Affinity and sub-class distribution of IgG-class antibodies following vaccination with a live rubella virus vaccine

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Thirty-three seronegative adolescents were vaccinated with a live rubella vaccine. Serum samples were collected 2 weeks, and 1, 2, 6 and 12 months later. Antibodies were analysed by using ELISA and monoclonal antibodies to different subclasses of human IgG. A proportion (10 out of 33, 30%) of the vaccinees developed low amounts of rubella IgG-class (both IgG1 and IgG3 isotypes) antibodies. There was no difference between the high and low responders when the IgG-class antibodies were measured at low serum dilutions suggesting that the affinity of the antibodies was probably equal. Our results point out a possible pitfall, development of low amounts of antibodies, in the serological evaluation of the success of rubella vaccination.

Keywords: Antibody affinity; ELISA; immunoglobulin G; rubella

Considerable differences in the seroconversion rate and persistence of vaccine induced antibodies have been reported for different rubella vaccines. Following infection antibody affinity has been suggested to be useful in the evaluation of the period between infection and serum sampling. IgG1 is the main isotype of antibodies directed against rubella, although recently, the contribution of IgG3 was reported to be significant in the early phase of antibody response. We have evaluated the amounts, relative affinity and sub-class distribution of rubella IgG antibodies in seronegative adolescents vaccinated with a live rubella vaccine.

Serial serum samples were available from 33 rubella seronegative students or school girls 2 weeks, and 1, 2, 6 and 12 (only 15 samples) months after vaccination with a live rubella vaccine (Cendevax, Smith-Kline, Belgium). Rubella IgG and IgM antibodies were analysed using a sucrose density gradient purified virion antigen in enzyme-linked immunosorbent assay (ELISA). Monoclonal antibodies to two IgG isotypes: IgG1 and IgG3 (clones BAM 15 and BAM 08, respectively, Seward Laboratories, UK) were used. The bound antibodies were detected by peroxidase-labelled anti-mouse antibodies (Dakopatts a/s, Denmark). Serum dilutions of 3 x 10^-2 for IgM and 1 x 10^-2 for IgG were chosen from the linear part of standard dilution curves for the analyses of total specific antibody levels. The results were expressed as enzyme-immuno units (EIU). In our previous study the rubella IgG antibody levels in a normal population peaked at about 80 EIU (mean 79 EIU, standard deviation, s.d., 23). Thus, persons with antibody levels lower than 10 EIU (mean - 3 s.d.) or lower than 55 EIU (mean - 1 s.d.) were considered seronegative or to be low responders, respectively. All the vaccinees were seronegative but developed a detectable IgG antibody response after vaccination. The low responders had significantly lower antibody levels (p < 0.005; Student's t-test) at six months after vaccination than the majority of the group. Different patterns of antibody response were detectable as early as one or two months before vaccination. There were no significant differences in the development of IgM antibodies. The IgG and IgM antibody levels showed no significant differences six months after vaccination at low serum dilution (3 x 10^-2). However, as the dilution increased (>= 1 x 10^-3) a considerable difference was noted in the IgG but not in the IgM-class antibodies. The analysis of the IgG1 and IgG3 antibodies revealed a pattern almost identical to that seen with the total IgG antibodies. The determination of antibodies by ELISA reflects either the total amount of antibodies or both antibody affinity and amount depending on the serum dilution and availability of the antigen in the solid phase. Our ELISA applies an antigen excess and is thus suitable for the evaluation of both the parameters. The vaccinees divided into two groups; those with high antibody levels and those with low. The difference between the two groups was at its greatest six months after vaccination. We did not find differences in the estimated antibody affinity between the two groups. IgG1 and IgG3 are the main IgG subclasses detectable after an acute rubella infection. In the low responder group also the IgG1 and IgG3 subclass
analyses showed low antibody levels. The estimated affinity of the subclass antibodies was about equal to that of the total rubella IgG antibodies. Thus, it is unlikely that differences in the subclass distribution or antibody affinity could explain the differences in the antibody reactivity.

Polyclonal activation occurs in rubella[2,13] and might explain the phenomenon described. Another explanation is that of an antigen-driven IgM antibody response[14] causing suppression of IgG antibody production[15,16]. The low responders had higher IgM antibody levels than the high responders, however, the differences were not statistically significant.

Serological evaluation of rubella vaccination (by Cendevax) may give contradictory results[2,17,18] since some individuals develop low amounts of IgG antibodies. Revaccination of the low responders should help to evaluate the protectivity of the first inoculation.

References
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Figure 1 Development of IgG (a) and IgM (b) antibodies following vaccination with a live rubella virus vaccine. Serum samples were drawn 0.5, 1, 2, 6 and 12 months after vaccination and the results are given as mean ± standard deviation. ●, High responders; ○, low responders

Figure 2 Titration of rubella virus antibodies 6 months (1 month for IgM) after vaccination. The serum samples were tested at dilutions of (1 x 10⁻³) for IgM, 1 x 10⁻³, 3 x 10⁻³, 1 x 10⁻², 3 x 10⁻², and 1 x 10⁻¹ and the results are given as mean ± standard deviation. ●, High responders; ○, low responders; a, IgG; b, IgG₁; c, IgG₂; d, IgM

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