Antibody response in school children to live rubella vaccine (Cendehill strain)

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Summary: The efficacy of an attenuated rubella virus vaccine, Cendevax, was tested on 65 school children. Forty-nine of them (75%) had pre-existing antibodies and in these there was no increase in the HAI antibody titres after administration of the vaccine. Sixteen children (25%) had no demonstrable rubella HAI antibody prior to vaccination. From the latter group, postvaccination serum samples were available from only 11, and 10 of these seronegative children showed seroconversion after vaccination. The geometric mean HAI titre was 1:180. Seven of the 10 postvaccination serum samples had complement-fixing antibodies and specific IgM antibodies were detected by the immunofluorescence test in 8. No correlation was observed between the CF and the IgM antibodies.

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Following the first demonstration by Parkman *et al.*[10] that safe and effective live rubella virus vaccine could be prepared by attenuation of the virus through serial passage in primary African green monkey cell cultures, several live rubella virus vaccines have been developed by others using primary or established cell lines. At present several such vaccines are available commercially but only two of these—"Meruvax" and "Cendevax" have been approved for clinical use in Canada. "Meruvax", manufactured by Merck, Sharp and Dohme, was developed by further passage of the HPV-77 virus strain of Parkman *et al.*[10] in duck embryo cell cultures. "Cendevax" was developed by Smith, Kline and French from a rubella strain which had been passaged 53 times in rabbit kidney cell culture.

Although these two vaccines, as well as the others, have been evaluated by different investigators,[11, 12] it was considered desirable to appraise the efficacy of the Cendevax vaccine which has been used in Ontario in connection with the rubella vaccination program for primary school children. Evaluation of the vaccine was based on the antibody response of the vaccinees and on the excretion of the virus in the throat.

Materials and methods

Sixty-five children in grades 4 and 5 were available for this study. After prevaccination serum samples had been obtained, each vaccinee received 1000 to 1800 tissue culture infectious doses of vaccine in 0.5 ml. quantities subcutaneously. Eight weeks later postvaccination serum samples were collected and paired sera were tested for antibody response. Three serological tests were employed for the estimation of antibody titres: hemagglutination inhibition (HAI), complement fixation (CF) and immunofluorescence (IF).

Hemagglutination inhibition test

The method employed was that advocated by the Center for Disease Control, Atlanta, Georgia[16] and was used without modification.

Complement fixation test

Rubella CF antigen was prepared from packed infected BHK21 cells by the alkaline extraction method, as described by Schmidt and Len-

nette.[14] All paired sera from the cases of seroconversion were tested by the CF test, using microtitre technique.

Immunofluorescence test

The IF method for the detection of rubella-specific antibody in the IgM fraction of the immunoglobulins was developed in this laboratory and has been described in detail elsewhere.[5] All postvaccination sera which showed increase in antibody titre were tested for the presence of rubella-specific IgM antibodies.

Tests for virus excretion

Throat washings from those with no pre-existing serum rubella antibodies were tested for the presence of rubella virus. The first specimen was collected one week, and the second two weeks after vaccination. The throat washings were inoculated into primary African green monkey kidney cell cultures, incubated for nine days at 36°C. and then challenged with echovirus 11.[9] Three blind passages were made before it was concluded that no rubella virus was present.

Results

Sixteen out of the 65 children (25%) showed no demonstrable rubella HAI antibodies in their prevaccination serum samples. The antibody titre of the remaining 49 sera (75%) ranged from 1:64 to 1:2048, the geometric mean titre being 1:120 (Table I). None of the vaccinees in this group with pre-existing HAI antibodies showed any increase in the antibody titre after vaccination.

Of the 16 initially seronegative vaccinees, postvaccination serum samples were available from only 11. Ten of these showed seroconversion with HAI titres ranging from 1:64 to 1:1024 (Table II). The geometric mean titre of these 10 was 1:180. The complement-fixing antibodies were demonstrable in seven out of 10, with the titres ranging from 1:4 to 1:16 and rubella-specific IgM antibodies were detected in eight out of 10 postvaccination serum samples.

Virus isolation was attempted from the throat washings collected from children who did not have antibodies in the prevaccination serum sample. Rubella virus was not recovered from any of them after three blind passages in African green monkey kidney cell cultures.

Comments

Seroconversion, using the same Cendevax rubella vaccine, was observed by Grant *et al.*[3] in 97% of the susceptible vaccinees. In our study, the percentage of seroconversion is somewhat lower, 10 cases out of 11 (90.9%). This discrepancy is most likely explained by the much smaller number of subjects used in our study.

The geometric mean titre of postvaccination sera of the 10 in whom seroconversion was noted was 1:180, which is considerably higher than 1:21 and 1:70.4, as reported by Karchmer *et al.*,[6] and Schiff, Rauh and Rotte,[13] respectively.

It is of interest that there was no increase in the HAI titre after vaccination in 49 vaccinees who had pre-existing antibodies, although such a rise has occasionally been observed by others in vaccinees with pre-existing HAI titres.[4, 6]

The complement-fixing antibodies were demonstrable in seven out of 10 vaccinees, and this supports the observations made by Schmidt and Lennette,[15] although it has been reported by others[1, 7] that attenuated rubella

Table I
Rubella HAI titres in school children, Grades 4 and 5, prior to administration of live rubella vaccine

Number* negative	Number with HAI titre						Number positive	Total number tested
	1:64	1:128	1:256	1:512	1:1024	1:2048		
16(24.6%)	2	1	7	24	11	4	49(75.4%)	65

*Negative at 1:8 dilution

Table II
Antibody response in susceptible school children to live rubella vaccine

Subject	Reciprocal of HAI titres		Reciprocal of CF titres		Specific IgM antibody
	Prevaccination	Postvaccination	Prevaccination	Postvaccination	Postvaccination
1	Negative*	1024	Negative**	16	–
2	Negative	256	Negative	Negative	–
3	Negative	256	Negative	8	+
4	Negative	128	Negative	4	+
5	Negative	256	Negative	4	+
6	Negative	64	Negative	8	+
7	Negative	64	Negative	8	+
8	Negative	64	Negative	Negative	+
9	Negative	256	Negative	16	+
10	Negative	256	Negative	Negative	+

*Negative at 1:8 dilution
**Negative at 1:4 dilution

live vaccine does not induce complement-fixing antibody. The failure on the part of others to demonstrate the complement-fixing antibody response may well be due to the nature of the complement-fixing antigen used.

The rubella-specific IgM antibodies were detected in eight out of 10 postvaccination sera.[2]

In natural rubella infection the specific IgM antibodies are, as a rule, detectable for about three weeks after appearance of the rash or approximately seven weeks after exposure. In vaccinees the specific IgM antibodies were demonstrable eight weeks after the administration of vaccine. This longer persistence of the IgM antibodies in vaccinated persons is undoubtedly due to a longer survival of attenuated virus in the body[8] and, therefore, more prolonged antibody stimulus. Failure to detect the rubella-specific IgM antibodies in two of the vaccinees could conceivably be due to a more rapid antibody response in these two individuals and, therefore, to a shorter period of persistence of the virus in the body. Unfortunately, earlier serum samples were not available to substantiate this assumption.

No apparent correlation between the complement-fixing and the rubella-specific IgM antibodies was observed, as is shown in cases 1, 8 and 10 (Table II). The diagnostic significance of the IgM antibodies has been discussed elsewhere.[5]

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Résumé

Réaction d'anticorps au vaccin anti-rubéoleux vivant (souche Cendehill) chez des écoliers

Nous avons évalué chez 65 enfants l'efficacité d'un vaccin (Cendevax) à base de virus atténué de la rubéole. Chez 49 des sujets (75%), on notait la présence d'anticorps pré-existants, et chez eux, l'administration du vaccin n'a pas augmenté le titre d'anticorps HAI. Les 16 autres enfants (25%) n'avaient pas d'anticorps HAI avant la vaccination. Dans ce dernier groupe, on n'a pu obtenir des échantillons de sérum post-vaccinal chez 11 d'entre eux: 10 de ces enfants séronégatifs révélaient une séroconversion après vaccination. Le titre géométrique moyen des HAI était de 1/250. Sept des 10 échantillons de sérum post-vaccinal avaient des réactions de fixation du complément. Des anticorps IgM ont été découverts chez huit sujets par la méthode d'immunofluorescence. Nous n'avons noté aucune corrélation entre les anticorps CF et les IgM.

Les demandes de tirés-à-part doivent être adressées au Dr. N. A. Labzoffsky, chef du Laboratoire des virus, Ontario Dept.of Health, Box 9000, Terminal "A", Toronto, Ontario.

References

1. DUDGEON JA, MARSHALL WC, PECKHAM GS, et al: Clinical and laboratory studies with rubella vaccines in adults. *Br Med J* 1: 271, 1969
2. BROWN GC, O'LEARY TP: Fluorescent-antibody marker for vaccine induced rubella antibodies infection and immunity. Infection and Immunity 2: 360, 1970
3. GRANT L, BELLE EA, PROVAN G, et al: Trials with a live attenuated rubella virus vaccine, Cendehill strain. *J Hyg (Camb)* 68: 505, 1970
4. HALSTEAD SB, CHAR DFB, DIWAN AR: Evaluation of three rubella vaccines in adult women. *JAMA* 991: 211, 1970
5. IWAKATA S, RHODES AJ, LABZOFFSKY NA: The significance of specific IgM antibody in the diagnosis of rubella employing immunofluorescence technique. *Can Med Assoc J* 106: 327, 1972
6. KARCHMER AW, HERRMANN KJ, FRIEDMAN JP, et al: Comparative studies of rubella vaccines. *Am J Dis Child* 197: 118, 1969
7. MEYER HM JR, PARKMAN PD, HOBBINS TE, et al: Clinical studies with experimental live rubella virus vaccine (strain HPV-77). Evaluation of vaccine-induced immunity. *Am J Dis Child* 115: 648, 1968
8. Rubella vaccination of pregnant women. Morbidity and Mortality Weekly Report 100: 20, 1971
9. PARKMAN PD, BUSCHER FL, ARTENSTEIN MS: Recovery of rubella virus from army recruits. *Proc Soc Exp Biol Med* 111: 225, 1962
10. PARKMAN PD, MEYER HM JR, KIRSCHSTEIN RL, et al: Attenuated rubella virus. 1. Development and laboratory characterization. *N Engl J Med* 275: 569, 1966
11. Proceedings of the International Conference on Rubella Immunization. *Am J Dis Child* 118: No. 1, 1969
12. Proceedings of the International Rubella Conference on Protection for the Postpubertal Female. *Can J Public Health* 62: Sept., 1971
13. SCHIFF GM, RAUH JL, ROTTE T: Rubella vaccine evaluation in a public school system. *Am J Dis Child* 203: 118, 1969
14. SCHMIDT NJ, LENNETTE EH: Complement-fixing and fluorescent antibody responses to an attenuated rubella virus vaccine. *Am J Epidemiol* 351: 91, 1970
15. SCHMIDT NJ, LENNETTE EH: Rubella complement-fixing antigens derived from the cellular and fluid phase of infected BHK-21 cells: extraction of cell-associated antigen with alkaline buffers. *J Immunol* 97: 815, 1966
16. Standardized rubella hemagglutination inhibition test. Immunology Series No. 2. Procedural Guide. U. S. Department of Health, Education and Welfare/Public Health Service 1970