Mumps vaccine virus genome is present in throat swabs obtained from uncomplicated healthy recipients

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Abstract

Seven children were followed for up to 42 days post-vaccination with live mumps vaccine and 37 throat swabs were obtained serially. Viral genomic RNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) in the phosphoprotein (P) and hemagglutinin-neuraminidase (HN) regions. Virus isolation was also attempted. Genomic differentiation of detected mumps virus genome was performed by sequence analysis and/or restriction fragment length polymorphism (RFLP). No adverse reaction was observed in these children. Although mumps virus was not isolated from any of the samples, viral RNA was detected in four samples from three vaccine recipients, 18, 18 and 26, and 7 days after vaccination, respectively. Detected viral RNA was identified as the vaccine strain. Our data suggests that vaccine virus inoculated replicates in the parotid glands but the incidence of virus transmission from recipients to other susceptible subjects should be low. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Mumps vaccine; Viral RNA genome; Healthy recipients

1. Introduction

Mumps vaccine is widely accepted as safe and efficacious, although vaccine related parotitis has been reported at an incidence of 2–3% of the recipients and aseptic meningitis occurred at an incidence of one case among 1000–2000 recipients of measles-mumps-rubella (MMR) combined vaccine in Japan [1–3]. In these cases, mumps virus was isolated or viral RNA was detected from throat swabs or cerebrospinal fluid and the virus was identified as the vaccine strain [4,5]. Transmission of mumps vaccine virus was reported from a vaccine recipient, who had parotitis with fever, to her susceptible sibling [6]. However, to our knowledge, there is no information about the presence of mumps vaccine virus or genomic RNA in healthy recipients in the absence of adverse reactions. Therefore, we investigated the isolation of mumps virus and the detection of mumps vaccine virus genome in throat swabs from vaccine recipient for the better understanding to clarify the probability of transmission from healthy vaccine recipients.

2. Subjects and methods

We studied seven healthy children (aged 2–4 years of age, three boys and four girls) who received either of the two different strains of mumps vaccine licensed in Japan. We designated the day of immunization as day 0 and during the 42 days post-inoculation, 37 throat swabs were obtained serially at least 4 times in each recipient including one sample just before vaccination. Cases 1, 2, 3, and 4 received the Miyahara strain (Kaketsuken, Kumamoto) and cases 5, 6, and 7 got the Hoshino strain (The Kitasato Institute, Tokyo).

Virus isolation was performed independently in two different laboratories (Kitasato Institute and Kagawa Prefectural Institute of Public Health). 0.1 ml of sample
was inoculated on a monolayer of Vero cells. Samples showing a viral-specific cytopathic effect (CPE) were confirmed by a neutralization test using antiserum to mumps virus while those not showing CPE after three passages were considered as negative for virus isolation.

Nested RT-PCR was done in the P and HN regions, as previously reported [4,5]. Briefly, total RNA was extracted from 200 μl of the clinical samples. Mumps genomic RNA was reverse-transcribed with AMV reverse transcriptase (Life Sciences Inc., St Petersburg, Florida) at 50°C for 1 h. Five μl of viral cDNA was amplified by PCR in a total volume of 50 μl mixture as recommended by the manufacturers, using 1.25 units of Taq DNA polymerase (TaKaRa Taq, TaKaRa BIOMEDICALS, Tokyo). The first three cycles were at 92°C for 2 min, 55°C for 3 min, then 72°C for 2 min. These were followed by 30 cycles (denaturation at 93°C for 1 min, reannealing at 58°C for 1 min, and extension at 72°C for 2 min) with a final additional extension period of 5 min at 72°C. For the nested PCR experiments, 1 μl of the first PCR product was mixed in a total of 50 μl PCR reaction mixture and the samples were subjected to PCR using the same thermal cycle program. Nested PCR amplified 183 nucleotides of the P gene and 520 nucleotides of the HN gene between the sets of primers. Genomic differentiation between vaccine strain and wild strains was performed by sequence analysis of the P gene in all RT-PCR positive samples. Restriction fragment length polymorphism (RFLP) of the HN gene was performed using two restriction enzymes, Afl II and Sca I [4,5]. PCR product was electrophoresed through low-melting-temperature 1% agarose gel and the specific DNA band was excised. We purified the PCR products and analysed the DNA sequence bidirectionally with a Dye Terminator Sequencing Kit (Applied Biosystems Japan Inc., Tokyo, Japan) using an automated nucleotide analyser, the 377 DNA Sequencer (Applied Biosystems, Foster City, CA).

3. Results

None of the recipients developed any adverse reactions (e.g. swelling of the parotid gland, fever, headache, vomiting, hearing loss, urticaria or local reaction). No virus was isolated from any of the 37 samples. The results of RT-PCR are summarized in Fig. 1. Mumps viral RNA was detected in four samples obtained from three children. In case 3, viral RNA was detected once, 18 days after vaccination. In case 4, two samples obtained at day 18 and day 26 were positive. A throat swab obtained from case 7 at 7 days after vaccination was positive. In the remaining samples from the above three cases viral RNA was not detected by RT-PCR examination. In cases 1, 2, 5 and 6, viral RNA was not found in any of the samples obtained.

4. Discussion

In Japan, five mumps vaccine strains (Urabe Am9, Torii, Miyahara, Hoshino, and NK-M46) were licensed and now three strains of live attenuated vaccine strains

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Fig. 1. Detection of mumps virus genome from serial throat swabs obtained from vaccine recipients. The Miyahara mumps vaccine was administrated to cases 1–4 and the Hoshino strain was inoculated in cases 5–7. Open circles indicate negative results for RT-PCR and the closed circles positive findings.

The results of sequence analysis of the P gene are shown in Fig. 2. The Miyahara strain of mumps vaccine was inoculated in cases 3 and 4, and the viral RNA sequences of the three positive samples obtained from these two recipients were identified as the Miyahara strain from their characteristic nucleotide positions. A positive RT-PCR product in case 7, immunized with the Hoshino strain, was similarly identified as the Hoshino strain. Moreover, using RFLP analysis in the HN region, the PCR product obtained from case 7 was cleaved into two fragments after digestion with Afl II and Sca I, identical to the RFLP pattern of the Hoshino vaccine strain. These digesting patterns of the RFLP profiles differed from those of wild strains (data not shown).

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Fig. 2. Sequence analysis of PCR products in the P gene. The nucleotide position was numbered from the first ATG codon. Sequence data of the four vaccine strains used in Japan (the Urabe AM9, Torii, Miyahara, and Hoshino) and wild strains was compared with the results reported by Yamada et al [4]. Cases 3 and 4 were inoculated with the Miyahara strain and case 7 with the Hoshino strain.
(the Torii, Miyahara, and Hoshino) are currently available. In 1989, MMR trivalent vaccine containing the Urabe Am9 strain was introduced into the Japanese recommended immunization program. However, once the MMR vaccine was widely employed, a high incidence of aseptic meningitis after vaccination was reported [3], while swelling of the parotid gland, with or without fever, was observed in a few percentages of vaccine recipients approximately 3 weeks after vaccination [1]. These adverse reactions might be related to infection with live attenuated vaccine virus. Therefore, we studied the potential role of vaccine virus in clinical problems associated with vaccine administration.

Only one case of transmission of the Urabe mumps vaccine virus between siblings has been reported [6]. In this paper we report, for the first time, the presence of mumps vaccine virus genomic RNA in throat swabs from non-complicated healthy recipients. Positive for RT-PCR means the presence of mumps virus genome but it does not always imply the presence of infectious mumps virus. We examined virus isolation together with RT-PCR but no infectious mumps virus was isolated. Our data suggests the potential risk of transmission of vaccine virus. Viremia probably occurs not only in patients with natural mumps infection but also in vaccine recipients and the detection of vaccine genome in the throat suggests that the vaccine virus replicates in parotid or other salivary glands following the viremic phase. However, the transmission of vaccine virus from recipients to other susceptible subjects is extremely rare in our clinical experience. For this reason, we believe that the magnitude of virus excretion is very low and the risk of virus transmission is almost negligible, a view supported by the fact that no infectious mumps virus was isolated despite the presence of mumps vaccine RNA.

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References