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Expression of Baboon Endogenous Virus in Exogenously Infected Baboon Cells

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Strains of low-passage, fetal diploid, baboon (*Papio cynocephalus*) fibroblasts were susceptible to exogenous infection with three independent isolates of baboon endogenous virus, as measured by an immunofluorescence assay specific for viral p28. Infectivity of the M7 strain of baboon endogenous virus for baboon cells of fetal skin muscle origin was equivalent to that for human and dog cells in that similar, linear, single-hit titration patterns were obtained. The assay for supernatant RNA-dependent DNA polymerase, however, showed that baboon cells produced only low levels of virus after infection compared with the production by heterologous cells. The results showed that baboon endogenous virus was capable of penetrating baboon cells and that viral genes were expressed in infected cells. Replication of complete infectious virus was restricted, however, indicating that in this primate system homologous cells differentially regulated the expression of viral genes.

Type C baboon endogenous viruses (BaEV) have been isolated by cocultivation of baboon cells in vitro with cells of heterologous species; this has been necessary because homologous cells are generally nonpermissive for replication of BaEV and cultures of most baboon tissues do not produce the virus (3, 9, 16, 17). Only a few examples of chronic or induced release of virus in baboon cell cultures have been reported (12; Lavelle and Foote, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978 S167, p. 240).

The nature of the restriction on BaEV replication by homologous cells has not been clearly defined. Most strains of cells have produced insignificant amounts of extracellular viral RNA-dependent DNA polymerase (RDDP) after exogenous infection (17). Some strains have also resisted focus induction by BaEV pseudotypes of sarcoma virus (17) and plaque formation by pseudotypes of vesicular stomatitis virus (14). Both of these findings suggest that restriction occurred at the level of adsorption or penetration. Other workers, however, found that BaEV pseudotypes of sarcoma virus did induce foci in cultures of baboon cells (10), indicating that postpenetration restriction of virus also occurs.

Using a fixed-cell immunofluorescence assay for the major internal structural polypeptide (p28) of BaEV, we found that baboon fibroblasts were susceptible to infection by the virus, but that only small amounts of complete virus were produced, as measured by supernatant RDDP assays. The latter finding is in agreement with reports that baboon cells restrict endogenous virus (17); but the observation that homologous cells respond to infection by stable expression of viral genes is new. Results of this study are presented here.

The cells and viruses used are identified in Table 1. The table shows that three strains of baboon cells tested were susceptible, as determined by p28 immunofluorescence assays, to three independent isolates of BaEV. These cells were uniformly negative for expression of endogenous viral p28 before infection.

The infectivity of BaEV for cells of heterologous species is also shown in Table 1. The results are in agreement with those previously reported (3, 16, 17).

The specificity of the staining reaction for BaEV p28 was demonstrated by inhibition of the reaction after absorption of antiserum with purified BaEV p28 but not with purified envelope glycoprotein (gp70), Rauscher leukemia virus p30, or bovine serum albumin. Reaction with BaEV-infected cells was not detected with goat antisera to simian sarcoma virus p30, Mason-Pfizer monkey virus p27, or normal goat serum (data not shown).

To compare the susceptibility to BaEV of baboon cells with that of heterologous cells, we carried out a series of virus titration experiments utilizing the immunofluorescence assay. The results in Fig. 1 show that titrations of M7 virus in baboon (Fig. 1A), human (Fig. 1B), and dog (Fig. 1C) cells were similar, were linear, and appeared to have single-hit patterns. Figure 1C also shows titration of baboon embryo (WBE) virus in dog

TABLE 1. *Susceptibility of baboon and heterologous cells to exogenous infection by BaEV*

Type of cells[a]	Viral p28 immunofluorescence for the following infecting virus:[b]		
	M7	WBE	2162-2
Baboon			
FSM	+	+	+
Fetal thymus	ND[c]	+	+
Juvenile kidney	ND	+	+
Human			
A204	+	+	+
Embryo	+	ND	ND
Rhesus monkey, DBS-FRhL-1	+	+	ND
Mink, CCL-64	+	+	ND
Dog, Cf2Th	+	+	ND
Rat, NRK	−	−	ND
Mouse, BALB/c, clone A31	−	−	ND
Cat, CRFK	−	ND	ND

[a] Baboon (*Papio cynocephalus*) cell strains were derived from skin muscle (FSM) cells and thymus of an 80-day-old fetus (7404530) and from the kidney of a juvenile animal. Human rhabdomyosarcoma (A204) cells (8) were obtained from W. K. Yang, Oak Ridge National Laboratory, and human embryo fibroblasts were from R. E. Hand, Oak Ridge National Laboratory. Rhesus monkey lung (DBS-FRhL-1) cells (18) were obtained from Charles Sherr, National Cancer Institute. Mink lung (CCL-64) cells were from the American Type Culture Collection. Fetal dog thymus (Cf2Th) cells and cat kidney (CRFK) cells were obtained through the Office of Program Resources and Logistics, Virus Cancer Program, National Cancer Institute. Normal rat kidney (NRK) cells (5) and mouse cells (BALB/c, clone A31) (1) were gifts from R. W. Tennant, Oak Ridge National Laboratory. DBS-FRhL-1, Cf2Th, and CRFK cell lines were propagated in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 2 mM glutamine, and antibiotics. CCL-64 cells were maintained in RPMI 1640 medium, and all other cells were maintained in McCoy 5A medium; both media contained the same supplements as Dulbecco medium (see above). All cell types were tested for mycoplasma contamination and found to be negative. The species of origin of baboon (FSM) and rhesus monkey (DBS-FRhL-1) cells were verified by metaphase scanning, trypsin Giemsa banding, and isozyme analyses through the generosity of W. D. Peterson, Jr., and the late C. S. Stulberg, Child Research Center of Michigan, Detroit.

[b] The M7 strain of BaEV (3) was produced by chronically infected A204 cells obtained from W. K. Yang. BaEV 2162-2 was isolated from the placenta of a 50-day-old baboon embryo by cocultivation with human fetal fibroblasts and was propagated in the same cells. WBE virus was spontaneously and chronically released from a culture of baboon embryo cells (WBE 1129) (12). Subconfluent monolayers of cells in 60-mm dishes containing glass cover slips were treated with 25 μg of DEAE-dextran per ml in medium without serum for 1 h at 37°C, washed, and incubated with filtered virus in medium without serum for 2 h at 37°C. Unattached virus was removed, and cells were washed, incubated in complete medium for 48 to 72 h, and assayed for virus infectivity by the immunofluorescence assay. Goat antisera to the p28 protein of BaEV (M7 virus), fluorescein-conjugated porcine anti-goat serum, and rhodamine counterstain were obtained through the Office of Program Resources and Logistics, Virus Cancer Program, National Cancer Institute. Cells on cover slips were washed in phosphate-buffered saline and fixed in cold acetone. They were incubated first with antiserum to viral p28 (1:120 dilution in phosphate-buffered saline) for 30 min at 37°C, washed, and then treated with a mixture of fluorescein-conjugated antiserum (1:100) and rhodamine (1:30).

[c] ND, Not determined.

cells. The amount of infectious virus is less than that of M7 virus, but the titration pattern is similar. Thus, by assay for intracellular viral p28, fetal skin muscle (FSM) cells were as susceptible as heterologous cells to infection by BaEV.

Production of viral protein was further examined by homologous competition radioimmune assays for viral p28 and gp70 in cultures of chronically infected baboon and canine cells. Cultures were also assayed for supernatant viral RDDP, which is indicative of complete virus production. Chronic infections were established by inoculation of cultures with M7 virus, followed by serial subcultures. As Table 2 shows, viral p28 and gp70 specificities were found both intra- and extracellularly, indicating that viral *env* gene as well as *gag* gene products were expressed in baboon cells. The presence of antigens in supernatant fluids indicates that virus particles were also produced. However, in agreement with other reports (16, 17), we found that cultures of baboon cells produced less viral RDDP and infectious virus than did heterologous cells after infection. Table 2 shows that the level of RDDP in fluids from infected baboon cultures was insignificant, in contrast to that in dog cultures.

Thus, the results of both chronic and acute infections of baboon cells confirm their susceptibility to exogenous infection by BaEV and also demonstrate a restriction which appears to influence virus production. Failure of cells to produce significant levels of extracellular polymerase is one consequence of the restriction, but the mechanism is not known. From what is known about the biosynthesis of type C viral polypeptides (6), *gag* and *env* precursor proteins were presumably synthesized, and restriction could result from modified translational processing of a *gag-pol* precursor. Other explanations, such as

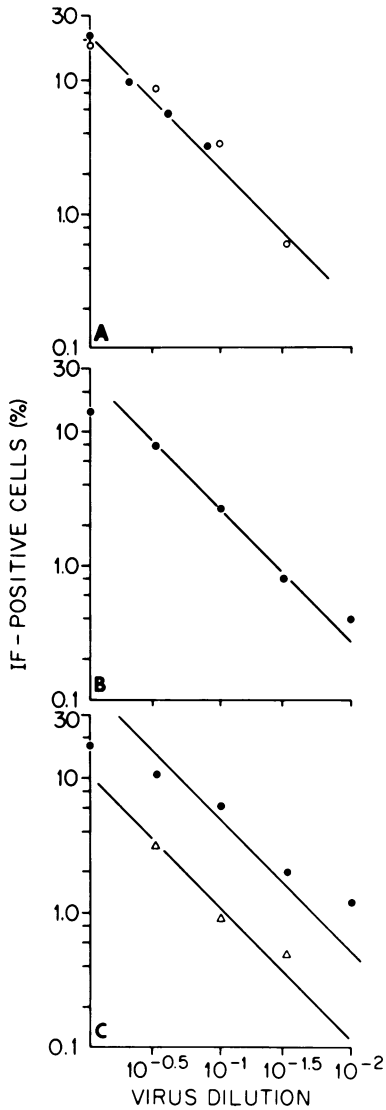

FIG. 1. *Titrations of BaEV in baboon and heterologous cells. Dilutions of M7 and WBE viruses were adsorbed to subconfluent monolayers of DEAE-dextran-treated cells for 2 h. Inoculum was removed, and cells were incubated in complete medium for 72 h. Cover slips were fixed and assayed for percentage of immunofluorescence (IF)-positive cells (4) determined from at least 1,000 total cells. (A) Baboon FSM cells and M7 virus (two separate titrations are shown); (B) A204 cells and M7 virus; (C) dog Cf2Th cells and M7 virus* (●) *and WBE virus* (△). *Slopes of the lines are drawn at 45°.*

transcriptional processing, must also be considered.

Differential expression of simian sarcoma-associated virus in a clone of infected human cells has been described (11), and our results are similar to those reported previously in that viral *gag* and *env* products were expressed but synthesis of polymerase was deficient. In the baboon system, restriction seems to be the rule rather than the exception, and it appears to be directed by host regulatory mechanisms at an endogenous virus. Restriction of endogenous retrovirus by primate cells may have a bearing on the difficulty of isolating endogenous viruses from human and many other primate cells (16) and, hence, on the difficulty of determining their relationship with neoplastic states.

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TABLE 2. *Radioimmune and RDDP assays for viral protein expression*

Type of cells	Viral antigen (ng/ml)[a]				RDDP (cpm × 10^{-3})[b]
	Cell associated		Supernatant		
	p28	gp70	p28	gp70	
BaEV-infected[c]					
Baboon FSM	257	3,176	153	174	3
Dog Cf2Th	1,680	2,240	312	1,840	150
Uninfected					
Baboon FSM	<17	NT[d]	NT	NT	2
Baboon WBE	110	680	59	44	13
Dog Cf2Th	<29	140	<12	<20	2

[a] Radioimmune assays were performed as described previously (15). Goat antiserum to viral p28 (M7 strain of BaEV) was prepared with antigen purified from virus by agarose-guanidine hydrochloride column chromatography (7); antiserum to gp70 was prepared with Tween-ether-disrupted virus. Viral p28 and gp70 were isolated from detergent-disrupted virus by phosphocellulose chromatography as described previously (2). The above antisera and purified virus were obtained from the Office of Program Resources and Logistics, Viral Oncology, National Cancer Institute. A 10% (vol/vol) lysate of cells was prepared for assays of cell-associated antigens. Lysates contained 10 to 12 mg of total protein per ml. Supernatant antigens were pelleted from 12 ml of tissue culture fluids and resuspended in 1 ml of detergent buffer (15).

[b] The methods of Ross et al. (13) were used for preparation of samples and Mn^{2+}-dependent enzyme reactions. Control negative reactions employed samples from uninfected baboon or heterologous cells and from reaction mixtures without enzyme. The mean background, 800 ± 200 cpm (1 standard deviation), was subtracted.

[c] DEAE-treated cells were exposed to filtered virus and serially subcultured. Radioimmune and RDDP assay values were obtained at 48 to 72 h after a subculture. At the time of assay, both FSM and Cf2Th cultures were 65 to 75% immunofluorescence positive.

[d] NT, Not tested.

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