J. Hyg., Camb. (1981), **87**, 131
Printed in Great Britain

Are many women immunized against rubella unnecessarily?

BY P. P. MORTIMER, J. M. B. EDWARDS, A. D. PORTER

Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

R. S. TEDDER, J. E. MACE,

Department of Virology, The Middlesex Hospital Medical School, Mortimer Street, London W1P 7PN

AND A. HUTCHINSON

Lintonville Medical Group, Ashington, Northumberland

(*Received* 26 *February* 1981)

SUMMARY

Radial haemolysis (RH) was used to test sera for immunity to rubella from 1317 patients attending a general practice. One hundred and forty-one (10·7%) were treated as susceptible and offered an attenuated virus vaccine (RA 27/3). Pre-immunization sera from 43% of these patients were reactive at low levels in RH (< 15 international units rubella antibody per ml).

Pre- (S1) and post- (S2) immunization sera from 66 vaccinees were studied in detail. Antibody was detected by RH, haemagglutination inhibition (HI) and enzyme-linked immunosorbent assay (ELISA), and the specific IgM response was measured by a solid-phase M-antibody capture radioimmunoassay (MACRIA). The vaccine-induced IgM response was only detected if the S1 serum was non-reactive by all tests for rubella antibody. It was weaker than that seen following wild virus infection. It could be detected reliably for six weeks, and in most cases for nine weeks, after immunization. In contrast, patients with S1 specimens reactive by RH, HI or ELISA never showed an IgM response in the S2 specimen despite 'significant' antibody rises often being present.

It was considered that an IgM response to RA 27/3 was the best indicator of pre-immunization susceptibility to rubella. The failure of many vaccinees to make an IgM response implied that a significant proportion were already immune. It is suggested that the threshold for a report of immunity to rubella could be lowered from 15 i.u. antibody per ml and so fewer women immunized without vaccine being withheld from those who need it.

INTRODUCTION

The need to immunize adult women against rubella has been recognized since 1972 (CMO, 1972), but the campaign to identify and immunize non-immune women has been intensified in the last two years. In 1980 the Public Health Laboratory

Service (PHLS) screened 600000 sera for rubella antibody and recommended the immunization of approximately 78000 women (unpublished data). The work is likely to continue on this scale, not only because the antibody status of many women of childbearing age remains unknown but also because at least 25% of girls entering this group each year have not received rubella vaccine at school (CMO, 1976).

Of the methods of sero-testing available, two, haemagglutination inhibition (HI) and radial haemolysis (RH), have been widely used in Britain. In spite of efforts to standardize these methods and to improve laboratory performance, including a widely accepted agreement to fix a threshold for a report of immunity at 15 international units (i.u.) rubella antibody per ml, several factors have tended to make the results inaccurate, particularly where sera of low reactivity are concerned. This has encouraged a conservative approach to the interpretation of screening tests in which sera with weak reactions (often of dubious specificity), are frequently reported as non-immune. Unfounded claims that low levels of antibody are not protective have encouraged this attitude.

At present many women reported as susceptible to rubella clearly have antibody (though below 15 i.u. per ml) and the development of a sensitive test for rubella IgM has offered an opportunity to review the immune status of these women. On the assumption that patients who are truly susceptible to rubella will make a primary response to vaccine characterized by the production of specific IgM whereas those who are actually immune will make no IgM, the accuracy of screening methods and the validity of the 15 i.u. per ml cut-off value can be investigated.

In this study specimens collected from women before and after rubella immunization were examined both by RH and HI, and by a new enzyme-linked immunosorbent assay (ELISA) test for anti-rubella IgG. In addition the post-immunization specimens were tested for anti-rubella IgM. The pre-immunization test findings are reviewed in the light of the anti-rubella IgM results on the post-immunization specimens, and changes in methods of screening for immunity to rubella are suggested.

MATERIALS AND METHODS

During 1979 and 1980 samples were taken from women aged 15–40 years in an urban general practice in the North of England. About 2 ml of venous blood were drawn from each patient, posted to the Virus Reference Laboratory and tested by RH. Out of 1317 women, 141 (10·7%) were found to have < 15 i.u. per ml rubella antibody and 106 of these accepted vaccine (RA 27/3, *Almevax*, Wellcome).

Two groups of women were selected as the basis for further tests: group I, those whose pre-immunization specimen (S1) was RH-reactive, though more weakly than the 15 i.u. per ml control: group II, those whose S1 specimen was RH-unreactive. A post-immunization (S2) specimen was obtained between the 20th and 80th day from 43 out of 61 women in group I. Sufficient serum for further tests was available from 33. An S2 specimen was obtained within the same interval from 60 out of the 80 women in group II. Specimens from the 33 of these most nearly matched both in interval to collection of S2 specimen and in age with the 33 available in group I were selected for further tests. The mean (and range) of

Table 1. *Means and ranges of RH, HI and ELISA results on pre-immunization (S1) and post-immunization (S2) specimens from patients grouped according to the presence or absence of RH antibody in S1 specimens*

		Pre-immunization (S1)			Post-immunization (S2)		
		RH zone diameter (mm)	HI reciprocal titre	ELISA absorbance	RH zone diameter (mm)	HI reciprocal titre	ELISA absorbance
Group I	Mean	5·3*	16**	0·58*	8·6	55	0·93
(S1 RH-positive,	Range	4·0–7·5	10–80	0·23–0·88	5·0–11·0	10–320	0·59–1·19
< 15 i.u. per ml)	Number	33	33	31	33	33	31
Group II	Mean	All negative	< 10	0·28	8·9	105	0·77
(S1 RH-negative)	Range	—	< 10–20	0·13–0·44	6·0–12·0	40–640	0·31–1·35
	Number	33	33	31	32	33	31

* Arithmetic mean. ** Geometric mean, calculated with < 10 scored as 5.

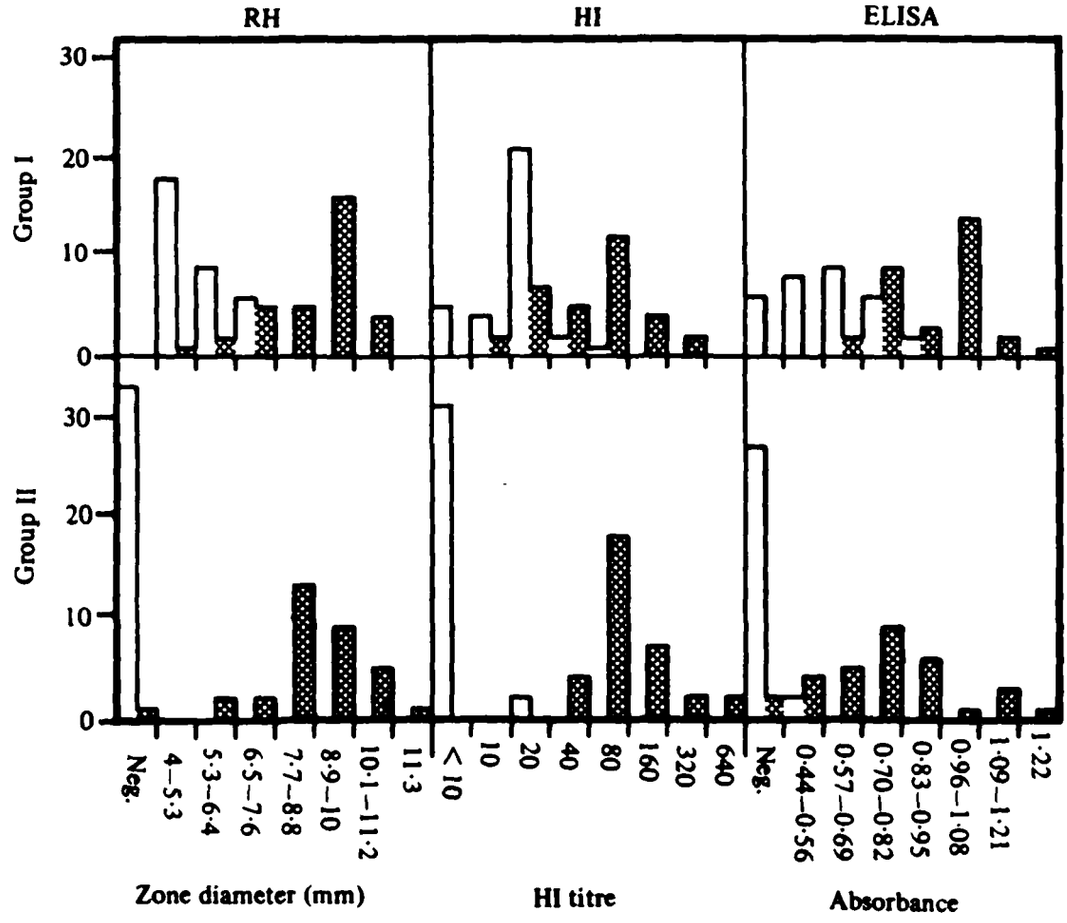

Fig. 1. Number of pre-immunization, □(S1) and post-immunization, ▩ (S2) specimens from patients in groups I and II reacting at each strength in RH, HI and ELISA tests.

intervals from immunization to S2 collection was 45·4 days (20–68) for the 33 group I patients and 44·0 days (21–84) for the 33 group II patients. The mean (and range) of the ages of the 33 in group I was 29·5 years (15–40) and of the 33 in group II 27·5 years (17–36).

The RH method was that of Kurtz and colleagues (1980). The HI method was that of Pattison & Mace (1973), in which overnight incubation of serum dilutions with haemagglutinin is used to increase the sensitivity of the test. The ELISA was an indirect test based on horseradish peroxidase-labelled anti-human IgG (*Rubazyme*, Abbott). Enough serum remained to use this assay on specimens from 31 of the 33 patients in each group.

An M-antibody-capture radioimmunoassay (MACRIA) (Mortimer *et al.* 1981), was used to detect anti-rubella IgM. In this test the IgM in the specimen is taken up onto a bead coated with anti-human μ chain serum and tested for specificity using rubella haemagglutinin followed by ^{125}I-labelled rabbit anti-rubella IgG.

The control for RH and HI tests was a freeze-dried serum, 2/74, prepared by the Division of Microbiological Reagents of the PHLS. It was used diluted to 15 i.u. per ml and gave zones of lysis in RH of mean diameter 7·0 mm and a titre by HI of 1 in 40. The ELISA test was controlled as recommended by the manufacturer: the threshold absorbance value for a positive result was 0·44. For the MACRIA a pool was prepared of sera strongly reactive for rubella-specific IgM. This was assigned a strength of 100 arbitrary units and was used as the standard. It was diluted in human serum devoid of rubella antibody to provide readings for a range of specific IgM concentrations from 0·3 to 100 units (Mortimer *et al.* 1981).

Table 2. *Correlations between three methods of testing pre-immunization (S1) specimens from 66 patients*

Radial haemolysis versus haemagglutination inhibition

	HI	
	Reactive (1 in ⩾ 10)	Unreactive
RH, Group I (positive, < 15 i.u. per ml)	28*	5
RH, Group II (negative)	2	31

Radial haemolysis versus enzyme immunoassay

	ELISA		
	Antibody-positive	Antibody-negative	Not tested
RH, Group I	25	6	2
RH, Group II	2	29	2

Haemagglutination inhibition versus enzyme immunoassay

	ELISA		
	Antibody-positive	Antibody-negative	Not tested
HI-reactive	26*	2	2
HI-unreactive	1	33	2

* Includes three specimens, HI 1 in 40, which would have been reported as immune to rubella.

Table 3. *Relationship between the RH, HI and ELISA reactivity of pre-immunization (S1) specimens and anti-rubella IgM result of the corresponding post-immunization (S2) specimen (66 patients)*

		S2 anti-rubella IgM (MACRIA)		
		Positive	Equivocal	Negative
S1 RH	Positive (group I)	—	—	33
	Negative (group II)	29	2	2
S1 HI	Reactive	—	—	30
	Unreactive	29	2	5
S1 ELISA*	Positive	—	—	27
	Negative	27	2	6

* Four patients not tested.

RESULTS

The results of antibody tests on the 66 patients from groups I and II are presented in Table 1 and Fig. 1. The segregation of the patients into groups on the basis of RH tests on S1 specimens was broadly confirmed by the results of HI and ELISA tests. The correlation between RH, HI and ELISA results on S1 specimens is shown in Table 2.

A close inverse relationship existed between the presence or absence of RH antibody in the S1 specimen and of an IgM response in the S2 specimen (Table 3).

https://doi.org/10.1017/S0022172400069308 Published online by Cambridge University Press

Table 4. *Relationship between the anti-rubella IgM result and the interval to collection of the post-immunization (S2) specimen in 31 group II (S1 RH-negative) patients**

	Interval to collection of S2 (days)		
	20–39	40–59	⩾ 60
Mean**	8·5	2·4	1·2
Range	1·3–41·0	0·9–8·6	0·6–1·8
Number	9	18	4

* Two patients who were HI-positive (titre 20) and ELISA-positive were excluded from the original 33.

** Arithmetic mean of results in MACRIA units per ml.

The 35 S2 specimens that were MACRIA-negative came from patients whose S1 specimens were rubella-antibody-positive by one or more of the three tests. The 31 S2 specimens that were MACRIA-positive or equivocal came from the patients whose S1 specimens were negative in all the tests. Table 4 relates the MACRIA result to the interval between immunization and collection of the S2 specimen in these patients. The two group II patients whose S1 specimens had rubella antibody in HI and ELISA tests made no IgM response to vaccine.

DISCUSSION

It is widely accepted that, in order to minimize the number of incorrect reports of immunity to rubella, a few patients with small amounts of antibody who are probably immune must be classified as susceptible. False positive reactions are a recognized hazard in HI tests, and the choice of a control serum with a strength as high as 15 i.u. per ml was made with the intention of avoiding them. A recent quality control study has shown that most rubella screening tests in Britain are now done by RH in which false positive reactions, if they occur at all, are rare (S. E. Reed, personal communication). It is now possible, therefore, to consider whether the criterion for a report of immunity could be reduced to below 15 i.u. rubella antibody per ml without loss of specificity.

In this investigation 61 out of 141 (43·3%) women treated as susceptible (< 15 i.u. per ml) were found to have some RH antibody. Sera from 33 of these women were intensively studied and the presence of antibody was confirmed by HI in 28 and by ELISA in 25 (out of 31). None of the patients with antibody by one or more of the tests made an IgM response after immunization, whereas most of those with undetectable antibody did (Table 3.). The failure to produce an IgM response and the detection of antibody in pre-immunization sera, albeit at low level, indicate that these women may not have needed immunization. Low levels of antibody are likely to confer protection against viraemia and clinical illness induced by either vaccine or wild virus, and if the titres remain stable, these women could be considered immune. Although unlikely, it is possible that low levels of antibody occur in a group of patients in whom immunity following a natural infection is waning and who will eventually lose antibody. If this is so then boosting with RA 27/3 vaccine might be considered to prevent a return to susceptibility.

Interesting analogies can be drawn between the artifical rubella infection

Table 5. *'Significant' rises in group I and II patients' antibody levels between pre-immunization (S1) and post-immunization (S2) specimens identified by radial haemolysis (RH), haemagglutination inhibition (HI) and enzyme immunoassay (ELISA)*

	Significant rise by		
	RH*	HI**	ELISA***
Group I	12/33	8/33	11/31
Group II	31/33	33/33	29/31

* ⩾ 4 mm increase in zone diameter. ** ⩾ fourfold rise in titre. *** S2 absorbance:S1 absorbance ⩾ 1·65 (manufacturer's criterion).

induced by vaccine and the natural infection. The responses made by the vaccinees who already had antibody in the S1 specimen are probably comparable with those of immune patients closely exposed to natural rubella, in whom a rise in antibody titre, often referred to as re-infection, is common. In every one of the 33 group I patients studied there was an increase in antibody levels in all tests after immunization. A proportion of these changes were of a magnitude that is often regarded as diagnostic of rubella (Table 5). The fact that rubella vaccine evokes these increases in the absence of a specific IgM response shows that such rises do not necessarily imply that a primary infection has occurred and that, alone, they cannot be diagnostic of rubella.

The outstanding problem of immunizing adult women against rubella is to decide how to manage those patients who find themselves pregnant at or just after vaccination. This problem would increase in size if the new American policy of vaccinating without preliminary sero-testing were to be widely followed in Britain. It is still unclear whether a primary infection due to vaccine is totally benign to the fetus. While such information as exists suggests that this is the case (IPAC, 1981) the evidence for RA 27/3 vaccine is meagre (Banatvala *et al.* 1981). However, it is much less likely that the immunization of a woman who already had some antibody and who did not make a specific IgM response would be a risk to the fetus.

In this survey pre-immunization (S1) antibody was always associated with a negative MACRIA response in S2. Thus women whose fetuses are least at risk can be identified either by detecting rubella antibody in a pre-immunization specimen, at whatever level it may be present, or by testing a post-immunization specimen to exclude an IgM response. Because IgM responses following immunization are up to tenfold weaker than following natural infection a sensitive test such as MACRIA must be used. Ideally the specimen should be collected four to five weeks after immunization when the specific IgM level is highest, but IgM remains detectable by MACRIA for about nine weeks (Table 4). This degree of sensitivity would be important if a woman failed to recognize promptly that she was pregnant.

We favour the continuance of sero-testing before immunization of adult women wherever laboratory facilities permit, but our results indicate that a significant proportion of those women currently reported as susceptible to rubella are immune, and that there may be scope for lowering the threshold of the antibody screening test to as low, perhaps, as 5 i.u. per ml. For this, the sensitivity of the methods would have to be increased, and it is doubtful whether this could be done for HI without loss of specificity (Harris *et al.* 1980). Experiments with RH, on the other

hand, have shown increased sensitivity when larger well sizes and a lower concentration of red cells in the gel are used. The bigger areas of haemolysis obtained make it much easier to recognize weak reactions. The ELISA apparently needs no modification to detect antibody below a concentration of 15 i.u. per ml, but further studies with weakly reactive sera are needed to confirm this.

In this study the proportion of patients recommended for immunization who had some-pre-existing antibody was higher than expected. If this is reflected throughout Britain an increase in the sensitivity of screening tests could result in a significant reduction in the number of women recommended for immunization. It would be possible to achieve this if all laboratories adjusted the sensitivity of their tests. Alternatively, weakly reacting sera (< 15 i.u. per ml) could be re-tested by more sensitive methods, locally or in a reference laboratory, before recommendations to immunize were made. There would be considerable benefit from defining more accurately the group of women who need to be immunized, particularly in reducing the number who might be inadvertently given rubella vaccine when they were pregnant.

We thank Miss Ann Boutland who organized the survey, Mrs Joyce Thompson who collected the specimens and Mrs Marian Joy who typed the manuscript.

REFERENCES

BANATVALA, J. E., O'SHEA, S., BEST, J. M., NICHOLLS, M. W. N. & COOPER, K. (1981). Transmission of RA 27/3 rubella vaccine strain to products of conceptions. Lancet **i**, 392.

CMO(1972)4. Chief Medical Officer, Department of Health and Social Security.

CMO(1976)4. Chief Medical Officer, Department of Health and Social Security.

HARRIS, R. E., SMITH, K. O., GEHLE, W. D. & DUPUY, J. (1980). Rubella immunity: comparison of haemagglutination inhibition and radioimmunoassay antibody methods. *Obstetrics and Gynaecology* **55**, 603–7.

KURTZ, J. B., MORTIMER, P. P., MORTIMER, P. R., MORGAN-CAPNER, P., SHAFI, M. S. & WHITE, G. B. B. (1980). Rubella antibody measured by radial haemolysis. Characteristics and performance of a simple screening method for use in diagnostic laboratories. *Journal of Hygiene* **84**, 213–22.

IPAC (1981). Rubella prevention: recommendation of the Immunization Practices Advisory Committee, Centers for Disease Control. *Morbidity and Mortality Weekly Report* **30**, 37–47.

MORTIMER, P. P., TEDDER, R. S., HAMBLING, M. H., SHAFI, M. S., BURKHARDT, F. & SCHILT, U. (1981). Antibody capture radioimmunoassay for anti-rubella IgM. *Journal of Hygiene* **86**, 139–53.

PATTISON, J. R. & MACE, J. E. (1973). Rubella screening tests. *Journal of Clinical Pathology* **26**, 161.