Laboratory and Epidemiology Communications

Picornavirus-Like Cytopathic Effects on RD-18S Cell Lines Were Induced by Human Coronavirus 229E Not Picornaviruses

Yohei Matoba1*, Yoko Aoki1, Shizuka Tanaka1, Kazue Yahagi1, Tsutomu Itagaki2, Yoko Matsuzaki3, and Katsumi Mizuta1

1Department of Microbiology, Yamagata Prefectural Institute of Public Health, Yamagata 990-0031; 2Yamanobe Pediatric Clinic, Yamagata 990-0301; and 3Department of Infectious Diseases, Yamagata University Faculty of Medicine, Yamagata 990-9585, Japan

Communicated by Makoto Takeda

We have used the HHVe6MRG plate, containing HEF, HEp-2, VeroE6, MDCK, RD-18S, and GMK cell lines, for virus isolation to clarify the epidemiology of acute viral respiratory infections in children in Yamagata, Japan, since January 2004, as part of the National Epidemiological Surveillance of Infectious Diseases, Japan (NESID), based on the Infectious Diseases Control Law (1). We also prepared HMV-II and LLC-MK2 cell lines as separate 96-well tissue culture plates (Greiner Bio-One, Frickenhausen, Germany) since 2008 and 2011, respectively, to isolate parainfluenza viruses (2-4). However, we have not yet isolated any human coronaviruses (HCoVs), which are generally considered as common pathogens, using these systems. To confirm whether HCoVs circulate in Yamagata, we performed a study to detect HCoV-229E, -HKU1, -NL63, and -OC43 using a molecular method, i.e., reverse-transcription (RT)-PCR, and sequence analysis using stored frozen respiratory specimens (5). This study revealed that all 4 HCoVs had been in circulation in Yamagata between 2010 and 2013 and had even caused occasional local outbreaks (5).

When we compared the results of the above recent molecular study with those obtained from routine virus isolation in 2010, we noticed that 6 specimens (756-Yamagata-2010, 775-Yamagata-2010, 777-Yamagata-2010, 882-Yamagata-2010, 1060-Yamagata-2010, and 1127-Yamagata-2010), for which we observed undetermined picornavirus-like cytopathic effects (CPEs) on RD-18S cell lines, also showed HCoV-229E-positive results for RT-PCR (5). We had previously isolated enteroviruses and rhinoviruses, which showed so-called picornavirus-like CPEs on RD-18S cell lines, such as the granular rounding of cells and subsequent cell destruction, in our laboratory up to 2010. Thus, we attempted to identify whether the 6 isolates were enteroviruses or rhinoviruses using only RT-PCR with primers such as EVP4, OL68-1, AN88, AN89, 040, and 011, (6-8), which are commonly used in our laboratory. However, we failed to identify these isolates, and they were thereafter stored without identification. We did not consider the possibility that they were HCoVs at that time. Accidentally, a combination of findings from the molecular screening of HCoVs and routine virus isolation sug-
HCoV-229E genomes. We had additional 7 unidentified finally confirmed that all these 5 strains contained COR229ENUC-R1R for sequence analysis (9). We also used the primers COR229ENUC-R2F and suggested that these 6 isolates were HCoV-229E strains. We therefore attempted to confirm whether these 6 strains were HCoV-229E.

We confirmed that 5 strains isolated using RD-18S cell lines in 2010 were stored in a deep freezer; however, 1 strain (1127-Yamagata-2010) was unfortunately lost. We again inoculated these stored viral fluids onto RD-18S cell lines using a maintenance medium without crystallized trypsin (1) and confirmed the presence of picornavirus-like CPEs (Fig. 1). We then extracted RNAs from the viral fluid, amplified them with HCoV-specific primers, and performed sequence analysis as reported previously (5). We also used the primers COR229ENUC-R2F and COR229ENUC-R1R for sequence analysis (9). We finally confirmed that all these 5 strains contained HCoV-229E genomes. We had additional 7 unidentified viral fluids on RD-18S cell lines that were stored in the freezer for a number of years since 2001–2008. We also confirmed that 1 of these strains (452-Yamagata-2008) contained the HCoV-229E genome. We have registered sequence data for the above 6 isolates under GenBank accession numbers LC005736–LC005741.

Fig. 1. Cytopathic effects of HCoV-229E (A–D) and poliovirus type 2 (E, F) on RD-18S cell lines. Granular rounding of cells and subsequent cell destruction, which were virtually indistinguishable from those caused by picornaviruses, were observed using a maintenance medium without crystallized trypsin (A and E, manifiguration ×100; B and F, ×200). Syncytia formations were clearly observed (C, ×100; D, ×200), whereas they were not found on poliovirus-inoculated cells (G, ×100; H, ×200) using a maintenance medium containing trypsin.

ACKNOWLEDGMENTS

We thank the medical staff and people of Yamagata Prefecture for their collaboration in specimen collection for the national epidemiological surveillance of infectious diseases in Japan.

Conflict of interest None to declare.

REFERENCES