

Booster Effect of Rubella Vaccination in College Students with Pre-existing Low Antibody Titers in Japan

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ABSTRACT. The object of this study was to clarify which titers are suitable for boosting immunity by rubella vaccination with the Matsuura strain. From among 435 college students, 76 students (mean age, 18.3 years old; range, 18 to 20 years old) who had rubella HI antibody titers of $\leq 1:32$ were enrolled in this study. After vaccination, we measured HI, ELISA-IgG and IgM titers and avidity. Among these students, who had pre-existing low antibody titers of 1:8, 1:16 and 1:32, 83%, 39% and 10% with the HI assay, and 83%, 50% and 33% with ELISA respectively, there was a significant increase in the titers. Immunity by vaccination could be boosted effectively in students with a pre-existing titer of 1:8. However, for women and high-risk people such as medical or educational workers or students, HI antibody titers $\leq 1:16$ would seem to be appropriate in Japan.

Key words ① rubella ② vaccination ③ booster effect

Although rubella is a relatively mild disease, rubella infection can lead to problems with congenital rubella syndrome (CRS) in the next generation due to fetal infection. Rubella and CRS are preventable with vaccination. In Japan, between 1977 and 1994, rubella vaccination was required for only female students in junior high school to prevent CRS. However, in 1995, regulations were changed to include all young children aged 12 to 90 months, both boys and girls, to prevent rubella epidemics. Since 2006, immunization of children between 12 to 23 months old and approximately six years old, before entry to elementary school, with an MR (measles and rubella combined) vaccine has become a requirement. And catch-up vaccination for adolescents aged 12 to 18 years old will start in 2008. However, we believe that a decrease in the number of adolescents and young adults susceptible to rubella in Japan is necessary to eliminate rubella and CRS, because approximately 3.5 million are still susceptible among people aged 15 to 30 years old¹⁾. In 2004, a research group supported by the national government recommended that women without antibody against rubella or with low antibody titers determined after measuring rubella antibody at antenatal screening, which is usually implemented in Japan, should be vaccinated to decrease the risk of CRS²⁾. In other words, not only women with a negative antibody of $<1:8$ as determined by the hemagglutination inhibition (HI) assay, but also women with low titers of $\leq 1:16$ should be vaccinated to prevent CRS due to infection or reinfection during pregnancy. Rubella reinfection during pregnancy, however, is rarely associated with CRS³⁾⁻⁶⁾. There have been few reports about the criteria for preventing reinfection. The purpose of this study was to

determine which antibody titer or titers would be most suitable for boosting immunity with vaccination.

SUBJECTS AND METHODS

The collection and use of human materials for the present study were approved by the Ethics Committee on Human Subjects of Kawasaki Medical School. Informed consent for this purpose was obtained from the subjects. In Kawasaki College of Allied Health Professions, antibodies against rubella, measles, mumps and varicella are measured to identify susceptible individuals, because we wish to prevent infections with these viruses during studying in our hospital. The rubella antibody was measured with the HI assay (Denka Seiken Co., Tokyo). From among the college students, healthy students with HI antibody titers of $\leq 1:32$ were enrolled in this study after their informed consent was obtained. They were asked to present us with written verification of their past history of vaccination and rubella infection as diagnosed by a physician. The IgG and IgM antibodies in their sera were also measured with an enzyme-linked immunosorbent assay (ELISA) kit (Denka Seiken Co., Tokyo). All of the students were confirmed to be negative for IgM antibody, because recent rubella infection was excluded. The students were vaccinated with a rubella vaccine (lot. R1008, Biken, Osaka, Japan) including 1000 pfu of the Matsuura strain, which has been used since 1976 in Japan. Identification of the E1 and NS4 genes in the Matsuura strain showed one point amino acid variation from the progenitor virus in the NS4 region⁷⁾. Blood was drawn from seven to nine weeks after the vaccination to analyze the antibodies as measured by HI and ELISA (Denka Seiken Co., Tokyo), and the avidity of ELISA-IgG antibodies (Dade Behring, Marburg, Germany). All assay protocols, cut-offs and the resulting interpretation were carried out according to the manufacturer's instructions.

AVIDITY INDEX

To determine the IgG titer and avidity, samples (initial dilution, 1:231) were placed in the antigen and control wells, respectively, and then were incubated at 37 °C for 1 h. After this, one sample was washed with an ordinary washing buffer provided by the manufacturer and the other was washed with a washing buffer supplemented with 8M urea (Nakalai, Kyoto, Japan) twice for 5 min each time. Then, after being washed twice for 2 min each time with the ordinary washing buffer, the subsequent procedures were done in accordance with the instructions of the manufacturer. The percent avidity was calculated as (urea-treated OD / untreated OD) \times 100.

RESULTS

The distribution of HI antibody titers in the 435 students is shown in Figure 1. The distribution of the immunized subjects was normal. The mean and median titers were both 1:64. The numbers of students with titers of <1:8, 1:8, 1:16 and 1:32 were 31 (7.1%), 17 (3.9%), 36 (8.3%) and 80 (18.4%) of the total, respectively. The subject students in a few departments could not be participated in this study, because they did not fit our schedule in this study. As a result, 76 students were enrolled, 22 students had negative HI antibody titers, that is, <1:8. Of the remaining 54 students, 6 students had titers of 1:8, 18 students had titers

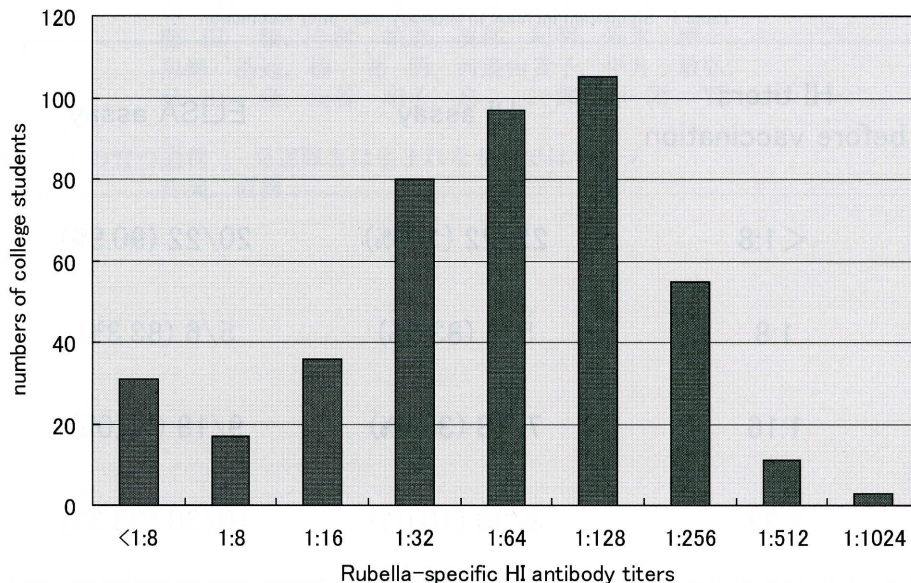


Fig. 1. Distribution of HI antibody titers •
 The mean and median titers were both 1:64. The numbers of students with titers of <1:8, 1:8, 1:16 and 1:32 comprised 6.4%, 2.3%, 5.0% and 12.9% of the total, respectively.

of 1:16 and 30 students had titers of 1:32. Among the 76 subjects, 43 students (56.6%) had had one rubella vaccination before, and 8 students (10.5%) had an unknown vaccination history. Nine students (11.9%) had a past history of the infection, and 8 students (10.5%) had an unknown infection history. Among 22 students with negative HI antibody, 9 (41%) students had neither a past history of infection nor of vaccination, 8 (36%) had a past history of vaccination, 4 (18%) had a history of rubella infection, and 3 (14%) students had a history of both.

1) Significant increase in antibodies after vaccination

The definition of a significant increase in antibodies was seroconversion or a ≥ four-fold rise in HI antibody titers, and seroconversion or a ≥ two-fold rise in ELISA titers. The ratio results which increased significantly in the antibody titers are shown in Table 1. With the HI assay, there was a significant increase in 22/22 (100%), 5/6 (83.3%), 7/18 (38.9%) and 3/30 (10.0%) after vaccination in students with titers of <1:8, 1:8, 1:16 and 1:32, respectively, before vaccination. With ELISA, there was a significant increase in 20/22 (90.9%), 5/6 (83.3%), 9/18 (50.0%) and 10/30 (33.3%) after vaccination in students with titers of <1:8, 1:8, 1:16 and 1:32, respectively, before vaccination.

2) IgM antibody detection and a high avidity index after vaccination

After vaccination, the IgM antibody was detected in 14 (63.6%) of the 22 students who had a negative HI antibody titer of <1:8. Seven students had a negative titer and the remaining one had + for the IgM antibody. The IgM antibody could not be detected in those who had pre-existing HI antibody. Among the seven students without a response for the IgM antibody after vaccination, three students had a past history of vaccination, and two of these students also had a past history of infection.

Sera with avidity index values of >50% were classified as samples containing high avidity of the rubella

Table 1. Significant increase in antibody titers after vaccination

HI titers before vaccination	HI assay	ELISA assay
<1:8	22/22 (100%)	20/22 (90.9%)
1:8	5/6 (83.3%)	5/6 (83.3%)
1:16	7/18 (38.9%)	9/18 (50.0%)
1:32	3/30 (10.0%)	10/30 (33.3%)

The definition of a significant increase in antibodies was seroconversion or a \geq four-fold rise in HI antibody titers, and seroconversion or a \geq two-fold rise in ELISA titers.

IgG antibody, whereas sera with values of <30% were classified as low. Among the 22 students who had a negative HI antibody titer before vaccination, 3 (13.6%) students showed a high avidity index after vaccination. Two of these students with a high avidity index had a documented past history of vaccination, but the remaining one had neither a history of vaccination nor of infection. The IgM antibody could be detected in only one student.

3) Rate of revaccination required after vaccination

When the criterion for vaccination was an HI antibody titer of <1:8 or \leq 1:8, the required revaccination rate was 0%. However, when it was \leq 1:16 or \leq 1:32, the required revaccination rates were 9/46 (19.6%) and 38/76 (50.0%), respectively, because their antibody titers were not over the titer of such criteria after vaccination.

DISCUSSION

To confirm a diagnosis of rubella, the IgM antibody with the ELISA assay is usually employed. However, in Japan, the sensitivity of the HI assay is the same as that of the ELISA assay⁸). Commercially, the cost of the HI assay is almost one-third that of the ELISA assay. So, the HI assay is usually chosen to measure the antibody when a physician wishes to determine whether or not a patient is susceptible to rubella. Rubella antibodies have been measured in approximately 80% of pregnant women in Japan. To prevent CRS due to infection or reinfection for the next baby, a research group supported by the Japanese government has recommended that women with an HI antibody titer of \leq 1:16; that is, women with negative or low titers, should be vaccinated after delivery. However, many doctors have been unsure that this criterion should be applied to general people, male workers or students in hospitals. The purpose of this study was to determine

at what titer pre-existing antibody titers could be efficiently boosted by vaccination. In this study using the Matsuura strain, based on the HI assay, 100% of the students who had an HI antibody titer of <1:8 and 83% of those who had a pre-existing titer of 1:8 showed a significant increase in antibody titers. However, 39% of the students with a 1:16 titer and 10% of students with a 1:32 titer also showed a significant rise in the titers. On the other hand, with the ELSA assay, 91% of the students who had an HI antibody titer of <1:8, and 83% and 50% of those who had a pre-existing titer of 1:8 and 1:16, respectively, showed a significant increase in antibody titers. However, 33% of the students with a 1:32 titer showed a significant rise in the titers. There have been a few reports regarding the booster effect. In postpartum vaccination for women with a pre-existing low antibody titer, Horstmann et al.⁹⁾ reported that 5 (16%) of 31 women who had HI antibody titers of 1:8 or 1:16 exhibited a four-fold or greater increase in titers after vaccination with the Cendehill strain. Isacson et al.¹⁰⁾ reported that 19 (46%) of 41 women who had HI antibody titers of 1:10 or 1:20 showed a four-fold or greater response after vaccination with HPV-77 DK₁₂, HPV-77 DE₅ or Cendehill. Grillner et al.¹¹⁾ also reported that 16 (8.7%) of 196 women who had an HI antibody titer of 1:20 showed a significant increase after vaccination with HPV-77 DE₅, Cendehill or RA27/3. In elementary school children¹²⁾, 39 (57%) of 68 children with 1:20 or 1:40 titers exhibited a four-fold or greater increase in the titer after revaccination with RA27/3 or HPV-77 DE₅ three years after the primary vaccination. The differences in the rates might be associated with differences in vaccine strains. The sequence in the E1 region of the Matsuura strain was clearly different from those in HPV77¹³⁾ or RA27/3¹⁴⁾. The rates were apparently lower than the seroconversion rates of >90% in susceptible subjects. In this study, however, the antibody response with the Matsuura strain among those who had a pre-existing low antibody titer was thought to be relatively good, although the assay for evaluation or age was different.

Among the 22 students who had exhibited a negative HI antibody titer before vaccination, the IgM antibody was not detected in 7 (31.8%) students after vaccination. No response of the IgM antibody after vaccination would imply the presence of partial immunity or secondary vaccine failure, because detection of IgM usually means primary infection. There has been one report of not finding the IgM antibody in any previously immunized subjects despite the lack of HI antibody¹⁵⁾. However, in this study, among eight students previously vaccinated, the IgM antibody was detected in five after vaccination. Among seven students without the IgM antibody, only one had a past history of vaccination. Two had a past history of vaccination and of infection and two had neither a past history of vaccination nor of infection. Although the numbers were small, we think that no response of the IgM antibody is an unsuitable criterion for partial immunity or secondary vaccine failure. Hamakar et al.¹⁶⁾ reported that the avidity assay is more sensitive and specific than measurement of IgM antibody titers to differentiate primary rubella infection from reinfection, which indicates partial immunity. High avidity is an indication of strong affinity between antigen and antibody, and suggests that memory cells still exist despite a lack of the antibody, since avidity is weak in the early stage of infection, increases with the course of the disease and is maintained for a long period. In this study, high IgG antibody avidity levels after vaccination were recognized in 3 (13.6%) of the 22 students who did not have pre-existing antibody. Among the three students with high avidity, two had a past history of one-dose vaccination, while the remaining one had neither a history of vaccination nor of rubella infection. Secondary vaccine failure or waning immunity after subclinical infection was thought to be involved in these cases. We consider the avidity test to be a better method than measurement of IgM antibody levels for determining partial immunity or secondary vaccine failure.

When the criteria were titers of $\leq 1:16$ and $\leq 1:32$ for vaccination, the subject numbers increased by 2.1-fold and 3.5-fold in comparison with the numbers with a titer of $<1:8$, respectively. In addition, 20% and 50% of the subjects with titers of $\leq 1:16$ and $\leq 1:32$, respectively, required revaccination, because the antibody titer after vaccination did not satisfy the criterion. Therefore, it is considered that a criterion of 1:8 or $\leq 1:8$ should be used for general people. However, considering the past one-dose-vaccination schedule in Japan, and that half of those who had a 1:16 titer showed a booster effect with the ELISA assay, we think the criteria of $\leq 1:16$ titers would be appropriate for women and high-risk people such as medical or educational workers or students.

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