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Growth Characteristics of Burkitt Somatic Cell Hybrids *in Vitro*[1]

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SUMMARY

Burkitt somatic cell hybrids (D98/HR-1) synthesize both the A and B forms of glucose 6-phosphate dehydrogenase, which were originally derived from the two fused human cells. The A form is from D98 cells and the B form was supplied by the P3J-HR-1 cells. The formation of a heteropolymer of glucose 6-phosphate dehydrogenase in the hybrid proves that a hybrid was obtained. Selection of hybrid cells with lower numbers of chromosomes has taken place after several cell passages. The properties of the hybrid and parental cell lines differ in their ability to grow in soft agar, but all showed little dependence on serum concentration.

INTRODUCTION

Under certain conditions, heterokaryons resulting from cell fusion have developed into replicating somatic cell hybrid cell lines containing chromosomes of both parental cell lines and expressing the sum total of the genotypic information contained in the nuclei of the hybrid cells. Somatic cell hybrids of polyoma virus-transformed hamster cells and mouse cells contained the virus genome and appear to have acquired some properties of the transformed cell (1). Recently, somatic cell hybrids of Burkitt lymphoblastoid cells were prepared (6). Clones of the human hybrid cell lines (D98/HR-1) contained EBV[2] DNA (4) but were negative for EBV-specific antigens and virus particles. When the D98/HR-1 cells were treated with iododeoxyuridine, EBV was induced as determined by immunofluorescence tests, electron microscopy (7), and hybridization of nucleic acids (4). The work reported here describes the properties of the hybrid cells as compared to the 2 parental cell lines.

MATERIALS AND METHODS

Cell Lines. The human cell line, D98/AH-2 (14), was maintained in Eagle's medium supplemented with 10% fetal calf serum, 100 units of penicillin per ml, 100 μg of streptomycin per ml, 1 μg of Fungizone per ml, 10 units of mycostatin per ml, and 0.075% $NaHCO_3$ in 8-oz glass prescription bottles at 37°. The D98/AH-2 cells are hypoxanthine-guanine phosphorylbosyl transferase deficient and are unable to grow in Eagle's medium supplemented with 10% fetal calf serum, 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine. The suspension Burkitt lymphoblastoid cell line HR-1, a male line (8), was maintained in Roswell Park Memorial Institute Medium 1640 supplemented with 10% fetal calf serum, antibiotics as already described, and 0.075% $NaHCO_3$ in 8-oz glass prescription bottles at 35°.

The D98/HR-1 hybrids, clones 1, 2, 3, and 8, were also maintained in Eagle's medium supplemented with 10% fetal calf serum, 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine with antibiotics as already described and 0.075% $NaHCO_3$ in 8-oz glass prescription bottles or 250-ml plastic tissue culture flasks (Falcon Plastics Co., Oxnard, Calif.) (6, 7).

G6PD Electrophoretic Assay. Cells were washed 3 times in phosphate-buffered saline (pH 7.4) after trypsinization (except HR-1, which did not require trypsinization). Approximately 0.8 to 1.0×10^8 cells were homogenized in 1 ml 0.05 M Tris (pH 7.5) and frozen before electrophoresis (12). Electrophoresis was carried out as described (13) for 16 hr at 2-4° in an alkaline Tris, borate, EDTA-buffered starch gel. The gels were stained in a 100-ml solution of 0.025 M Tris HCl (pH 7.4), 50 mg NAD, 35 mg nitro-blue tetrazolium, 3 mg phenazine methosulfate, 200 ml 60% sodium lactate, and 1.0 ml 0.5 M KCN and incubated for 2 to 4 hr at 32° (14).

Chromosome Counts. Hybrid cell cultures were prepared for chromosome analysis according to the modification (10) of the method of Moorhead *et al.* (9). Briefly, cell cultures were treated with 50 μg of Colcemid per ml approximately 24 hr after the fluids on the cultures were changed and the cultures were incubated for 1.5 additional hr. Chromosome preparations were stained in Giemsa.

Ability to Grow in Soft Agar. D98 and hybrid cells were prepared at a concentration of 10^3 cells in 0.5 ml of 0.33% Noble agar containing 20% fetal calf serum, 0.225% $NaHCO_3$, and antibiotics as already described. The suspensions were mixed with 4.5 ml of additional agar preparation in tissue culture plates (Falcon Integrid). HR-1 cells were prepared at a concentration of 500 cells/plate in a similar manner. The cells were incubated at 37° in an atmosphere of 5% CO_2; the number of cell colonies was counted 10 to 14 days later and the cloning efficiency was determined.

Serum Requirement of Cells. To determine the serum requirement of D98, HR-1, and D98/HR-1 cells, 10^5 cells were seeded into duplicate 1-oz glass prescription bottles and maintained in Eagle's medium (Roswell Park Memorial Institute Medium 1640 for HR-1) containing 2, 5,

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[2] The abbreviations used are: EBV, Epstein-Barr virus; HR-1, P3J-HR-1; G6PD, glucose 6-phosphate dehydrogenase; SV40, simian virus 40.

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and 10% fetal calf serum. The cells were incubated at 37° and cell counts were performed at 24, 48, and 72 hr after seeding.

RESULTS

G6PD Electrophoretic Assay. The different electrophoretic forms of G6PD expressed in D98 and HR-1 homogenates were used to characterize the somatic cell hybrids (Fig. 1). D98 homogenates were run in Channels 1 and 8, HR-1 homogenates were run in Channel 2, an artificial mixture of D98 and HR-1 homogenates was run in Channel 3, and homogenates of the 4 hybrid clones were run in Channels 4 to 7. The D98 G6PD phenotype was the characteristic A electrophoretic band found in cells from Negro patients (D98 cells may be contaminated with HeLa cells derived from a Negro patient) (3, 11). The artificial mixture demonstrated both A and B forms of G6PD. Both A and B forms and a presumptive hybrid enzyme characteristic of a dimeric enzyme were found in all 4 clones examined.

Chromosome Counts. D98/HR-1 clones 1 and 2 were examined for total number of chromosomes at various passage levels and the range of chromosome numbers and the model numbers were determined. Chromosome analysis in an earlier study suggested that the D98 and HR-1 cells had been hybridized (6). The range in the number of chromosomes decreased in clones 1 and 2 from early to later passage levels as shown in Table 1. There also appears to be a gradual decrease in the model number of chromosomes in clone 2 but not in clone 1, even though the upper range of the number of chromosomes decreased in both clones.

Ability to Grow in Soft Agar. The parental cell lines D98 and HR-1 as well as hybrid clones were examined for their ability to grow in soft agar since this ability is often a characteristic of transformed cells. All cell lines tested were capable of growing in soft agar. The D98 cells had a cloning efficiency of 9.4%; the HR-1, 50%; and D98/HR-1 clones 1 and 3, 28 and 26%, respectively (Table 2). The 2 hybrid clones had a cloning efficiency approximately 3 times that of the D98 cells in multiple experiments performed.

Table 1

Number of chromosomes in D98/HR-1 hybrid cells

Clone	Subculture[a] level	No. of chromosomes[b] Range	Modal no.
1	12	72-123	NC[c]
	24	70-108	92-94
	37	64-101	93-97
2	5	80-134	100-108
	10	81-116	97-99
	21	72-110	94-102
	29	83-102	94-95

[a] All clones were grown in Eagle's medium supplemented with 10% fetal calf serum, 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin, and 1.6×10^{-5} M thymidine.

[b] Data were obtained by examining 40 metaphases.

[c] NC, no clear modal number.

Serum Requirements of Cells. Since transformed cells are less dependent on serum concentrations in growth medium than normal cells, we examined the D98 and HR-1 parental cell lines and the 4 clones of D98/HR-1 for this property. The cells were grown in medium containing 2, 5, and 10% fetal calf serum and cell counts were performed at 24, 48, and 72 hr after seeding in bottles. The results are shown in Chart 1. The D98, HR-1, and D98/HR-1 clones 1, 2, 3, and 8 cells showed little dependence on serum concentrations in the growth medium (D98/HR-1 clone 1 is graphed as representative of the hybrid cells). Medium containing 5% fetal calf serum gave results similar to medium containing 2 or 10%.

DISCUSSION

The D98/HR-1 hybrid cells, as previously shown, contain chromosomes from both parental cell lines, D98 and HR-1 (5). There appears to have been some selection of cells

Table 2

Cloning efficiency of D98/HR-1 cells in soft agar

Cell line	% cloning efficiency
D98[a]	9.4
HR-1[b]	50
D98/HR-1 Clone 1[a]	28
D98/HR-1 Clone 3[a]	26

[a] One thousand cells were seeded per plate.

[b] Five hundred cells were seeded per plate.

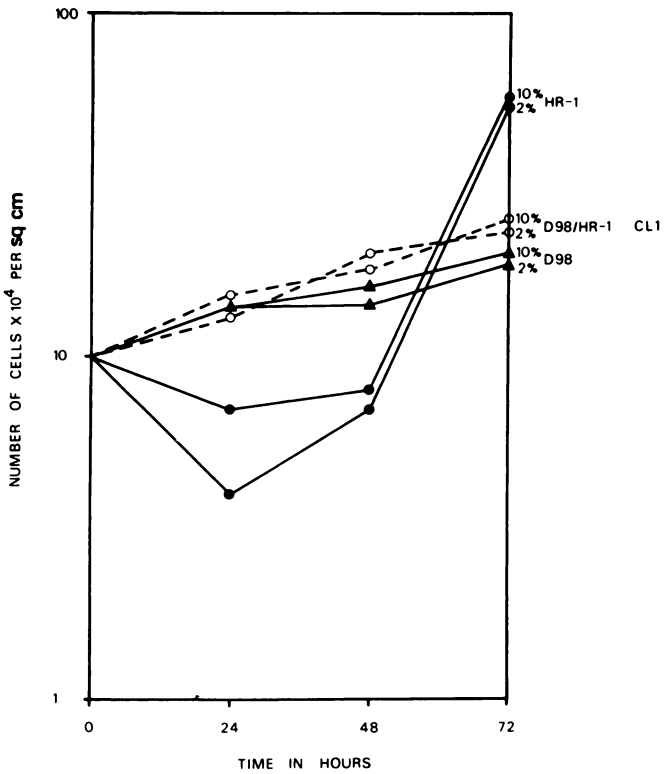

Chart 1. Growth of D98, HR-1, and D98/HR-1 cells as a function of serum concentration. *Cl 1*, clone 1.

during passage. The modal number of clone 1 remained the same over several passage levels while the modal number has decreased in clone 2 (Table 1). Chromosomal loss is a characteristic of hybrid cells on passage. However, in both clones, selection has been against those cells containing higher numbers of chromosomes. This may be due to some instability of these cells or perhaps due to a slower rate of growth. The cells were passaged approximately once per week.

There is some evidence that cells containing a higher number of EBV genome equivalents as measured by DNA-cRNA hybridization (4) are being preferentially selected. D98/HR-1 clone 1 cells contained 11 EBV genome equivalents per cell in early passage levels (before Passage 20) (4). These have recently been reexamined at Passage 32 and now contain 24 genome equivalents per cell (5). Since the data reflect the average number of EBV genome equivalents in a pool of cells, the increase in the average suggests that those cells containing higher levels of EBV DNA are selected for and those cells containing lower amounts or no EBV DNA are selected against. If selection is partially based on rate of growth, it may be that those cells containing higher amounts of EBV DNA grow faster than those cells with less virus genome equivalents.

All clones of D98/HR-1 exhibit different growth characteristics in culture when compared with the D98 monolayer parent cell line. The hybrid cells, as well as the D98 parent, are epithelial-like in morphology (2, 7). However, the D98/HR-1 cells appear to have totally lost contact inhibition, as indicated by the formation of multilayered foci. This is not the case for D98 cells, which do not form foci and appear to be at least somewhat contact inhibited (7). In addition, the cloning efficiency of D98 cells is about 3 times less in soft agar than 2 clones of D98/HR-1 cells tested. The growth of the hybrid clones, as well as both parental cell lines, was not dependent on serum concentration, which is characteristic of transformed cells. There was a lag in growth of HR-1 cells presumably due to the low concentration of cells used for this study.

Based on the history of the D98 cells (possible HeLa contamination), it is clear that they are transformed. However, the acquisition of the ability to grow in soft agar with a 3-fold efficiency increase as well as ability to spontaneously form multilayered foci *in vitro* was acquired only after hybridization with another transformed cell, HR-1. The expression of transformation characteristics has also been observed in somatic cell hybrids of SV40-transformed rat cells and normal mouse cells (16). SV40 tumor antigen was expressed in the hybrid cells. In addition, the normal mouse cells were not able to grow in soft agar while the hybrid cells were capable of growing with an efficiency of 1 to 4%. This suggests that the SV40 genome was capable of altering the growth characteristics of the hybrid cells (16). One possible explanation for the results obtained in these studies may be that the EBV genome, once having become associated with the D98 cell genome, enhanced the attributes described and may be responsible for the changes in the properties of the resulting hybrid cells.

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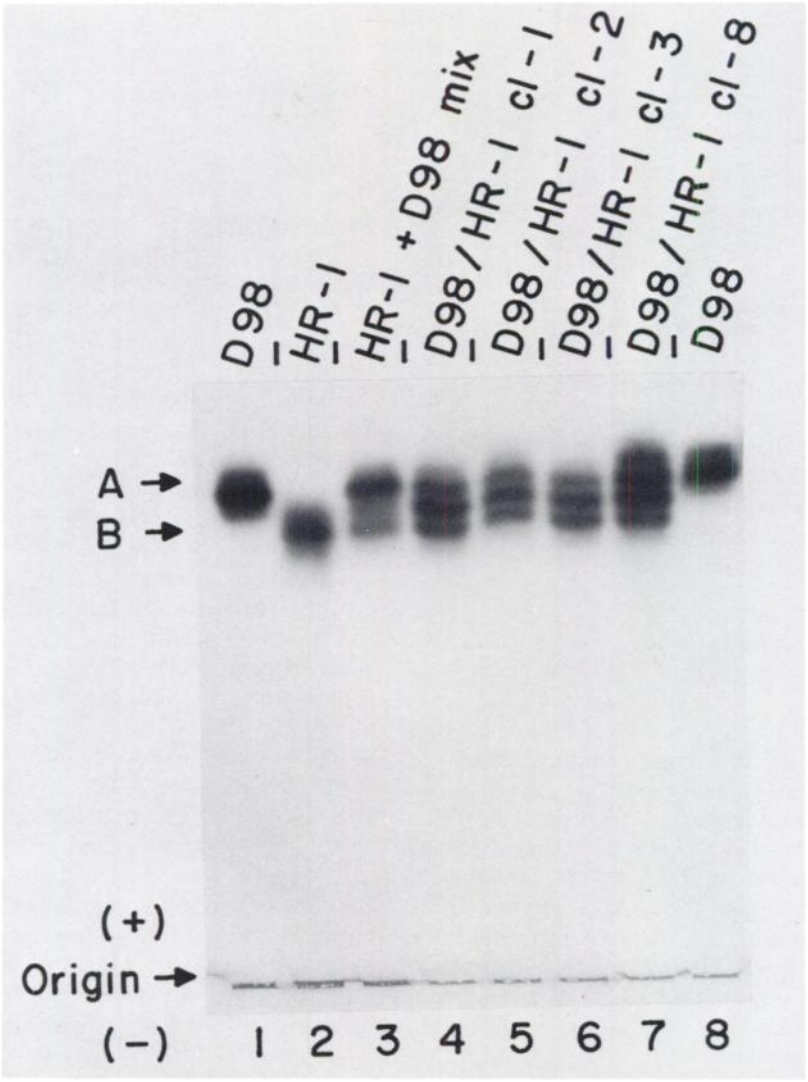

Fig. 1. G6PD zymogram. *Channels: 1*, D98; *2*, HR-1; *3*, HR-1 + D98 mixture; *4*, D98/HR-1 clone 1 (*cl 1*); *5*, D98/HR-1 clone 2 (*cl 2*); *6*, D98/HR-1 clone 3 (*cl 3*); *7*, D98/HR-1 clone 8 (*cl 8*); and *8*, D98. The fast electrophoretic form corresponds to G6PD A (D98) and the slow form to G6PD B (HR-1). The cell hybrid clones (*Channels 4* to *7*) demonstrate both parental forms and a presumptive hybrid (heteropolymer) enzyme.