ORIGINAL ARTICLE

Laboratory diagnosis of vaccine-associated measles in Zhejiang Province, China

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Abstract *Background/Purpose:* Along with the improving vaccine coverage, suspected vaccine-associated measles has been reported in Zhejiang Province, China. In order to maintain the accuracy of the measles surveillance system, it is critical to discriminate between measles vaccine and wild-type virus.
Methods: Eight suspected cases of vaccine-associated measles were reported in Zhejiang Province during 2011 and 2014. Sera collected within 4 days and throat swabs collected within 6 days after rash onset were tested with immunoglobulin M and measles virus (MeV) RNA to confirm MeV infection. In order to further identify the vaccine-associated cases, throat swabs with positive MeV RNA were tested using an allelic discrimination real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay developed in this study, RT-PCR-restriction fragment length polymorphism (RFLP) recommended by the National Measles Laboratory, and RT-PCR followed by sequencing and genotyping.
Results: Combining anti-measles immunoglobulin M and RNA testing, eight cases were confirmed as MeV infection. Of the eight, two were identified as vaccine-associated cases by the allelic discrimination rRT-PCR assay, and one was identified by RT-PCR-RFLP. Subsequent sequencing and genotyping confirmed that the sequences of the two cases were identical to that of the Chinese vaccine strain. The developed allelic discrimination rRT-PCR was 10 times more sensitive than the RT-PCR-RFLP assay when RNA standards generated from three genotypes of MeV were tested.

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Conclusion: Vaccine-associated measles has been identified in Zhejiang. The developed allelic discrimination rRT-PCR assay is rapid and sensitive, which will facilitate the surveillance for vaccine-associated measles.

Introduction

Measles is a highly contagious disease, which resulted in millions of deaths in the pre-vaccine era. The morbidity and mortality of measles have declined considerably since the 1960s, when the attenuated or killed measles vaccine was developed and used successfully throughout the world.[1,2] After decades of improved immunization coverage and decreasing incidence, the elimination of measles became the goal of the World Health Organization (WHO) in its four regions by 2015.[3] To achieve this goal in China, WHO has recommended a measles mass immunization campaign (MMIC) in addition to two doses of routine vaccination.

Measles-containing vaccines, the most effective means to control and interrupt measles infection at present,[4] have been used globally for ~50 years. Although the vaccines have been proved to be safe and effective, previous studies have found that the live attenuated vaccine may cause measles-like symptoms, such as moderate fever, rash, and conjunctivitis, in 5–15% of recipients.[5,6] With the apparent drop in the incidence of wild-type measles, the epidemiology and clinical pattern of the disease have changed, and vaccine-associated measles has been reported in many countries.[7–10] In order to ensure the accuracy of the measles surveillance system during the period of measles elimination, vaccine-associated measles should be distinguished from wild-type measles virus (MeV) infection when cases are reported.

Differentiation of vaccine-associated measles based on clinical symptoms or laboratory methods routinely used in MeV surveillance, such as immunoglobulin M (IgM) testing, virus isolation, and RNA amplification, is unworkable or unreliable. Sequencing combined with genotyping has always been the most accessible way to identify vaccine-associated cases[11]; however, this method is time consuming, and not every clinical specimen contains sufficient amount of viral RNA to perform sequencing analysis.[12] A reverse transcriptase polymerase chain reaction-restriction fragment length polymorphism (RT-PCR-RFLP) assay has been developed and recommended for discriminating between measles vaccine and wild-type strains by the Chinese National Measles Laboratory.[13] The process of the RT-PCR-RFLP is divided into RT-PCR plus enzyme digestion and gel electrophoresis, which are not only labor intensive but can easily cause crossover contamination as well. A loop-mediated isothermal amplification assay has also been developed for differentiating vaccine from wild-type MeVs in Japan.[12] This assay is simpler than RT-PCR-RFLP, but its sensitivity for detecting wild-type MeV strains is ~10 times lower than it is for detecting vaccine-type strains.[12]

In this paper, we describe the differentiation process of eight suspected vaccine-associated measles monitored in Zhejiang Province, China. We have developed an allelic discrimination real-time RT-PCR (rRT-PCR) assay and applied it to discriminate between measles vaccine and wild-type strains. Our research provides data for the laboratory diagnosis of vaccine-associated measles case and contributes to the accuracy of the measles case surveillance system.

Methods

Suspected vaccine-associated measles

Eight suspected vaccine-associated measles were reported in Zhejiang Province, China during 2011 and 2014. All the suspected cases were vaccinated with live attenuated measles and rubella combined vaccine 4–11 days prior to the onset of rash. Patients presented with symptoms of fever and rash, which are consistent with measles clinically (Table 1). Cases 1 and 7 were reported to present with typical Koplik's spots. Cases 2–4 and 6 presented with symptoms of cough, conjunctivitis, and coryza (3C). Case 1 was also reported to have enlarged lymph nodes. Cases 1, 2, and 6 had been hospitalized prior to onset of illness (Table 1).

Specimens

Clinical specimens of eight suspected vaccine-associated cases were used in this study. Serum samples collected within 4 days after rash onset were tested by enzyme-linked immunosorbent assay kit (Virion\Serion, Würzburg, Germany). Throat swab specimens collected within 6 days after rash onset were analyzed by a multiplex rRT-PCR for MeV and rubella virus (RV) routinely used in Zhejiang Provincial Center for Disease Control and Prevention (CDC),[14] as well as used for virus isolation.[15] Afterwards, throat swabs with positive MeV RNA were tested using the allelic discrimination rRT-PCR developed in this study, RT-PCR-RFLP recommended by the National Measles Laboratory,[13] and RT-PCR followed by sequencing and genotyping.[16]

RNA extraction

Total RNA was extracted from clinical specimens or cell culture supernatants using the High Pure Viral Nucleic Acid Kit (Roche, Welwyn Garden City, UK). Briefly, 200 μL of clinical specimen or cell culture supernatant was added to a lysis buffer containing poly (A) and proteinase K and