

A-549 is a Suitable Cell Line for Primary Isolation of Coxsackie B Viruses

J.R. Otero,^{1*} L. Folgueira,¹ G. Trallero,² C. Prieto,¹ S. Maldonado,¹ M.J. Babiano,¹ and I. Martinez-Alonso¹

¹Clinical Virology Unit, Microbiology Department, Hospital "12 de Octubre", Madrid, Spain

²Enterovirus Reference Laboratory, Centro Nacional de Microbiología, Majadahonda, Madrid, Spain

A common receptor for coxsackie B virus and adenovirus has been described recently in cells of human and murine origin. Since the established cell line A-549 is suitable for adenoviruses, the potential use of A-549 cells for the isolation of coxsackie B viruses from clinical samples was investigated. All throat swabs sent to the laboratory between April 1998 and June 1999 were inoculated onto monolayers of MRC-5 and A-549 cells in tubes, and the enterovirus isolates obtained were typed. From April to June 1999, A-549 cells were compared prospectively to Buffalo green monkey (BGM) cells, considered as the most susceptible cell line for isolating coxsackie B viruses. Fifty-six out of 171 enterovirus isolates (33%) displayed a cytopathic effect (CPE) in the A-549 monolayer only, 48 isolates (28%) in the MRC-5 monolayer only, and 67 isolates (39%) in both cell lines. Most isolates that showed CPE in A-549 cells only (48 out of 56, 86%) were coxsackie B viruses, belonging to four different serotypes (B1, B2, B4, and B6). When BGM and A-549 cells were inoculated in parallel, both recovered the same number of coxsackie B isolates ($n=20$), and the CPE was noted on approximately the same day. In conclusion, growth in A-549 but not MRC-5 cells identified coxsackie B viruses in most cases. A-549 was comparable to BGM for primary isolation of coxsackie B viruses. *J. Med. Virol.* 65:534–536, 2001. © 2001 Wiley-Liss, Inc.

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INTRODUCTION

Samples are submitted often to the clinical virology laboratory with a vague request for viral culture, without specifying the investigation of a particular agent. Considering the diversity of cell lines available for growing clinically significant viruses and the lack of precise standardisation, clinical virology laboratories

usually select their own combinations of cell lines to be used in routine, depending on technical and economical factors. In our laboratory, a diagnostic virology facility in a teaching hospital, samples received with such a non-specific request are inoculated into a general cell culture system, incorporating only two cell lines, the MRC-5 strain of foetal lung fibroblasts and the established epithelial cell line A-549. This choice was based on the evidence that many of the viruses isolated most commonly, including herpes simplex virus, cytomegalovirus, varicella-zoster virus, adenovirus, para-influenza viruses, Respiratory Syncytial virus and a number of enteroviruses, can be isolated readily by using this combination [Smith et al., 1986; Woods and Young, 1988; Wiedbrauk and Johnston, 1993; Brumback and Wade, 1996; Huang and Turck, 2000]. Following this inoculation protocol with throat swabs, the observation of an enteroviral cytopathic effect (CPE) in MRC-5 cells was frequent and expected, since this is a classical cell line for enteroviruses [Lee et al., 1965; Bell and Cosgrove, 1980; Dagan and Menegus, 1986; Wiedbrauk and Johnston, 1993; Rotbart, 1999]. The simultaneous growth in MRC-5 and A-549 cells was also a common finding. However, more intriguing was the observation of this CPE in the A-549 monolayer only. The exact nature of these isolates was investigated in order to define the use of the A-549 cell line for primary isolation of enteroviruses and, more specifically, coxsackie B viruses.

MATERIALS AND METHODS

Cell Lines

The MRC-5 strain of foetal lung fibroblasts, received regularly from Vircell Laboratories (Granada, Spain) at passage number 26 to 30, was passaged further for use in the following 2-week to 1-month period. A-549, an established epithelial cell line derived from a human

*Correspondence to: J.R. Otero, Servicio de Microbiología, Hospital "12 de Octubre," Avda. de Córdoba s/n, 28041, Madrid, Spain. E-mail: jotero@arrakis.es

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lung carcinoma (American Type Culture Collection, ATCC-CCL 185, at passage number 77), and Buffalo green monkey kidney cells (BGM, kindly provided by M. D. Bermúdez, Centro Nacional de Microbiología, Majadahonda, Madrid) were also maintained and prepared in our laboratory. The growth medium was medium 199 (Sigma-Aldrich Co., Ltd., Irvine, UK) for MRC-5 cells or Eagle Minimum Essential Medium (Sigma-Aldrich) for A-549 and BGM cells, with Hepes, 10% foetal calf serum, L-glutamine, and antibiotics. The maintenance medium was Basal Medium Eagle (Gibco BRL, Life Technologies, Ltd., Paisley, Scotland) with Hepes, 2% foetal calf serum, L-glutamine, and antibiotics.

Isolation Procedures

Throat swabs, received in 2 ml of viral transport medium (ViralPack, Biomedics S.L., Madrid, Spain), were inoculated in a volume of 0.2 ml to monolayers of MRC-5 and A-549 cells in tubes. From April to June 1999, a third tube with a monolayer of BGM cells was added. All tubes were incubated at 37°C in stationary phase and scored daily for CPE for 7 days or until CPE developed. Enteroviral isolates were submitted to the Enterovirus National Reference Laboratory (Centro Nacional de Microbiología, Majadahonda, Madrid) to be typed by the standard method of virus neutralization [Melnick and Wimberly, 1985].

RESULTS

Between April 1998 and June 1999, 171 enteroviral isolates were recovered from throat swabs. Considering growth in A-549 and MRC-5, 56 isolates (33%) displayed an enterovirus CPE in the A-549 monolayer only (group 1), 48 isolates (28%) in the MRC-5 monolayer only (group 2), and 67 isolates (39%) in both cell lines (group 3). All isolates in group 1, 23 isolates in group 2, and 21 isolates in group 3 were typed, and the results are shown in Table I. Most isolates in group 1 (48 of 56, 86%), were coxsackie B viruses that belonged to four different serotypes (B1, B2, B4, and B6). The mean number of days to the appearance of CPE in A-549 cells for group 1 isolates was 4 (range 2-7). It was also interesting to observe that, in those instances when

both A-549 and MRC-5 cells were positive (group 3), 37 out of 67 isolates (55%) were detected 1 to 4 days earlier (mean 1.8 days) in A-549 cells.

Early in the study, it was evident that most isolates in group 1 were coxsackie B viruses. Therefore, BGM cells were added to the general cell culture system to be compared with A-549 cells. Thus, three tubes with, respectively, monolayers of A-549, MRC-5, and BGM cells were inoculated from April to June 1999, a period of traditionally high incidence of enterovirus infection in Madrid. Ninety enteroviral isolates (12%) were recovered from 746 throat samples collected during this 3-month period. It was observed that all isolates ($n=20$, 22%) growing in A-549 but not MRC-5 cells (group 1) also displayed CPE in BGM cells. All these isolates were identified as coxsackie B viruses (2 coxsackie B1, 7 coxsackie B2, 4 coxsackie B4, 5 coxsackie B6, and 2 isolates reported as coxsackie B viruses of an unidentified serotype). The CPE in A-549 and BGM cells was noted on the same day, or no more than one day later. To compare further the susceptibility of both cell lines, a known clinical sample with coxsackie B6 virus was titered in parallel in A-549 and BGM monolayers. The titre obtained by the Spearman-Kärber method was slightly higher in A-549 (5.10^7 TCID₅₀/ml) than in BGM cells ($5.10^{6.5}$ TCID₅₀/ml). Considering that members of the coxsackie B3 and B5 serotypes were not isolated in our study, the T.V. Dee strain of coxsackie B3 (kindly provided by Dr. A.M. van Loon, National Institute of Public Health, Bilthoven, The Netherlands) was inoculated to A-549 monolayers, with the result of a rapid manifestation of CPE. Unfortunately, a coxsackie B5 strain was not available.

DISCUSSION

The general rules of growth of enteroviruses in the different cell lines are well established. In the case of coxsackieviruses, some serotypes in group A (A1, A19, and A22) depend absolutely on inoculation of suckling mice for isolation [Schmidt et al., 1975], but members of this group can be isolated in primary monkey kidney cells, human embryonic fibroblasts, or RD cells [Lee et al., 1965; Wecker and Ter Meulen, 1977; Schmidt

TABLE I. Identification of Enterovirus Isolates Growing in A-549 and MRC-5 cells

Group 1 Growth in A-549		Group 2 Growth in MRC-5		Group 3 Growth in A-549 and MRC-5	
SEROTYPE ^a	(Number of isolates)	SEROTYPE	(Number of isolates)	SEROTYPE	(Number of isolates)
Coxsackie B1	(3)	ECHO 2	(1)	ECHO 6	(1)
Coxsackie B2	(20)	ECHO 7	(7)	ECHO 7	(2)
Coxsackie B4	(8)	ECHO 9	(3)	ECHO 11	(7)
Coxsackie B6	(15)	ECHO 11	(3)	ECHO 17	(4)
Coxsackie A16	(3)	ECHO 17	(8)	Polio 1, Sabin-like	(4)
Unidentified CBV	(2)	Coxsackie A9	(1)	Unidentified EV	(3)
Unidentified EV	(5)				

^aEV, enterovirus; CBV, coxsackie B virus.

et al., 1978). Coxsackie B viruses are more readily grown in cell cultures, and Johnston and Siegel [1990] proposed a rapid identification based on growth in primary monkey kidney and HEp-2 cells but not RD cells. However, primary monkey kidney cells are expensive or unavailable to many laboratories, and Hep-2 and RD cells are difficult to manage due to rapid growth and deterioration. It is currently accepted that BGM is the most susceptible cell line for coxsackie B viruses [Schmidt et al., 1978; Menegus and Hollick, 1982; Dagan and Menegus, 1986]. A-549 cells were not even considered in a recent review [Rotbart, 1999], although Woods and Young pointed out their potential utility in 1988. A common receptor for coxsackie B viruses and adenoviruses (called the Coxsackie-Adenovirus receptor, CAR) has been described recently in the membrane of cells of human and murine origin [Bergelson et al., 1997]. In this context, it is not surprising to find that A-549 cells, described originally as excellent for adenoviruses [Smith et al., 1986], are also suitable for coxsackie B viruses.

The data obtained demonstrate that, whereas most enteroviral isolates that display CPE on A-549 but not MRC-5 cells (group 1) were identified as coxsackieviruses (in most cases group B), none of the isolates growing in MRC-5 or in both MRC-5 and A-549 (groups 2 and 3) was a coxsackie B virus. However, it is true that the scarcity of neutralisation reagents allowed the identification of only 44 out of 115 (38%) of these non-group 1 isolates. Interestingly, when A-549 and BGM were compared, the same number of coxsackie B isolates was recovered from both cell lines, and the CPE appeared on approximately the same day. The first CPE of coxsackie B virus in A-549 cells was detected later than in previous studies evaluating BGM cells [Dagan and Menegus, 1986; Menegus and Hollick, 1982], but the mean time for CPE detection may have been prolonged falsely because the tubes were not scored for CPE during the weekend. Some researchers have found a small number of coxsackie B virus isolates, ranging from 7% to 23%, growing in MRC-5 cells [Menegus and Hollick, 1982; Dagan and Menegus, 1986]. The absence of coxsackie B virus isolates in MRC-5 cells in this study could be related to the short incubation period (7 days), but even accepting that some coxsackie B virus isolates will grow in MRC-5 cells if the incubation period is long enough, the identification of a probable coxsackie B virus will be anticipated by an earlier CPE in A-549 cells.

In conclusion, the A-549 cell line seems suitable for primary isolation of coxsackie B viruses, and it was shown as comparable to BGM for this purpose. Although BGM is accepted as the most appropriate cell line for coxsackie B viruses, this is its only major contribution to diagnostic virology. However, A-549 cells could be more convenient in practice, considering the wide spectrum of susceptibility to other viruses that are important clinically.

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