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Outcome of asymptomatic infection with rubella virus during pregnancy

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SUMMARY

We have tried to detect prenatal infection in 34 infants whose mothers were re-infected with rubella virus during pregnancy and in six infants whose mothers had primary subclinical rubella during pregnancy. Two methods of assessment were used: first, serum obtained soon after birth was tested for IgM antibody; secondly, serum obtained after the age of 8 months was tested for specific IgG.

The 34 women with re-infections had increases in IgG antibody titre but no IgM response. No evidence of prenatal infection was found in 33 of their 34 infants. One infant was found to have IgG antibody at the age of 11 months. This infant was IgM-negative at birth and had a rubelliform rash at the age of 5½ months; it therefore probably contracted post- rather than pre-natal infection. Fetal infection from maternal re-infection during pregnancy is probably rare.

The six women with primary subclinical rubella produced both IgG and IgM classes of antibody. Three of their six infants showed serological evidence of intrauterine infection. One, infected when its mother was 8 weeks pregnant, had clinical evidence of congenital rubella. Primary subclinical rubella during pregnancy therefore carries a significant risk of fetal infection.

Because of the difference in outcome, great care should be taken to distinguish between primary infection and re-infection when investigating symptomless increases in antibody titre after contact with rubella during pregnancy.

INTRODUCTION

Acute rubella is followed by lifelong production of antibody and usually by a high degree of immunity. This immunity, however, is not always complete. Although second attacks with a rash are rare, up to 6% of adults with antibody derived from previous natural infection may show increases in titre of at least fourfold, unaccompanied by symptoms, after close contact with cases of the acute disease (Horstmann *et al.* 1970; Evans, Niederman & Sawyer, 1971; Vesikari, 1972). Persons whose immunity is due to vaccination are more susceptible and may show rises in titre in up to 80% of cases, depending on the type of vaccine and

the circumstances of re-exposure (Wilkins *et al.* 1969; Chang, DesRosiers & Weinstein, 1970; Horstmann *et al.* 1970; Davis *et al.* 1971). These changes in titre are thought to be due to purely local re-infection, since virus has sometimes been isolated from the throat but never from the blood although viraemia has been sought by many workers.

Is symptomless re-infection a risk to the fetus? Boué, Nicolas & Montagnon (1971) described three women with rises in IgG antibody titre, but with no IgM response, following contact during early pregnancy with cases of acute rubella. Each woman produced a healthy infant in whom IgM antibody was not detected. These observations support the generally held view that a symptomless rise in the titre of pre-existing IgG antibody, without IgM, is characteristic of re-infection, which, being purely local and unaccompanied by viraemia, is unlikely to harm the fetus. Re-infection may not always be harmless, however, since at least three infants with congenital infection have been decribed whose mothers had apparently possessed antibody before the commencement of pregnancy (Eilard & Strannegård, 1974; Forsgren, Carlström & Strangert, 1979; Partridge, Flewett & Whitehead, 1981).

Unlike re-infection, primary subclinical rubella is thought to be a significant risk to the fetus. This suspicion, founded on occasional observations of individual cases, has been reinforced by retrospective surveys. In a summary of the first 4 years of the National Congenital Rubella Surveillance Programme, Sheppard *et al.* (1977) found that 42% of mothers of infants with confirmed or suspected congenital rubella could not recall having had a rash during pregnancy, although many of them remembered having been in contact with the disease. In American surveys 19–50% of mothers of confirmed cases of congenital rubella have been unaware of any rash (Avery *et al.* 1965; Cooper *et al.* 1965).

The risk to the fetus from symptomless maternal infection can only be assessed by prospective serological surveys which include both mother and child. In a study of the effect of normal human immunoglobulin the Public Health Laboratory Service Working Party on Rubella (1970) observed 119 pregnant women who had rises in antibody titre of fourfold or more and who allowed their pregnancies to go to term. In 48 of these mothers the infection was subclinical. Sixty of the infants were followed up by Peckham (1974), who found persistent HI antibody in 53% of those children whose mothers had experienced clinical rubella but in only 19% of those whose mothers had been without symptoms. These studies appeared to show that rubella was less dangerous when unaccompanied by a rash, but did not distinguish between primary subclinical rubella and re-infection.

We describe here a serological study of 40 women who apparently had symptomless infection during pregnancy after contact with clinically diagnosed cases of rubella. We have confined the study to mothers and babies from whom we had specimens which allowed us to distinguish between primary infection and re-infection in the mother, and to detect or exclude infection in the infant. Women exposed during the second and third trimesters have been included because previous studies of rubella with a rash have shown that the fetus can be infected at any time during pregnancy, although the risk varies at different stages. (Cradock-Watson *et al.* 1980). Thirty-four of these women were thought to be suffering from re-infection because they possessed antibody at, or soon after, the

time of contact and showed increases in titre of at least fourfold by at least two out of three tests. The other six women were thought to be experiencing primary subclinical infection because they had no antibody in their initial serum specimens but produced antibody after contact.

We have tried to detect fetal infection in two ways, using the procedures desribed in our previous work (Cradock-Watson *et al.* 1980). First, we have sought specific IgM antibody in cord blood, or in blood taken soon after birth. Secondly, we have looked for persistence of IgG antibody after the age of 8 months, when maternal antibody should have disappeared.

MATERIALS AND METHODS

Women with probable re-infection

We studied 34 women who were apparently re-infected at various times during pregnancy. Of these, 23 were in close contact with clinically diagnosed cases of rubella in their own homes, and 11 had briefer or less intimate contacts outside the home. Exposure to rubella occurred during the first 13 weeks in 22 cases, between 13 and 16 weeks in 7 cases, and at later times in the remainder. The diagnosis in the index case was always purely clinical, since we were never able to obtain specimens for laboratory confirmation. From four women we possessed an initial serum taken before exposure, and from 24 we obtained the first specimen within 9 days afterwards. In other cases the first serum was taken 11–16 days after exposure. Subsequent specimens were obtained at various times between 20 days and 5 months after contact. All sera were titrated by haemagglutination-inhibition (HI) and by immunofluorescence (IF) for IgG antibody (Cradock-Watson, Bourne & Vandervelde, 1972). The first serum and whichever later one showed the greatest rise in titre were also titrated for IgG antibody by radioimmunoassay (RIA) (Kangro, Pattison & Heath, 1978). The first serum was additionally tested for antibody by radial haemolysis (RH) (Kurtz *et al.* 1980). Sera which showed increases in titre were tested for specific IgM by fractionating the serum on a sucrose density gradient and then testing the fractions by the long incubation HI method and by IF.

These 34 women produced one stillborn and 33 liveborn infants.

Women with probable primary subclinical infection

We made similar studies on six women who apparently had primary rubella but did not notice any rash. They were exposed to clinically diagnosed cases of rubella at various times between the eighth week and the last month of pregnancy. From five women we possessed a serum taken before exposure, and from one we obtained the first serum 14 days after contact. Later specimens were taken at various times up to 9 weeks after contact. Each woman produced a liveborn infant.

Infants

Single specimens of serum obtained from 35 of the 40 infants at various ages up to 11 weeks were tested for IgM antibody. Two methods were used: (1) 32 sera were fractionated on sucrose density gradients and the peak IgM fraction was tested by IF; (2) 33 sera were tested by RIA without prior fractionation. In a

Table 1. *Results of four different tests for rubella antibody in the initial serum specimens from 34 pregnant women who had symptomless increases in titre after contact with cases of rubella*

No. of patients	HI antibody (i.u./ml)*	No. of patients with stated radial haemolysis result				Range of IgG antibody titres by	
		⩾ 15 i.u.	< 15 i.u.	negative	NT	IF	RIA
21	30–480	15	4	0	2	8–1024	100–35000
4	⩾ 15, < 30	3	1	0	0	16–64	300–1600
9	< 15	0	4	5	0	< 8–64	< 100–1600

NT = not tested.

* A standard serum containing 15 international units (i.u.) of rubella antibody per ml has an HI titre of about 20.

Table 2. *Results of three different tests for rubella antibody in nine women from Table 1 who possessed low titres of antibody not detectable by HI*

Patient	Radial haemolysis (i.u./ml)	IgG antibody titre by	
		IF	RIA
M.J.	Positive (< 15)	16	200
K.W.	Positive (< 15)	< 8	200
E.F.	Positive (< 15)	32	100
M.C.	Positive (< 15)	64	200
K.H.	Negative	32	1600
J.F.	Negative	16	< 100
S.E.	Negative	8	100
V.L.	Negative	32	< 100
J.C.	Negative	8	< 100

previous comparison these two methods showed good agreement (Cradock-Watson *et al.* 1979). Subsequent serum specimens from 34 of these infants at ages between 8 months and 2 years were tested for specific IgG antibody by IF. We did not test these follow-up sera by RIA since in a previous study of children whose mothers had had rubella during pregnancy we did not find any sera which were IF-negative but RIA-positive (Cradock-Watson *et al.* 1980).

RESULTS

Women with probable re-infection

Rubella antibody was detected by at least one of four methods in the first serum of all 34 women with probable re-infection (Tables 1, 2). Twenty-five of these sera contained more than 15 international units (i.u.) of HI antibody per ml (HI titres ⩾ 20) and were also positive by IF and RIA. Twenty-three of these 25 sera were additionally tested by RH: all were positive, but five gave small zones corresponding to less than 15 i.u./ml (Table 1). In nine women the first serum contained less than 15 i.u. of HI antibody per ml. By RH four of these nine sera were positive, but gave small zones, and five were negative. The presence of antibody in these nine sera was confirmed by IF in 8 cases and by RIA in 6 cases (Table 2). One woman

was IF-negative (< 8) but had an RIA titre of 200 and a weakly positive RH test. Three women were positive by IF alone, and negative by RIA (< 100) and RH. These nine women with low antibody titres were exposed to rubella at various times between 3 and 17 weeks after the last menstrual period and were first bled either before contact (2 cases) or within 8 days afterwards. The post-exposure sera were tested by HI, IF and RIA. All 34 women showed increases in antibody titre of at least fourfold by at least two of the three tests. The 25 women with HI antibody in the first serum all showed significant rises in HI titre: three showed a fourfold rise, three a sixfold rise, nine an eightfold rise, and 10 showed rises of 16-fold or more. The nine with no detectable HI antibody subsequently had levels ranging from 15 to ⩾ 960 i.u./ml (titres 20 to ⩾ 1280). In seven of these patients the antibody level increased fourfold or more and in two cases it rose from < 15 to 15 i.u./ml. IF titrations of IgG antibody were performed on paired sera from all 34 patients: titres after exposure to rubella ranged from 64 to 16000, confirming rises of 4- to 256-fold in 33 cases and twofold in one case. RIA titrations were done in 32 cases, giving titres of 500 to 104000 and confirming rises of 4·7- to 128-fold in 25 patients and less than fourfold in seven.

IgM antibody was not detected in any of the women in this group when density gradient fractions were tested by the long incubation HI method. IF staining of the peak IgM fraction was negative in 33 cases. In one case, however, the peak IgM fraction was weakly positive when tested by IF (titre = 2) and RIA (titre = 250). This was the only woman in the series whose immunity was known to have been due to the administration of rubella vaccine (RA 27/3), which had been given 3 years previously. Her own child developed clinical rubella when the mother was 7 months pregnant.

Women with probable primary subclinical infection

Six women had no detectable antibody in the first serum. All were negative by HI and IF, and all of five who were tested were also negative by RH and RIA. After exposure to rubella all six women produced high titres of antibody, easily detected by HI, IF and RIA. All six patients also produced IgM antibody which was easily detected when density gradient fractions were tested by HI.

Infants whose mothers had probably been re-infected during pregnancy

The serological results from 34 infants are summarized in Table 3. All of 29 infants (including 1 stillborn) who were tested soon after birth were IgM-negative. Twenty-seven out of 28 who were tested after the age of 8 months were IgG-negative. One healthy infant, whose mother had been in close contact with rubella when 10 weeks pregnant, possessed antibody at the age of 11 months (HI ⩾ 960 i.u./ml, IgG by IF = 2048). However, this child, who had been IgM-negative at birth, developed a rubelliform rash at the age of 5½ months and therefore probably contracted post- rather than pre-natal infection. If so, we can conclude that none of the infants whose mothers were re-infected during pregnancy showed any serological evidence of congenital infection.

Table 3. *Number of infants with IgM and/or persistent IgG antibody following probable maternal re-infection with rubella virus during pregnancy*

IgM antibody	Persistent IgG antibody			Total
	+	−	NT	
+	0	0	0	0
−	1	22	6	29
NT	0	5	0	5
Total	1	27	6	34

NT = not tested.

Infants whose mothers had probably had primary subclinical infection during pregnancy

Three out of six infants were IgM-positive by both IF and RIA and were therefore infected *in utero*. They subsequently remained IgG-positive. They had been at risk from maternal rubella at 8 weeks, 31 weeks, and during the last month of pregnancy. One of them, at risk at 8 weeks, had thrombocytopenia and osteopathy, but the other two showed no clinical signs of infection. The other three infants in this group, all normal and healthy, were IgM-negative and subsequently IgG-negative and were evidently not infected.

DISCUSSION

When assessing the dangers of subclinical rubella during pregnancy it is essential to distinguish between re-infection and primary infection. The presence or absence of existing antibody is crucial to this distinction and therefore it is unwise to rely on a single test such as HI which cannot distinguish between a low titre of antibody and non-specific inhibitors of agglutination. In the present series of patients a positive HI test always indicated antibody, but this was a matter of luck since in routine tests for immune status it is not uncommon to find individuals with positive HI tests who evidently have no antibody when retested by RH or IF. Equally, a negative HI result does not always indicate absence of antibody, which may be present in so low a titre that it can only be detected by more sensitive and specific methods.

By applying additional tests we confirmed the presence of antibody in the 25 women with positive HI tests; also we were able to detect antibody in 9 of the 15 women with negative HI tests and to be reasonably sure that there was no antibody in the other six. Our patients were tested for specific IgM because their lack of symptoms raised doubts about the type of infection. The IgM results corresponded almost exactly with the initial presence or absence of IgG antibody. Ig M was produced in all six women who had no IgG antibody at the time of contact; it was not detected by the conventional method in any of the 25 women with positive HI tests, although a trace was found by IF and RIA in the one patient whose initial antibody had been elicited by immunization. Nor was IgM detected in any of the nine women in whom HI was negative but other more sensitive tests were positive. In this way we classified our patients into 6 with primary subclinical infections and 34 who were probably re-infected. A similar inverse relationship

between pre-existing antibody and IgM response was observed by Mortimer *et al.* (1981) after the administration of RA 27/3 rubella vaccine.

Tests on the infants confirmed that primary subclinical rubella carries a risk of fetal infection, but because the number of patients in this group was small we could not assess the risk numerically or compare it with that which accompanies rubella with a rash. In contrast, in cases of maternal re-infection we found no evidence of transmission to the fetus and we suspect that fetal infection in these circumstances is rare. We emphasize, however, that we do not know how often symptomless increases in titre occur in the general community, nor do we know the relative frequency of re-infection and primary subclinical disease.

In any individual case a conclusion about the type of infection may affect the decision whether or not to terminate the pregnancy; consequently the results of tests should be interpreted with care. Clearly the first specimen of blood should be taken at, or soon after, the time of exposure. The presence of IgG antibody, even in very low titre, within 10 days of contact implies that primary infection occurred in the distant past. A subsequent rise in IgG titre, without the appearance of IgM, probably indicates re-infection. If the first serum is taken too late, and contains antibody, it is impossible to know whether this antibody indicates previous immunity or is appearing in response to recent primary infection. The distinction then depends largely on the presence or absence of IgM. A strong IgM response almost certainly indicates primary infection and a risk of spread to the fetus. Apparent absence of IgM is less easily interpreted: it does not necessarily exclude primary infection, since the response may be missed if specimens are not taken at the right time, or the test may fail for technical reasons.

Our general conclusion that re-infection is harmless to the fetus applies to women whose immunity is due to natural infection; it should not be assumed to include those whose immunity is the result of immunization. The one woman in our series whose immunity was known to have been vaccine-induced was also the only individual to show a trace of IgM when re-infected. This observation suggests that vaccinees may frequently produce IgM antibody, albeit in very small amounts, when re-infected. It is not known, however, how often they do so or whether this indicates possible spread to the fetus. In the United Kingdom women with vaccine-induced immunity will probably always be fewer than women with immunity from natural disease, but their susceptibility to re-infection is likely to be greater. Wild virus will continue to circulate, and the outcome when it re-infects vaccinees during pregnancy is a problem which requires further study.

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