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Is trisomy cause or consequence of murine T cell leukemia development? Studies on Robertsonian translocation mice

(chromosome 15/dimethylbenz[*a*]anthracene/Moloney leukemia virus/trypsin–Giemsa chromosome banding)

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ABSTRACT **Trypsin–Giemsa banding studies on T cell leukemias induced in Robertsonian translocation mice by dimethylbenz[*a*]anthracene and Moloney leukemia virus show a trisomy of chromosome 15 even in cases in which chromosome 15 has undergone centromeric fusion with chromosomes 1, 5, or 6. These results suggest that the duplication of gene(s) located on chromosome 15 is of critical importance for murine T cell leukemia development.**

Trisomy of chromosome 15 was identified as a regular nonrandom chromosomal change in murine T cell leukemias. It has been found in spontaneous thymic lymphomas and in T cell lymphomas induced by diverse agents such as x-rays, the chemical carcinogen dimethylbenz[*a*]anthracene, radiation leukemia virus, and Moloney virus (1–5). In CBAT6T6 mice that carry a 14;15 translocation we have shown that the active region of chromosome 15 is in the distal (translocated) part of chromosome 15 (5). In unpublished experiments we have found no trisomy 15 in non-T cell leukemias induced by the Friend, Rauscher, or Abelson viruses, or in mineral oil-induced plasmocytomas.

The question arises whether trisomy 15 is a cause or a consequence of thymic lymphoma development. On a causal hypothesis, it might be presumed that the dosage of a critically important gene on the distal part of chromosome 15 is crucially important for the normal responsiveness of the T lymphocyte to growth control, and overdosage of this gene would lead to uncontrolled T cell proliferation. Alternatively, trisomy is merely a consequence of lymphoma development. It can be noted that primary T cell lymphomas contain few chromosomal anomalies other than trisomy 15 and, if they do, it is usually a second trisomy—e.g., of chromosome 17 (2). It could be argued that a whole variety of chromosomal anomalies is generated at random during the development of T cell leukemia, but whereas cells with trisomy 15 would be viable, most other trisomies and other anomalies would lead to competitive disadvantage or cell death.

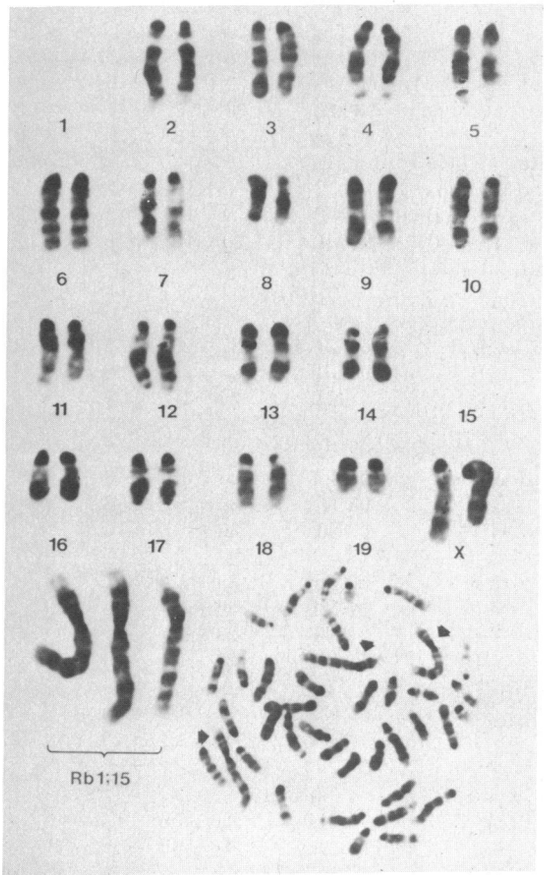

FIG. 1. Trypsin–Giemsa-banded karyotype and metaphase plate of a leukemic cell originating from the thymus of a Moloney leukemia virus-inoculated Rb(1;15)Ct mouse. Note the presence of the trisomic Rb1;15 chromosome (arrows).

MATERIALS AND METHODS

Mice. Mouse stocks carrying Robertsonian translocations between chromosome 15 and chromosome 1, 4, 5, or 6 were obtained from Mary Lyon (Harwell) and Henry Harris (Oxford). Breeding nuclei of the translocation stocks were maintained by brother–sister mating. F_1 hybrid animals were produced by crossing Robertsonian translocation-carrying males with normal C57BL or (A × C57BL) F_1 females.

Lymphoma Induction. Thymic lymphomas were induced by inoculating newborn mice with Moloney leukemia virus or by four feedings of young adults with 1 mg of dimethylbenzanthracene (dissolved in 0.1 ml of polyethylene glycol, molecular weight 400). The mice developed thymic lymphomas, generalized lymph node and spleen enlargement, or both about 180 days after the start of dimethylbenzanthracene feeding and 100 days after Moloney virus inoculation, respectively.

Chromosome Analysis. Chromosome spreads were prepared from enlarged organs (thymus, spleen, or both, mesenteric lymph node) and analyzed by (trypsin–Giemsa) banding (6). The percentage of θ (Thy-1) antigen-positive cells was determined in a micro cytotoxicity assay described elsewhere (7).

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Table 1. Banding analysis of T cell lymphomas induced in the Robertsonian translocation mice Rb1;15, 4;15, 5;15, and 6;15 and their F_1 hybrids by Moloney virus and dimethylbenzanthracene

Lymphoma designation and mouse stock*	θ-positive cells,† %	Modal chromosome no.‡	Trypsin–Giemsa banding analysis of no. of chromosomes 15	
			Rb	Normal
	Rb1;15			
YRb1;15 A	NT	36 + 3B	3	
DRb1;15 B	90 Thy	36 + 3B	3	
YRb1;15-				
×C57BL A	<100 Spl, ML	39 + 1B	1	2
B	100 Thy	38 + 2B	2	1
C	80 Spl	39 + 1B	1	2
D	100 Thy	39 + 1B	1	2
E	100 Thy	38 + 2B	2	1
F	100 ML	39 + 1B	1	2
G	90 ML	39 + 1B	1	2
H	90 Thy	39 + 1B	1	2
YRb1;15-				
×(A×C57BL) A	NT	39 + 1B	1	2
B	NT	39 + 1B	1	2
C	90 ML	39 + 1B	1	2
	Rb4;15			
YRb4;15-				
×C57BL A	60 ML	37 + 2B	2	1
B	90 ML	38 + 2B	2	1
C	NT	39 + 1B	1	2
	Rb5;15			
DRb5;15 A	100 Spl	36 + 3B	3	
B	95 Thy	36 + 3B	3	
C	NT	36 + 3B	3	
YRb5;15-				
×C57BL A	90 Thy	38 + 2B	2	1
B	90 ML	39 + 1B	1	2
C	NT	39 + 2B	2	1
D	90 ML	39 + 1B	1	2
DRb5;15-				
×C57BL A	NT	39 + 1B	1	2
B	NT	39 + 1B	1	2
	Rb6;15			
DRb6;15 A	NT	36 + 3B	3	
B	100 Thy	36 + 3B	3	
YRb6;15-				
×C57BL A	100 Thy	38 + 2B	2	1
B	100 Thy	39 + 1B	1	2
C	100 ML	38 + 2B	2	1
D	100 Spl	38 + 2B	2	1
E	NT	39 + 1B	1	2
F	NT	39 + 2B	2	1
DRb6;15-				
×C57BL A	100 Thy	39 + 1B	2	1
B	NT	38 + 2B	2	1

* Nomenclature: Each horizontal line corresponds to a different lymphoma. The first letter describes the mode of induction: Y for Moloney virus, D for dimethylbenzanthracene. This is followed by the host genotype symbol Rb1;15, 4;15, 5;15, or 6;15 for the translocation homozygotes and the same symbols followed by × for the outcross to C57BL or (A × C57BL) F_1 hybrids. The last letter—A, B, C, etc.—is the serial designation of independently induced lymphomas.

† NT, not tested; Thy, thymus; Spl, spleen; ML, mesenteric lymph node.

‡ B indicates biarmed chromosomes.

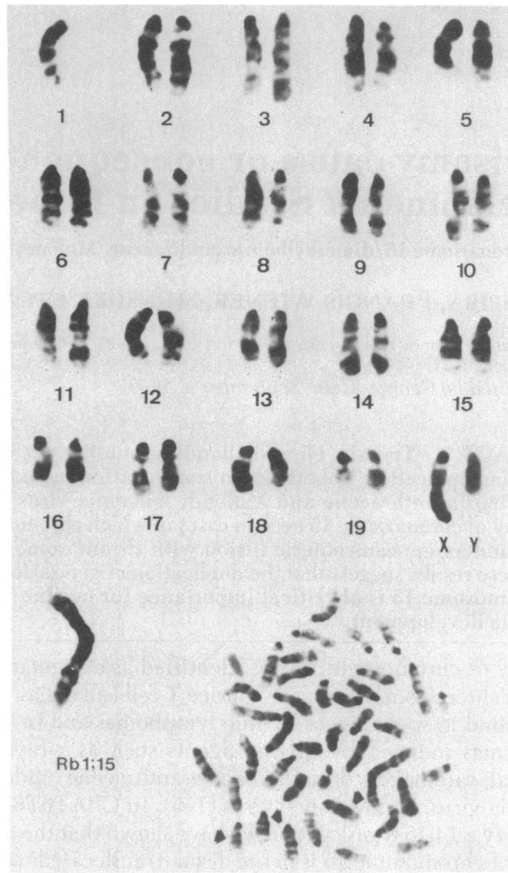

FIG. 2: Trypsin–Giemsa-banded karyotype and metaphase plate of a leukemic cell originating from the mesenteric lymph nodes of a Moloney leukemia virus-inoculated Rb(1;15)Ct × C57BL F_1 mouse. Note the presence of two normal chromosomes 15 and one Rb1;15 chromosome (arrows).

Five karyotypes were prepared from magnified photos for each tumor and at least ten additional metaphase plates with high banding quality were studied directly under the microscope. Chromosome identification followed the recommendations of the Committee on Standardized Genetic Nomenclature for Mice (8).

RESULTS

Two tumors were analyzed from the homozygous Rb1;15 translocation mice, three from Rb5;15, and two from Rb6;15 (Table 1). All seven tumors had 36 acrocentric and 3 biarmed chromosomes. Banding analysis revealed that the extra biarmed chromosome arose through the duplication of the Robertsonian translocation carried by the stock. Representative banding patterns, are shown in Figs. 1 and 2.

To examine whether there was any difference in the likelihood of duplication between a normal chromosome 15 and a chromosome 15 involved in a Robertsonian translocation, we have also analyzed 28 lymphomas induced in F_1 hybrids between a Robertsonian translocation mouse and normal C57BL or (A × C57BL) F_1. As shown in Table 1, all 28 tumors had a 15 trisomy. In 16 tumors, there were 39 acrocentric and one biarmed chromosome. Banding analysis showed the presence of two normal chromosomes 15 and one Robertsonian chromosome 15 translocation. In the remaining 12 tumors there were 38 or 39 acrocentric chromosomes and 2 biarmed elements. Banding analysis showed the presence of one normal chromosome 15 and duplication of the Robertsonian translocation. There were thus three copies of chromosome 15 in all

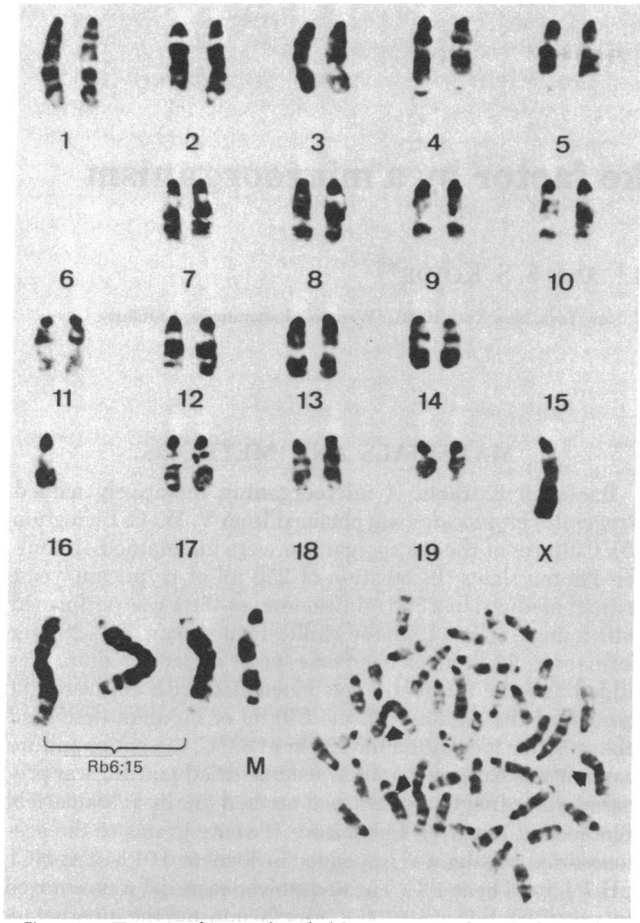

FIG. 3. Trypsin–Giemsa-banded karyotype and metaphase plate of a leukemic cell originating from the thymus of a dimethylbenzanthracene-induced leukemia in a Rb(6;15)Ald mouse. Note the presence of three Rb6;15 chromosomes and one marker (M). The marker is probably composed from the missing chromosome 16 and part of the second X chromosome.

cases. Although the normal chromosome 15 was duplicated twice as often as the translocated 15 in the F_1 tumors, the significance of this finding is uncertain, in view of the relatively small number of tumors analyzed. Banding analysis of a representative F_1 tumor is shown in Fig. 3.

DISCUSSION

These findings show that trisomy 15 is a regular feature of thymic lymphoma development, not only in mice with normal karyotypes but also in Robertsonian 15 translocation mice as well. The fact that 15 is attached to another chromosome apparently "forces" the partner chromosome involved in the translocation to become trisomic as well. The present material shows that even the largest chromosome, no. 1, can behave as a "fellow traveler" in this system, and the same is true for the other chromosomes entering the test, nos. 1, 5, and 6.

These findings argue against the possibility that trisomy 15 is due merely to secondary selection from amongst a variety of potential trisomies in the course of thymic leukemia development. It suggests, on the contrary, that the duplication of some critical 15-associated element is causally involved in thymic leukemogenesis. Apparently, the increased gene dosage of the critical segment is so important for the development of thymic leukemia that duplication of attached genetic material will have to be "tolerated," even if it involves the largest chromosome.

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