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Establishment of a Lymphoblastoid Cell Line and Isolation of an Epstein-Barr-Related Virus of Gorilla Origin

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A B-lymphoid cell line was established from a normal gorilla. The cells contained Epstein-Barr virus-related antigens, and herpesvirus particles were demonstrated by electron microscopy. DNA-DNA reassociation kinetics revealed 30 to 40% hybridization to Epstein-Barr virus with 50 genomes per cell. Examination of the viral nuclear antigen with gorilla sera showed this to be a unique isolate termed *Herpesvirus gorilla. H. gorilla* transformed gibbon B-lymphocytes in vitro.

Since 1976, Epstein-Barr virus (EBV)-related lymphotropic herpesviruses have been isolated from normal and leukemic baboons (*Papio papio, Papio anubis, Papio hamadryas*) (1), normal chimpanzees (*Pan troglodytes*) (3), and an orangutan (*Pongo pygmaeus*) with monomyelocytic leukemia (15). These viruses are associated with and transform lymphocytes of B-cell origin and are antigenically related to the EBV of humans, and their DNA hybridizes approximately 40% with that of EBV. Although the viruses are related to each other, they can be distinguished on the basis of their nuclear antigens (NA) as demonstrated by using an anticomplement immunofluorescence (ACIF) assay (13). The present report describes the isolation of an antigenically unique virus (*Herpesvirus gorilla*) from a normal gorilla (*Gorilla gorilla*) and its characterization as a fifth member of the EBV family of lymphotropic herpesviruses.

These studies were initiated with 10-ml samples of peripheral blood collected in sterile preservative-free heparin from each of two female gorillas (obtained through the courtesy of Harold McClure, Yerkes Primate Center, Atlanta, Ga.). The mononuclear cell fraction was collected after density centrifugation on Ficoll-Hypaque gradients and cultured at 10^6 cells per ml in RPMI-1640 medium supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 50 U of penicillin per ml, and 50 μg of streptomycin per ml. After 4 to 5 weeks of incubation at 37°C with weekly media changes, cell replication was evident in cultures from both gorillas. The cells could be subcultured after 6 weeks. Shortly thereafter, cultures from one animal showed foamyvirus cytopathology and were lost. Cells from the second gorilla, Machi, continued to replicate, and a cell line was established.

Since chimpanzee lymphoid cell lines have been established which contain *Herpesvirus pan* (3), and since chimpanzee and gorilla cells are difficult to distinguish from each other, it was essential to verify the gorilla nature of the Machi cell line. Cytogenetic examination revealed that the modal number of chromosomes ($2n$ = 48) and their distribution was typical for gorillas, with satellites present on most acrocentric chromosomes. After q-banding with quinacrine mustard, brilliant fluorescence was observed at the satellites on the short arms of the acrocentric chromosomes. This technique distinguishes gorilla cells from those of chimpanzee origin, in which it is the short arms that fluoresce and not the satellites (6). Machi cells could also be distinguished from chimpanzee cells by an examination of surface glycoproteins using the neuraminidase-galactase oxidase sodium [^{3}H]borohydride labeling technique (2). Results of this assay showed a distribution of surface glycoproteins that was clearly different from CH888 cells, which are B-cells of chimpanzee origin (3), and resembled that of B-lymphoid cell lines of other primate species (17) (Fig. 1). After three months in culture, the surface marker characteristics of Machi cells were examined. The cells were negative for both sheep erythrocyte and activated complement receptors. Direct immunofluorescence microscopy revealed that the cells expressed surface immunoglobulin G (IgG), and by radial immunodiffusion it was determined that cultures initiated at a density of 10^6 cells per ml

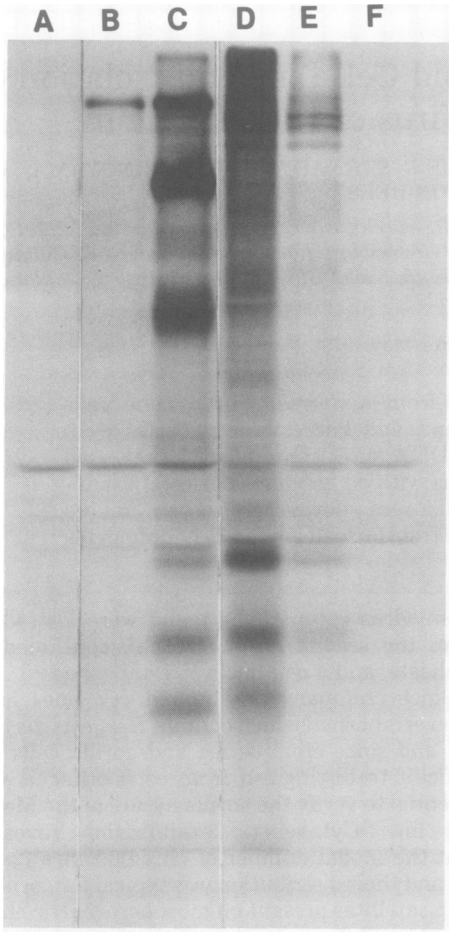

FIG. 1. *Comparison of surface glycoproteins of Machi cells (gorilla B-cells, D to F) and CH888 (chimpanzee B-cells, A to C). The cells were radiolabeled as described (2, 17) using sodium [^{3}H]borohydride at 0.5 mCi per 3 × 10^7 cells per ml alone (A and F) or after pretreatment with either galactose oxidase (5 U/ml for 30 min at 37°C; B and E) or neuraminidase (25 U/ml) and galactose oxidase (5 U/ml for 30 min at 37°C, C and D). The labeled surface glycoproteins were separated on acrylamide gels in the presence of sodium dodecyl sulfate and analyzed by fluorography (17). The surface glycoproteins in C and D range in molecular weight from 27,000 to 220,000.*

released IgG at a level of 4.7 μg/ml of medium after 72 h. These cells were also shown to have Fc receptors by indirect immunofluorescence using heat-aggregated goat IgG and fluorescein-conjugated rabbit anti-goat IgG. These findings of surface and released IgG, the presence of Fc receptors, and the lack of sheep erythrocyte receptors are compatible with Machi cells being of B-cell origin.

Testing of Machi cells by indirect immunofluorescence, using human sera positive for antibodies to EBV, revealed cells positive for intracellular antigens at a level of 1 to 2%. Treatment with 5-iodo-2′-deoxyuridine (60 μg/ml for 3 days) resulted in increased levels of antigen-positive cells (to 10 to 15%), and herpesvirus particles were observed by electron microscopy in about 5% of the cells. Previously, we had shown that treatment of cells with 5-iodo-2′-deoxyuridine in the presence of phosphonoacetic acid (100 μg/ml) selectively stimulated viral early antigens (EA) (7). Serological analysis of the EA of different lymphotropic herpesviruses indicated that these antigens were related but could be distinguished in certain cases by specific naturally occurring discordant sera. In the present study, 5-iodo-2′-deoxyuridine–phosphonoacetic acid-treated Machi cells and similarly treated Raji cells (as source of EBV EA) were examined using human and gorilla sera. Eight human sera had geometric mean titers of 104 against EBV EA and 57.4 against *H. gorilla* EA; conversely, 12 gorilla sera had geometric mean titers of 101.6 against *H. gorilla* EA and only 22.1 against EBV EA ($P < 0.05$), suggesting differences between the EA of EBV and *H. gorilla*. Machi cells were shown to have an NA demonstrable by ACIF staining, as has been shown for EBV-positive cells (16). This NA was detected by nearly all gorilla sera tested. These same positive gorilla sera did not react with the NA of EBV, *H. pongo*, *H. pan*, or *H. papio* by ACIF, indicating a high degree of antigenic specificity for *H. gorilla* NA (Table 1). *H. gorilla* NA was also detected by ACIF with some, but not all, human, orangutan, and gibbon sera positive for antibodies to the NA of one or more of the other primate lymphotropic herpesviruses. An NA has also been demonstrated for both EBV and *H. papio* by use of an acid-fixed nuclear binding immunofluorescence assay after a biochemical purification of the antigen (9, 10). In the present study, *H. gorilla* NA was purified by DNA-Sepharose column chromatography and assayed by the acid-fixed nuclear binding technique using gorilla, human and baboon sera; EBV and *H. papio* NA were similarly prepared and examined. Results suggested that the NA of *H. gorilla* and *H. papio* were related but distinguishable from each other and from the NA of EBV (Table 1). A comparison of gorilla sera antibody titers to *H. gorilla* NA using the ACIF and acid-fixed nuclear binding techniques did not show a correlation ($r = 0.01$), suggesting that at least for this virus the two techniques are detecting different antigenic determinants.

To examine the relationship between the DNAs of *H. gorilla* and EBV, a [^{3}H]thymidine-

TABLE 1. *Demonstration of a unique H. gorilla NA by using ACIF and acid-fixed nuclear binding techniques*

Sera	NA (no. positive sera/no. tested)[a]					
	EBV		*H. gorilla*		*H. papio*	
	ACIF	AFNB[b]	ACIF	AFNB	ACIF	AFNB
Human	24/24	22/24	9/24	6/24	0/24	14/24
Gorilla	0/12	0/12	11/12[c]	10/12	0/12	12/12
Baboon	0/39	0/39	0/39	4/38	0/39	6/39

[a] All sera were tested at a dilution of 1:10.
[b] AFNB, acid-fixed nuclear binding.
[c] Gorilla sera demonstrated only *H. gorilla* nuclear antigen by ACIF. Tests against *H. pan* and *H. pongo* nuclear antigen were negative.

labeled DNA probe was prepared from Raji cells after superinfection with the P3HR-1 strain of EBV (18) and used in hybridization experiments with Machi cellular DNA. Results of these DNA-DNA reassociation kinetic studies revealed that *H. gorilla* hybridized 30 to 40% to EBV DNA and was present in Machi cells at about 40 to 50 genome copies per cell (Fig. 2).

To test *H. gorilla* for transforming activity, Machi culture fluids, concentrated 100-fold by ultrafiltration and ultracentrifugation, were inoculated onto gibbon (*Hylobates lar*) lymphocytes, which are known to be susceptible to transformation by EBV, *H. papio*, and *H. pongo* (14). Lymphocytes were tested in a microwell assay employing 2×10^5 lymphocytes per well (5) and were observed for 6 weeks. Cells from both gibbons tested showed signs of transformation (cell clumping and increased metabolism) within 14 days, and lymphoid cell lines were established. No transformation was observed in uninoculated cultures. These cells have a modal chromosome number of $2n = 44$ and normal gibbon karyotypes. They have surface properties characteristic of B-lymphocytes (surface IgG and lack of sheep erythrocyte receptors) and have an NA with *H. gorilla*-type specificity.

Machi cells were also tested for the possible presence of type C RNA viruses. A 20% extract was prepared at 2.5 months of culture age and tested in a complement fixation assay with a broadly reacting goat serum that has antibody to the p30 determinants shared by the known mammalian type C viruses (4). This serum had indicated the presence of type C virus in rhesus monkey carcinoma cells (12) before its isolation (11). Testing of Machi cell extracts with this serum has given low-level positive results. Machi cells are currently being cocultivated with a variety of cell lines in an attempt to isolate a possible gorilla retrovirus.

H. gorilla is the most recent EBV-related

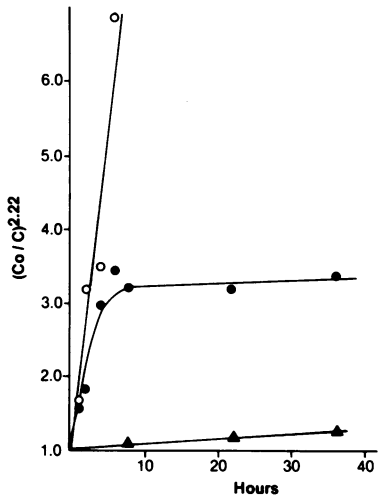

FIG. 2. *DNA-DNA reassociation kinetics between EBV DNA and DNA from Machi cells.* (○) *Raji cell DNA (50 genomes per cell);* (●) *Machi cell DNA;* (▲) *Simpson cell DNA (EBV negative). DNA-DNA reassociation kinetics were performed as described for Herpesvirus pongo (14).* ^{3}*H-labeled EBV DNA was obtained from Raji cells after superinfection by the P3HR-1 strain of EBV in the presence of 100 μCi of [*^{3}*H]thymidine per ml (18).* ^{3}*H-EBV DNA was used at 0.3 genomes per cell, and all DNA was sonicated to make randomly sheared fragments. After incubation at 66°C, the degree of reassociation was measured by S1 nuclease. EBV-negative human cells, Simpson, were used as controls.*

lymphotropic herpesvirus isolated from primates. All five of these viruses are related to each other antigenically, but differences have been observed in EA and to a larger extent in NA, as determined by naturally occurring discordant sera. Additionally, the DNAs of all the simian viruses share a degree of homology with EBV DNA. For clearer differentiation of these viruses from each other, monospecific sera and individual DNA probes are required. Such im-

portant biological reagents are not yet available, but efforts are underway to develop them. Similar to EBV, the EBV-like simian viruses appear to be widespread in their host species. There is no definite evidence that any of the simian viruses is oncogenic, but *H. papio* has shown a serological association with spontaneous B-cell lymphomas of captive baboons (8).

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