PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 407-410 (1976)

Growth Characteristics of Bovine Herpesvirus 1 (Infectious Bovine Rhinotracheitis) in Human Diploid Cell Strain WI-38 (39221)

F. J. MICHALSKI, A. DIETZ, AND G. D. HSIUNG

Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut 06510, and Virology Laboratory, Veterans Administration Hospital, West Haven, Connecticut 06516

Bovine herpesvirus 1, infectious bovine rhinotracheitis virus (IBR) has been reported to be capable of replicating in a wide range of cell types including human cells (1-9). In addition to bovine cells (1, 2), nonprimate cells including porcine (3), caprine, ovine, equine (4), murine, avian (5), and rabbit (6) were susceptible to infection with IBR. Recently we found that IBR virus not only could infect hamster cells but also could malignantly transform primary Syrian hamster embryo cells, when conditions were favorable (10). Since certain lots of bovine serum were found to be contaminated with IBR virus (5), it was of interest to investigate what effect this bovine herpesvirus might have on some commonly used cell types, such as the human diploid fetal lung cell strain, WI-38, in case they should be accidentally infected through the use of contaminated bovine serum. This cell strain was chosen because it has been used in the production of live poliovirus vaccine and for several other vaccines under experimental study. This paper presents a detailed study on the growth characteristics of IBR virus in WI-38 cells under various laboratory conditions.

Materials and methods. Cell cultures. Monolayer tube cultures of WI-38 cells at passage levels 20-26 were purchased from Flow Laboratories. All cultures were maintained in Eagle's basal medium containing 2-5% fetal bovine serum, which was replaced every 7 days. Primary bovine embryonic kidney cell suspension (BEK) was also purchased from Flow Laboratories. They were planted in tubes and grown in confluency in a modified growth medium, HK, with 10% fetal bovine serum (11). The confluent cell sheets were maintained in Eagle's basal medium containing 2% bovine serum and were inoculated in parallel with the WI-38 cells.

Virus strain. A Colorado strain of IBR virus, originally isolated from the oral cavity, was obtained from Dr. P. W. Chang of the University of Rhode Island, and passaged six times in BEK cells (2). Virus was assayed in BEK cell cultures by the serial tenfold dilution method, using four tubes per dilution.

Growth curves. Monolayer cultures were inoculated with IBR virus at various input multiplicities and adsorbed for 30 min at room temperature. Unadsorbed virus was removed by washing three times before replacement of maintenance medium and incubation. Total virus yield, both cell-associated and extracellular virus, was determined by assay of the pooled contents of duplicate infected culture tubes frozen at −70° at various time intervals after inoculation. Assay of cell associated virus was accomplished both by dispersing infected monolayers with trypsin-versene solution and inoculating the infected cells into BEK cells, and by washing the infected monolayer and then freezing it at −70° before assay. The extracellular virus was measured by inoculation of serial tenfold dilutions of supernatant fluids into BEK cells.

Virus yield in young and old cultures. The same lot of WI-38 cells, at passages 23-26, was infected in a similar manner at both 7 and 14 days after the cells were seeded. Fresh maintenance medium was replaced immediately after virus adsorption. BEK cells were infected in parallel. After 48 hr of infection, the culture tubes were frozen at −70° followed by assay of each sample for virus infectivity titers, using the same lot of BEK cells.

Results. Growth of IBR virus in WI-38 cells at high input multiplicity. Comparative growth curves of IBR virus in WI-38 and BEK cells infected at high input multiplicities are represented in Fig. 1. Equal

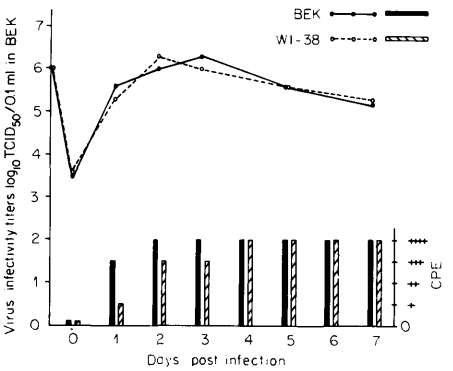

FIG. 1. Growth curves of IBR virus in BEK and WI-38 cell cultures at an input multiplicity of approximately 2.

amounts of virus were inoculated into each culture tube of both cell systems. At input multiplicities of about two or greater, the virus infectivity titers were comparable in the two cell systems, and the highest virus titer was reached in 2–3 days. The development of cytopathic effect (CPE) took longer in the WI-38 cells. It was also found in the latter that the increase in virus infectivity titer was greater in the cell-associated portion than in the cell-free portion. Serial passage of IBR virus in WI-38 cells infected at an input multiplicity of approximately 10 resulted in good virus growth for 12 serial passages (Table I), but did not achieve the consistently high titers that were always obtained in the BEK cells, even after 12 passages.

Lack of persistency of IBR virus in WI-38 cells at low input multiplicities. At a lower input multiplicity, about 0.02, an almost 3 log difference in virus titer was found between BEK and WI-38 cells (Fig. 2). The peak titer in BEK was reached at 2 days. In WI-38 cells there was a rise in virus infectivity titer, indicative of growth, with a peak at 2 days, but this peak was lower than that in BEK. Comparison of the extent and initial appearance of CPE showed that complete cell destruction occurred in 3 days in BEK, whereas only incomplete CPE was observed in WI-38, reaching a maximum of 2+ or 50% destruction in 4 days. The CPE remained at this level for 7 days and, if the medium was changed, the cells recovered. By 5–6 weeks no evidence of virus-induced CPE was observed. Virus infectivity titers gradually declined until, by 5 weeks after infection, no infectious virus was detectable, even when the infected WI-38 cells were cocultivated with the IBR sensitive BEK cells. The cultures that had recovered after 6 weeks (Fig. 2) were found to be susceptible to reinfection with IBR virus. Similar virus titers were obtained 72 hr after infection with IBR virus from the recovered cultures, which had been previously infected, and from the noninfected control cultures of the same age.

Difference in susceptibility to IBR virus upon aging of WI-38 cells. WI-38 cells at 7 and 14 days after seeding, designated as young and old cultures, were infected at different input multiplicities of IBR virus. The amount of CPE was recorded at 3, 7, and 14 days after infection (Table II). After 3 days, CPE was evident in young cultures with an input multiplicity of 0.0002, while in

TABLE I. SERIAL PASSAGE OF IBR VIRUS IN WI-38 CELLS.

Number of passages in WI-38 cells	Days after infection	Degree of CPE	Virus infectivity titer in BEK (log $TCID_{50}$/0.1 ml)
Inoculum			6.8[a]
1	2	3+	4.6
3	3	3+	3.0
5	4	2+	4.0
7	2	4+	5.6
9	2	4+	5.0
12	3	4+	5.6

[a] Total inoculum log $TCID_{50}$.

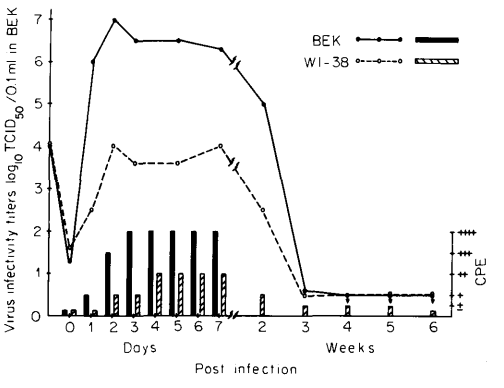

FIG. 2. Growth curves of IBR virus in BEK and WI-38 cell cultures at an input multiplicity of approximately 0.02.

old cultures only when an input multiplicity of 0.25 or greater was used. Apparently the young cultures were more sensitive than the old to infection with IBR virus, as indicated by virus-induced CPE. However, in the young cultures the initial CPE at lower inocula disappeared as the cells regrew by 7 and 14 days.

To better quantitate the difference in sensitivity, the yield of IBR virus in young and old bovine cells and WI-38 cells was then compared. Representative experiments are illustrated in Table III. At an input multiplicity of approximately 2, complete cellular destruction was observed in both young and old BEK cell cultures. In the young WI-38 cells the CPE was half of that in bovine cells, while in the old WI-38 cells only 1+ CPE was observed. Virus infectivity titer varied slightly in both the young and old BEK cells, but in the young WI-38 cells there was about one log higher virus yield than in old WI-38 cells.

Discussion. IBR virus was found capable of replication in the human diploid cell strain, WI-38, in direct proportion to the input multiplicity of infection. At input multiplicities greater than 1, IBR appeared to replicate well in WI-38 cells, although serial passage did not lead to a virus yield comparable to that obtained in the bovine cells. However, following infection at lower input multiplicities, minimal initial cell destruction was observed followed by rapid cell regrowth when the medium was changed. Virus could not be detected in the WI-38 cells 5 weeks after initial infection, even when the infected cells were cocultivated with BEK cells. Since persistent infections were established following infection of hamster cells with low input multiplicities of IBR virus, (unpublished data), it was of interest to note that the same phenomenon did not occur in the WI-38 cells. Thus, even if bovine serum was contaminated with IBR virus at a low level, there would be little likelihood that IBR virus would persist in the human cells, although the possibility that a fraction of the viral genome remained in these cells cannot be completely ruled out.

During the initial course of these experiments on the growth of IBR virus in WI-38 cells, inconsistent results were obtained until it was discovered that the WI-38 cells vary in susceptibility to IBR virus after aging *in vitro* for as little as 1 week. Since all cultures were changed with the same lot of medium immediately after virus adsorption and maintained in the same medium thereafter, the pH could not be a factor for the

TABLE II. EFFECT OF AGING OF WI-38 CELL CULTURES ON SUSCEPTIBILITY TO IBR VIRUS.

Inoculum log $TCID_{50}$	Input multiplicity	Virus induced cytopathic effect (days postinfection)					
		Young culture[a]			Old culture		
		3	7	14	3	7	14
7.0	25	4+	4+	4+	4+	4+	4+
6.0	2.5	2+	4+	4+	2+	3+	4+
5.0	0.25	1+	1+	1+	1+	1+	1+
4.0	0.02	1+	1+	0	0	0	0
3.0	0.002	1+	0	0	0	0	0
2.0	0.0002	1+	0	0	0	0	0

[a] Young culture, 7 days old; old culture, 15 days old.

TABLE III. YIELD OF IBR VIRUS IN YOUNG AND OLD WI-38 CELLS.[a]

Experiment number	CPE				Virus infectivity titer (log $TCID_{50}$/0.1 ml)			
	BEK		WI-38		BEK		WI-38	
	Young	Old	Young	Old	Young	Old	Young	Old
1	4+	4+	2+	1+	6.0	5.6	5.5	4.5
2	4+	4+	2+	1+	6.3	6.5	4.3	3.5
3	4+	4+	2+	1+	7.0	6.5	5.6	4.0

[a] Young culture, 7 days old; old culture, 14 days old.

differences in susceptibility of these cultures. The sensitivity of WI-38 cells to IBR virus infection also varied from lot to lot. Therefore it is possible that discrepancies in results reported by different laboratories may be influenced by the age of the cultures used and the culture lot tested.

Summary. IBR virus was found to replicate in WI-38 cells. At a high input multiplicity the virus yield was comparable to that obtained in bovine cells, but comparable degree of CPE took longer to achieve. At a low input multiplicity of IBR virus, such as may be encountered in virus contaminated bovine serum, virus yield was only about 1% of that in bovine cells, with 50% of the cells showing CPE, followed by cell regrowth. Infectious virus was not recoverable from the regrown cells by 5 weeks after initial infection, and these regrown cells were susceptible to reinfection with IBR virus. Aging of WI-38 cells in cultures for as little as 1 week reduced IBR virus yield to 90% less than the yield from the same lot of cells inoculated 7 days earlier.

This investigation was supported by NIH Contract No. FDA 233-74-1035 from the Food and Drug Administration, and USPHS Research Grant No. AI-08648-07 from the Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. the excellent technical assistance of Susan Rake is appreciated.

1. Madin, S. H., York, C. J., and McKercher, D. G., Science **124,** 721 (1956).
2. Jasty, V., and Chang, P. W., Amer. J. Vet. Res. **30,** 1325 (1969).
3. Schwarz, A. J. F., Zirbel, L. W., Estela, L. A., and York, C. J., Proc. Soc. Exp. Biol. Med. **97,** 680 (1958).
4. McKercher, G. D., Advances Vet. Sci. **5,** 299 (1959).
5. Kniazeff, A. J., *in* "Contamination in Tissue Culture" (J. Fogh, ed.), pp. 233–242. Academic Press, New York (1973).
6. Armstrong, J. A., Pereira, H. G., and Andrewes, C. H. Virology **14,** 276 (1961).
7. Rouhandeh, H., Nature (London) **200,** 386 (1963).
8. Cabasso, V. J., Brown, R. G., and Cox, H. R., Proc. Soc. Exp. Biol. Med. **95,** 471 (1957).
9. Cheatham, W. J., and Crandell, R. A. Proc. Soc. Exp. Biol. Med. **96,** 536 (1957).
10. Michalski, F., and Hsiung, G. D., Proc. Soc. Exp. Biol. Med. **148,** 891 (1975).
11. Hsiung, G. D. "Diagnostic Virology." Yale University Press, New Haven (1973).

Received July 11, 1975. P. S. E. B. M. 1976, Vol. 151.