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

CAROLINE K. Y. FONG,1,2* AND MARIE L. LANDRY1,2,3

Virology Reference Laboratory, Veterans Affairs Medical Center, West Haven, Connecticut 06516,1 and Departments of Laboratory Medicine2 and Internal Medicine,3 Yale University School of Medicine, New Haven, Connecticut 06510

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 Disease 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

* Corresponding author.
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.

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.

REFERENCES
6a. Johnson, R. W. Personal communication.
Revised Nomenclature of *Alloiococcus otitis*

The species named *Alloiococcus otitis* by Aguirre and Collins (1, 2) should be renamed *Alloiococcus otitidis*. Rule 12c of the Bacteriological Code (3) states that a specific epithet must be treated as an adjective, as a substantive in apposition in the nominative case, or as a substantive in the genitive case. "A. otitis" would belong to the second category ("otitis," grammatical category ("otitis," grammatical category), meaning "ear inflammation"), but such a combination does not make sense from a grammatical point of view. Rather, the genitive case, i.e., *Alloiococcus otitidis* ("of the ear inflammation"), analogous to *Neisseria meningitidis* or *Pseudomonas pseudomallei*, must be applied to this new species.

REFERENCES

A Case of Infectious Bovine Rhinotracheitis Virus Contamination

The recent article on contamination of commercially supplied A549 cells by infectious bovine rhinotracheitis virus (IBRV) (2) points out the risk of adventitious viral agents in biological materials. In the case cited above, there were multiple isolates of an agent which caused a cytopathic effect (CPE) somewhat resembling the CPE caused by herpes simplex virus. The agent was also present in two un inoculated control tubes, suggesting an adventitious agent. The Viral and Rickettsial Disease Laboratory (VRDL), California Department of Health Services, had a recent experience with IBRV which involved only a single specimen and which probably have remained unidentified except for reference to the report cited above.

In April of 1991 the VRDL received from a county public health laboratory a cell culture-passage specimen from a tracheal aspirate obtained from a 71-year-old male for identification. The isolate, submitted in African green monkey kidney cells, was reported to cause an enterovirus-like CPE and to be neutralized by Lim and Benyesh-Melnick (LBM) pools ABE and H. This combination of pools does not identify a virus, and an enterovirus was considered unlikely diagnosis, on the basis of the type of CPE. The agent was identified as a herpesvirus-like virus by electron microscopy. However immunofluorescence (IF) assays for herpes simplex virus types 1 and 2, varicella-zoster virus, and cytomegalovirus were negative. Further investigation into the origin of the specimen revealed that it had been initially isolated by a third laboratory in commercially prepared primary rhesus monkey kidney (PRK) pools and then passed into primary rhesus monkey kidney cells. The possibility of herpes B virus, originating from the monkey cells, was ruled out by testing done on an aliquot of infected cell culture material sent to the Southwest Foundation for Biomedical Research, San Antonio, Tex. A variety of immune sera to simian herpesviruses were tested by indirect IF, and all were negative. Likewise, normal rabbit and human sera were negative in the indirect IF assay. There was weak staining with human herpesvirus 6 immune and negative control sera (Granbio) and with one rabbit preimmune serum. Upon becoming aware of the report by Fong and Landry (2), Dr. Fong was contacted, and she agreed to test our isolate and subsequently identified it by IF. Repeat testing of the LBM pools with the isolate at the VRDL resulted in no neutralization even with a very low virus dose.

IBRV is a known contaminant of fetal bovine serum (1). The commercial supplier of the fetal bovine serum and the PRK cells was informed that IBRV, most likely originating from PRK cells or serum purchased from them, had been identified. They indicated that they were aware of IBRV contamination and have incorporated culture of serum samples and filtration of serum to 0.1 μm in order to control this potential problem. Our experience points out the difficulty of identifying a rare adventitious virus, especially when there is only a single isolate, and also the need for a complete history of agent passage in cell culture for reference laboratories to work efficiently with submitted specimens.

The need to recognize different types of CPE and to consider adventitious viruses when unusual CPE occurs or when an isolate cannot be identified remains an important potential problem of which diagnostic and reference virology laboratories must be aware.

REFERENCES

David Schur
Marge Dondero
Juanita Dennis

Viral and Rickettsial Disease Laboratory
Division of Laboratories
California State Department of Health Services
2151 Berkeley Way
Berkeley, California 94704