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Propagation and Isolation of Group A Coxsackieviruses in RD Cells

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The RD cell line, derived from a human rhabdomyosarcoma, supported replication of a number of group A coxsackieviruses, including types A5 and A6 which heretofore have been propagable only in suckling mice. A number of the group A coxsackievirus types which replicated in RD cells had higher titers in this cell line than in other cell culture systems. In tests on a limited number of clinical specimens, RD cells were slightly less sensitive than suckling mice for isolation of group A coxsackieviruses, but they did permit the recovery of certain virus types which previously could be isolated only in suckling mice. Group B coxsackieviruses replicated poorly or not at all in RD cells.

R. L. Crowell and B. Goldberg (Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V44, p. 208) reported that the RD cell line, established by McAllister et al. (2) from a human rhabdomyosarcoma, supported the replication of most of the group A coxsackieviruses, including some types which heretofore have not been propagable in cell culture systems. Studies were conducted in this laboratory to further explore the sensitivity of the RD cell line for propagation of the group A coxsackieviruses, and for isolation of certain group A virus types from clinical materials.

MATERIALS AND METHODS

Cell cultures. The RD cell line was obtained from Paul Arnstein of this laboratory at the 185th passage level. Growth medium consisted of 90% Eagle minimal essential medium and 10% fetal bovine serum. For subpassage, cells were dispersed with a mixture containing a 0.125% concentration of trypsin and a 1:2,000 dilution of versene, and cells from each culture bottle were diluted in growth medium to a volume 12 times that of the original culture volume (1:12 split). Tube cultures were seeded with approximately 100,000 cells in 0.6 ml of growth medium and incubated at 36 C. Medium was replaced after 1 day, and the cultures were used on day 2 after planting. For virus propagation, tube cultures were maintained on 2 ml of Leibovitz medium no. 15 (1) supplemented with 2% inactivated fetal bovine serum.

Stock virus. Stock virus preparations of group A coxsackievirus types 1 to 24 consisted of 20% suspensions of infected suckling mouse muscle, as did those of group B coxsackievirus types 2 and 6. Group B coxsackievirus types 1, 3, 4, and 5 were 20% suspensions of infected suckling mouse brain. The identity of each stock virus preparation was confirmed by neutralization tests (4) against reference equine antisera from the Research Resources Branch (RRB), National Institutes of Health.

Propagation of laboratory strains of group A coxsackieviruses in RD cells. Stock virus preparations of each immunotype were inoculated into two tube cultures of RD cells in a volume of 0.2 ml per tube and carried through three serial passages in this cell line. Subpassages were made as soon as the cultures showed a 3- or 4-plus viral-specific cytopathic effect (CPE), or, if no CPE was seen, blind passages were made at 7- to 8-day intervals. Third passage material of immunotypes which produced CPE in RD cells were titrated in this cell system, and the identity of the virus was confirmed by neutralization tests against homotypic immune hamster serum. The hamster antisera to group A coxsackieviruses were produced in this laboratory, and their identity was confirmed by neutralization tests against reference viruses from RRB. For virus types showing no CPE in RD cells, the third-passage material was examined for infectivity by inoculation into suckling mice.

Virus isolation attempts. Ten stool specimens and one throat washing which had previously yielded group A coxsackieviruses in suckling mice, and which had been stored at −70 C for varying lengths of time, were processed (4) and inoculated in parallel into RD cells (0.25 ml into each of two tube cultures) and into suckling mice less than 1 day of age. Two litters of six mice each were used for isolation attempts, and they were inoculated with 0.02 ml by the intracerebral route and 0.03 ml by the intraperitoneal route; mice were observed for 14 days for signs of illness. Virus recovered in each host system was identified by neutralization tests in RD cells.

RESULTS

Replication of group A coxsackieviruses in RD cells. Results of attempts to propagate laboratory strains of group A coxsackieviruses in RD cells are shown in Table 1. The table

shows the titers, in suckling mice, of the stock virus preparations which were passaged into RD cells and the infectivity titers in RD cells after three passages of the viruses in this host cell system. Titers of RD cell-propagated virus are also compared to those of stock viruses of the same immunotype prepared in other cell culture systems in this laboratory.

Virus types A1, A11, A13, A15, A18, A19, A20, A22, and A24 failed to produce CPE or to replicate in RD cells. Interestingly, a number of these types, viz., A11, A13, A15, A18, A20 and A24, replicate and may be isolated in HeLa and other continuous cell lines of human origin (3). However, virus types A5 and A6, which have not been propagable in other cell culture systems, replicated in RD cells. Virus type A17, which has a low titer and is difficult to work with in other cell culture systems, had a higher titer in RD cells. A number of the virus types which replicated in RD cells had higher titers in this cell line than in other cell culture systems.

The group B coxsackieviruses replicated poorly, or not at all, in RD cells.

Isolation of group A coxsackieviruses in RD cells. Results of parallel isolation attempts in RD cells and suckling mice on stool specimens and the throat washing previously found to contain group A coxsackieviruses are shown in Table 2. All except two of the strains were reisolated on the initial passage of the clinical specimens into suckling mice. One coxsackievirus type A4 strain was isolated in RD cells but

TABLE 1. *Propagation of laboratory strains of coxsackieviruses in RD cells*

Coxsackievirus type	Strain	Titer		Stock virus prepared in other cell cultures	
		Suckling mice	RD cells	Cell type[a]	Titer
A1	Tompkins	6.0[b]	—[c]		
A2	Fleetwood	7.0	7.0[d]	HuAm	5.0[d]
A3	Olson	7.5	6.5	HuAm	5.0
A4	Texas	8.0	6.5	HuAm	4.5
A5	NIH-H_1	8.0	5.0		
A6	Gdula	8.0	4.5		
A7	Parker	6.5	6.5	RhMK	6.5
				HFDK	4.5
A8	HIH-H_2	7.5	5.5	HuAm	6.0
A9	Bozek	6.0	6.0	RhMK	6.5
				HFDK	4.5
A10	Traynor	7.0	7.0	HuAm	5.5
A11	Belgium-1	6.5	—	HFDK	5.0
A12	Texas-12	7.5	7.5	HuAm	3.5
A13	Flores	6.0	—	HFDK	5.0
A14	G-14	7.0	5.0	HFDK	6.0
A15	G-9	6.0	—	HFDK	4.5
A16	G-10	6.0	5.5	RhMK	5.5
				HFDK	4.5
A17	G-12	5.5	3.5	HFDK	2.0
A18	G-13	6.0	—	HFDK	5.5
A19	NIH-8663	6.0	—		
A20	IH-35	3.5	—	HFDK	5.5
A21	Kuykendall	5.5	5.0	HFDK	5.5
A22	Chulman	5.5	—		
A24	Joseph	6.0	—	HFDK	4.0
B1	Conn-5	5.5	—	RhMK	7.0
B2	Ohio-2	5.5	—	RhMK	6.0
B3	Nancy	4.5	2.5	RhMK	6.5
B4	JVB	5.0	—	RhMK	6.5
B5	Faulkner	3.6	3.0	RhMK	6.5
B6	Schmitt	4.0	1.5	RhMK	4.5

[a] HuAm; Primary human amnion cells; RhMK, primary rhesus monkey kidney cells; HFDK, human fetal diploid kidney cell strain.

[b] Log_{10} mean lethal dose per 0.02 ml.

[c] —, No replication.

[d] Log_{10} mean tissue culture dose per 0.1 ml.

TABLE 2. *Comparative sensitivity of suckling mice and RD cells for isolation of certain group A coxsackieviruses from clinical specimens*

Coxsackievirus type	Strain	Year	Reisolation results in	
			Suckling mice	RD cells
A2	LaRa	1967	Pos. P1, day 3[a]	Pos. P2, day 6[b]
A4	KeDu	1965	Neg.	Pos. P2, day 6
A4	DaDo	1971	Pos. P1, day 4	Pos. P2, day 4
A5	SaMi	1972	Pos. P1, day 3	Neg. P3
A5	TyHa	1967	Pos. P1, day 4	Pos. P2, day 4
A6	ElTo	1963	Pos. P1, day 4	Neg. P3
A6	FrEn	1972	Neg.	Neg. P3
A10	DeUn	1965	Pos. P1, day 4	Pos. P1, day 5
A10	SuSh[c]	1970	Pos. P1, day 4	Pos. P2, day 4
A10	ArTu	1971	Pos. P1, day 4	Pos. P1, day 4
A16	GeNo	1966	Pos. P1, day 10	Pos. P3, day 6

[a] Positive (Pos.) on initial passage (P1) of stool suspension into suckling mice; signs of illness appeared on day 3. Neg., Negative.

[b] Positive on the second passage (P2) in RD cells; viral cytopathic effect appeared on the day 6.

[c] Isolated from throat washing; other isolates were from stool specimens.

not suckling mice, and one specimen originally containing type A6 virus was negative in both host systems. Specimens containing virus types A2, A4, A5, A6, and A10 produced overt signs of infection in mice at 3 to 4 days after inoculation; the type A16 isolate required 10 days. Most of the strains required a second passage in RD cells before a clear-cut viral CPE was apparent. Isolation attempts on an A5 and an A6 strain which were positive in suckling mice were negative in RD cells; however, the suckling mouse isolates of these strains could be propagated and identified in RD cells.

DISCUSSION

Our results on propagation of group A coxsackieviruses in RD cells differed from those reported by Crowell and Goldberg (Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V44, p. 208) for certain virus types. The latter investigators found that virus types A11, A15, A18, and A19 were propagable, whereas our results with these types were negative. On the other hand, we found that virus type A17 replicated in RD cells, whereas Crowell and Goldberg did not. Whether RD cells with different passage histories will prove to vary in their viral susceptibilities remains to be determined, and this will be an important factor affecting the general utility of this cell line for work with group A coxsackieviruses.

The use of RD cells in this laboratory has extended the number of group A coxsackievirus types which can be propagated and for which neutralization tests can be conducted in cell cultures, leaving only types A1, A19, and A22 which require suckling mice. The fact that infectivity titers for some of the virus types are higher in RD cells than in other cell culture systems is also an advantage.

Although a number of the lower-numbered group A coxsackievirus types have been adapted to growth in human amnion cells, they have been isolated from clinical materials only in suckling mice. Our results on a limited number of clinical specimens suggest that, whereas RD cells may be slightly less sensitive than suckling mice for isolation of these immunotypes, virus may be recovered in RD cells if more than one passage is made. Thus, for laboratories lacking facilities for suckling mice, the use of multiple passages in RD cells may provide an alternative host system for isolation of some of the group A coxsackieviruses which have previously required suckling mice.

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