NATURE OF DIVIDING CELLS IN THE REGENERATING LIVER OF XENOGENEIC RADIATION CHIMERAS

V. M. Faktor, É. V. Chernyshova, I. V. Uryvaeva, and N. G. Krushchov

UDC 576.312.37:576.35

Chromosome analysis of cells isolated from the regenerating liver of 14 xenogeneic mouse–rat radiochimeras showed that of 763 metaphases examined 95 were donor in origin. One of this latter group of metaphases was tetraploid. Most probably the cells of donor karyotype isolated from the liver of the radiation chimeras are Kupffer cells or cells from extramedullary foci of hematopoiesis. Polyploid cells of donor karyotype may belong to the megakaryocytic line of differentiation or may be polyploid cells of an inflammatory focus.

KEY WORDS: xenogeneic radiochimeras; megakaryocytes; donor karyotype; polyploidy; Kupffer cells.

Interesting results of a cytogenetic analysis of cells of mouse radiation chimeras were published in 1966 [4]. A suspension of tagged spleen cells of a syngeneic F_1 ($C3HT_6$) donor was injected into lethally irradiated C3H mice. Of 110 metaphases analyzed in preparations from the liver regenerating after CCl_4 poisoning, a T_6 chromosome marker was present in 98, indicating the donor origin of these cells. In each of 2 tetraploid metaphases among 80 chromosomes there were two donor markers. This fact is particularly interesting because polyploidy is a characteristic feature of the parenchymatous cells of the liver.

The object of this investigation was to verify these findings of cells originating from the donor's hematopoietic stem cells in the liver of xenogeneic (mouse–rat) radiation chimeras and to study their possible nature. Unlike in the investigation cited above [4], in the present work xenogeneic (mouse–rat) mouse radiation chimeras were used and the liver cells were induced to proliferate by partial hepatectomy.

EXPERIMENTAL METHOD

Fourteen xenogeneic radiochimeras were used. These were obtained as follows. Male F_1 (CBA × $C57BL_6$) mice weighing 20-22 g were irradiated in a dose completely destroying the hematopoietic tissue (850 R). The recipients received an intravenous injection of 25×10^6 rat bone marrow cells 24 h later. To confirm the chimerism, in some cases the bone marrow cells were karyotyped. Partial hepatectomy was performed on the radiochimeras 21-30 days after transplantation of the bone marrow in order to stimulate proliferation of the liver cells. About two-thirds of the liver was removed under ether anesthesia. The postoperative mortality among the chimeric animals was higher (6 of 20 animals) than would correspond to the usual 100% survival of normal animals. The experiment ended 42-75 h after the operation. Colchicine was injected intraperitoneally in a dose of 1 μg/g 4 h before the animals were sacrificed. Preparations of chromosomes of the liver cells were obtained as described previously [1]. The ploidy of mitosis was determined by counting the number of chromosomes and, in some cases, approximately from the size of the metaphase plate. Pieces of liver were removed at operation and at the end of the experiment for histological analysis, fixed in Carnoy's fluid, and embedded in paraffin wax. The chromosome preparations were stained with carbol-fuchsin and liver sections with Mayer's hematoxylin and azure-eosin.

Laboratory of Cytology and Histogenesis, Institute of Biology of Development, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 9, pp. 103-106, September, 1974. Original article submitted November 5, 1973.

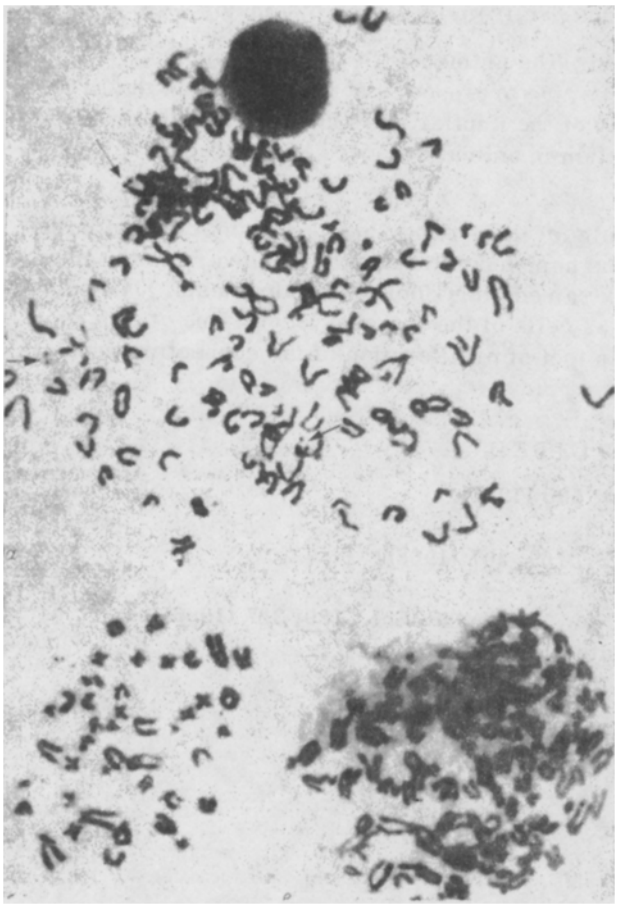

Fig. 1. Dividing cells isolated from the regenerating liver of xenogeneic radiochimeras (stained with carbol-fuchsin; 500 ×): a) polyploid metaphase plate; mouse karyotype, acentric chromosomal fragments marked by arrows; b, c) diploid and tetraploid metaphase plates: rat karyotype.

EXPERIMENTAL RESULTS

The results of chromosome analysis of cells isolated from the regenerating liver are summarized for all 14 animals studied. Altogether 763 mitoses were examined; 668 metaphases had the mouse karyotype, and of this number 128, 281, 255, and 4 metaphases corresponded to 2n, 4n, 8n, and 16n, respectively. A rat karyotype was found in 95 metaphases, of which 94 were 2n and 1 was 4n.

The presence of mouse polyploid cells in the preparations reflected division of the parenchymatous cells of the liver stimulated by removal of part of the organ. It is well known that in mice of this age nearly all the hepatocytes are polyploid. The rates of regeneration of the liver in the chimeric animals were slower than normal. Only a few dividing hepatocytes were observed in the sections taken 42-50 h after partial hepatectomy, whereas in normal animals at this time proliferation is usually maximal. By 72 h after the operation the number of mitoses among the parenchymatous cells was increased, but was always less than in normal mice. In all animals mouse polyploid metaphases usually had chromosomal aberrations induced by irradiation (small acentric fragments; Fig. 1a). The long persistence of unstable chromosomal aberrations [7] – for 3-4 days after irradiation – is explained by the fact that thaoughout this time most of the hepatocytes did not divide by mitosis. This fits in closely with the view of the stability of the hepatocyte population in adult animals.

The presence of cells of the rat karyotype (Fig. 1b, c) in the liver is evidence primarily of the survival of the donor's hematopoietic stem cells and of their functioning in the body of the mouse. The question arises: what cells had the rat genotype? First, it can be postulated that the liver of the irradiated animals, like the spleen, becomes the site of formation of hematopoietic foci. No information on this problem could be found in the literature. On histological analysis of sections stained with azure-eosin foci of infiltration could be found among the parenchymatous cells or near the blood vessels. Some of them were undoubtedly of inflammatory nature and some consisted of undifferentiated cells, frequently dividing by mitosis. These clusters of cells are most probably hematopoietic foci. Megakaryocytes were identified in some of them.

The rat cells isolated from the liver in some cases could thus be proliferating hematopoietic cells. The solitary tetraploid cells of donor origin, also found by Hard and Kullgren [4], could be young forms of megakaryocytes. Differentiation of megakaryocytes is accompanied by polyploidization.

The second source of the rat karyotype in the preparations could be cells of the reticulo-endothelial system of the liver. Kupffer cells, although they divide intensively, cannot maintain themselves for long. Under various conditions of stimulation of their function precursors of liver macrophages were found among the bone marrow cells [3, 6] and lymphocytes of the thoracic duct [5]. Proliferation of Kupffer cells in the regenerating liver is usually intensified. Consequently, the Kupffer cells of the liver may also have the rat karyotype. One other possibility must also be mentioned, although it cannot yet be confirmed – maintenance of the population of parenchymatous cells of the liver on account of bone-marrow precursors. The discovery of tetraploid forms of donor origin among cells isolated from the liver of the radiochimeras would seem to support this hypothesis. However, polyploidy cannot be regarded as a unique feature of the epithelium of the liver. Polyploid forms besides megakaryocytes are also found among cells of the in-

flammatory focus which, in radiation mouse chimeras, may arise from the donor's hematopoietic cells [2].

The great heterogeneity of the material must be noted. The animals differed in the intensity of the inflammatory reaction in the liver, the degenerative changes, the presence of hematopoiesis in the liver tissue, the degree of proliferative and macrophagal activity of the Kupffer cells and, finally, the proliferative activity of the parenchyma. The causes of this variation in animals exposed to several factors will be evident.

The following conclusion can be drawn from the results of these experiments and information in the literature. In all probability cells of donor origin (from the hematopoietic tissue) isolated from the liver of the radiochimeras are Kupffer cells and also cells of extramedullary foci of hematopoiesis. The polyploid forms of donor karyotype must evidently be classed as cells of the megakaryocytic line of differentiation or as polyploid cells such as are frequently found in foci of proliferation of the connective tissue in inflammation.

LITERATURE CITED

1. I.V. Uryvaeva and V. M. Faktor, Tsitologiya, No. 4, 530 (1971).
2. D. Barnes and N. Khrushchov, Nature, 218, 599 (1968).
3. J. D. Boak, G. H. Christie, et al., Proc. Roy. Soc. B., 169, 307 (1968).
4. R. Hard and B. Kullgren, Science, 152, 349 (1966).
5. J. G. Howard, J. L. Boak, and G. H. Christie, Ann. New York Acad. Sci., 129, 327 (1966).
6. R. G. Kinsky, G. H. Christie, et al., Brit. J. Exp. Path., 50, 438 (1969).
7. P. C. Nowell, D. E. Craig, et al., Radiat. Res., 24, 108 (1965).