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Hybridization of Burkitt Lymphoblastoid Cells

Abstract. *Burkitt lymphoblastoid cell lines have been fused to mouse and human cell lines with the use of inactivated Sendai virus. The heterokaryons have developed into somatic cell hybrids of both parental cell types. Chromosome analyses confirm that cells now growing in selective medium are hybrids. Initial observations of preparations of the hybrid cells reveal that 5'-iododeoxyuridine can induce continued synthesis of Epstein-Barr virus antigens by these hybrid cells.*

Heterokaryons of several cell types have been produced by using inactivated Sendai virus to initiate cell fusion (*1*). When cells transformed by certain oncogenic viruses, such as simian papovavirus 40 (SV40), were fused to potentially susceptible cells, virus synthesis was initiated, and infectious SV40 was recovered from the cells (*2*).

A herpesvirus, the Epstein-Barr virus (EBV), has been associated with a number of lymphoblastoid cell lines derived from patients with Burkitt's lymphoma (BL) (*3*). A small percentage of the cell population of several of these lines contain antigens that can be detected with serums from BL patients by the indirect immunofluorescent test (*4*). In addition, there is evidence from cloning and nucleic acid hybridization experiments that the EBV genome is at least partially integrated in the host cell genome (*5*, *6*).

The fusion of Burkitt lymphoblastoid cells has previously been found to be difficult in this laboratory and others (*7*). We have attempted to produce heterokaryons of Burkitt lymphoblastoid cells that grow only as suspension cultures in vitro, with cells capable of growing on glass to determine if somatic cell hybrids can be developed. We have also investigated the possibility of recovering the EBV from the heterokaryons of human-human and human-mouse cells. It would be useful to obtain infectious virus in order to study the relation between EBV and Burkitt's lymphoma.

The Burkitt lymphoblastoid cell line studied was the EB_3 line containing EBV antigens in 1 to 4 percent of the cells. The other parental cell line was a mouse L cell line, LM(TK$^-$)CL1D (CL1D) (*8*), which is unable to grow in selective medium [Eagle's or F-12 medium supplemented with 10 percent fetal calf serum, $1 \times 10^{-4}M$ hypoxanthine, $4 \times 10^{-7}M$ aminopterine, and $1.6 \times 10^{-5}M$ thymidine (HAT)].

The fusion procedure was as follows: 2×10^6 CL1D cells were seeded into tissue culture plates, and were grown for 24 hours at 37°C. The EB_3 cells (1×10^7 cells in 0.2 ml of F-12 medium containing no serum) were washed with saline buffered with phosphate. Sendai virus (400 hemagglutinating units in 0.2 ml), inactivated with β-propiolactone, was added to the center of the cell monolayer and to the EB_3 cell suspension. Both the CL1D and EB_3 cells were cooled on ice for 5 minutes. The cell suspension was then placed on the monolayer cultures, and cooled for 5 minutes on ice; the cultures were then placed in an atmosphere of 5 percent CO_2 for 3 to 4 hours at 37°C. Normal F-12 medium supplemented with 10 percent fetal calf serum was added, and was replaced 3 days later by HAT medium. The cultures were maintained in selective HAT medium which was renewed every 3 days.

Chromosome spreads were prepared by a modification (*9*) of the method of Moorhead *et al.* (*10*). Sixty coded metaphases were analyzed for each cell line. The chromosomes of the parental human cell line EB_3 were diploid, consistent with previous reports (*11*).

There were wide variations in the total chromosome number and in the number of biarmed chromosomes, especially in the CL1D line (Table 1). The CL1D cells had chromosome numbers of 48 to 52, with a modal number of 51; the number of biarmed chromosomes generally had a range of 10 to 13, although a few cells contained 14 or 15. The modal number of biarmed chromosomes was 11 to 12. The chromosome number of the EB_3/CL1D hybrid, passage 15 (10 months after fu-

Table 1. Chromosome analysis of parental mouse (CL1D) and hybrid mouse-human (EB_3/CL1D) cell lines. Chromosome counts in italics were not considered to be significant.

	Number of cells with chromosome numbers of											Number of cells with biarmed chromosome numbers of							
	44	46	47	48	49	50	51	52	53	54	55	10	11	12	13	14	15	16	17
LM(TK$^-$) CL1D		*1*	*2*	3	*5*	5	7	4		*2*		8	21	16	9	*2*	*2*		
EB_3/CL1D						*1*	2	8	13	4	2		*3*	14	13	9	11	7	*3*

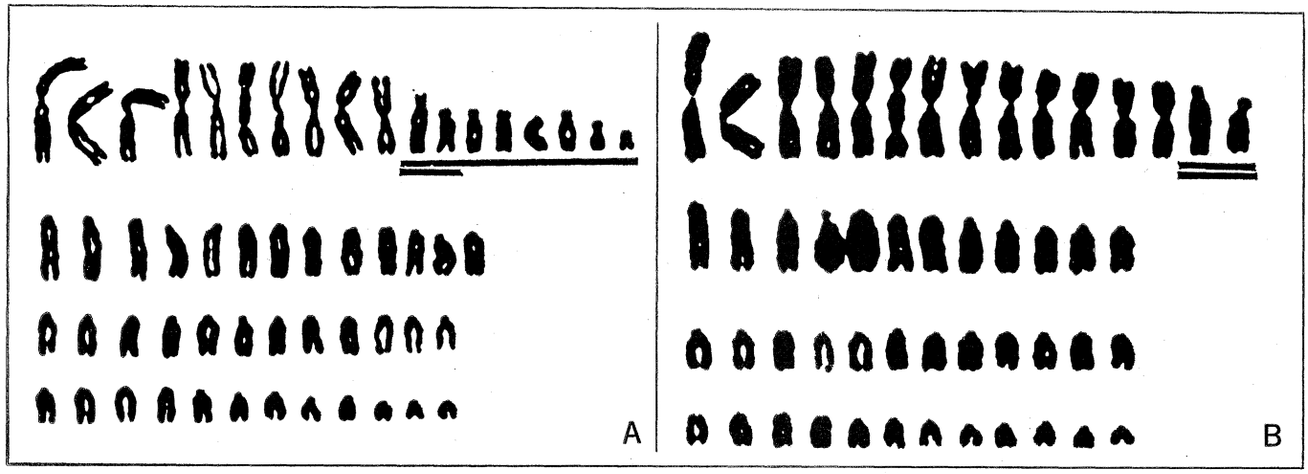

Fig. 1. Karyotype of EB_3/CL1D (A) with eight submetacentric chromosomes and CL1D (B) with two such chromosomes. The two chromosomes farthest to the left in the underlined group of eight in (A) are the same two underlined in (B).

sion), was 51 to 55, with a modal number of 52 to 53. These hybrid cells had 12 to 16 biarmed chromosomes, although cells with 11 or 17 such chromosomes were occasionally identified. In addition, the EB_3/CL1D cells contained from 4 to 8 submetacentric, and 9 to 10 metacentric, chromosomes, while CL1D cells contained only 1 to 2 submetacentric, but 11 to 13 metacentric, chromosomes (Fig. 1). No parental CL1D cells were found with more than 2 submetacentric chromosomes, even when the slides were reexamined.

Under no circumstances were we able to grow CL1D cells in the selective medium. Several attempts to do so were made during the course of the study, but the cells consistently died within 1 to 2 weeks after the addition of HAT medium to the culture. Cultures of fused CL1D cells were tested as controls, and these were totally inhibited as well.

When the EB_3/CL1D cells were fixed in acetone and examined by immunofluorescence at 1, 4, and 11 months after fusion, no EBV antigens were detected. No virus particles were found in the cells by electron microscopy.

We then attempted to hybridize the Burkitt cell line P3J-HR1 (HR1) and a human sternal marrow cell line D98/AH-2 (D98) (clonal derivative of D98/AG with 8-azahypoxanthine–resistance marker), that is killed in HAT medium. At the time of cell fusion, approximately 5 to 10 percent of the cells in the lymphoblastoid suspension synthesized detectable EBV antigens. Two of eight clones have been analyzed to date (Table 2). The chromosome number for HR1 was bimodal, with cells containing 46 or 47 chromosomes. The range of chromosome numbers for the D98 was 61 to 63, with a modal number of 61 to 62. Clone 2 of the human-human hybrid (D98/HR1), passage 5 (4 months after fusion), had cells with a range of chromosomes numbers of 98 to 110. However, the modal number appears to fall somewhere between 100 and 107.

Table 2. Chromosome analysis of human-human hybrid cell lines and parental lines (D98 and HR1).

Chromosome numbers	Number of cells in			
	HR1	D98	D98/HR1 clone 2	D98/HR1 clone 7
44	1	1		
45	2			
46	13			
47	33			
48	1			
51		1		
52		1		
56		1		
57		1		
58		2		
61		14		
62		20		
63		10		
80			1	
82			1	
89			3	
92			1	1
93			1	
94			1	
95			1	
97			1	
98			2	
99				1
100			3	
101			5	
102			5	
103			6	4
104			5	1
105			6	4
106			3	6
107			5	8
108			2	19
109			1	9
110			1	1
111				2
112				1
113				1
134			2	
138				1

The chromosome number for cells in clone 7, passage 5 (4 months after fusion), was 103 to 111, with a modal number of 108. All the clones isolated have been growing for 8 months, and are morphologically different from the parental lines. The cells in clone 7 are now replicating poorly, while the cells in clone 2 and others are still passaged on a weekly basis. The modal number of chromosomes has decreased in all clones examined; it is not known if the loss of chromosomes is selective, or whether chromosomes from both parental cells are being lost randomly.

Cell cultures of D98/HR1, clones 2 and 7, were fixed in acetone and examined 2 and 5 months after fusion by immunofluorescence; no EBV-specific antigens were detected. However, preliminary data suggest that EBV antigens can be induced in the hybrid cells with 5′-iododeoxyuridine by methods described for EBV (*12*) and other viruses (*13*).

From the data presented here, it is suggested that Burkitt lymphoblastoid cells have been hybridized with both human and mouse cells. The hybrids apparently retain at least some of the genome of EBV. Whether the recovery of infectious EBV from the hybrid cells is possible is not yet clear.

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5,6-Trans-25-Hydroxycholecalciferol: Vitamin D Analog Effective on Intestine of Anephric Rats

Abstract. *A new compound, 5,6-*trans*-25-hydroxycholecalciferol, has been synthesized and tested for its biological activity. Like 1,25-dihydroxycholecalciferol, it stimulates intestinal calcium transport in anephric rats, whereas 25-hydroxycholecalciferol does not. But this analog has little if any activity in stimulating mobilization of calcium from the bone of anephric rats.*

The first serious attempt to synthesize analogs of vitamin D_2 was made by Windaus and co-workers. They explored the possibility of producing antirachitic substances from $\Delta^{5,7}$ sterols having differing side chains. Two analogs of ergosterol, 22-dihydroergosterol and 7-dehydrocholesterol, upon irradiation yielded two new biologically active compounds known as vitamin D_4 (*1*) and vitamin D_3 (*2*), respectively. Since these initial experiments, vitamin D–modified chemicals have been tested for biological activity in various ways. One of the most important group of analogs is the dihydrotachysterols, which have little antirachitic activity (180 I.U./mg) but display preferential activity in mobilizing calcium from bone (*3*).

Verloop *et al.* (*4*) were the first to report the synthesis of 5,6-*trans*-ergocalciferol [9,10-*seco*-(5*E*,7*E*,22*E*)-5,7,-10(19,22)-ergostatetraene-3β-ol] by iodine-catalyzed isomerization of vitamin D_2 under neutral conditions in a nonpolar solvent. They reported that the ultraviolet absorption maximum shifted from 265 nm for vitamin D_2 (5,6-*cis*-ergocalciferol) to 272 nm for the 5,6-*trans* isomer. Similarly, Inhoffen *et al.* (*5*) studied this isomerization and reported the synthesis of 5,6-*trans*-vitamin D_3.

In light of the finding that 25-hydroxycholecalciferol (25-OHD_3) must be hydroxylated at C-1 by the kidney (*6*, *7*) to 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] before it can induce either intestinal calcium transport (*8*) or bone calcium mobilization (*9*), it was of interest to investigate the biopotency of the 5,6-*trans* isomer of 25-OHD_3 because it was similar in appearance to 1,25-$(OH)_2D_3$ (Fig. 1). Compared to 25-OHD_3, the 5,6-*trans* isomer has its A ring rotated 180°, with the 3β-hydroxyl function in the same geometrical position as the 1-hydroxyl of 1,25-$(OH)_2D_3$. It seemed possible, therefore, that the 5,6-*trans*-25-OHD_3 may well be able to substitute for 1,25-$(OH)_2D_3$.

Ten milligrams of 25-OHD_3 was dissolved in 10 ml of a mixture of Skellysolve B (a light petroleum fraction redistilled at 67° to 69°C) and diethyl ether (9 : 1, by volume) and 50 μl of a solution of iodine in Skellysolve B (0.1 mg/1 ml) was added. After 1 hour at 25°C, the reaction was terminated with solid $Na_2S_2O_3$ and washed with water, and the product in the Skellysolve B–ether phase was dried over anhydrous Na_2SO_4, according to procedure of Verloop *et al.* (*4*). The solvent was evaporated under nitrogen, and the product was redissolved in 1 ml of a mixture of Skellysolve B and diethyl ether (7 : 3, by volume). The sample was applied to a multibore silicic acid (15 g) column, measuring stepwise in diameter 1.2, 0.8, and 0.4 cm (*10*). The column was eluted with a hyperbolic gradient generated by having 230 ml of Skellysolve B and diethyl ether (7 : 3, by volume) in a mixing chamber and 400 ml of Skellysolve B and diethyl ether (3 : 7, by volume) in a holding chamber. Diethyl ether (300 ml) was added to the holding chamber after it emptied. One hundred fractions, 5 ml each, were collected, and the ultraviolet absorption spectrum of each tube was taken to determine the elution position of the 5,6-*trans*-25-OHD_3. The isomer was collected and chromatographed once again on the multibore silicic acid column.

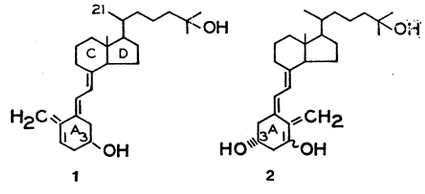

Fig. 1. Structure **1** is 5,6-*trans*-25-OHD_3; structure **2** is 1,25-$(OH)_2D_3$.

The ultraviolet spectrum of the isomer showed the characteristic maximum at 273.5 nm and a minimum at 232 nm (*4*, *5*) for the 5,6-*trans* triene system. The mass spectrum of the analog showed a molecular ion at *m/e* 400 similar to 25-OHD_3 and fragments at *m/e* 271 and 253 (271 − H_2O) which are characteristic for loss of the side chain, and at *m/e* 136 and 118 (136 − H_2O) which are characteristic for the A ring plus C-6 and C-7. Gas-liquid chromatography of the 5,6-*trans*-25-OHD_3 showed only one component, which is consistent with a previous report for 5,6-*trans*-vitamin D_3 (*11*).

Antirachitic activity for the 5,6-*trans*-25-OHD_3 was measured by the antirachitic line test assay method described in *The United States Pharmacopeia* (*12*) and was found to be one-tenth that of vitamin D_3.

For intestinal calcium transport measurements, weanling male albino rats (Holtzman, Madison, Wis.) were housed individually in hanging wire cages and given free access to food and water. They were fed for 3 weeks on a purified diet deficient in vitamin D and low in calcium (0.02 percent) (*13*) and then divided into five groups. One group received only the ethanol