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A Marker Chromosome in Transplantable Rat Leukemia, R3149.* (31998)

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While marker chromosomes have been described for human chronic granulocytic leukemia(1,2) and for leukemia in mice(3), there have been few reports of persistent marker chromosomes for leukemia in rats. Nowell *et al*(4) investigated some methylcholanthrene induced leukemias in rats and noted no consistent or specific chromosome changes. Moloney and co-workers(5) also studied leukemia in rats. These animals had received radiation, oral methylcholanthrene, or both agents. Cytogenetic studies from such leukemic rats revealed no consistent chromosome changes. Marker chromosomes for leukemia in rats have been but briefly noted by Russian investigators(6), who described 2 small marker chromosomes in one of 3 leukemias studied. The present studies document a large marker chromosome in a stable transplantable rat leukemia.

Chromosome studies were carried out on inbred Fischer rats inoculated with the Dunning R3149 acute leukemia(7). The origin of the leukemia, which arose in a female Fischer rat, will be described by Dr. Dunning elsewhere(8). This rat leukemia is considered by Dunning to be an acute monocytic leukemia which resembles her IRC 741 rat leukemia(9). With the help and cooperation of Dr. Dunning we have studied the chromosomal constitution of the R3149 rat leukemia. Our interest in the chromosomes of this leukemia began when we observed the presence of abnormal mitotic figures in blood smears from leukemic animals.

Materials and methods. Young adult Fischer rats (Charles River Breeding Laboratories, North Wilmington, Mass.) were used for these studies. A piece of leukemic spleen (2 × 2 cu mm) from the donor rat was inserted under the lateral abdominal skin of the recipients with the use of a trocar. The in-

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oculated animals were observed for the development of a tumor at the site of inoculation and for the appearance of leukemia in the peripheral blood. Blood counts were performed and stained blood smears were examined for differential counts, leukocyte alkaline phosphatase(10) and for peroxidase (11).

All rats dying after implantation were autopsied and the presence of leukemic splenic enlargement ascertained. Tissues were fixed in 10% buffered formalin and sections were routinely stained with hematoxylin and eosin.

Chromosome preparations were obtained directly from the bone marrow, liver and spleen as described by Nowell, Ferry, and Hungerford(4). Rats were pretreated with 0.75 mg of colchicine 2 hours prior to sacrifice by ether anesthesia. Suitable metaphase preparations were examined, photographed, and karyotyped. Classification of the rat chromosomes followed the recommendation of Nowell, Ferry, and Hungerford(4).

Results. All rats inoculated with R3149 leukemic tissue developed leukemia. The average survival after leukemic inoculation in 57 adult rats was 8.4 days and ranged from 6 to 11 days. There was no sex difference in survival noted.

Leukemic cells in large numbers were seen in the blood usually 2 or 3 days prior to death. White blood counts became elevated during this time. The highest whitecell count noted to date has been 315,000 per cu mm. This R3149 leukemia is characterized by the presence of large monocytoid cells in the peripheral blood. Cytochemical staining showed that these leukemic cells were consistently lacking in leukocyte alkaline phosphatase while the normal granulocytes present remained rich in this enzyme. Most of the leukemic cells were also peroxidase negative. However, a very few of the large cells showed a slight positive peroxidase reaction. All blood smears obtained on the day of death demonstrated leukemia and evidence of a probable hemolytic anemia, *i.e.*, increased polychromasia and the presence of small to moderate numbers of nucleated red cells.

At autopsy, the leukemic spleen was in-

FIG. 1. Metaphase and karyotype from spleen of leukemic male Fischer rat. Giant marker chromosome indicated by arrows. The leukemic cell is female.

variably enlarged 2 or 3 times normal, while the liver was enlarged about 1½ times normal. Histologic examination showed there were always massive metastases to the liver, lungs, and almost entire replacement of spleen by these abnormal cells.

The striking chromosome finding in this leukemia is shown in Fig. 1; the giant marker chromosome is indicated by arrows. The normal Fischer rat karyotype contains 40 autosomes and 2 sex chromosomes. The leukemic cells were found to be always female (XX). The sex chromosomes of normal female Fischer rat cells appeared consistently to be X_tX_t, as classified for other strains of rats (4). The female leukemic cell maintains the X_tX_t pattern even when transplanted to male rats. Since it was not always possible to distinguish separately the X_tX_t chromosomes from the other medium sized acrocentric chromosomes, the sex chromosomes were placed with this group in karyotypes. Hence the chromosomes in the second row of the karyotypes in Fig. 1 include the X_tX_t chromosomes.

In all of the metaphase preparations from leukemic tissues, the R3149 leukemic cell could be easily identified due to the presence of the single giant metacentric chromosome. This conspicuous "marker" was found in all animals that were leukemic. It was never seen in normal Fischer rat karyotypes. Arm measurements of this marker made it unlikely to be an isochromosome; the short arm total length ratio for 20 markers was approximately 1:3.

Most of the leukemic metaphases with marker chromosomes were pseudoeuploid; 125 of 140 (89%) metaphases in the leukemic

TABLE I. Chromosome Counts of Leukemic R3149 Cells.

Tissue	No. of animals	No. of metaphases	No. of chromosomes 41	42	43	45	46
Bone marrow	7	140	11	125	4		
Spleen	4	80	5	70	3	1	1

bone marrows and 70 of 80 (87%) in the leukemic spleens contained 42 chromosomes (Table I). Other chromosomal aberrations were observed but they appeared to be minor and not constant.

The incidence of the giant marker chromosome in the spleen, liver and bone marrow cells of 26 inoculated rats was examined as a function of time after leukemic inoculation. Twenty consecutive metaphase plates per specimen were scored for the presence of the marker. These findings are presented in Table II. The results show early invasion of the spleen with proliferative leukemic cells. Leukemic invasion of the spleen differed temporally from that of the bone marrow. Although all spleen preparations contained the marker chromosome, some early bone marrow preparations did not. In preparations taken 6 days after inoculation, in 2 spleens as many as 90% of the metaphases contained the marker chromosome; whereas in the most infiltrated bone marrow of the sixth day, only 20% of the metaphase plates contained the marker chromosome. By the seventh day after inoculation, and later, a substantially high percentage of the dividing cells in the spleen, liver and bone marrow contained the marker chromosome.

Discussion. In our cytogenetic study of R3149 rat leukemia, we have observed the presence of a stable, easily visible marker chromosome in a persistently female cell. This large submetacentric chromosome is larger than the largest pair of chromosomes of the normal Fischer rat karyotype. The size of this chromosome, in the face of euploidy, may or may not represent extra DNA. The only other consistent marker chromosomes that have been reported for rat leukemia have been much smaller in size(6). However, those small "markers" usually appeared in metaphases with 45 chromosomes. Hence, they too could represent extra DNA. The large marker seen here may have arisen by translocation. Labeling of chromosomes with H^3 thymidine may help to demonstrate this and will be done.

Whether the present abnormal large chromosome is a primary factor in the etiology of R3149 leukemia is not known. Rich, Tsuchida and Siegler(3) have studied the causal relationship between chromosome alterations and virally induced leukemia in mice. In their induced leukemia, it appeared that in many animals, leukemic changes preceded detectable chromosomal changes. They believe that in their murine leukemia, chromosomal changes were not causal but were a consequence of the neoplastic transfor-

TABLE II. Appearance of Leukemic Metaphases After Inoculation.

Total	Sex	Generation No.	Days after inoculation	Tissues examined*	% Metaphases (20 plates)
1	♀	17	12	S	100
			12	L	100
6	♂	2,5,9,36	8	BM	95,100,100,40,55,75
			8	S	85,100
4	♀	18	8	BM	85,85,100
			8	S	85
4	♂	5,27	7	BM	20,60,75,90
			7	S	80,90
			7	L	80
4	♀	39,40	7	BM	30,60,75
			7	S	40,90,100,100
4	♂	27,34,39	6	BM	5,10,0
			6	S	45,70,90
3	♀	27,34	6	BM	0,5,20
			6	S	55,90

* S—spleen, L—liver, BM—bone marrow.

mation. In the present study, while we are transplanting leukemic cells with an unusual chromosome picture, we might also be transplanting a virus which could be the primary causal factor. To date, a number of laboratories have been unable to pass R3149 with use of cell free filtrates(8). No viral particles have as yet been seen after electron microscopic search(12). At present, we consider this leukemia to be a stable mutant and not presently viral associated. It is pertinent to note that this leukemia arose in a female rat(8). The persistence of these leukemic cells as female, even in male rats supports speculation on their origin as a stable mutation and is strong evidence against continuing viral association and multiplication.

The presence of the stable marker chromosome for this leukemia, R3149, should permit further studies of the effects of chemotherapy on this disorder. Dunning and Curtis(9) have reported that this leukemia could be cured by chemotherapy with nitrogen mustard and other related compounds. The effect of such therapy and other agents on the incidence and fate of this marker chromosome will be of interest.

Summary. This report describes a stable marker chromosome for the transplantable Dunning rat leukemia R3149 in Fischer rats. The karyotype of this acute leukemia was female and pseudoeuploid with a giant submetacentric marker chromosome.

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Amorphous-Crystalline Mineral Changes During Endochondral and Periosteal Bone Formation.* (31999)

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The inorganic portion of skeletal tissue has been shown to contain two separate mineral components, amorphous (non-crystalline) calcium phosphate and crystalline apatite(1-4). Bone from young animals, in general, was found to be richer in amorphous mineral than in crystalline apatite, while bone from older animals was always found to contain more calcium phosphate in the crystalline rather than the amorphous state(3,4). These recent quantitative findings are compatible with the earlier electron microscopy studies suggesting that non-crystalline mineral particles may be deposited prior to apatite crystallites during early bone calcification(5-8).

Synthetic, non-crystalline calcium phosphate can be prepared as either a stable(9,10) or a metastable(11,12) salt. The metastable form of amorphous calcium phosphate will spontaneously convert in aqueous media into crystalline apatite *via* dissolution and reprecipitation by means of an autocatalytic, kinetic mechanism(12). Since the water lability be-

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