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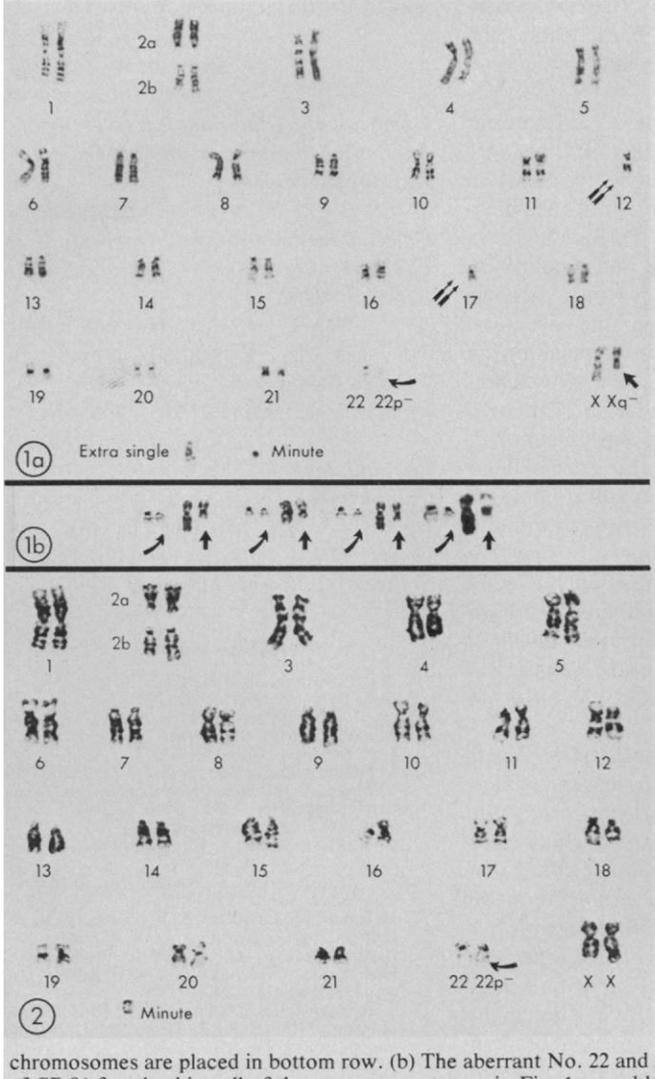
Establishment of a Cell Line with Associated Epstein-Barr–Like Virus from a Leukemic Orangutan

Abstract. *An Epstein-Barr virus like herpesvirus has been isolated from a lymphoid cell line derived from an orangutan with spontaneous myelomonocytic leukemia. Herpesvirus has not previously been isolated from this species of higher ape.*

We have isolated and partially characterized a herpesvirus similar to Epstein-Barr virus (EBV) (*1*) produced by a lymphoid cell line derived from an orangutan (*Pongo pygmaeus*) with subacute myelomonocytic leukemia. The orangutan was a 13-year-old female, born in the wild, and housed at the Los Angeles Zoo for the past 10 years. The diagnosis of myelomonocytic leukemia was made in April 1976 by hematological and cytochemical studies of bone marrow and peripheral blood smears and was confirmed at necropsy in October 1976. The animal received neither chemotherapy, radiotherapy, nor blood transfusion.

Six months before the animal died, leukocytes were obtained from defibrinated peripheral blood by separation on a Ficoll-isopaque mixture at 20°C (*2*). The cultures were maintained in RPMI 1640 media containing 20 percent fetal bovine serum, 2 m*M* glutamine, 50 μg per milliliter of gentamicin, and 25 μg per milliliter of fungizone and incubated at 37°C in humidified atmosphere containing 5 percent CO_2. During the first 10 days of culture, the medium was also supplemented with 20 percent autologous plasma.

After an initial 4-week period of slow growth, the cells began proliferating rapidly in suspension and reached a doubling time of approximately 20 hours.



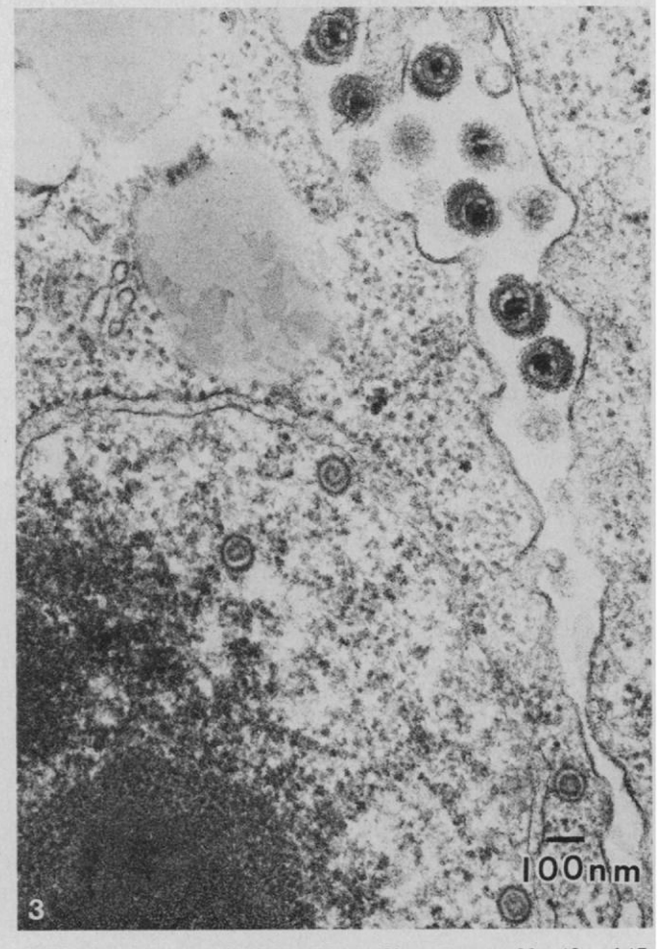


Fig. 1. (a) Karyotype of CP-81 cell (♀). Chromosomes No. 12 and 17, monosomic (double arrows). One homolog of No. 22 has a deletion in the short arm, 22 p⁻ (curved arrow), and one X chromosome is minus a portion of its long arm, X q− (short arrow). Additional nondefined chromosomes are placed in bottom row. (b) The aberrant No. 22 and X chromosomes from four additional cells of CP-81. Fig. 2. Karyotype of CP-81 female skin cell of the same orangutan as in Fig. 1, a and b, showing only a deleted No. 22 chromosome 22 p⁻ (curved arrow) and a minute chromosome. Fig. 3. CP-81, EM micrograph showing extracellular herpesvirus particles (×75,000).

The cultures consisted mainly of two cell sizes: one 8 to 11 μm and the other 15 to 22 μm in diameter. The cells showed a tendency to clump and grow as large aggregates in liquid as well as on soft agar medium (*3*). The cell line designated CP-81 has not produced tumors in hamsters, NIH mice, and rats treated with antithymocyte serum (ATS) but has produced tumors in ATS-treated NIH Swiss nude mice (*4*).

Morphologically the cells were mononuclear and blastlike in appearance, with considerable pleomorphism. Their ultrastructural appearance was also consistent with poorly differentiated stem cells. The cells did not respond to mitogenic stimulation. Cultures exposed to 1:10 to 1:10,000 dilutions of phytohemaglutinin-M or pokeweed (Difco, 5 ml reconstituted) for 20 to 72 hours did not show any increase or decrease in the [^{3}H]thymidine uptake as compared to the untreated cultures. By the immuno-cytoadherence test virtually all cells have fragment crystallizable (fc) receptors (*5*) and bind firmly to preformed soluble complexes of sheep erythrocyte and antibody. However, there is no binding to either complexes of sheep erythrocyte antibody and complement (EAC rosette) nor to sheep erythrocytes alone (E rosette) (*6*). By direct immunofluorescence, plasma membranes of 6 to 7 percent of cells stain for markers consisting of human kappa light chains from immune gamma globulin (*7*). With immunoperoxidase staining (*8*), however, about 90 percent of the cells show cell surface staining for human kappa light chains.

Karologic examination was done on the orangutan's cultured blood cells and skin fibroblasts by conventional staining as well as by the G banding technique (*9*). According to Turleau's nomenclature (*10*), one homolog of chromosome pair No. 22 of both skin- and blood-derived cells exhibited deletion of the short arm and possibly a portion of the centromere (curved arrow Figs. 1a and 2). This abnormality of No. 22 chromosome is dissimilar to the Philadelphia chromosome in human leukemia, where a portion of the long arm of chromosome No. 22 is usually translocated to another chromosome, most often No. 9 (*11*). The CP-81 cells exhibit 12 to 40 percent polyploidy not observed in the skin fibroblasts. A prominent marker chromosome abnormality, noted only in hematogenous cells, in addition to the deletion of No. 22 is the deletion of a large portion of the long arm of one x-homolog (short arrow, Figs. 1, a and b). Further, in the hematogenous cells there is an increased prevalence of monosomy of chromosome Nos. 12 and 17 (double arrow, Fig. 1). We cannot, of course, determine from our limited data whether these chromosomal abnormalities are related to the etiology of the orangutan's disease. These studies do, however, confirm the orangutan karyotype ($2n = 48$) of the cultured cells; the lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PD) isoenzyme patterns (*12*) of both lymphocyte and skin cultures are also orangutan-specific.

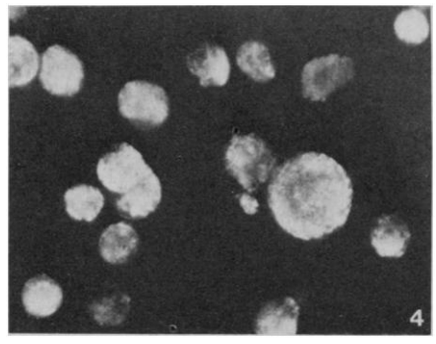


Fig. 4. CP-81 cells showing immunofluorescence stained nuclei with human serums reactive with EBV nuclear antigen (EBNA).

Herpesvirus particles, intranuclear and intracytoplasmic in location, are seen by electron microscopy (EM) in thin sections of <1 percent of the hematogenous cultures (Fig. 3). Budding from the nuclear membrane and localization of virions within cytoplasmic vacuoles are consistent with the fine structural features of herpesvirus replication in other animals. However, the virus is predominantly seen in the larger of the two cell sizes present in suspension cultures. Negative staining of the extracellular particles harvested from culture fluid also confirms their herpesvirus morphology. The virus, as determined by negative stain EM, bands at a density of 1.27 g/ml after the culture fluid is filtered (0.45 μm Millipore) and concentrated (4000×), mixed with cesium chloride (density 1.3 g/ml), and centrifuged (183,000*g*) for 44 hours (*13*).

Acetone-fixed CP-81 cells show 3 percent positive immunofluorescence staining for intracellular antigens with virus capsid antigen (VAC) and early antigen (EA) positive human, baboon (*14*, *15*), as well as with autologous serum. About 90 percent of the cell nuclei stain with human serums reactive with EBV nuclear antigen (EBNA) (*14*) (Fig. 4) and with chimpanzee serum reactive with chimpanzee EBV-like virus (*16*). The orangutan's own serum also gave EBNA staining. The presence of a nuclear antigen that reacts with antiserum to chimpanzee virus nuclear antigen suggests a close relation between the orangutan and chimpanzee isolates. Cell-free virus was found to transform gibbon lymphocytes in vitro leading to the formation of suspension cell lines.

There is no evidence that this cell line is producing type C virus by EM, reverse transcriptase assay with synthetic template primer polyriboadenylate · oligodeoxythymidylate (polyrA · oligodT) (*17*), or by complement fixation against a highly reactive mammalian interspecies p30 antiserum (*18*). Also no type C virus has been rescued by cocultivation with several mammalian cells or activation treatment with 5′-iododeoxyuridine (*19*). However, the cells are susceptible to exogenous infection with baboon leukemia virus (*20*), RD-114 virus (*21*), and amphotropic mouse leukemia virus (*22*).

This is the first herpesvirus isolate and the only reported case of myeloproliferative disease in the orangutan. We believe that this cell line will be an important resource for further study.

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Adrenergic Stimulation of Taurine Transport by the Heart

Abstract. *A high-affinity transport system that is specific for β-amino acids has been delineated in rat hearts. This system transports the cardiotonic sulfonic amino acid taurine. β-Adrenergic stimulation increases the transport capacity without effect on α-amino acid uptake, as does stimulation with adenosine 3',5'-monophosphate or theophylline. The existence of such an uptake system for taurine in the heart accounts for the high intra- to extracellular concentration gradient that is maintained, and suggests that cardiac stress is associated with increased taurine uptake. This may explain why taurine is the only amino acid to be markedly elevated in congestive heart failure. Taurine is a modifier of calcium fluxes in the heart, as are β-adrenergic agonists. The presence of this uptake system suggests a link between β-adrenergic stimulation of calcium and taurine fluxes.*

One of the major mechanisms whereby the heart increases its output under work stress is the β-adrenergic system. Prolonged stimulation of this system causes an increase in heart mass—cardiac hypertrophy—and, if the stress is severe and long-lasting, eventual congestive heart failure. Stimulation of the β-adrenergic system thus causes the physiological changes summarized as the "fight or flight" syndrome, but a high level of stimulation for a prolonged period causes pathological changes leading to cardiac hypertrophy and decreasing cardiac efficiency. An understanding of the cellular changes occurring at different levels of β-adrenergic activation is therefore crucial for an elucidation of the biochemical basis for cardiac hypertrophy.

One of the major actions of agonists of the β-adrenergic system in increasing cardiac contractility is to increase the flux of calcium into the cell. Another modifier of calcium fluxes in the heart is the sulfonic amino acid taurine (*1*). Taurine is present in mammalian heart in large quantities, comprising in excess of 50 percent of the total free amino acid pool (*2*). The content of taurine in the heart is maintained remarkably constant, and in the rat is invariant over a wide range of conditions. It is of interest, therefore, that marked elevations in taurine concentration occur in congestive heart failure in humans and experimental animals (*3*). We have examined the relation between taurine content and cardiac stress, and now propose that a transport system for taurine exists in the heart that is modulated by the level of β-adrenergic activation. The existence of such a system may explain why the concentration of taurine is markedly elevated in hearts in the process of congestive failure.

The precise functions of taurine in the heart are undelineated, but taurine is known to alter the kinetics of calcium, leading to a decreased rate of exchange with extracellular fluid, and an increased affinity of intracellular structures for calcium (*2*). The concentrations of taurine attained in the heart are determined by the balance of any metabolic and transport processes that are operative. Although the rat heart can synthesize taurine, the quantitative importance of this is unclear (*4*). The only metabolic pathway for taurine for which evidence is available is conversion to isethionic acid (*5*). However, this pathway proceeds at a very low rate, or possibly is absent in the heart, and has no appreciable influence on taurine balance. No transport process has been shown for taurine in the intact heart, but in view of the fact that the myocardium/serum ratio of taurine is in excess of 200, it is likely that an active transport process exists. Such transport systems for taurine have been demonstrated in Erhlich ascites cells (*6*), platelets (*7*), and fractions of brain tissue enriched in glial and synaptosomal fragments (that is, crude synaptosomes) (*8*).

This study was designed to determine whether metabolic or transport processes affecting taurine concentration are modified by cardiac stress. We used isoproterenol, a β-adrenergic agonist, to produce a high output of stress on the heart. Preliminary studies in vivo revealed that isoproterenol given to rats for periods of up to 10 days produced a cardiac hypertrophy accompanied by a marked increase in total taurine content of the heart (*9*). No alteration occurred in the rate of taurine synthesis, as measured either by the overall conversion of cysteine to taurine, or by the activity of cysteamine dioxygenase (E.C. 1.13.11.19), an enzyme involved in the biosynthesis of taurine in the heart. Increases were observed, however, in the rate of influx of taurine. The effect of isoproterenol on taurine influx has therefore been examined in the isolated, perfused rat heart.

In the heart perfused by the Langendorff technique, the rate of taurine influx was constant for more than 20 minutes when taurine was perfused at concentrations of 25 to 200 μM (Fig. 1). [^{3}H]Taurine (specific activity, 1.4 mc/mmole) was perfused through the heart, and the radioactivity eluting from the heart was monitored (Fig. 1). Uptake was determined as the difference be-

Table 1. Effect of isoproterenol on amino acid influx in the heart. Data are expressed as the mean ± standard error of four hearts. The amino acids were perfused in 0.05 mM concentrations. Other conditions were as in Fig. 1 (*12.*).

Treatment	Influx [nanomole per gram (dry weight) per minute]				
	Taurine	β-Alanine	Aminoisobutyric acid	Leucine	Serine
Control	15.0 ± 1.0	16.0 ± 1.3	13.4 ± 1.3	28.5 ± 3.8	53.3 ± 3.5
Isoproterenol-stimulated	19.2 ± 0.2*	19.7 ± 1.0*	12.3 ± 0.5	30.6 ± 0.8	57.3 ± 1.3

*$P < .05$.