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Vaccination of adults with Wistar RA 27/3 rubella vaccine

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SUMMARY

Thirty-three adults were vaccinated subcutaneously with Wistar RA. 27/3 (live attenuated) rubella vaccine at the Wellcome Research Laboratories, Beckenham. All subjects with pre-vaccination haemagglutinating-inhibiting antibody titres of 1/20 or less and three of seven subjects with pre-vaccination titres of 1/40 showed at least fourfold rises of titre. Reactions encountered were mild and of short duration.

INTRODUCTION

It has been estimated that the extensive rubella epidemic in the United States in 1963–4 resulted in the birth of between 10,000 and 20,000 congenitally abnormal infants, in addition to many abortions and still-births (Cooper & Krugman, 1966). Thus the protection of women of child-bearing age against rubella is important, although the method by which it should be achieved remains in dispute. Vaccination of schoolgirls aged 11–13 years has been advocated but it is not yet clear that rubella vaccines will provide a 30-year duration of immunity and periodic booster doses may be required. Since there is at present no definite evidence to show that any rubella vaccine is not teratogenic if administered in early pregnancy, there is a tendency to avoid the vaccination of adult women.

This report describes the vaccination of 33 adults aged 18–65 years at the Wellcome Research Laboratories, Beckenham, with Wistar RA. 27/3 strain (live attenuated) rubella vaccine. Seronegative subjects and subjects with low antibody titres were included. The attenuation and characteristics of this vaccine have been described in detail elsewhere (Plotkin, Cornfeld & Ingalls, 1965; Plotkin, Farquhar, Katz & Ingalls, 1967). Wistar RA. 27/3 is the only rubella vaccine prepared in WI-38 human diploid cells and the only one which is regularly immunogenic when administered by the intranasal route.

METHOD

Eleven males and 22 females were vaccinated subcutaneously. All the males and 13 of the females were involved in the production and safety testing of rubella vaccine, or required entry into production and testing areas. The remaining nine female subjects, unconnected with vaccine production, took part in the clinical trial as volunteers.

Serology

Rubella haemagglutinating inhibiting (HAI) antibody titrations were carried out according to the method described by Stewart *et al.* (1967). The titration method was modified to utilize manganous chloride and heparin for the removal of non-specific inhibitors (Mann, Rossen, Lehrich & Kasel, 1967; Feldman, 1968; Plotkin, Bechtel & Sedwick, 1968) and pigeon red cells were substituted for day-old chick erythrocytes (Peetermans & Huygelen, 1967). The United States National Reference Serum, with a geometric mean HAI antibody titre of 1/100 following kaolin treatment (E. B. Seligmann, personal communication), in our hands gave a mean titre of 1/235 in 24 estimations. The difference could be accounted for by the different techniques employed to remove inhibitors. The distribution of pre-vaccination HAI antibody titres was as follows: < 1/10 (17), 1/10 (4), 1/20 (4) 1/40 (7), 1/80 (1). Fourfold increases in titre following vaccination have been reported in subjects with pre-vaccination titres up to 1/64 (Dudgeon, Marshall, Peckham & Hawkins, 1969). Since the protection afforded by low antibody concentrations appears uncertain, subjects with low antibody titres were vaccinated.

Safety precautions

A careful explanation was given of the possible reactions following vaccination, risks of vaccination during pregnancy, and upon pregnancy occurring within the following 6–8 weeks. Informed consent was obtained from all subjects. As a security measure latex and tube agglutination pregnancy tests (Prepurex, Prepuerin, Wellcome Reagents Ltd.) were carried out on urine specimens from all women of child-bearing age before vaccination. Subjects were not told that a pregnancy test was being performed unless they enquired, since it was thought undesirable for subjects to be able to place reliance on pregnancy testing if they were uncertain about pregnancy. This test was repeated 8–10 weeks after vaccination.

Vaccine

Two lots of Wistar RA. 27/3 strain live attenuated rubella vaccine were used in this study at dose titres of $10^{2\cdot94}$ and $10^{2\cdot5}$ respectively, as assayed by the indirect method in monkey kidney tissue cultures. Vaccine was produced on WI-38 cell cultures and, after stabilization, was lyophilized. Uninoculated control cells were shown to be of the normal human karyotype, susceptible to viruses and not oncogenic in hamsters. No extraneous agent was detected in the virus harvests when tested in both tissue cultures and in animals.

Reactions

All subjects were seen 3 weeks after vaccination, and if necessary as symptoms occurred. Serial weekly differential white cell and platelet counts were carried out in a group of eight subjects.

RESULTS

Development of HAI antibody

All 25 subjects with pre-vaccination HAI titres of 1/20 or less responded to vaccination with at least fourfold increases in titre and no difference could be determined in the response between subjects with no detectable antibody and those with low titres. Similarly, no differences in antibody responses were evident between subjects given either of the lots of vaccine. Serial weekly HAI antibody

Table 1. *Development of rubella HAI antibody after subcutaneous vaccination with Wistar RA. 27/3 rubella vaccine (pre-vaccination titres* < 10, 10, 20*-reciprocals)*

	Days after vaccination							
Study group	10	14	21	28	35	42	90	180
No. of subjects with some increase in antibody titre/no. examined	1/4	5/8	7/7	8/8	—	—	—	—
No. of subjects with ⩾ 4-fold increase in antibody titre/no. examined	— —	1/8	6/7	8/8	4/4	8/8	—	—
G.M.T.	—	23	126	174	269	247	247	—
Median titre	—	20	160	160	160/320	160/320	320	—
Late responder–prevaccination titre < 1/10	20	10	10	10	< 10	40	80	—
Total group								
G.M.T.	—	—	91	—	—	159	124	119
Median titre	—	—	80	—	—	160	160	80
Modal titre	—	—	80/160	—	—	160	160	80
No. of subjects in total group	—	—	14	—	—	25	17	7

G.M.T. = geometric mean titre.

titrations in a group of nine subjects (study group, Table 1) showed that antibody almost always first developed between the 10th and 21st days after vaccination, but increases in antibody titre occurred up to the 35th day after vaccination. The results of antibody development in one additional subject whose response to vaccine was markedly delayed are in complete contrast to the foregoing. This subject, who has no history of rubella or maternal contact with rubella, suffers no general medical disease and experienced no reaction to vaccination. The development of rubella specific immunoglobulins in this subject is being compared with those of other vaccinees and will form the basis of a separate report.

In three of seven subjects with pre-vaccination titres of 1/40 a fourfold increase was seen. One of these exhibited clear symptoms of arthralgia of 3 days' duration, starting on the 18th post-vaccination day, while another developed vague pain in the limbs on the 7th post-vaccination day, which persisted for 2 weeks. In one subject with a pre-vaccination titre of 1/80 no change in titre and no reactions were seen.

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Reactions

Reactions after vaccination are shown in Table 2. Of the five subjects with pre-vaccination titres of 1/40 or 1/80 who did not show a four-fold increase in titres, only one reported any reaction to vaccination. This subject recorded a mild sore throat on the 3rd and 10th post-vaccination days.

Table 2. *Reactions to vaccination of* 33 *subjects with RA.* 27/3 *rubella vaccine* (*subcutaneous administration*)

Reactions	Non-immune (Total = 28)	Immune (Total = 5)
No reactions	10	4
Post-vaccination:		
Sore throat	7	1
Occurring during post-vaccination days:		
1–7	2	—
7–16	4	—
Both	1	1
Fever	2	—
Rash	2	—
Lymphadenopathy	4	—
Cough and coryza	5	—
Local discomfort at injection site	3	—
Myalgia	3	—
Joint involvement	7	—

Table 3. *Joint involvement after vaccination*

Total number of subjects = 7										Joints involved				
Subjective symptoms	Subjective and objective symptoms									Small joints of hands and feet		Knee joint		General
4	3									2		4		1
	Days after vaccination													
Subject/involvement	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A/bilaterial effusions to knees	.	×	×	×	×	×	×	×	×	×	×	×	×	.
B/pains in thighs/knees	.	.	×	×	×	.	.	.	.	.	.	.	×	.
C/generalized joint pain	.	.	×	×	.	.	.	.	.	×	×	×	×	.
D/pain in knee	.	.	.	×	×	×	×	.	.	.	.	.	.	.
E/pain in hands	.	.	.	.	.	.	.	.	.	.	×	×	.	.
F/pain in wrists	.	.	.	.	.	.	.	.	.	.	×	×	×	.
G/pain in knees	.	.	.	.	.	.	.	.	.	×	×	×	×	.

None of the 28 susceptible subjects who were vaccinated experienced a severe or incapacitating reaction. The reactions that did occur were similar in type to those reported following rubella, but were of short duration and mild in nature.

Sore throat occurred in two phases: the first centred on the 3rd post-vaccination day, and the second on the 10–16th post-vaccination days. The incidence of

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lymphadenopathy in this study was low. This is not unexpected since assessments were made from subjective reports rather than frequent clinical examination of those vaccinated. Joint symptoms developed in 7 (25%) subjects, in 3 of whom there was an effusion into a knee joint. The small joints of hands or feet were involved in 2 subjects, the knee joints in 4, while 1 subject suffered generalized joint pain. Joint symptoms were again reported in two phases between the 9th and 14th days, and between the 17th and 20th days post-vaccination. Symptoms were usually limited to a few days' duration (Table 3). Serial weekly differential

Table 4. *Report of serial platelet counts in eight subjects vaccinated with Wistar RA. 27/3 rubella vaccine subcutaneously*

	Platelet counts in 7 subjects responding serologically to vaccine			Plate count in 1 immune vaccinated subject	
	Mean	Range	change (%)	Count	change (%)
Before vaccination	180,300	150,000–234,000	—	*	*
After vaccination					
7 days	142,857	109,000–160,000	−21·1%	188,000	—
14 days	152,286	119,000–176,000	−15·6%	187,000	−0·5%
21 days	190,556	119,000–305,000	+6·7%	182,000	−2·7%
28 days	183,143	149,000–238,000	+1·7%	183,000	−2·6%
35 days	177,433	127,000–205,000	−1·7%	174,000	−7·4%
42 days	147,571	119,000–175,000	−18·3%	163,000	−13·3%

* No result obtained.

white cell counts showed no abnormalities. However, certain trends are visible in the weekly platelet counts which were carried out in a small group of subjects. These data require amplification, since relatively small numbers of subjects were involved. Nevertheless, sharp falls in platelet counts occurred between the 7th and 14th days after vaccination, followed by some compensatory increase in counts in the 3rd post-vaccination week. (Table 4).

Oski & Naiman (1966) measured platelet counts for 35 days after administration of live measles vaccine, and found a maximum fall 3–7 days after vaccination, which was attributed to decreased platelet production. This finding is similar to the early fall in platelet count in our subjects. However, thrombocytopenia as a complication of natural rubella and measles is seen shortly after the appearance of the rash, and is believed to result from increased platelet destruction. We found a further fall in platelet count 8 weeks after vaccination, but this is later than might be expected, and its significance is therefore not clear.

DISCUSSION

Vaccination of adults with subcutaneously administered Wistar RA 27/3 (live attenuated) rubella vaccine is clearly effective in stimulating circulating antibody production in adult men and women. No difficulty was found in avoiding the vaccination of a pregnant woman in this small group of subjects where a

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careful explanation of the hazards of vaccination in pregnancy was given and a pre-vaccination pregnancy test was also performed. Nevertheless it is clear that the potential teratogenicity of vaccine is likely to cause difficulties in vaccination of larger groups of women of child-bearing age in different communities. The administration of oral contraceptives to such women may provide an additional safeguard.

Since three of seven of our subjects with pre-vaccination HAI antibody titres of 1/40 developed fourfold increases in titre after vaccination, it is clear that vaccination of such susceptible, but seropositive subjects, might place them at risk if they were pregnant. More information is needed to determine the clinical handling of these subjects who are not immune to subcutaneously administered vaccine, but who might be immune to natural infection.

The differing techniques used in the removal of non-specific inhibitors and the varying sensitivities of the technique in different laboratories are clearly of critical importance to the handling of subjects with low titres and there is much to commend the interpretation of such titres against a national or international standard rubella reference serum.

The subjective recording of reactions to vaccination on a daily basis tends to accentuate the apparent reactivity. With the exception of joint involvement, reactions to vaccination are unlikely to be of clinical significance. Joint involvement occurred less frequently than was reported after administration of HPV-77 vaccine (Cooper *et al.* 1969), and it was usually of short duration.

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