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Selective Isolation of Poliovirus in Recombinant Murine Cell Line Expressing the Human Poliovirus Receptor Gene

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Sixty-eight laboratory strains representing 49 enterovirus, 10 adenovirus, and 3 reovirus serotypes were inoculated in a recombinant murine cell line expressing the human poliovirus receptor gene (Lα cells). Only polioviruses caused cytopathic effect over a 10-day period. Likewise, only polioviruses were isolated, by use of Lα cells, from 168 fecal specimens from children from developing countries. These results suggest that the recombinant Lα cells can be used for selective isolation of poliovirus from clinical specimens.

Poliovirus infection in humans is usually subclinical or associated with only nonspecific symptoms; a typical paralytic disease is seen in less than 1% of cases (10). In partially immune human populations, the latter proportion may be even smaller and the virus can circulate widely before being noticed and notified (6, 7). Therefore, a comprehensive epidemiological control of poliomyelitis should include virological surveys in addition to thorough investigation of all clinically suspected cases (7, 18).

The Americas, most of Europe, and some other countries are free of indigenous wild-type poliovirus circulation, but poliovirus infection is still endemic in many developing countries of Africa and Asia (18). Potential import of polioviruses from poliovirus-endemic countries to the virus-free zones can be monitored by screening immigrant children for poliovirus excretion. Putative circulation of poliovirus in a population can be investigated, for instance, by examining fecal specimens from preschool-aged children living in areas suspect for poliovirus. Poliovirus-infected people, whether symptomatic or not, shed considerable amounts of poliovirus into feces for several weeks (10). The virus may survive in sewage and other environmental locations for long periods. Therefore, screening of environmental specimens for poliovirus is yet another method to assess putative circulation of poliovirus in a population (13, 14). All of these approaches are rather tedious, which limits the number of specimens that can be processed and, hence, weakens the coverage of the surveillance. Other enteric viruses, often present in fecal specimens from children and almost regularly found in sewage, require laborious identification to exclude the presence of polioviruses. They may also interfere with or mask the presence of polioviruses and bring about falsely negative results in screening. A further problem in most countries results from the use of the live oral poliovirus vaccine (OPV) in regular immunizations. Abundant OPV-derived attenuated polioviruses may mask the putative presence of wild polioviruses (6, 19).

New techniques are desirable for the monitoring, as well as for the laboratory diagnosis, of poliomyelitis. The latter is improved by the recently developed poliovirus-specific immunoglobulin M assays based on the μ-capture principle (15), but these assays cannot replace isolation of the virus strains in the surveillance. PCR is a powerful method to demonstrate the presence of viral genomes and has also been applied to detect poliovirus and other enteroviruses in both clinical and environmental specimens (1, 2, 4, 8, 16). The method is, however, relatively sophisticated and not yet in wide use in laboratories engaged in diagnostic services or poliovirus surveillance. Virus isolation is also often preferred in the surveillance because of the common need to characterize the virus further, especially for its genetic origin: i.e., vaccine versus wild type, genotype of wild virus, etc. (18).

The HeLa cell surface receptor for polioviruses, a protein of the immunoglobulin superfamily, was identified some years ago (9, 11). A recombinant murine cell line transfected with the human poliovirus receptor cDNA (Lα cells) was established by Koike and coworkers and shown to be susceptible to poliovirus (9). We have obtained this cell line through the courtesy of A. Nomoto and tested its susceptibility to various human enteric viruses. Stationary tube cultures of Lα cells and of the regularly used continuous monkey kidney cell lines (GMK and Vero, from a local cell bank) or the human lung cancer cell line A549 (from T. Hyypiä, University of Turku, Finland) were rinsed with phosphate-buffered saline and inoculated with 100 μl of stock preparations of 68 laboratory strains of human pathogenic enteric viruses. The stocks stored at −70°C had been prepared by freezing and thawing cultures with almost complete cytopathic effect (CPE) and clarifying the lysates by low-speed centrifugation. End point titers of enterovirus stocks in susceptible cells were in the range of 10^6 to 10^8 50% cell culture infectious doses ($CCID_{50}$) per ml; those of adenoviruses and reoviruses were in the range of 10^3 to 10^4 $CCID_{50}$ per ml. Samples of all virus strains were inoculated into the cells at dilutions from 10^0 to 10^{-2}. After 30 min of adsorption at 36°C, 1 ml of the minimum essential medium (local product) supplemented with 2% fetal calf serum was added, the cultures were incubated at 36°C, and potential replication of the inoculated viruses was monitored by daily microscopy. If no CPE was found, the cultures were fed with fresh medium on day 7 and the incubation was continued for another week. Cells from tubes showing any change in morphology, as compared with uninoculated controls, were frozen and thawed and then subcultured, and the putative virus was identified by pools of monotypic neutralizing antibodies. Adenoviruses were identified by a commercial group-specific latex agglutination kit (Adenolex; Orion Diagnostica, Helsinki, Finland). All polioviruses replicated in Lα cells with distinct cytolytic CPE in 3 days, whereas none of the other enteric viruses tested caused reproducible changes in the morphology of Lα cells within a 10-day observation period (Table 1). All

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TABLE 1. Selectivity of Lα cells for poliovirus propagation

Serotype and strain of virus[a]	CPE in Lα cells at: 3 days	10 days
Poliovirus type 1		
Brunhilde	+	+
Mahoney	+	+
Sabin	+	+
Poliovirus type 2		
MEF-1	+	+
Sabin	+	+
Poliovirus type 3		
Finland 84	+	+
Leon	+	+
Sabin	+	+
Saukett	+	+
Coxsackievirus A (9–11, 13, 16, 18, and 21)	–	–
Coxsackievirus (B1–B6)	–	–[b]
Echovirus (1–9, 11–22, 24–30, and 32–33)	–	–
Enterovirus (68, 69, and 71)	–	–
Adenovirus (1–10)	–	–
Reovirus (1–3)	–	–[c]

[a] Most virus reference strains used were obtained in the 1960s and 1970s from the National Institute of Allergy and Infectious Diseases, Bethesda, Md. (5), either directly or via the World Health Organization Enterovirus Reference Centre, Copenhagen, Denmark, and used in these studies after one to five passages at the KTL National Public Health Institute. Exceptions are poliovirus type 1 Mahoney, coxsackievirus B2, enterovirus types 68, 69, and 71 from the American Type Culture Collection and the Sabin strains of the three poliovirus serotypes from the National Institute for Biological Standards and Control, Potters Bar, England.

[b] Coxsackievirus B4 showed slight CPE at 12 days and grew upon subculture.

[c] Reovirus type 2 showed atypical CPE at 14 days and grew upon subculture.

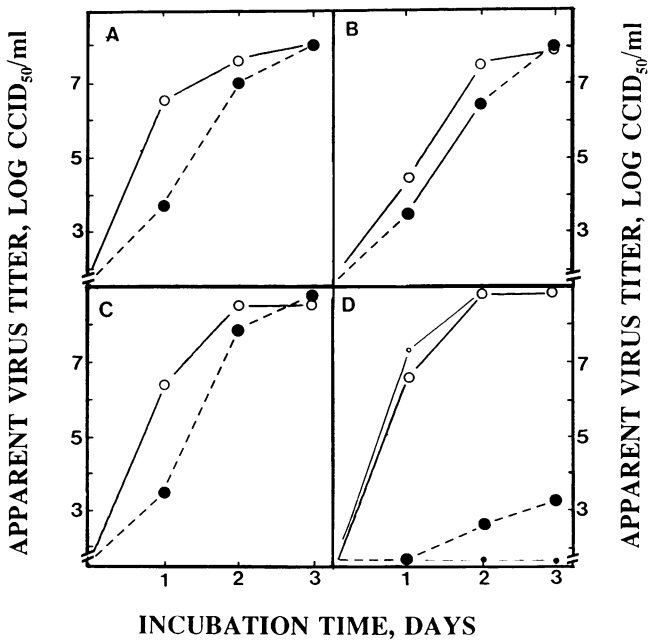

FIG. 1. Kinetics of the appearance of CPE due to selected enteroviruses in Lα and GMK cells. Serial 10-fold dilutions of stock preparations of poliovirus type 1 strain Mahoney (A), strain Sabin (B), and type 3 strain Saukett (C) and high-titer preparations of coxsackievirus B4 (D) (large circles) and B5 (D) (small circles) were inoculated in microtiter cultures of the two cell lines. Four parallel wells of each cell type were used for each dilution. CPE was monitored daily, and apparent titers were calculated. ●, Lα cells; ○, GMK cells.

virus strains readily replicated in one or more of the primate cells tested.

Our results (described above) suggested that Lα cells are highly selective for polioviruses, as judged by a short-term follow-up of inoculated cultures. However, after 12 days of incubation, cultures inoculated with coxsackievirus B4 or reovirus type 2 showed emerging CPE and replication of these two viruses could be confirmed after a subculture in Lα cells. A number of cultures inoculated with other nonpoliovirus viruses also showed some late signs of cell degeneration, but subculture revealed no replicating virus in these cases. Since a blind passage is a normal routine in diagnostic laboratories, it is obvious from these results that a slowly growing isolate from Lα cells should not be considered a poliovirus before specific identification. In further studies, we conducted parallel titrations of purified high-titer preparations of coxsackievirus B2 and B4 (gift from M. Roivainen, KTL National Public Health Institute, Helsinki, Finland) and stock preparations of three poliovirus strains (type 1 Mahoney and Sabin and type 3 Saukett) in Lα and GMK cells.

Poliovirus-induced CPE appeared in Lα cells about one day later than in GMK cells, but the end point titers of all three polioviruses were identical in the two cell lines. In contrast, coxsackievirus B4 showed only a very low titer in the Lα cells, 5 to 6 orders of magnitude lower than that in the GMK cells (Fig. 1). In other experiments, some CPE was also observed in Lα cell cultures inoculated several days before with a high-titer preparation of coxsackievirus B2.

The experiments described above demonstrated that CPE in Lα cells may be occasionally caused by some nonpoliovirus enteric viruses if these viruses are present in large amounts. Since clinical specimens usually contain much lower concentrations of viruses, we then tested the performance of Lα cells in practical screening of clinical specimens. Seventy fecal specimens from Pakistani children with paralytic poliomyelitis (kindly provided by Mubina Agboatwalla, Karachi, Pakistan) and 98 specimens from healthy children arriving in Finland from countries with endemic poliovirus infection were investigated. The Pakistani specimens had been frozen at −20°C for 1 to 4 months before testing. Suspensions (ca. 10%) of fecal material in Hank's balanced salt solution containing 0.5% bovine serum albumin were extracted with chloroform and inoculated, as 100-μl aliquots of 1:1, 1:2, and 1:10 dilutions, in tube cultures of Lα and GMK cells, and the first set of specimens was also inoculated into HEp-2c cells. Cultures were incubated for about 2 weeks at 36°C. Cytopathic viruses were identified as described above. One blind passage was made, and cultures remaining unaffected after a further 2 weeks of incubation were considered negative. Sensitivity of Lα cells in poliovirus isolation was at least as good as that of the primate cells (Table 2). All specimens yielding a poliovirus in any of the cell lines tested were positive in the Lα cells, while both GMK and HEp-2c cells missed one or more poliovirus strains. The specificity of Lα cells was also excellent for the present set of specimens: none of the 21 nonpoliovirus viruses isolated in the primate cells caused CPE in Lα cells.

Since both wild-type and OPV-derived polioviruses replicate in Lα cells, the use of these cells does not solve the problem of potential masking of wild-type poliovirus circulation in the presence of abundant live vaccine viruses. PCR as such has been used successfully for specific amplification of a known wild poliovirus genotype (20), and PCR followed by restriction fragment length polymorphism analysis is a promising approach for a more general application (3, 17). However, at

TABLE 2. Performance of different cell cultures in isolation of viruses from 168 fecal specimens from poliomyelitis patients (specimen collection I) or healthy immigrants (II)

Virus isolated	Specimen collection	No. of specimens with indicated viral isolation result in cell line:			Cumulative no. of specimens yielding virus
		Lα	GMK	HEp-2c	
Poliovirus	I	43	41	39	43
	II	20	20	NT[a]	20
Enterovirus (not poliovirus)	I	0	11	3	11
	II	0	3	NT	3
Adenovirus	I	0	2	1	2
	II	0	5	NT	5
No virus	I	27	16	27	
	II	78	70	NT	

[a] NT, not tested.

present the latter method may not be able to detect a wild strain present as a minority population among abundant vaccine strains.

Our results with clinical specimens suggest that the use of the recombinant Lα cells, rather than one or more lines of primate cells, in poliovirus surveillance would simplify the work by reducing the number of isolates to be identified without a decrease in sensitivity. Our preliminary results suggest that the specificity of Lα cells in environmental screening, if not as excellent as suggested by the clinical material described above, will also here considerably reduce the number of cytopathic agents to be identified by costly and tedious serum pool neutralization. As for virological diagnosis of suspected poliomyelitis, it is advisable to use other cell lines as well because nonpoliovirus enteroviruses may cause paralytic disease occasionally.

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ADDENDUM

While the manuscript was in preparation, Pipkin and coworkers published a paper suggesting similar selectivity for laboratory strains of enteric viruses of an independently established poliovirus receptor-expressing murine cell line (12).

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