Isolation of Vaccine-Derived Measles Viruses from Children with Acute Respiratory Infection

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The measles elimination project led by the World Health Organization (WHO) has been moving toward the target of eliminating measles in the WHO Western Pacific Region. In Japan, prefectural public health institutes play a key role for the laboratory diagnosis of measles virus (MV) infection, which is based on PCR, virus isolation, and genotyping. Microscopic examination of viral-sensitive cell lines during routine virus isolation from nasopharyngeal specimens has been used to detect the morphological changes typical for the growth of respiratory viruses. Here, we describe the unexpected isolation of vaccine-derived MVs from the two unrelated 1-year-old boys with acute respiratory infection. The nasopharyngeal specimens were obtained from one patient in February 2007 and from another in December 2012. Incidentally, the two children had received measles-rubella vaccination 9 or 11 days before the sampling. The isolates from two children induced morphological changes of the viral-sensitive cell lines, such as syncythia formation (cell fusion). We finally identified the isolates as vaccine-derived MVs by sequence analysis and immunological methods with anti-measles nucleoprotein antibodies. As no typical symptoms of MV infection were observed in either patient, the vaccine-derived MVs were isolated not as causative pathogens but by chance. In fact, there was no suspected case of secondary MV infection in either patient, thereby excluding the possibility that vaccine-derived MVs spread from human to human. Our experiences suggest the possibility of vaccine-derived MV isolation by cell cultures and the difficulty in identifying MVs in specimens from patients other than clinically suspected measles cases.

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Introduction

The measles elimination project led by the World Health Organization (WHO) has been moving toward the target of eliminating measles in the WHO Western Pacific Region, including Japan, by 2012 and a total of 32 out of 47 prefectures in Japan had reported less than 1 case/million population, one criterion of measles elimination, by the end of 2012 (NIID 2013). Prefectural and municipal public health institutes and the National Institute of Infectious Diseases (NIID) have established a collaborative network for laboratory diagnosis based on polymerase chain reaction (PCR), virus isolation and genotyping in Japan (NIID 2013), and this network plays a key role in the confirmation of measles virus (MV) infection in the final stage of elimination.

In Yamagata, we have actively monitored suspected measles cases, and reported an outbreak of measles caused by genotype D9 at a junior high school in 2004 and a case of measles caused by genotype D9 imported from Thailand in 2009 (Mizuta et al. 2005; Aoki et al. 2009) (Fig. 1). We finally realized our goal of "no cases of MV infection" for the first time in 2011 (Aoki et al. 2012) and we were also able to report no new cases of MV infection in Yamagata, in 2012. As part of our activities, we noted the importance of the differential diagnosis of viral exanthematous diseases along with measles, rubella, enterovirus and other infections (Aoki et al. 2012; NIID 2013).

Our search of the relevant literature identified no reports of the isolation of vaccine-derived MV, proved by cell culture. We succeeded in isolating vaccine-derived MV from two children without clinical suspicion of MV infection in Yamagata, Japan in 2007 and 2012. Since these are rare cases, we herein report our experiences.

Subjects and Methods

We routinely use a 96-well microplate, which includes six cell lines for virus isolation from nasopharyngeal specimens from patients

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with acute respiratory infections as part of the national surveillance of viral infectious diseases in Japan based on the Infectious Diseases Control Law (Mizuta et al. 2008). The six cell lines include human embryonic lung fibroblast (HEF), human laryngeal carcinoma (HEp-2), African green-monkey kidney cells (VeroE6), Madin-Darby canine kidney cells (MDCK), human rhabdomyosarcoma (RD-18S) and African green-monkey kidney cells (GMK). We also used the Vero/hSLAM cell line, which stably expresses the principal cellular receptor for MV (the human signaling lymphocyte activation molecule:

hSLAM), for the isolation of MV from patients with suspected MV infection (Mizuta et al. 2005; Aoki et al. 2009, 2012). For identification of MV, we used a commercial antiserum for neutralization testing (Denka Seiken, Tokyo, Japan) and monoclonal antibodies "Antimeasles, nucleoprotein, clone 83KKII" (Merck Millipore Corporation, Billerica, MA) and "Goat anti-mouse IgG-h+1 FITC" (Bethyl Laboratories, Inc., West FM). We also carry out molecular analysis as described previously (Mizuta et al. 2005; Aoki et al. 2009)



Fig. 1. A phylogenetic tree of measles viruses isolated in Yamagata, Japan in 2001-2012. Reference strains registered in GenBank (accession numbers are shown after the strain name) are also shown. Yamagata strains are indicated by circles, with the two isolates described in this study indicated by open circles. Measles viruses have generally been typed into genotypes A-G, and the two isolates in this study belonged to genotype A (vaccine type), whereas other isolates in Yamagata had been typed into genotypes D or H. The tree was based on the nucleotide sequences (456 bps) of the nucleoprotein and was constructed by the neighbor-joining method. Branch lengths are proportional to the number of nucleotide differences. Only significant bootstrap values are shown. The marker denotes a measurement of the relative phylogenetic distance.

Results

The use of the microplate allowed us to isolate a variety of respiratory viruses such as influenza, parainfluenza, respiratory syncytial (RS) virus, human metapneumovirus, mumps, enterovirus, rhinovirus, and adenovirus (Mizuta et al. 2008). In general, observation of the cell culture images under a microscope after specimen inoculation, allows us to determine the respiratory viruses present, based on morphological changes (cytopathic effects: CPEs) in sensitive cell lines, without difficulty. However, we found unfamiliar CPEs and syncythia formation (cell fusion) in the HEF,



Fig. 2. Morphological changes (Cytopathic effects) and immunofluorescent images of vaccine-derived measles viruses. Syncytia formation (cell fusion) was observed on (A) VeroE6, (B) GMK, and (C) HEF cell lines for the MVi/Yamagata. Jpn/9.07 strain and on (D) VeroE6 and (E) Vero/hSLAM cell lines for the MVi/Yamagata.Jpn/50.12 strain. MVi/Yamagata.Jpn/50.12 strain-inoculated VeroE6 cells were stained by the indirect immunofluorescent method (F), whereas VeroE6 cells without inoculation were not stained (G). The immunofluorescent analysis indicates that the cytopathic effects are due to the growth of measles virus.

HEp-2, VeroE6, and GMK cell lines with the specimens from the two children, who had been diagnosed with acute respiratory infection or pharyngitis.

Patient 1 was a 1-year-old boy who presented at the Yamanobe Pediatric Clinic on 27 February, 2007 with fever, rhinorrhea and a slightly reddened pharynx. Although there was an influenza outbreak at his nursery at that time, he was found to be negative for influenza virus by rapid test kit. We collected a nasopharyngeal specimen on 28 February and subsequently performed virus isolation. We observed syncytia formation in the GMK and VeroE6 cells on the 5th and 14th day after specimen inoculation, respectively. After the second passage, we also observed syncytia formation in HEF, VeroE6 and GMK cell lines (Fig. 2). Without an accurate vaccine history, we considered the CPEs to be due to a paramyxovirus, which often causes syncytia formation in sensitive cell lines, and we performed reverse transcription PCR (RT-PCR) targeting parainfluenza viruses, RS virus, mumps virus and human metapneumovirus. However, we failed to amplify any genome. Only after we consulted several clinical virologists with the pictures of the CPEs over an approximately three-month period we were able to identify the isolate as MV (MVi/Yamagata. Jpn/9.07). We heard that the patient had received a measles-rubella vaccination (MEARUBIK, Lot No. MR013, The research foundation for microbial diseases of Osaka University, Osaka, Japan) on 19 February, only after we had identified the virus.

Patient 2 was a 1-year-old boy who was clinically diagnosed with pharyngitis on 9 December, 2012 on the basis of symptoms of fever, rhinorrhea, and nasal obstruction. We collected a nasopharyngeal specimen on 11 December and thereafter performed virus isolation. We suspected a CPE in the VeroE6 cell line on the 7th day after specimen inoculation, and passaged this CPE-suspected culture fluid to the six cell lines described above. Syncytia formation was observed in the HEF, HEp-2, VeroE6 and GMK cell lines as well as the Vero/hSLAM cell line, which was prepared separately (Fig. 2). In this case, as we knew he had received a measles-rubella vaccination (MEARUBIK, Lot No. MR188) on 30 November, we could differentiate the isolate from the paramyxoviruses described above, and succeeded in identifying the isolate as MV (MVi/Yamagata. Jpn/50.12).

We confirmed both strains as MV by neutralization test and an indirect immunofluorescent method (Fig. 2.). Both strains were also confirmed as genotype A vaccine-derived strains by sequence analysis of the N gene (Fig. 1); MVi/ Yamagata.Jpn/9.07 and MVi/Yamagata.Jpn/50.12 strains had 100% and 99% similarity to the vaccine strain CAM-70 (accession number: U03650), respectively. We registered the sequence data with GenBank (accession numbers AB781070 and AB781069, respectively).

Discussion

Cluster differentiation 46 (CD46) acts as a receptor for

vaccines and laboratory-adapted strains of MV, whereas SLAM is the principal cellular receptor for MV (Yanagi et al. 2009). All vaccine strains derived from genotype A MV, can use not only SLAM but also CD46 as a cellular receptor and grow well in Vero and other cell lines beside Vero/hSLAM (Gerlier and Valentin 2009). Thus, theoretically, our virus isolation system can isolate genotype A MVs such as vaccine-derived MV, whereas it is difficult to isolate wild type MV (genotypes B-G) in our system without the Vero/hSLAM cell line.

In both patients, the specimens were collected from patients with acute respiratory infection or pharyngitis and not from suspected measles cases. In Patient 1, we never suspected that MV was responsible for the CPEs and syncytia formation due to the absence of a vaccine history during the laboratory examination. In Patient 2, as we had experience with Patient 1 as well as the patient's vaccination history, we considered the possibility of MV in the differential diagnosis. Because both patients had no typical symptoms of MV infection and there was no suspected case of secondary MV infection in either case, we considered that vaccine-derived MVs were isolated not as causative pathogens but by chance.

Finally, our experiences suggest both the possibility of vaccine-derived MV isolation in cell cultures and the potential difficulties in the identification of MVs from patients other than those clinically suspected of measles.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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