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Chance Isolation of Non-Pathogenic Vaccine-Derived Measles and Rubella Viruses from Children with Acute Respiratory Infections

Yoko Aoki¹, Yohei Matoba*, Shizuka Tanaka¹, Kazue Yahagi¹, Sueshi Ito², Hiroshi Yoshida², Tsutomu Itagaki³, and Katsumi Mizuta¹

¹Department of Microbiology, Yamagata Prefectural Institute of Public Health, Yamagata; ²Department of Pediatrics, Tsuruoka Municipal Shonai Hospital, Yamagata; and ³Yamanobe Pediatric Clinic, Yamagata, Japan

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To study the epidemiology of viral acute respiratory infections in children, we have been isolating viruses from nasopharyngeal specimens including 6 cell lines (HEF, HEp-2, VeroE6, MDCK, RD-18S, and GMK) using a microplate method for over a decade as part of the National Epidemiological Surveillance of Infectious Diseases in Japan (NESID) based on the Infectious Diseases Control Law (1). We introduced the LLC-MK2 cell line in October 2011, mainly to isolate para-influenzavirus type 4 (2). When images of the cytopathic effects (CPEs) show typical morphological changes in sensitive cell lines, we can estimate the causative virus and readily carry out identification steps using a neutralization test, hemagglutination inhibition test, reverse-transcription PCR (RT-PCR), and sequence analysis (1). However, we struggle to identify isolates when the CPEs are not typical or are unfamiliar, and we cannot estimate the viral agents producing the morphological changes.

We previously described an example of this difficulty in which it took approximately 3 months to identify isolates as measles virus (MV) vaccine-derived strains (3). Until that time, we had usually isolated MVs from patients with suspected MV infection using a Vero/hSLAM cell line (4,5). We never suspected that the isolates from children with acute respiratory infections and with no clinical suspicion of MV infection would be MV vaccine-derived strains. These strains were able to grow not only in Vero/hSLAM cells, but also in other cell lines such as HEF, Vero E6, and GMK (3). Thus, sharing such difficult and rare cases of virus isolation-related viral identification is important to improve laboratory diagnoses. Here we describe 4 cases from which we isolated MV and rubella virus (RV) vaccine-derived strains in Yamagata, Japan in 2015, although there was no relation between the clinical symptoms of each patient and isolation of vaccine-derived strains.

We described the clinical background, isolated virus, and vaccination history of Cases 1–4 (Table 1). In case 1, we suspected a small syncytia formation in the VeroE6 cell line on the 8th day after nasopharyngeal specimen inoculation. After the second passage, we clearly observed syncytia formation in GMK and HEp-2 cell lines as well as in VeroE6 and Vero/hSLAM cell lines. Although there was no confirmed history of vaccination, we considered the CPEs to be due to an MV vaccine-derived strain, and succeeded in identifying the isolate as genotype A vaccine-derived MV strain based on our previous experience (3). After that, we confirmed that the patient had received a Measles-rubella (MR)-vaccine.

In cases 2 and 3, we suspected CPEs in the VeroE6 cell line for both cases on the 8th day after nasopharyngeal specimen inoculation, and passaged this CPE-suspected culture fluid into the 6 cell lines comprising the microplate method. However, we could only observe CPEs in the VeroE6 cell line (Fig. 1). Based on our experience, we initially suspected that the CPEs were due to adenovirus, human metapneumovirus, or parainfluenza virus. However, these viruses were later ruled out by rapid test kits and RT-PCR. Although there was no information related to vaccination history, we speculated that the CPEs were due to a vaccine-derived RV strain. A previous vaccine-derived RV strain isolated from a 1-year-old child in 2012 in Yamagata

Fig. 1. Morphological changes (cytopathic effects) of vaccine-derived rubella viruses on VeroE6 cells (×100).
showed round CPEs in VeroE6 cells similar to those observed for Cases 2 and 3. We succeeded in identifying the isolates as vaccine-derived RV strains by RT-PCR and sequence analysis using primers E1-2F, E1-2R, E1-7F, and E1-12R (6). We also used an indirect immunofluorescent method using commercial antisera: anti-rubella virus capsid antibody ab34749 (Abcam, Cambridge, UK) and goat anti-mouse IgG (H+L) FITC (Bethyl Laboratories, TX, USA). We later confirmed that both cases had received the same lot of MR vaccine. A comparison of the 739 nucleotide E1 fragment according to the RV nomenclature revealed that the sequences of the 2 isolates, were completely identical. The interval for another case in 2012, in the 12th–26th days post-vaccination, which is longer than that of our previous 2 cases (3), suggests that the MV vaccine-derived strain was shed during the 9th–11th days post-vaccination. In contrast, the intervals for Cases 2–4 suggest that the RV vaccine-derived strain is shed during the 12th–26th days post-vaccination, which is longer than that for MV. The interval for another case in 2012, in which an RV vaccine-derived strain was isolated (data not shown), was 22 days. This finding also supports the notion that the RV vaccine-derived strain remains alive in immunized children for more than 3 weeks. These experiences indicate that laboratory technologists involved in virus isolation should recognize the possibility of isolating vaccine-derived MV and RV strains in routine cell cultures from 1-year-old patients likely to have received an MR vaccine, but not displaying clinical symptoms of MV or RV infections. Laboratory technologists in public health institutes should obtain adequate information of vaccination history before conducting virus detection, including virus isolation using cell culture techniques and PCR.

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Conflict of interest None to declare.

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