INHIBITION OF FORMATION OF STROMAL CELL COLONIES OF HUMAN BONE MARROW BY A FACTOR FORMED in vitro BY PERIPHERAL BLOOD LEUKOCYTES

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Treatment of a monolayer culture of human bone-marrow cells with human peripheral blood leukocytes or with medium in which leukocytes have been cultured inhibits growth of colonies of fibroblasts. This effect is unconnected with immunologic incompatibility of the cells in culture, for autologous blood cells have the same action as homologous. The factor inhibiting growth of fibroblasts does not inhibit growth of other cells or, in particular, of macrophages.

KEY WORDS: Culture of human bone-marrow cells; colonies of fibroblasts; leukocytes, their inhibitory action.

Colonies consisting of clones of fibroblasts are formed in monolayer cultures of guinea pig, rabbit, mouse, and human bone marrow. The number of colonies formed depends on the number of stromal precursor cells and on their cloning efficiency. The number of colonies is 10^{-4}–10^{-5} of the number of explanted cells [1].

This investigation showed that the addition of peripheral blood leukocytes or nutrient medium in which these leukocytes have been cultured, to a monolayer of human bone marrow inhibits colony formation.

EXPERIMENTAL METHOD

Fragments of ribs resected at operations on patients with tuberculosis were used as the source of bone-marrow cells. The ribs were split open and the bone-marrow cells washed out with medium No. 199 from a syringe. The suspension was pipeted, filtered through Kapron tissue, and cultured in Carrel's flask in 5 ml of medium of the following composition: medium No. 199 plus 20% human group AB (IV) serum. The number of bone-marrow cells seeded in each flask varied from $5 \cdot 10^4$ to $3 \cdot 10^6$.

Peripheral blood leukocytes were added to the flasks along with the bone-marrow cells in the number of $2 \cdot 10^6$–$4 \cdot 10^6$ per flask. Leukocytes were obtained from the blood of healthy donors or, in one experiment, from the blood of the patient from whom the rib was resected. Blood with heparin (20–30 units/ml) was allowed to settle for 1 h at room temperature, sometimes with the addition of 1–2% gelatin. The cells were washed twice with Hank's solution to remove the plasma. The medium in the Carrel's flask was changed once after 48 h. Nonadherent cells were sedimented by centrifugation and again added to flasks with the cultures.

To test the inhibitory activity of the medium in which the leukocytes were cultured, a modified method of Iscove et al. [2] was used. Washed leukocytes were added to CMRL medium or medium No. 199 at 38° C, containing 0.5% agar and 15% human serum. The cells were added in a dose of $4 \cdot 10^6$ cells to 1 ml medium. The suspension was poured into Roux flasks, and when the agar had solidified, an equal volume of the same medium without agar was added. The medium was removed after 6–7 days and kept in a frozen state. It was added in doses of 0.5 ml to the flasks at the time of seeding and when the medium was changed. The cultures

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TABLE 1. Number of Fibroblast Colonies in Culture of Bone-Marrow Cells after Addition of Peripheral Blood Leukocytes or Medium in Which Leukocytes Were Cultured (in % of control)

	Number of bone-marrow cells		
	$2 \cdot 10^5$	$5 \cdot 10^5$	$1 \cdot 10^6$
Homologous leukocytes, $2 \cdot 10^6$	20	43	12
	—	32	14
Homologous leukocytes, $4 \cdot 10^6$	—	5	—
Autologous leukocytes, $2 \cdot 10^6$	—	22	9
Culture medium of leukocytes, 10%	—	7	22
			9
			26
			9

were fixed on the 14th day with 96% alcohol and stained with azure-eosine, after which colonies of fibroblasts were counted under a binocular loupe (magnification 12.5 × 2), counting those that contained more than 50 cells.

EXPERIMENTAL RESULTS

A linear relationship between the number of explanted cells and the number of colonies formed was observed during growth of between $5 \cdot 10^4$ and $(1.5\text{-}2) \cdot 10^6$ cells in the Carrel's flasks. The number of colonies per 10^5 explanted cells (the colony-forming efficiency) varied with different individuals: from 3 to 27 according to the results of five experiments. Explanation of bone-marrow cells in a dose of $3 \cdot 10^6$ per flask led to growth of a monolayer of fibroblasts.

Addition of peripheral blood cells led to marked inhibition of growth of the fibroblast colonies (Table 1), as reflected not only in a decrease in the number of colonies, but also a sharp decrease in their size. The colonies were loose in texture, honeycombed, and sometimes ring-shaped; the cells were not arranged in a smooth layer, and they were poorly adherent to the glass. Inhibition of growth of the fibroblasts by the use of autologous peripheral blood leukocytes was just as marked as when homologous cells were used. Consequently, the effect was not due to immunologic incompatibility between the bone marrow and blood cells in culture.

The medium in which the blood cells were cultured had just as strong inhibitory action on growth of the fibroblasts as the cells themselves. Large numbers of macrophages were observed in such flasks, often forming large clusters. Keeping the conditioned medium for 2-3 months in a frozen state, with frequent thawing, did not modify its inhibitory activity.

The results are evidence that during cultivation of peripheral blood cells in vitro a factor inhibiting growth of human bone-marrow fibroblasts appears in the medium. The effect of inhibition was seen both when the peripheral leukocytes and bone-marrow cells were grown together in monolayer culture and when the medium in which the peripheral blood leukocytes were cultured was used. The presence in the medium of a factor inhibiting growth of fibroblasts was not accompanied by inhibition of the growth of other cells. In monolayer culture, for instance, an increase in the number of macrophages was observed, whereas addition of the conditioned medium to the agar culture of bone-marrow cells had a stimulant action on growth of granulocytic and macrophagal colonies. The relationship of this factor inhibiting growth of fibroblasts to the factor stimulating growth of granulocytic colonies in agar is not clear. Nor is it known which cells of human peripheral blood were responsible for the inhibitory effect and whether it is exhibited on cells of other animals.

LITERATURE CITED

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