COMPARATIVE EVALUATION OF HETEROLOGOUS FEEDERS DURING CLONING OF HUMAN BONE MARROW STROMAL FIBROBLASTS

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UDC 612.419.014.2:612.6]-085.23-063

KEY WORDS: bone marrow; stromal fibroblasts; cloning; feeder.

Methods of cloning stromal bone marrow fibroblasts from laboratory animals and man that have been developed up to the present time enable their role to be studied in hematopoiesis, immunogenesis, and osteogenesis under normal and pathological conditions [1-5]. The results of cloning human bone marrow stromal fibroblasts with explantation of about 10^7 nucleated cells are very variable, and this makes their analysis difficult. The use of a feeder can reduce the number of cells from a sample of bone marrow under investigation required for explantation by an order of magnitude; consequently, by increasing the multiplicity of seeding, this increases the accuracy of the determination.

It was shown previously that a heterologous feeder, prepared from rabbit or rat bone marrow cells can be used for cloning human bone marrow stromal fibroblasts [6]. The efficiency of colony formation by human bone marrow stromal fibroblasts under such conditions of culture was almost the same as when a feeder from human bone marrow cells was used.

The aim of this investigation was a comparative evaluation of heterologous feeders from rabbit and rat bone marrow cells when cloning human bone marrow stromal fibroblasts.

TABLE 1. Efficiency of Colony Formation by Stromal Fibroblasts from Femoral Marrow of Children with Congenital Dislocation of the Hip

Serial No.	No. of explanted bone marrow cells	Amount of feeder		Cloning efficiency, per 10^5 nucleated cells	
		rabbit	rat	on rabbit feeder	on rat feeder
1	$5 \cdot 10^6$	$2 \cdot 10^7$		4,5±0,1	
	$5 \cdot 10^6$		$2 \cdot 10^7$		1,8±1,2
2	$6,5 \cdot 10^6$	$2,4 \cdot 10^7$		3,8±0,7	
	$6,5 \cdot 10^6$		$2,4 \cdot 10^7$		1,1±0,6
3	$6 \cdot 10^6$	$2 \cdot 10^7$		2,3±0,2	
	$6 \cdot 10^6$		$2 \cdot 10^7$		0
4	$5 \cdot 10^6$	$2 \cdot 10^7$		3,2±0,4	
	$5 \cdot 10^6$		$2 \cdot 10^7$		1,4±0,9
5	$5 \cdot 10^6$	$1,6 \cdot 10^7$		4,9±0,6	
	$5 \cdot 10^6$		$2,7 \cdot 10^7$		1,6±0,5
6—8	$5 \cdot 10^6$	$2 \cdot 10^7$		2,6±0,6	
	$5 \cdot 10^6$		$2 \cdot 10^7$		2,0±0,9
9—30	$2,5 \cdot 10^7$	No feeder		0,1±0,5	

Legend. Mean values of cloning efficiency (M ± m): on rabbit feeder 3.7 ± 0.3, on rat feeder 1.4 ± 0.8, without feeder 0.1 ± 0.5 ($P < 0.001$).

Laboratory of Immunology, Kiev Research Institute of Orthopedics, Ministry of Health of the Ukrainian SSR. (Presented by Academician of the Academy of Medical Sciences of the USSR D. F. Chebotarev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 111-113, October, 1982. Original article submitted February 19, 1982.

 0007-4888/82/9410-1446$07.50

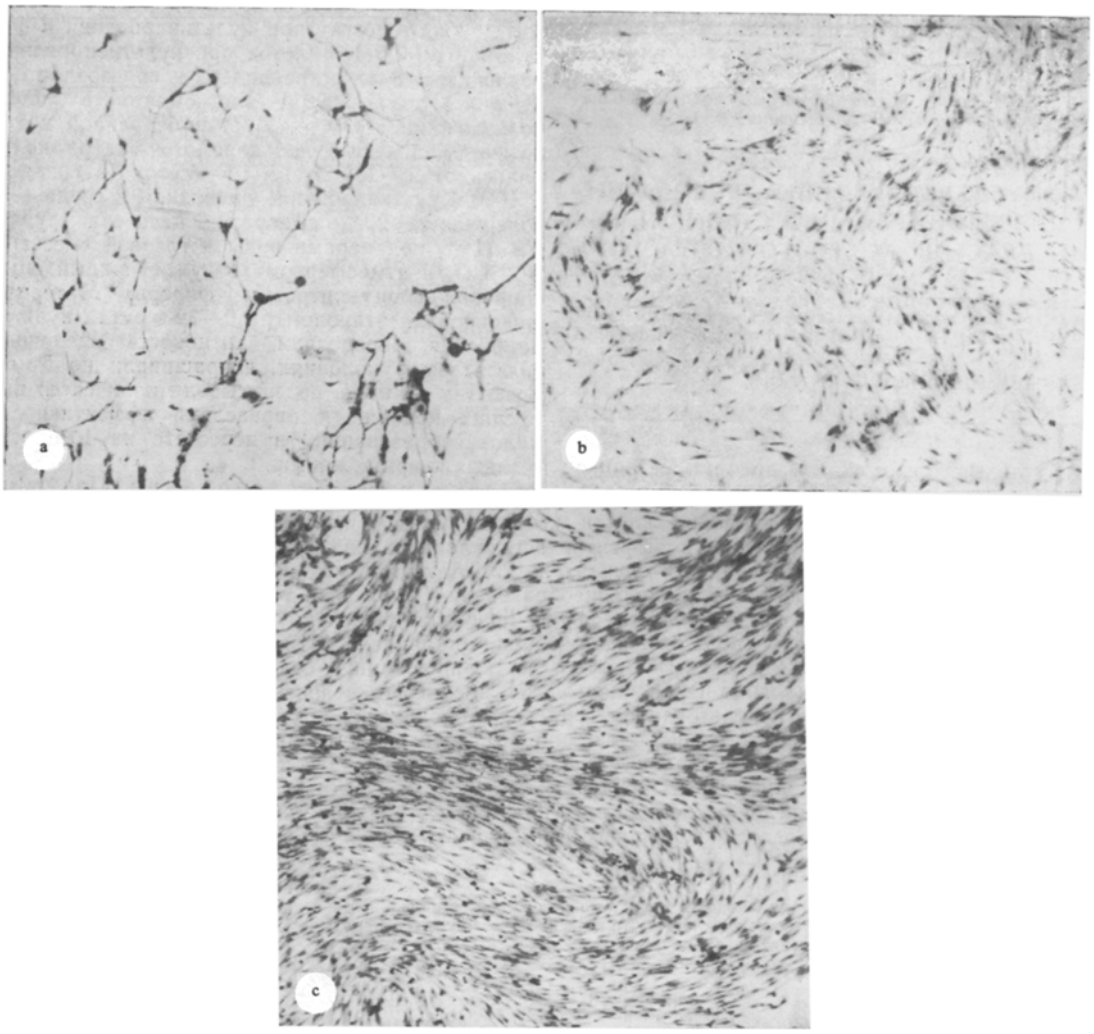

Fig. 1. Colonies of human bone marrow stromal fibroblasts cultured under different conditions: a) without feeder, b) with rat feeder, c) with rabbit feeder.

EXPERIMENTAL METHOD

The cloning efficiency of stromal fibroblasts from the femoral marrow of children aged 3-12 years with congenital dislocation of the hip was studied. Bone marrow from 30 children was investigated. The marrow was taken from the proximal metaphysis of the femur during operations and transferred to medium 199. A cell suspension was prepared by disintegrating the bone marrow in the medium by means of needles of decreasing diameter. The material was cultured in Roux flasks, the bottom with an area of 65 cm^2, on which on average 5×10^6 nucleated bone marrow cells were explanted for culture with feeder and 2.5×10^7 cells for culture without feeder. Bone marrow cells from the rabbit ileum and rat femur, irradiated with a dose of 5000 R, were used as the feeder. The quantity of feeder added varied from 2.7×10^7 to 1.6×10^7 cells (mean 2×10^7). The material was cultured in medium 199 containing 20% human group AB (IV) serum, under water vapor and a gas mixture consisting of 5% CO_2 in atmospheric air. Each experiment was accompanied by a feeder control. Material was fixed with ethanol on the 9th-12th day of culture, alkaline phosphatase activity was determined in colonies stained by the Romanovsky–Giemsa method, the number of growing colonies was counted, and the efficiency of colony formation was calculated per 10^5 seeded nucleated cells.

EXPERIMENTAL RESULTS

During culture of human bone marrow cells without feeder the cloning efficiency was 0 in 12 of 22 cases. Maximal efficiency of colony formation was 1, and this figure was never exceeded. The mean value was 0.1×10^{-5}.

The results of the experiments to clone human bone marrow stromal fibroblasts with the use of rabbit and rat feeders are given in Table 1. When human bone marrow was cultured with rabbit feeder the results showed great stability within the limits of the experiment. The mean efficiency of colony formation under these conditions was $(3.7 \pm 0.3) \times 10^{-5}$.

When human bone marrow was cultured with rat feeder the results were not stable. The efficiency of colony formation varied within the limits of the experiment from 0 to 5.6 and the mean value was 2.5 times less than when the same bone marrow samples were cultured with rabbit feeder.

The time of addition of the feeder had no effect on the efficiency of colony formation if it did not exceed 72 h from the time of explantation of the cells. Irradiation in the dose stipulated completely inhibits multiplication of rabbit and rat bone marrow cells while leaving their feeder properties intact.

A difference in the structure of the colonies also was found. When bone marrow was cultured without feeder or with rat feeder, the colonies which grew were small (not more than 1.5 mm in diameter), composed of a single layer, and phosphatase-negative. When bone marrow was cultured with rabbit feeder the colonies were large (diameter 5 mm or more), stratified, and 60-80% of them were phosphatase-positive. Colonies of human bone marrow stromal fibroblasts cultured without feeder and with rabbit or rat feeder are illustrated in Fig. 1.

Consequently, when cloning human bone marrow stromal fibroblasts the most effective feeder to use is that from rabbit bone marrow cells. It is easy and convenient to prepare and can be widely used.

The data obtained for the efficiency of colony formation by stromal fibroblasts from the femoral marrow of children aged 3-12 years with congenital dislocation of the hip can be used as control data when studying cloning of bone marrow stromal cells in inflammatory diseases of bone and other types of pathology in children of this age group.

The author is grateful to Professor A. Ya. Fridenshtein for scientific guidance and to E. A. Luriya and N. N. Kulagina, on the staff of the Immunomorphology Laboratory, N. F. Gamaleya Institute of Epidemiology and Microbiology, for technical assistance in the course of the work.

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