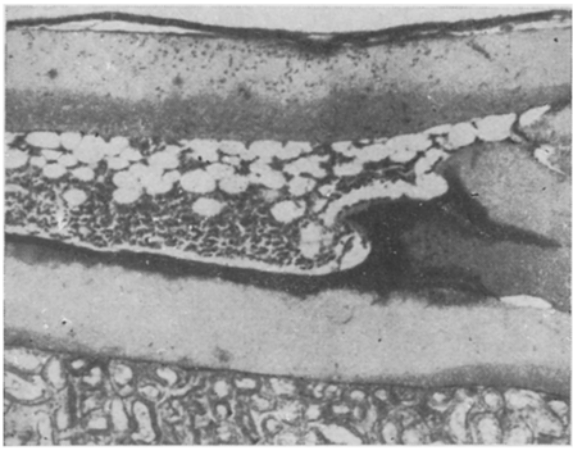


Fig. 2 Fig. 3

Fig. 2. Graft of bone marrow cell suspension after 1 month. Hematopoietic tissue between laminae of bone (B), located on surface of millipore filters (F); 400 ×.

Fig. 3. Graft of suspension of bone marrow cells beneath the kidney capsule after 2 months; 100 ×.

virtually indistinguishable in its structure from that formed at the site of transplantation of a bone marrow fragment. In control grafts (either without bone marrow cells or with irradiated bone marrow) after 2 months neither bone nor hematopoietic tissue was found. There is every reason to suppose that, in the same way as has been demonstrated for transplantation of bone marrow fragments, after transplantation of a suspension of bone marrow cells stromal tissue, including bone tissue, is built at the expense of the donor's cells, whereas hematopoiesis on the territory of the graft is established chiefly on account of repopulating recipient's cells.

The results show that if bone marrow is dissociated into isolated cells, the stromal cells responsible for transfer of the hematopoietic microenvironment remain viable and can carry out self-assembly of stromal structures which maintain the medullary microcirculation. Cells transferring the hematopoietic microenvironment can be removed from a suspension of bone marrow cells by incubation with iron carbonyl powder, from which it can be concluded that they can either phagocytose iron particles or cause them to adhere to their surface. For successful formation of a bone-marrow organ on heterotopic transplantation of a suspension of bone marrow cells, a certain minimal initial cell density and the proper spatial organization are required, and can be achieved by sandwiching the cells between millipore filters.

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