ORIGIN OF STROMAL MECHANOCYTES IN BONE MARROW CULTURES

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The strain of origin of fibroblasts growing in monolayer bone marrow cultures of semisyngeneic heterotopic grafts and radiochimeras was investigated by the indirect immunofluorescence method using an isoantiserum. All fibroblasts from bone marrow of complete radiochimeras were shown to be of recipient origin whereas histiocyte–macrophages in the same cultures were of donor origin. All fibroblasts in bone marrow cultures of heterotopic grafts belonged to the original donor, whereas 80% of the histiocytes were of recipient origin. This proves directly that the stromal mechanocytes and hematopoietic cells, and also the macrophages, belonged to different cell lines.

KEY WORDS: stromal mechanocytes; heterotopic grafts; radiochimeras; immunofluorescence.

It has been shown by the use of semisyngeneic heterotopic transplantation of bone marrow and also by grafting bone marrow of radiochimeras [4, 5] that stromal tissue is histogenetically independent of the hematopoietic cells. This has been shown to be true also of the stromal tissue of the spleen and lymph nodes [2, 3].

In this investigation the linear origin of stromal mechanocytes growing in cultures of bone marrow cells from heterotopic grafts and radiochimeras was determined by an immunofluorescence method using antiserum against mice of another strain (isoantiserum).

EXPERIMENTAL METHOD

Radiochimeras were obtained by irradiating (C57BL/6 × CBA)F_1 mice in a dose of 1100 R (on the RUM-3 cesium apparatus) and subsequently transplanting 10^7 bone marrow cells from CBA mice intravenously into them. Heterotopic bone marrow grafts were prepared by transplanting the contents of the femur of CBA mice beneath the renal capsule of (CBA × C57BL/6)F_1 mice [5]. Cells washed out of the medullary cavity of 8-month-old radiochimeras and 2-month-old heterotopic grafts, and also bone marrow cells of normal CBA and

TABLE 1. Cytotoxic Indices for Bone Marrow cells in Reaction with CBA–anti-C57BL/6 Isoantiserum

Source of bone marrow cells	Cytotoxic index
Intact (CBA × C57BL/6) F_1	1,0
Intact CBA	0,12
Radiochimera 1	0,12
» 2	0
Heterotopic grafts	0,45*

*Mixture of cells from 7 heterotopic graphs.

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TABLE 2. Determination of Strain of Origin of Bone Marrow Fibroblasts of Radiation Chimeras and Heterotopic Grafts by Indirect Immunofluorescence

Source of Cells for cultures	Number of fibroblast colonies with specific fluorescence*	Number of fibroblast colonies without specific fluorescence	% of specifically fluorescent histiocytes located between colonies †
Bone marrow of chimera 1	30	0	0
Bone marrow of chimera 2	30	0	0
Bone marrow of heterotopic grafts	0	30	80
Bone marrow of F_1 hybrids	50	0	100
Bone marrow of CBA mice	0	50	0

*All fibroblasts in composition of colonies gave specific fluorescence just as did fibroblasts outside colonies.
†Number of histiocytes counted in each experiment was not less than several thousands.

(C57BL/6) × CBA)F_1 mice were cultivated on cover slips in penicillin flasks [1]. The cultures were fixed on the tenth day [7].

The CBA–anti-C57BL/6 isoantiserum was prepared by repeated immunization of CBA mice with spleen cells from C57BL/6 mice. It was obtained from L. N. Fontalin.

Rabbit antimouse γ-globulin antiserum conjugated with fluorescein isothiocyanate (National Biochemicals Corporation, Cleveland, Ohio) and unlabeled rabbit antimouse γ-globulin antiserum obtained from K. L. Shakhanina were used.

The indirect immunofluorescence test was carried out by the method of Coons and Kaplan [7] on cells grown on coverslips with the isoantiserum (in a dilution of 1 : 10) and with the rabbit antimouse γ-globulin antiserum labeled with isothiocyanate (in a dilution of 1 : 5) mixed (1 : 1) with rhodamine-labeled albumin. The specificity of the reaction was verified by setting up the following controls: treatment with normal mouse serum and with labeled antimouse γ-globulun antiserum; treatment with anti-BL isoantiserum, with unlabeled antimouse γ-globulin antiserum, and with labeled antimouse γ-globulin antiserum; treatment with labeled antimouse γ-globulin antiserum.

Parallel with explantation, bone marrow cells also were used for the cytotoxic test in the presence of guinea pig complement with isoantiserum [8].

EXPERIMENTAL RESULTS

The cytotoxic indices for bone marrow cells when treated with isoantiserum in a dilution of 1 : 10 are given in Table 1.

The fibroblasts in the 10-day cultures were clearly distinguishable from macrophage–histiocytes. The fibroblasts formed colonies consisting of cells with a widely spread-out cytoplasm and a large oval nucleus. The fibroblasts were several times larger than the macrophages, which did not form colonies but were arranged between the colonies of fibroblasts. The results of the immunofluorescence reaction are given in Table 2.

The CBA–anti-C57BL/6 isoantiserum used was specific: In the presence of complement the antiserum caused death of cells of (CBA × C57BL/6)F_1 mice and gave an immunofluorescence reaction with them, whereas it did not react with cells of CBA mice. The results of the cytotoxic test with this antiserum showed that bone

marrow cells of 8-month-old radiochimeras were virtually entirely of donor origin, whereas about 50% of the bone marrow cells from the 2-month-old heterotopic grafts were of recipient origin, in agreement with results obtained by other workers [6].

All controls of specificity of the immunofluorescence reaction were positive, i.e., the fibroblasts and histiocytes did not fluoresce in the control reactions. The results of the immunofluorescence test indicate that bone marrow fibroblasts are histogenetically independent of macrophage-histiocytes and hematopoietic cells. In fact, all fibroblasts in cell cultures from radiochimeras bound antibodies against H-2 antigens of (CBA × C57BL/6)F_1 recipients but no histiocytes did so. All bone marrow fibroblasts of the radiochimeras were thus of recipient origin and all the histiocytes of donor origin. All fibroblasts growing from cells of the heterotopic grafts were of donor origin, whereas 80% of the histiocytes were recipient's cells and 20% donor's cells.

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ACID MUCOPOLYSACCHARIDES OF THE INNER ZONE OF THE RENAL MEDULLA IN ALBINO RATS KEPT UNDER DIFFERENT CONDITIONS

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In albino rats kept without water reactions for acid mucopolysaccharides in the interstitial tissue of the distal part of the inner zone of the renal medulla remained highly stable in experiments carried out under conditions of higher relative atmospheric humidity.

KEY WORDS: renal medulla; interstitial tissue; acid mucopolysaccharides.

Dry feeding experiments on rats have shown that the morphological picture of the inner zone of the renal medulla differed considerably depending on the relative atmospheric humidity. Corrections thus had to be introduced to the morphological picture of the renal medulla described by the writer previously [2, 3], given the concentration character of diuresis in rats.

The object of this investigation was to study the distribution of acid mucopolysaccharides (AMPS) in the renal medulla of albino rats during dry feeding at different levels of relative atmospheric humidity (φ).

EXPERIMENTAL METHOD

Male rats weighing 250-300 g were kept in single cages at 20-22°C without water, with an excess of dry food (cereals), with a water content of 4.8-5%. The 100 rats of group 1 were kept in a relative humidity of

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