JOURNAL OF CLINICAL MICROBIOLOGY, June 1992, p. 1611–1613
0095-1137/92/061611-03$02.00/0

Vol. 30, No. 6

An Adventitious Viral Contaminant in Commercially Supplied A549 Cells: Identification of Infectious Bovine Rhinotracheitis Virus and Its Impact on Diagnosis of Infection in Clinical Specimens

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Received 3 February 1992/Accepted 27 March 1992

The isolation and identification of an adventitious viral agent, infectious bovine rhinotracheitis virus, in one lot of A549 cells from a commercial supplier is described in this report. The presence of infectious bovine rhinotracheitis virus in A549 cells was unexpected and has caused problems in the diagnosis of infections in clinical specimens in three laboratories.

Contamination with adventitious viral agents in tissue culture cells, especially endogenous viruses in primary cells, has been known for many years; examples include simian viruses in monkey kidney cells (4), guinea pig herpesvirus in guinea pig kidney or embryo cells (6), and equine herpesvirus in horse kidney cells (5). In continuous cell lines, endogenous retroviruses have been reported (7). In addition, adventitious viral agents in bovine serum, which is an essential major component of cell culture medium, have been reported (1–3, 8–10). Since routine quality control procedures for bovine serum used for tissue culture medium now include screening for viruses of bovine origin, mycoplasmas, and other contaminants (1), adventitious viral contamination in tissue culture introduced by contaminated bovine serum is not frequently encountered. In this communication, isolation and identification of an infectious bovine rhinotracheitis virus (IBRV) contaminant from a commercially supplied A549 cell culture and its impact on the diagnosis of infections in clinical specimens are described.

During the week of 7 October 1991, one lot of A549 cells from a commercial supplier was inadvertently contaminated by a viral agent. Many laboratories received this lot of A549 cells for laboratory tests without knowledge of the contamination. A few days after specimen inoculation into this lot of A549 cells, six viral isolates from six separate specimens were obtained in our laboratory (lab A) (Table 1). These six specimens were three eye swabs obtained during an outbreak of epidemic keratoconjunctivitis, one throat swab, one lip lesion swab, and one rectal aspirate. The cytopathic effects (CPE) of these six isolates in A549 cells were similar, consisting of characteristic rounding with some syncytial formation which somewhat resembled the CPE induced by herpes simplex virus (HSV). On the basis of the characteristic CPE and specimen sources, immunofluorescence assays using monoclonal antibody to HSV-1 and HSV-2 (Syva Co., Palo Alto, Calif.), polyclonal antibody against HSV (Whittaker Bioproducts, Walkersville, Md.), monoclonal antibodies to varicella-zoster virus (Ortho Diagnostic Systems, Inc., Raritan, N.J.), and respiratory syncytial virus and adenovirus group antigen (Centers for Diseases Control) were performed repeatedly, but no viruses could be identified.

In the meantime, the second laboratory (lab B), with which we collaborate in many areas, had a virus isolate in the same lot of A549 cells from a bronchoalveolar lavage of a bone marrow transplant patient with an undiagnosed severe pneumonitis. The isolate could not be identified by routine immunofluorescence assays using monoclonal antibody to HSV-1 and HSV-2 (Syva Co.) and adenovirus group antigen (Centers for Disease Control) (Table 1). Because of the urgent situation, the laboratory (lab B) reported to the physician the possible isolation of HSV on the basis of CPE alone, which was not its routine procedure. Acyclovir treatment of this patient was initiated. Over the following 2 days, the laboratory personnel of lab B observed a similar atypical CPE in A549 cultures inoculated with specimens from six other patients, including a bone marrow aspirate, a throat swab, an esophagus biopsy specimen, and three stool specimens (Table 1), and in two uninoculated tubes of A549 cells. Contamination of A549 cells with an adventitious viral agent was then suspected. The commercial supplier and the patient's physician were notified that an adventitious agent was present in the A549 cell cultures.

During the same period, a third laboratory (lab C) sent an isolate to lab A for identification by electron microscopy, since lab C was unable to identify it by routine procedures. This isolate was also obtained from the same lot of A549 cells from the same commercial supplier. Lab C also kept uninfected cells from each lot for negative controls, but no spontaneous cell degeneration in their uninfected A549 control cell cultures in that particular lot was observed.

In order to identify the viral contaminant, electron microscopic examination of the supernatants of the infected cell cultures negatively stained with potassium phosphotungstate was performed, and a herpesviruslike particle was observed. Subsequently, six isolates grown in A549 cells, five from lab A and one from lab C, were fixed in 2% buffered glutaraldehyde, postfixed in osmium tetroxide, and embedded in Epon as described previously (3). Thin sections were cut, stained with uranyl acetate and lead citrate, and examined with an electron microscope. Herpesvirus particles with similar cellular changes were found in all six cases (Fig. 1). The virus was finally identified by direct immunofluorescence assay using fluorescein isothiocyanate-conjugated goat anti-bovine rhinotracheitis virus immunoglobulin G (Colorado Serum Co. Laboratories, Denver, Colo.).

Immediately after the identification of IBRV, the five

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TABLE 1. Isolation and attempted identification of isolates from clinical specimens in IBRV-contaminated A549 cells

Lab code	Specimen no.	Specimen source	Result of[a]:			
			IFA using antibody to:		EM examination	Reinoculation from original specimens
			HSV	Adenovirus		
A	91-8819	Eye	–	–	Herpesvirus	Adenovirus
	91-8820	Eye[b]	–	–	Herpesvirus	–
	91-8821	Eye[b]	–	–	ND	ND
	91-8849	Rectal aspirate	–	–	Herpesvirus	Adenovirus
	91-8853	Throat	–	–	Herpesvirus	–
	91-8854	Lip	–	–	Herpesvirus	–
B	91-1190	Throat	ND	ND	ND	ND
	91-1196	Stool	ND	–	ND	–
	91-1221	Esophageal biopsy	–	–	ND	ND
	91-1227	Stool	ND	–	ND	–
	91-1319	Bronchoalveolar lavage	–	–	ND	ND
	91-1704	Bone marrow	ND	–	ND	–
	91-2094	Stool	ND	–	ND	–
C	91-9335	Throat	–	–	Herpesvirus	ND

[a] IFA, immunofluorescence assay; EM, electron microscopic; ND, not determined; –, negative. All A549 cells exhibited CPE.
[b] Specimens obtained from the same patient.

original specimens from lab A were reinoculated into a separate lot of A549 cells, and adenovirus was isolated from two specimens and identified by immunofluorescent technique using monoclonal antibody to adenovirus common antigen (Centers for Disease Control). These two adenovirus isolates were not typed. The problems in laboratory diagnosis of infection in clinical specimens by using IBRV-contaminated A549 cells in the three laboratories described above are summarized in Table 1.

Since the presence of IBRV as a contaminant in A549 cells was unexpected, all three laboratories experienced problems identifying the isolates by routine procedures. In addition, only about 10% of the cultures (estimated by lab A and lab B) of this lot of A549 cells developed CPE induced by IBRV. Cells in an uninoculated A549 tube kept for a control in lab A did not develop CPE. An adventitious viral agent was not suspected until the discovery of cells in two uninoculated A549 tubes from lab B with similar viral CPE.

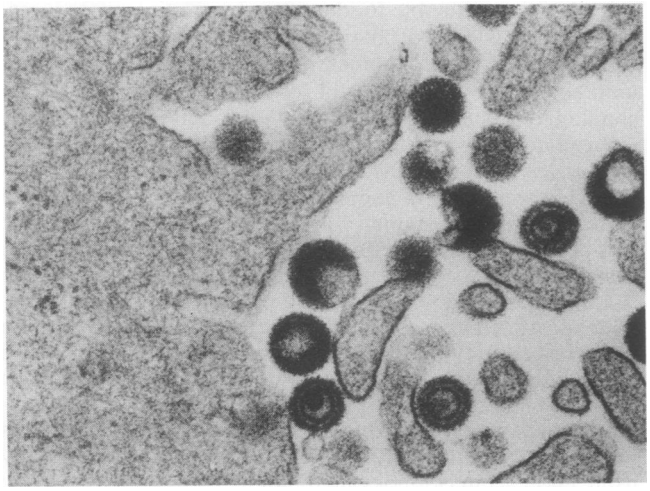

FIG. 1. Electron micrograph of an A549 cell infected with the contaminating IBRV, showing many herpesvirus particles in the extracellular space. Magnification, ×48,000.

Electron microscopic examination was an essential step in providing a quick primary identification of the unknown agent. According to the commercial supplier, the source of the IBRV contamination was thought to be the calf serum used in freezing the seed stocks of A549 cells. This calf serum had been tested by an independent laboratory for adventitious bovine viruses, but no virus had been detected. To prevent the reoccurrence of this problem, the company destroyed the existing stock of A549 cells, obtained a new starter seed from the American Type Culture Collection, removed the lot of fetal bovine serum used, and revised the standard operation procedure for sterility and viability testing of frozen seed stocks to include observation of cells for CPE (6a). Although quality control of bovine serum includes screening for viral agents, contaminants may not be detected if they are present in small amounts.

This report has demonstrated that the unexpected IBRV contamination in a lot of commercially supplied A549 cells led to an erroneous report of HSV-induced CPE and interfered with adenovirus isolation from clinical specimens. In addition, false-negative reports may have resulted, since labile viruses may fail to be isolated upon reinoculation of original specimens. The problems in laboratory diagnosis of infections in clinical specimens by using endogenous virus-contaminated cells are well illustrated.

We wish to express our thanks to G. D. Hsiung for her valuable suggestions and her gift of fluorescein isothiocyanate-conjugated anti-IBRV immunoglobulin G and Frank Michalski for the specimen, the valuable suggestions, and the relevant information obtained from his laboratory.

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