Chromosomes of Two Strains of a Feline Cell Line (F_1B) Permanently Shedding a C-Type Virus (36064)[1]

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(Introduced by N. A. Vedros)

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Viruses belonging to the C-type group of RNA viruses (1) have been found in most species of vertebrates so far examined for them (2). Cells harboring or shedding the virus, as seen in the electron microscope, have been from normal tissues or tumors and were found to be either diploid or aneuploid (3).

Although it appears that this type of virus is becoming a central figure in the etiology of cancer (4), and specific genes or genetic strains as well as specific chromosomal abnormalities, are implicated (5–8), no direct relationship has been established between the chromosome complement and the presence of C-type viruses (3, 9).

Nevertheless, existing karyological studies of cells harboring C-type RNA virus have involved mouse, chicken, rat, hamster, monkey, cat, and dog cells (3, 9–11).

During recent years, C-type RNA virus involvement in the sarcoma–leukemia complex in cats has been the object of increasing numbers of studies (11–13). F. de Noronha (personal communication) has data on chromosomes of canine and feline cells inoculated with feline sarcoma and leukemia viruses. In this instance, the feline cells exhibited "never distinct aberrations."

No report has come to our attention describing the karyotype of a cell line at various passage levels *in vitro* derived from a C-type virus bearing spontaneous tumor at biopsy, and which continues to produce the virus after passage in tissue culture. We describe the chromosomes of such a cell line derived from a tumor of a lymphomatous cat and of a subline thereof.

Materials and Methods. The cell line was initiated on August 20, 1968, from the swollen left submandibular lymph node of a 3-year-old female cat with clinically diagnosed lymphoma. Minced tissue was placed in Eagle's minimal essential medium with Earle's salts and with 10% fetal calf serum in each of 6 prescription bottles. The culture designated F_1B was the only one successful attempt to culture tissues from this specimen, the others having been destroyed after initiation, probably by the feline syncytia-forming virus which appears to be ubiquitous in adult cats (14, 15), but which is not demonstrable in the F_1B culture.

Here designated F_1B (R) this line has been maintained at the Viral and Rickettsial Disease Laboratory (Berkeley) in the above medium with antibiotics (penicillin, streptomycin, neomycin, bacitracin, and Fungizone), and a subline F_1B (N) has been cultivated at the Naval Biomedical Research Laboratory from the 10th passage onward in the same medium but antibiotic free. Search for C-type RNA virus particles was performed by electron microscopy of cells grown in monolayer as well as by detection of tritiated uridine-labeled particles in the supernatant growth medium by sucrose gradient sedimentation at a density range of 1.16 to 1.18 g/cm^3 characteristic of C-type RNA tumor viruses (16). Tests for mycoplasma followed standard techniques utilizing cell suspension growth on agar and in broth. For morphological observations, monolayers were grown on Leighton cover slips fixed and stained with May-Grünwald, and chromosome observations followed exposure of cells

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TABLE I. Presence of C-Type RNA Virus and Absence of Mycoplamsa in F_1B Cultures at Various Passage Levels.

Passage	Culture R	Original	N
1		C+[a]	
3		C+[a]	
10		C+[a]	
13	C+[a] ^{3}HUr+[d]		Myco—[c]; ^{3}HUr+
16			Myco—
19	C+[a]		^{3}HUr+
20			C+[a]
31	C+[a]		
56			Myco
57	C+[a]		
64			C+[b]
67	^{3}HUr+		C+[a]; ^{3}HUr+
69	Myco—		Myco—
98	C+[b]		
101	Myco—		

[a,b] C-type virus particles observed in the electron microscope by Drs. L. S. Oshiro and A. J. Hackett, respectively (unpublished data).

[c] Myco—, negative results in tests for mycoplasma by Dr. L. Hayflick.

[d] ^{3}HUr+, positive results of Dr. Hackett in detection of tritiated uridine-labeled particles in the supernatant by sucrose gradient sedimentation (unpublished data).

to Colcemid, hypotonic treatment, fixative, air drying (17), and staining in Giemsa.

Results. Samples of the original lymph node tissue, as well as of subsequent tissue culture passages of both lines, have consistently revealed C-type RNA-virus particles by electron microscopy, as well as by detection of tritiated uridine-labeled particles in the supernatant growth medium. Tests for presence of mycoplasmas at various passage levels gave negative results (Table I).

The earliest chromosome studies were made on F_1B (R) cells from the 12th tissue culture passage. The modal number of cells exhibited a normal, female karyotype, confirming the sex of the donor (Figs. 1 and 2). All metaphases clearly demonstrated the achromatic region in the short arms of one or both E_1 chromosomes. Neither chromosome nor chromatid aberrations were noted. Polyploidy was less than 1%; and 1–2% of the cells were polykaryocytes with 1–5 nuclei.

F_1B (N) cells were first studied at passage 17. Approximately 59% of the metaphases had chromosome and chromatid aberrations and the majority were hypodiploid. Leighton cover slip preparations, at passages 22 and 24, revealed many chromatid bridges between cells or between nuclei of cells, micronuclei and, in general, a heterogeneous array of morphologically distinct nuclei. At passage 32, F_1B (N) cells were still mostly hypodiploid, but 20.5% of the cells were now hypotetraploid and chromatid aberrations were still noticed, particularly in the latter cells. At passages 65 and 68, the highest studied for F_1B (N), all cells were hypotetraploid and 90% of the metaphases of the final sample had chromosome aberrations. Briefly, the course of events leading from diploidy to polyploidy was accompanied by chromosomal aberrations and nuclear reorganization reminiscent of those described previously in a bovine testicular cell line (18). The peculiar E_1 chromosomes mentioned above were no longer detectable (Fig. 3). The cause of these aberrations remains unknown.

In contrast, higher passage levels of F_1B (R) 67, 96, and 98 (Fig. 1) revealed hypo-

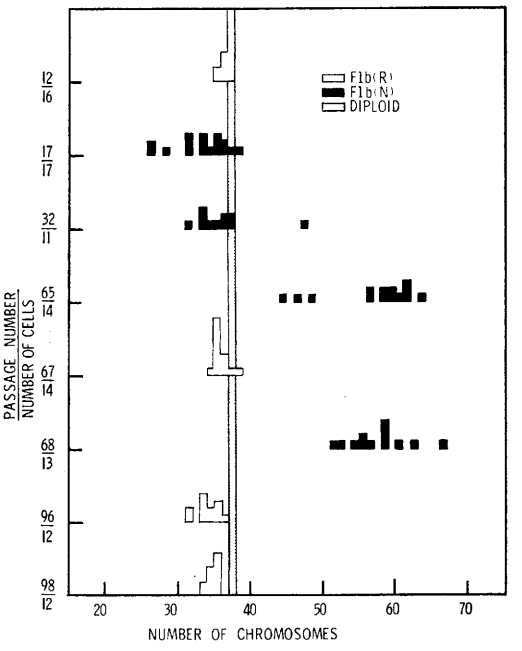


FIG. 1. Number of chromosomes in F_1B cells at different passage levels.

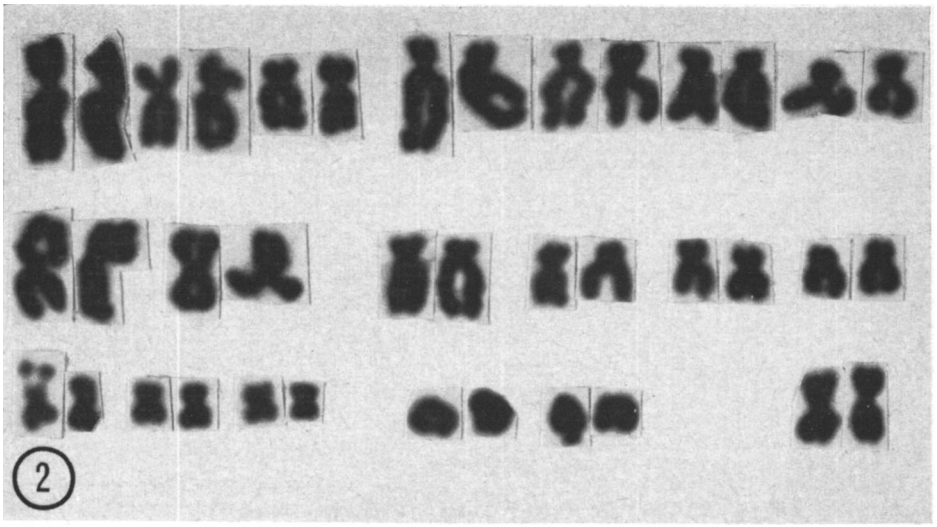


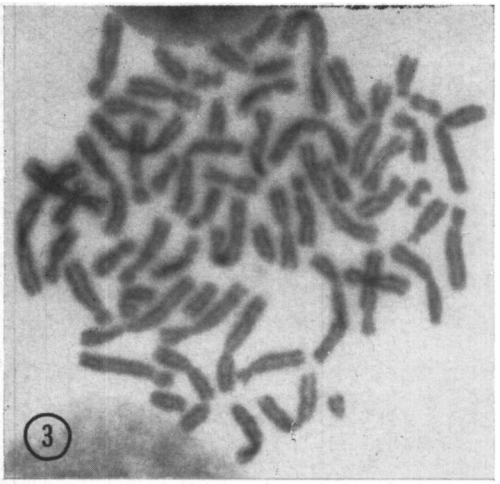
FIG. 2. Chromosomes of F_1B (R) cell, passage 12: E_1 chromosomes at lower left; X chromosomes, lower right.

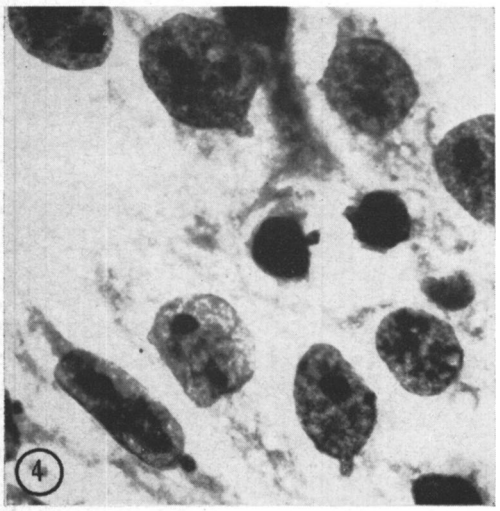
diploid cells with only 1–5% polyploidy. Polykaryocytes, however, in both lines F_1B (N) and (R) were no higher at passage 69 than they had been at passage 14, or 1–2%. Both lines retained the fibroblast-like or spindle-shaped cell morphology as first observed for F_1B (R) at passage 12.

What appeared at first to be an artifact was noticed infrequently in cells of F_1B (R) at passage 96. This was a small bleb-like protrusion on interphase nuclei (Fig. 4). However, 80 to 90% of the cells at passage 98 and 101 also revealed the nuclear protrusion. Interestingly, although chromosome and chromatid aberrations were infrequent (1%) in metaphases studied, over 90% of them at this passage contained one unusually long previously unnoticed subtelocentric chromosome and were clearly marked by at least one E_1 chromosome per metaphase (Fig. 5). The appearance of nuclear blebs concomitant with an excessively long chromosome in one of the lines (R) conforms to earlier reports of similar association in an established pig kidney cell line (19) and in certain cases of human neoplasia (20). Whether or not C-type viruses were associated with the cells in the latter studies is not known.

Discussion. It is doubtful that C-type RNA viruses of the kind which are leukemogenic in rodents produce microscopically detectable chromosome aberrations (3). The present study shows that C-type viruses of feline origin which are apparently identical in morphology and mode of production to the murine and avian leukemia viruses (21) may be produced by chromosomally normal feline cells as well as by descendants of these cells which become chromosomally altered in the course of cultivation *in vitro*. This would conform to a conjecture by Stich and Yohn (3)—namely, that abnormal karyotypes are not directly related to viral infection. The fact that the virus was seen abundantly within cells and budding from cells of the original tumor coupled with the observation of only a normal karyotype in early passage cells, which also revealed the virus, speaks against the necessity to assume that only karyotypically abnormal cells in the tumor were the virus producers and that these, in turn, enjoyed selective advantage *in vitro*.

Summary. Direct preparation of cells from a swollen lymph node of a cat with lymphosarcoma revealed the presence and shedding of C-type RNA virus particles. When grown *in vitro*, a line and subline of cells derived from the tumor continued to produce this virus. While the original tissue was diploid, both strains subsequently developed different and altered karyotypes, one hypodiploid and one hypotetraploid.





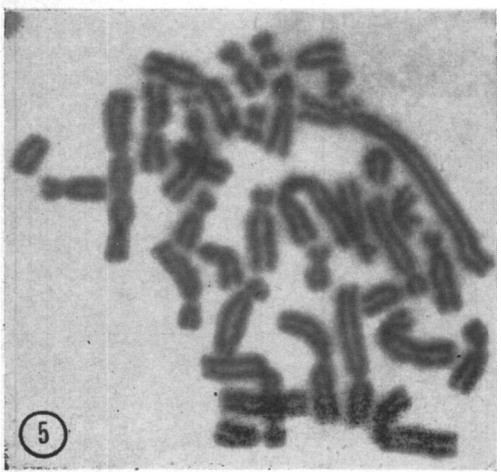


FIG. 3. Metaphase of F_1B (N) cell passage 65.

FIG. 4. Monolayer of F_1B (R) cells, passage 101. Note nuclear blebs at telo- and interphase.

FIG. 5. Metaphase of F_1B (R) cell with long subtelocentric chromosome.

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