EFFECT OF CURETTAGE OF THE MEDULLARY CAVITY ON BONE MARROW STROMAL PRECURSOR CELLS

Yu. V. Gerasimov and R. K. Chailakhyan

UDC 616.419-089.877-07:616.419-003.93-018.15-07

Changes in the number of stromal bone marrow precursor cells in guinea pigs after curettage of the medullary cavity were studied by cloning and monolayer cultures in vitro. Curettage was shown to remove about half of the fibroblast colony forming cells (FCFC) from the bone marrow. Later, the number of FCFC in the curetted limb fell to reach a minimum after 12 h. Starting from 24 h their number increased. By the 7th-12th day the number of FCFC reached the normal level, and by the 20th day it was 2.5 times higher than normal. The number of FCFC in the contralateral limb between 6 h and 20 days after curettage was 2-2.5 times greater than normal.
KEY WORDS: curettage; stromal cells; bone marrow cells; colonies of fibroblasts.

The study of interaction between the stroma of the hematopoietic organs and the hematopoietic cells colonizing these organs has made considerable progress through the development of the method of stromal cell cloning [3, 2, 5]. By this method it is possible to estimate changes in the number of stromal precursor cells, especially during regeneration of the hematopoietic tissue after mechanical curettage of the medullary cavity.

The object of this investigation was to study the number of stromal bone marrow precursor cells after curettage by means of cloning and monolayer cultures in vitro.

EXPERIMENTAL METHOD

Guinea pigs weighing 160-220 g were anesthetized with ether and the tibia curetted. Physiological saline was injected in a volume of 2-3 ml into the medullary cavity through a needle introduced via the proximal epiphysis. The contents of the medullary cavity were withdrawn, after which it was again washed out with 5 ml of physiological saline. Cloning [3, 2, 5] of the medullary stromal fibroblasts from the curetted and contralateral tibias was carried out immediately after curettage (at time 0) and also between 3 h and 20 days thereafter. In each experiment a mixture of cells obtained from three animals was used for explanation. Bone marrow cells of normal guinea pigs of the same body weight served as the control. The cells were cultured in Roux flasks (volume 100 ml, area of bottom 40 cm^2) and the density of the explanted cells was 10^6 per flask in 12 ml of medium. The culture medium consisted of: 80% medium 199, 15% bovine serum; 5% embryonic calf serum. To each flask were added 10^7 bone marrow cells irradiated with a dose of 4000 R as supplementary feeder. On the 12th-14th day the cultures were fixed and stained by Giemsa's method. The number of growing fibroblast colonies was counted under a binocular loupe. The results were evaluated by Student's criterion.

EXPERIMENTAL RESULTS

In each experiment the number of nucleated cells and of fibroblast colony forming cells (FCFC) in 10^6 explanted cells in the tibia and also the number of FCFC per 10^6 bone marrow cells were determined. These data are given in Table 1. At time 0 the curetted tibia still contained 20% of its cells. The number of cells remained low until the seventh day, when it began to increase, although by the 20th day it had not yet regained the control level. The number of cells in the contralateral tibia showed no significant changed compared with the control.

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. 86, No. 9, pp. 362-365, September, 1978. Original article submitted September 27, 1977.

0007-4888/78/8609-1243$07.50

TABLE 1. Changes in Number of Bone Marrow Cells and FCFC after Curettage (M±m)

Time of curettage	Source of cells	Number of nucleated cells ~~cells~~ × 10^6	FCFC × 10^6 cells	Number of FCFC in bone
Control	Tibia of intact animals	72±2,8	31±2	2193±137,6
Time 0	Curetted tibia	14±2,4	93±6	1290±220
	Contralateral tibia	59,2±5,2	63,3±15.9	3753±983
3 h	Curetted tibia	10,3±5,3	101±30	1013±289
	Contralateral tibia	61,3±13,3	23±2,5	1485±412
6 h	Curetted tibia	19±2,8	66±4	1110±208
	Contralateral tibia	82,5±8,1	49±3,6	4089±626
12 h	Curetted tibia	9±2,1	5,8±18	488±119
	Contralateral tibia	66±8	64,7±35,5	3715±1576
24 h	Curetted tibia	10,5±1,8	149±15	1417±159,5
	Contralateral tibia	69±8,3	77,8±6.1	5386±832
3 days	Curetted tibia	7,6±0,3	152±14	1161±122
	Contralateral tibia	82,3±20	84±8	7313±1993
7 days	Curetted tibia	17,3±2,4	150±29	2390±712
	Contralateral tibia	64±8,5	94±19	5717±1594
12 days	Curetted tibia	19±2,5	144±15	2629±318
	Contralateral tibia	68,3±13,7	67±5	4416±729
20 days	Curetted tibia	49±2,6	115±25	5369±940
	Contralateral tibia	83,4±10	70±12	5348±609

Legend. Each value obtained as a result of 3-17 experiments; 3 animals were investigated in each experiment, and cells from their bones were mixed and explanted in 3 parallel flasks.

After curettage about half the FCFC were removed from the bone marrow. Later the number of FCFC in the curetted tibia continued to decline, reaching a minimum after 12 h. Starting from 24 h the number of FCFC increased gradually, and by the 7th to the 12th day it was back to normal. By the 20th day the number of FCFC was higher still, 2.5 times greater than normally. The number of FCFC in the contralateral tibia after 6 h was still 2.5 times greater than their normal number.

After regeneration of the bone marrow and curettage of the medullary cavity, the blood clot was replaced by connective tissue, osteoblasts grew into it, trabeculae of coarsely woven bone were formed, and in the space between them sinusoids were formed, after which foci of hematopoiesis appeared in the regenerated reticular tissue [6, 7, 9, 11-14]. Changes in the number of bone marrow cells in the curetted bone, observed in the present investigation, were identical with the dynamics of regeneration described on the basis of morphological investigations. The main source of regeneration of the bone marrow cells after curettage, as was shown in [8, 11], was repopulating medullary cells. The present experiments showed that the number of FCFC remaining after curettage was significantly greater than the number of residual hematopoietic cells. The number of FCFC in the early stages after curettage thus represented a higher proportion of the total bone marrow cells. This proportion increased still further from the end of the first until the seventh day, parallel with the increase in the absolute number of FCFC in the bone. This indicates that the increase in the number of stromal precursor cells took place before repopulation of the territory of the curetted bone marrow by hematopoietic cells and it is in good agreement with results showing that the stromal cells are responsible for the formation of the hematopoietic microenvironment [3, 4, 1]. On the 20th day the absolute number of FCFC in the curetted bone of the guinea pigs was 2.5 times greater than in the control. According to the available data [15], in mice on the 11th day after curettage it may be even five times greater. When these results are evaluated it must be remembered that the FCFC of the bone marrow possess osteogenic properties [3, 4, 1] and that they evidently take part in bone formation in the medullary cavity after curettage.

The data on the effect of curettage on FCFC in the contralateral tibia indicate that immediately after trauma the number of stromal precursor cells in distant parts of the skeleton increased. Between 6 h and 20 days after trauma the number of these cells remained about 2-2.5 times greater than in the control. According

to Wilson et al. [15], the number of stromal precursor cells in the contralateral femur of curetted mice on the 11th day was increased by four times. These results agree with those of autoradiographic analysis of bone marrow [10], which showed that partial depopulation of the medullary cavity leads to stimulation of the proliferative activity of stromal cells in undamaged areas.

The mechanism and role of this systemic response of the stromal tissue have not yet been explained and are interesting for they indicate the existence of hitherto unknown interrelationships between different parts of the microenvironment of hematopoietic tissue mediated through humoral or nervous connections.

LITERATURE CITED

1. A. Ya. Fridenshtein, in: Hard Tissue Growth, Repair and Remineralization, Amsterdam (1973), p. 169.
2. A. Ya. Fridenshtein and K. S. Lalykina, Induction of Bone Tissue and Osteogenic Precursor Cells [in Russian], Moscow (1973).
3. A. Ya. Fridenshtein, R. K. Chailakhyan, and K. S. Lalykina, Cell Tissue Kinet., 3, 393 (1970).
4. A. Ya. Fridenshtein, R. K. Chailakhyan, N. V. Latsinik, et al., Transplantation, 17, 331 (1974).
5. R. K. Chailakhyan, A. Ya. Fridenshtein, and A. V. Vasil'ev, Byull. Éksp. Biol. Med., No. 2, 94 (1970).
6. S. Amsel, A. Maniatis, M. Tavassoli, et al., Anat. Rec., 164, 101 (1969).
7. P. J. Branemark, U.Breine, B. Johansson, et al., Acta Anat. (Basel), 59, 1 (1964).
8. B. G. Brecher, J. H. Tjio. W. W. Smith, et al., Blood, 48, 679 (1976).
9. W. H. Knospe, S. A. Gregory, S. G. Husseini, et al., Blood, 39, 331 (1972).
10. K. Meyer-Hamme, R. J. Haas, T. M. Fliedner, et al., Acta Haemat. (Basel), 46, 649 (1971).
11. H. M. Patt and M. A. Maloney, Exp. Hematol., 3, 135 (1975).
12. K. Rohlich, Z. Mikr -anat. Forsch., 49, 425 (1941).
13. B. Steinberg and S. Hufford, Arch. Path., 43, 117 (1947).
14. D. Van Dyke and N. Harris, Blood, 34, 257 (1969).
15. F. D. Wilson, L. O'Grady, et al., Exp. Hematol., 2, 343 (1974).

FORMATION OF DNA-PROTEIN CROSS-LINKAGES AND THEIR POSSIBLE ELIMINATION BY MUSTINE IN CULTURES OF NORMAL HUMAN FIBROBLASTS

V. V. Rupasov, V. I. Kukharenko, A. I. Gorin, K. N. Grinberg, and P. I. Tseitlin

UDC 612.398.145.1.014.46: 615.277.3/-085.23

To determine DNA-protein cross-linkages induced by mustine in cultures of human skin fibroblasts a radiometric method and fractionation of the cell lysate on hydroxyapatite were used. The formation of DNA-protein cross-linkages and their elimination during long-term culture of the cells after treatment with the mutagen were demonstrated.

KEY WORDS: mustine; DNA-protein; repair.

The formation of DNA-protein cross-linkages in the chromatin of eukaryotes is evidently a general type of injury which may arise through exposure to various physical and chemical mutagens: ionizing radiation [5], UV radiation [13], and alkylating compounds [5, 7-12, 14, 15]. According to our own observations, these injuries cause disturbances of the template properties of chromatin [4]. Methods used currently to detect DNA-protein cross-linkages in cells are indirect and do not provide an unequivocal answer to the question of the quantitative parameters which characterize this defect. In the present investigation, in which a radiometric method developed

Laboratory of Biophysics and Laboratory of Human Cytogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsniy, Vol. 86, No. 9, pp. 365-367, September, 1978. Original article submitted January 24, 1978.