Original Article

Persistence of Immunity Acquired after a Single Dose of Rubella Vaccine in Japan

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SUMMARY: To date, Takahashi, Matsuura, and TO-336 strains of live-attenuated rubella vaccine have been used in Japan. Japan implemented a single-dose rubella vaccination program until 2006. However, few reports are available on the persistence of immunity after this vaccination program. We collected 276 serum samples from January 2009 to December 2011 at Okafuji Pediatric Clinic and assessed the immune status of these samples against rubella virus during 1–10 years after vaccination with a single dose of Takahashi rubella vaccine. Regional outbreak of rubella did not occur during 1999–2011. The collected serum samples were tested for antibodies against the rubella virus by performing a standard hemagglutination inhibition (HAI) test. Our results showed that all the tested serum samples contained antibodies against the rubella virus 10 years after the vaccination. Geometric mean titer of HAI antibodies was 1:180 and decreased to 1:68 at 10 years after the vaccination. The levels of HAI antibodies decreased logarithmically with time after the vaccination. In conclusion, vaccine-acquired immunity after vaccination with a single dose of live-attenuated Takahashi rubella vaccine was retained for at least 10 years when rubella was under regional control.

INTRODUCTION

Rubella is a vaccine-preventable disease caused by rubella virus. Japan implemented a rubella vaccination program targeted toward girls studying in junior high schools in 1977 and introduced a measles-mumps-rubella (MMR) vaccine for children of both the genders aged 12–72 months in 1989. However, the MMR vaccine was withdrawn in 1993 because of adverse events associated with the mumps vaccine component. In 1994, the rubella vaccine strategy was modified to include rubella vaccination for children aged 12–90 months. Further, a 2-dose strategy involving a measles and rubella combined (MR) vaccine was introduced for children aged 1 and 6 years in 2006 (1).

The most globally used rubella vaccine is a live-attenuated vaccine containing the RA 27/3 strain of the rubella virus. Other strains used in live-attenuated rubella vaccines include Takahashi, Matsuura, TO-336, and BRD-2 strains (2). The RA 27/3 rubella vaccine have been well documented, but persistence of immunity has been controversial under the situation where rubella is well controlled.

In Japan, 5 rubella strains have been used in live-attenuated vaccines, of which 3 strains are used at present. Few reports are available on the persistence of immunity after rubella vaccination in Japan. Moreover, investigation of the persistence of immunity after a single dose of rubella vaccine is difficult because this vaccine is now given twice in the MR vaccine. Therefore, the present study investigated the persistence of im-

munity after administering a single dose of Takahashi rubella vaccine.

MATERIALS AND METHODS

Ethics statement: The study protocol was approved by the Ethics Committee of Kitasato Institute Hospital (approval no.: 11005 [retrospective study] and 11006 [prospective study]).

Subjects and samples: In all, 276 serum samples were collected from January 2009 to December 2011 at Okafuji Pediatric Clinic, Himeji city, which is located in the western part of Japan. The population of Himeji city is >500,000. In Himeji city, all rubella cases in children have been surveyed since 1981. Further, all rubella cases in Japan have been registered since 2008. The annual number of patients with rubella is shown in Fig. 1. No rubella outbreaks have occurred in Himeji city from 1999 to 2011. The serum samples were collected randomly from subjects aged 1–11 years who visited the Okafuji Pediatric Clinic for unrelated reasons. The subjects received the Takahashi rubella vaccine (monovalent or MR) at the Okafuji Pediatric Clinic when they were aged 1–5 years. Subjects with uncertain vaccination history and those receiving gamma globulin before 6 months of study initiation were excluded. Informed consent was obtained from the parents of the children before collecting the serum samples. Sera were prepared and stored at -70°C until further use.

Serological tests: The serum samples were tested for antibodies against the rubella virus by performing a standard hemagglutination inhibition (HAI) test. The samples were treated with 25% Kaolin for 20 min and with 8% goose RBCs for 60 min at 4°C . The samples were then diluted to a ratio of 1:8. Serial 2-fold dilutions of the samples were mixed with 4 units of HA antigen (Denka Seiken, Tokyo, Japan), followed by the addition of 0.25% goose RBCs. Titer of HAI antibodies was defined the highest serum dilution that inhibited hemag-

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Fig. 1. Number of notified rubella cases in children by year in Himeji. Notification was based on clinical diagnosis.

glutination.

Statistical analysis: Linear regression analysis was performed to directly assess whether a decrease in the titer of HAI antibodies occurred with time. Statistical analysis was performed using Stata 12 software (StataCorp LP, College Station, TX, USA), and *P* values less than 0.05 were considered statistically significant.

RESULTS

Geometric mean titer of HAI antibodies: Geometric mean titer (GMT) of HAI antibodies in all the children was more than 1:8, and immunity against the rubella virus was retained for 10 years after the vaccination. The annual change in the GMT of HAI antibodies after the vaccination is shown in Table 1. The GMT of HAI antibodies was 1:180 and decreased to 1:68 at 10 years after the vaccination.

Waning of HAI antibodies after vaccination with the Takahashi rubella vaccine: Results of regression analysis are shown in Fig. 2. Titers of HAI antibodies decreased logarithmically with time after vaccination with the Takahashi rubella vaccine.

DISCUSSION

Results of the present study indicated that immunity acquired after vaccination with a single dose of the live-attenuated Takahashi rubella vaccine was retained for at least 10 years even when rubella was under regional control. The titers of HAI antibodies decreased gradually with time. To our knowledge, this is the first study to report the persistence of immunity after vaccination with the Takahashi rubella vaccine.

Our results are compatible with those of several studies that determined the persistence of immunity after vaccination with the RA27/3 rubella vaccine (3–6). Christenson and Bottiger (3) reported antibody decay in

Table 1. Persistence of rubella HAI antibodies after vaccination

Time after vaccination (yr)	<i>N</i>	% HAI titer ≥ 8	GMT (HAI)
1	16	100	180.0
2	51	100	173.0
3	51	100	159.0
4	53	100	131.0
5	34	100	112.0
6	22	100	111.0
7	25	100	101.0
8	9	100	85.3
9	7	100	82.3
10	4	100	68.0

a cohort of 436 female subjects who were vaccinated with the RA27/3 rubella vaccine at the age of 12 years. They observed that of the 436 subjects, 96% were seropositive after 8 years of the vaccination and 94% were seropositive after 16 years of the vaccination. However, the GMT of HAI antibodies in these subjects declined from 1:110 to 1:18 after 16 years. Plotkin et al. (4) performed HAI test and reported that 1 of 29 vaccines was seronegative after 13 years of vaccination.

However, these studies were performed when infection with wild-type rubella virus was prevalent. O'shea et al. (5) reported that only 4 of 117 (3.4%) vaccines were seronegative after 10–21 years of rubella vaccination. However, they could not confirm the persistence of vaccine-acquired immunity in the absence of a circulating virus because of a potential reinfection. King et al. (6) reported that antibody levels resultant from rubella vaccine were likely to decline with advancing age in area free from natural rubella and whether repeated exposures or subclinical infections were needed to maintain immunity to rubella was an important issue. In contrast, Hillary and Griffith (7) suggested that reinfection with an antibody booster occurred less frequently in children vaccinated with the RA 27/3 rubella vaccine

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Fig. 2. Persistence of hemagglutination inhibition (HAI) antibodies to rubella after administration of the Takahashi strain vaccine. Circles represent individual titer levels. The solid line represents the best fit by linear regression ($y = 2^{7.21-0.00039X}$, $R^2 = 0.056$, $P = 0.001$).

and that this response was not observed among children included in their study.

Several studies have reported that antibodies do not persist for up to 10 years after vaccination with the RA 27/3 rubella vaccine (8,9). Johnson et al. (8) performed enzyme-linked immunosorbent assay and showed that seropositivity rate was significantly lower in 11- to 13-year-old children than in 4- to 6-year-old children (67% vs. 90%). In this study, all the children received the first dose of the vaccine at ≥ 15 months of age. LeBaron et al. (9) found that 9% subjects in the kindergarten group were seronegative before administering the second dose of the MMR vaccine.

Lin et al. (10) performed linear regression analysis and found a significant association between average antibody titers against the rubella virus and time after RA 27/3 rubella vaccination. In addition, they found that the mean annual antibody decay rate was -0.77 IU/ml. In the present study, the titer of HAI antibodies decreased logarithmically with time after the vaccination. Our results suggested that the titer of HAI antibodies resulting from the Takahashi rubella vaccine was retained ≥ 8 for 29.6 years.

The present study is important because it was conducted when rubella was under regional control. A rubella outbreak occurred in Japan in 2012 and 2013 (11), and 45 and 40 rubella cases, respectively, were reported in Himeji city during these outbreaks. Therefore, further studies should be performed to determine whether this outbreak affected the persistence of immunity after rubella vaccination.

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Conflict of interest None to declare.

REFERENCES

1. Nakayama T. Vaccine chronicle in Japan. *J Infect Chemother.* 2013;19:787-98.
2. Rubella vaccines: WHO position paper. *Wkly Epidemiol Rec.* 2011;86:301-16.
3. Christenson B, Bottiger M. Long-term follow-up study of rubella antibodies in naturally immune and vaccinated young adults. *Vaccine.* 1994;12:41-5.
4. Plotkin SA, Buser F. History of RA27/3 rubella vaccine. *Rev Infect Dis.* 1985;7:S77-8.
5. O'Shea S, Woodward S, Best JM, et al. Rubella vaccination: persistence of antibodies for 10–21 years. *Lancet.* 1988;2:909.
6. King Jr JC, Lichenstein R, Feigelman S, et al. Measles, mumps, and rubella antibodies in vaccinated Baltimore children. *Am J Dis Child.* 1993;147:558-60.
7. Hillary IB, Griffith AH. Persistence of rubella antibodies 15 years after subcutaneous administration of Wistar 27/3 strain live attenuated rubella virus vaccine. *Vaccine.* 1984;2:274-6.
8. Johnson CE, Kumar ML, Whitwell JK, et al. Antibody persistence after primary measles-mumps-rubella vaccine and response to a second dose given at four to six vs. eleven to thirteen years. *Pediatr Infect Dis J.* 1996;15:687-92.
9. LeBaron CW, Forghani B, Matter L, et al. Persistence of rubella antibodies after 2 doses of measles-mumps-rubella vaccine. *J Infect Dis.* 2009;200:888-99.
10. Lin CC, Yang CY, Shih YL, et al. Persistence and titer changes of rubella virus antibodies in primiparous women who had been vaccinated with strain RA 27/3 in junior high school. *Clin Vaccine Immunol.* 2012;19:1-4.
11. Ujiie M, Nabae K, Shobayashi T. Rubella outbreak in Japan. *Lancet.* 2014;383:1460-1.