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12. Supported in part by PHS grant (AM-10149). I thank D. Delekta, A. O'Neill, and P. Siersma for assistance.

14 July 1967

Herpes-Type Virus and Chromosome Marker in Normal Leukocytes after Growth with Irradiated Burkitt Cells

Abstract. *Cultured cells derived from male patients with Burkitt's lymphoma and harboring herpes-type virus particles were lethally irradiated. These irradiated cells induced normal peripheral leukocytes of female infants to grow within 2 to 4 weeks after mixed cultivation. Cells of a line free of this agent failed to stimulate growth. If either type of cell was cultured separately, it did not survive under the experimental conditions. Herpes-type viral antigen and C-group chromosomal marker previously described in cultured Burkitt cells were found in all of the female cell cultures that were obtained.*

A small number of cells in most continuous cultures derived from Burkitt lymphomas harbor herpes-like virus particles (*1–3*), as judged from electron-microscopic examination. Apparently, the same type of virus has also been found in some cell lines established from peripheral leukocytes of leukemic patients (*4*) or even of healthy donors (*5*). The virus-producing cells in these cultures can readily be detected by indirect immunofluorescence tests not only with serums from patients with Burkitt lymphoma, but with many other human serums, regardless of source (*6, 7*). By various procedures, it was shown that the fluorescent cells contain virus particles (*8*), that serums which yield positive immunofluorescence have antibodies to the viral capsid (*9*), and that the agent represents a hitherto-unknown member of the herpes group of viruses (*6, 10*). Whether this widely disseminated virus is merely a passenger in these cultures or whether it plays a role in the etiology of lymphomas, leukemias, or any other human illnesses is unknown. Attempts to transmit this agent to other host systems have not been successful, possibly because most of the virus particles are defective (*1, 3, 11*). Nor has it been possible to induce tumors in animals or to transform normal cells in vitro by addition of intact or disintegrated cell suspensions or by virus particles extracted from such suspensions. The presence of a chromosomal marker in cultured Burkitt cells has been described (*12, 13*); this marker consists of a subterminal constriction or achromatic gap on the long arm of one or both No. 10 chromosomes. There is some evidence that the same marker may also be found in some of the cell lines derived from leukemic patients (*13, 14*). The cause for this abnormality has not been established, but it could be due to the herpes-type virus associated with Burkitt and other cell lines, as indicated by our current finding that the herpes-type virus can be transmitted to normal peripheral leukocytes and that it may stimulate their growth.

Since infections by certain viruses of the herpes group are spread predominantly by close cell-to-cell contacts rather than by dissemination of virus via the fluid medium, it was desirable to explore the effects of nonreplicating Burkitt cells on other human cells on mixed cultivation with the use of the chromosomal sex of the two types of cells as marker for identification of outgrowing cell populations. The general techniques were as follows. Lethally x-irradiated (3000 to 6000 roentgens) cells from two Burkitt lines of cells derived from males served as "donors"; one harbored herpes-type virus particles in 3 to 5 percent of the cells (Jijoye), the other in none (Raji). The "recipient" cells were leukocytes of female infants; the cells were separated from 15 ml of peripheral blood after addition of 5 ml of a 6 percent dextran solution. The Burkitt cells were mixed immediately after irradiation or up to 6 days later with fresh or 1-day-old leukocyte suspensions (1 to 2 $\times$ 10^6 cells/ml, each) and incubated at 37°C for 2 to 24 hours in conical centrifuge tubes to permit close contact between the settled cells. Donor and recipient cells were handled separately in parallel, as controls. The mixed and individual cells were then seeded in equal numbers (2 to 4 $\times$ 10^6) onto monolayers of human diploid female fibroblasts (WI-38) which are known not to grow in suspension; these cultures were maintained by frequent renewal of the medium during a 2- to 8-week period. The media removed each time contained numerous floating cells which were used to set up suspension cultures, or to augment already existing ones of the corresponding series. These suspension cultures were observed for evidence of replication and eventual establishment of continuous lines.

Many facets of the culturing procedures remain to be analyzed. However, the available results are encouraging. In all seven experiments thus far, the normal peripheral leukocytes were induced to grow within 2 to 4 weeks after mixed cultivation with x-irradiated Jijoye cells (virus-positive); Raji cells (virus-negative) did not induce growth. Separate cultures of the leukocytes as well as of the x-irradiated Burkitt cells failed to survive for any length of time under our experimental conditions.

Five of the growing mixed cultures were maintained for 6 to 8 weeks before rapid replication began, after which subcultures could be made at 5- to 7-day intervals. Thus, sufficient numbers of cells for detailed analyses became available from these five cultures. From 100 to 300 cells in metaphase from each culture—Ba, Ry, McC, Na, and Re—were examined, and none contained a Y chromosome. Thus, certainly less than 0.3 to 1 percent of the cells, if any, could be of the donor type, and, at most, 0.03 to 0.05 percent of the total cell population would be expected to reveal immunofluorescence specific for the Burkitt-associated virus, since the Jijoye line contained from 3 to 5 percent stainable cells. Yet, when the five cultures were tested at

various intervals, each of them showed, on one or more occasions, at least 0.5 percent immunofluorescent cells (Table 1). Indeed, herpes-type virus particles were discovered in the Ry culture, the only one examined thus far by electron microscopy (*15*). The cells containing viral antigen in the Ry culture increased gradually to about 25 percent which was accompanied by a marked reduction in the cellular growth rate. After recovery of the culture, only 0.1 to 0.2 percent of stainable cells were observed. No fluorescence characteristic of this agent could be elicited in smears of fresh leukocytes made prior to their cultivation, nor in cells of control leukocyte cultures after several days of incubation. Serums of three of the infants who contributed leukocytes were tested for antibodies to the Burkitt-associated agent; the results were negative, indicating that they had not been infected with this virus. Thus, the herpes-type virus apparently was transmitted from the Jijoye cells to the leukocytes during mixed cultivation.

Further analysis of the five cultures (Table 1), now in their 3rd to 8th months of continuous growth, revealed that well over 80 percent of the cells contained 46 XX chromosomes, and, more intriguing, that between 5 and 16 percent of the female cells contained the same C-group chromosomal marker as in Burkitt cell lines (*12, 13*). A karyogram of a McC cell with this marker is shown in Fig. 1. Since this chromosomal abnormality has not been observed in normal peripheral leukocytes, there is little doubt that its appearance in the female cells of the five cultures is related to their exposure to the male x-irradiated Jijoye cells.

The foregoing observations may denote that the herpes-type virus found in cultured Burkitt cells induces a persistent chromosomal lesion and facilitates continuous growth of hematopoietic cells with a remarkable degree of frequency. The possibility of a release of a growth-promoting factor (*16*) from x-irradiated Jijoye, but not from Raji cells, remains to be explored.

Table 1. Analysis of cultures obtained from mixtures of x-irradiated male Jijoye cells and peripheral leukocytes of female infants. The cultures of Jijoye cells contained from 3 to 5 percent cells with viral antigen.

Cultures	Period of growth (mo.)	Cells with viral antigen (%)	Cells (No.)	Chromosomal analysis						Cells with C-group marker (%)
				Counts				Sex*		
				44	45	46	47	XY	XX	
Ba	8	0.5–1.0	65†	1	3	62	0	0	62	7.8
Ry	5	2–25§	100‡	1	9	90	0	0	90	8
McC	4	0.3–1.0	100	0	6	94	0	0	94	5
Na	3	0.3–0.5	100	2	14	82	2	0	84	16
Re	3	0.1–0.3	100	1	11	88	0	0	88	8

* Hypodiploid cells not included. † Additional 200 cells in metaphase were studied earlier, all being XX. ‡ Additional 100 cells in metaphase studied earlier. § This culture went through a "crisis" and shows now 0.1 to 0.2 percent positive cells.

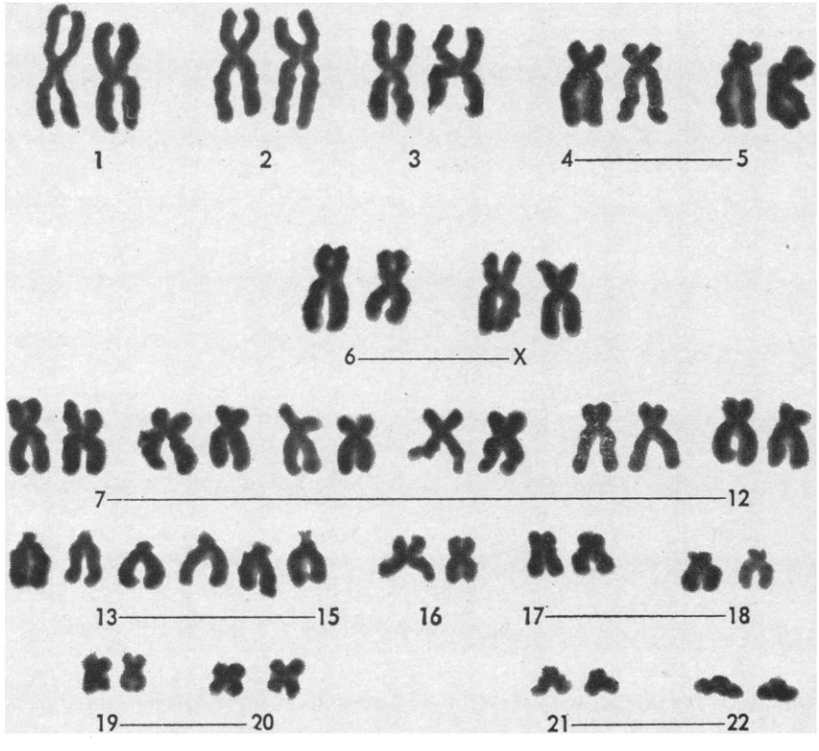

Fig. 1. Karyogram of a McC cell showing subterminal secondary constrictions in the No. 10 chromosome.

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17. NIH career award 5-K6-AI-22,683 to W.H. Supported by PHS grants CA-04568, HD-00588, 5T1-AI-104, and 5T1-A1-154 and contract PH43-66-477 within the Special Virus Leukemia Program of the NCI.

19 June 1967