Interferon Studies with Japanese and U.S. Rubella Virus Strains

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Summary

Japanese strains of rubella virus have been claimed not to be teratogenic, and tests on three Japanese strains showed that they induced high levels of interferon in human placental cell cultures obtained from conceptuses ranging from 13 to 24 weeks' gestational age, whereas two strains derived from the U.S.A. induced low levels. Both Japanese and U.S. strains induced similar but low levels in fetal lung cell cultures and leucocyte preparations. A representative Japanese strain and a U.S. strain were both interferon-sensitive. If indeed a strain can be shown to be non-teratogenic it could lead to an alternative, safer rubella vaccine.

Introduction

Although the teratogenicity of attenuated rubella virus strains incorporated in currently licensed vaccines is unknown, it has been shown that, if administered during pregnancy, vaccine strains may infect the conceptus (Phillips *et al.*, 1970; Larson *et al.*, 1971; Ebbin *et al.*, 1972; Vaheri *et al.*, 1972). Much fetal wastage has occurred since pregnancies have frequently to be terminated after inadvertent vaccination during pregnancy, often without earlier determination of the vaccinee's immune state (Mair and Buchan, 1972). A vaccine incorporating a virus strain which was not transmissible to the fetus would clearly be desirable, provided, of course, such a vaccine induced a satisfactory level of immunity.

Great interest was aroused by the claim that congenitally acquired rubella is rare in Japan, though the pattern of postnatally acquired infection differs little from that in most Western countries (Kono *et al.*, 1969). Thus, between 1966 and 1970, there were extensive epidemics of rubella in Japan, but only 50 cases of congenitally acquired rubella were reported. This contrasts with the reports that the 1963-4 epidemic of rubella in the U.S.A. resulted in the birth of some 10,000 to 20,000 congenitally deformed infants (Cooper and Krugman, 1966). Experiments in which pregnant rabbits were inoculated with representative Japanese and U.S. strains suggested that the Japanese strains

were not transmitted to the fetus, whereas U.S. strains caused fetal infection and damage (Kono *et al.*, 1969).

In previous studies on interferon production by different rubella virus strains, we observed that a Japanese strain, Ko-1, induced particularly high levels of interferon in placental cultures, whereas other virus strains, including vaccine strains, were generally poor interferon inducers in these cultures. Since we tested only one Japanese strain and did not determine whether this strain induced high levels in fetal cultures, we have compared the interferon-inducing capacity of three Japanese strains with two U.S. strains in human placental cultures, fetal lung cultures, and fetal leucocyte preparations. For comparison, cell cultures and leucocyte preparations were also inoculated with Sendai virus, since this virus is a good interferon inducer in human cells (Strander and Cantell, 1966; Smorodintsev, 1968; Lee, 1969). In addition, the interferon sensitivity of one Japanese and one U.S. strain was compared in placental and amnion cell cultures.

Materials and Methods

Cell Cultures.—Primary monolayer lung and placental cell cultures were prepared from seven "healthy" conceptuses removed by hysterotomy, cultures being prepared as previously described (Banatvala *et al.*, 1971). Fetuses ranged in gestational age from 10 to 24 weeks.

Leucocyte Preparations.—Blood was obtained by cardiac puncture within one to two hours of hysterotomy from three fetuses which ranged in gestational age from 18 to 22 weeks. In addition, cord blood was obtained from an infant at delivery. Leucocyte preparations were prepared as previously described (Banatvala *et al.*, 1971).

Virus Strains.—The derivation and passage history of virus strains are shown in table I. Viruses were generally inoculated into cell cultures at multiplicities of 1 and 10.

Interferon Assay.—Cell culture fluids were harvested 24 hours after inoculation, interferon assays being carried out in flat-bottomed microtitre plates (Linbro, Ltd.) using V3A cell cultures and Sindbis as challenge virus according to the method described by Banatvala *et al.* (1971). The British Standard human interferon preparation A69/19 was incorporated in each test and results of assays were expressed in international units of interferon.

Sensitivity to Interferon.—A Japanese strain (To-336) and a U.S.-derived strain (Brown) were tested for sensitivity to interferon in three placental and six amnion cell cultures. Cultures were incubated with dilutions of human interferon prepared in leucocytes which ranged from 5 to 500 IU in a roller drum at 36°C for 18 hours. This preparation was kindly provided by Professor K. Cantell, State Serum Institute, Helsinki. Cultures

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TABLE I—Derivation and Passage History of Rubella Virus Strains. Figures in Parentheses Indicate Number of Passages

Strain	Derivation	Passage History of Virus
Giguere Brown	1964; New Haven, U.S.A.; pharynx of infant with congenitally acquired rubella 1966; Baltimore, U.S.A.; pharynx of female with postnatally acquired rubella	V.M.K. (2), R.K.-13 (7), B.H.K.-21 (3) V.M.K. (4), R.K.-13 (3), Vero (4), B.H.K.-21 (1)
Ko-1 To-336 Hi-1	1967; Kochi, Japan; child with postnatally acquired rubella 1967; Japan; pharynx of child with postnatally acquired rubella 1969; Japan; urine of child with congenitally acquired rubella	V.M.K. (2), R.K.-13 (4), B.H.K. (5) V.M.K. (5), Vero (1), B.H.K. (7) V.M.K. (2), Vero (1), B.H.K.-21 (4)

V.M.K. = Vervet monkey kidney. R.K.-13 = Continuous line of rabbit kidney. B.H.K.-21 = Continuous line of baby hamster kidney. Vero = Continuous line of vervet monkey kidney.

TABLE II—Interferon Levels (IU/ml) Induced by Rubella Virus Strains and Sendai Virus in Placental Cell Cultures

Virus	Japanese Strains						U.S. Strains				Sendai	
	Hi-1		To-336		Ko-1		Brown		Giguere			
Multiplicity of Infection:	1	10	1	10	1	10	1	10	1	10	1	10
Gestational age (weeks):												
13	12.5	200	100	400	50	200	12.5	50	12.5	25	12.5	50
14	12.5	100	25	200	12.5	50	<6.25	<6.25	<6.25	12.5	12.5	75
16	25	200	50	200	100	200	25	50	25	25	50	50
17	25	100	50	200	25	100	6.25	25	12.5	25	25	100
18	25	200	50	200	100	200	12.5	50	6.25	50	50	50
22	25	100	50	400	25	150	12.5	25	12.5	25	50	50
24	25	25	25	200	25	50	6.25	25	<6.25	12.5	12.5	25
Range	12.5-50	25-200	25-100	200-400	12.5-100	50-200	<6.25-25	<6.25-50	<6.25-25	12.5-50	12.5-50	25-100
Geometric mean titre	20.5	110.4	45.3	243.8	37.1	116.9	7.9	21.9	6.1	22.6	20.5	53.0
No. of observations	7	7	7	7	7	7	7	7	7	7	7	7

Student's *t* tests were used for the statistical analyses included in results.

were then washed three times and inoculated with rubella virus at a multiplicity of 0.1. After two hours' adsorption, cultures were again washed three times and incubated at 36°C for 72 hours, after which the culture fluid was harvested and titrated for rubella virus in RK-13 cell cultures. Virus titres were compared with those in cultures which were not pretreated with interferon.

Results

In placental cultures, the three Japanese strains of rubella virus induced markedly higher levels of interferon than the two U.S. strains and Sendai virus (table II); occasionally U.S. strains failed to induce detectable interferon levels. At a multiplicity of 1, interferon titres induced by all three Japanese strains were statistically significantly different from those induced by U.S. strains at the 5% level of probability for Hi-1, 2% to 5% for Ko-1, and 1% for To-336. At a multiplicity of 10, differences between the Japanese and U.S. strains were all highly significant, being at the 1% level of probability. The highest levels of interferon were induced by the To-336 strain. The results of an experiment in which interferon levels induced by different rubella virus strains at multiplicities ranging from 0.1 to 10 were compared are shown in fig. 1.

In contrast, in lung cell cultures all rubella virus strains generally induced low amounts of interferon, this being particularly low for the strain Giguere (table III). At multiplicities of both 1 and 10, however, no statistically significant differences in interferon levels were detected between Japanese and U.S. strains. Though Sendai virus induced somewhat higher interferon titres than the various rubella strains, when compared statistically with both U.S. and Japanese rubella viruses, differences were not significant. Though the number of observations was small, both Japanese and U.S. strains induced somewhat similar levels of interferon in leucocyte preparations (table IV). Levels of interferon induced by rubella and Sendai viruses in placental and lung cultures as well as leucocyte preparations did not increase with fetal maturity.

FIG. 1—Interferon production in placental cultures of 13 weeks' gestation inoculated with increasing multiplicities of Japanese and U.S. rubella virus strains. ● = Brown. ▲ = Giguere. ▼ = Hi-1. x = To-336. ○ = Ko-1.

Both the To-336 and Brown strains were interferon-sensitive in both placental and amnion cell cultures, but although the Japanese strain induced far higher levels of interferon in placental cultures, the Brown strain was slightly more interferon-sensitive than To-336 (fig. 2).

Discussion

Our studies show that Japanese strains of rubella virus induce significantly higher levels of interferon in human placental cultures but not in fetal lung cultures or leucocyte preparations. Were these in-vitro findings to occur in vivo, they might explain why Japanese strains do not cause fetal damage. It is important, however, to stress that, so far as we are aware, the rarity of congenitally acquired rubella in Japan has not been substantiated by firm documentary evidence. It is quite possible, particularly in remote areas, that cases may not be diagnosed or

TABLE III—Interferon Levels (IU/ml) Induced by Rubella Virus Strains and Sendai Virus in Fetal Lung Cultures

Virus	Japanese Strains						U.S. Strains				Sendai	
	Hi-1		To-336		Ko-1		Brown		Giguere			
Multiplicity of Infection	1	10	1	10	1	10	1	10	1	10	1	10
Gestational age (weeks):												
13	12.5	N.T.	25	N.T.	25	N.T.	6.25	N.T.	12.5	N.T.	50	N.T.
14	<6.25	6.25	6.25	12.5	6.25	6.25	6.25	<6.25	6.25	12.5	25	25
16	<6.25	50	25	50	12.5	25	12.5	25	<6.25	12.5	25	50
17	25	50	25	50	25	50	12.5	37.5	25	25	12.5	50
18	25	25	12.5	12.5	12.5	25	12.5	12.5	12.5	25	6.25	12.5
22	<6.25	25	<6.25	12.5	<6.25	12.5	12.5	12.5	<6.25	<6.25	25	25
24	12.5	12.5	6.25	12.5	6.25	6.25	<6.25	6.25	<6.25	6.25	12.5	12.5
Range	<6.25-25	6.25-50	<6.25-25	12.5-50	<6.25-25	6.25-50	<6.25-12.5	6.25-37.5	<6.25-12.5	6.25-25	6.25-50	12.5-50
Geometric mean titre	5.1	22.3	9.5	19.8	8.6	15.7	6.9	9.8	3.8	7.1	18.3	25.0
No. of observations	7	6	7	6	7	6	7	6	7	6	7	6

N.T. = Not tested.

Student's *t* tests were used for the statistical analyses included in results.

TABLE IV—Interferon Levels (IU/ml) Induced by Rubella Virus Strains and Sendai Virus in Fetal and Neonatal Leucocyte Preparations

Virus	Japanese Strains						U.S. Strains				Sendai	
	Hi-1		To-336		Ko-1		Brown		Giguere		1	10
Multiplicity of Infection:	1	10	1	10	1	10	1	10	1	10		
Gestational age (weeks):												
18	100	N.T.	50	N.T.	200	N.T.	100	N.T.	50	N.T.	200	N.T.
22	25	N.T.	25	N.T.	N.T.	N.T.	100	N.T.	50	N.T.	25	100
24	12.5	12.5	6.25	12.5	12.5	25	12.5	25	12.5	12.5	12.5	50
Cord	6.25	12.5	6.25	12.5	12.5	25	12.5	25	12.5	12.5	25	100

N.T. = Not tested.

FIG. 2—Interferon sensitivity of To-336 and Brown strains. As reduction of virus yield was of a similar order in both placental and amnion cultures, results have been combined. Figures are numbers of observations. ● = Brown. ○ = To-336.

reported. Indeed, in most countries neither postnatally nor congenitally acquired rubella is notifiable, but some indication of its prevalence in Japan could be obtained on a basis similar to the National Congenital Rubella Surveillance Programme which has been instituted in the United Kingdom since January 1971. In this nationwide survey, paediatricians, medical officers of health, and clinics for the deaf notify cases centrally for a virological confirmation of the diagnosis.

The finding that the Brown strain of rubella virus could be recovered from congenitally infected offspring of rabbits infected in early pregnancy, whereas such Japanese strains as To-336 and Hi-1 failed to infect or damage the conceptus (Kono *et al.*, 1969), provided some experimental evidence to support the hypothesis that Japanese strains were non-teratogenic. However, we could not confirm these findings, being unable to induce infection or teratogenic effects in the offspring of rabbits infected with the Brown strain of virus. A possible explanation for this discrepancy may be that we used a different strain of rabbit (New Zealand white) from the Japanese workers who used Japanese NIBS rabbits. Apart from any likely difference in the properties of Japanese rubella virus strains, one must bear in mind that the rarity of congenitally acquired rubella in Japan, even if firmly established, might be explained by a difference in a host response which is peculiar to Japanese women.

We have previously shown that most strains of rubella are relatively poor interferon inducers in fetal cultures (Banatvala *et al.*, 1971). A further explanation for the high levels of interferon induced by Japanese strains might be that they were

contaminated with another virus which induced high interferon levels. However, inoculation of Japanese strains into numerous cell cultures and suckling mice, together with examination of material by electron microscopy, failed to show an extraneous virus.

Although serological studies suggest that all rubella virus strains are antigenically closely related (Banatvala and Best, 1969; Best and Banatvala, 1970), our studies show that strains do exhibit differences in their biological behaviour. Perhaps insufficient attention is being directed towards the development and characterization of virus strains which may have biological properties which may render them more suitable for vaccines than those currently licensed. If it was possible to develop better immunogenic vaccines and if they were also non-teratogenic, then one would be approaching an ideal vaccine.

We hope our observations may encourage prospective inquiries designed to obtain accurate data on the incidence of congenitally acquired rubella in Japan and perhaps other parts of the Far East. We also feel that, although there is not a really satisfactory animal with which to assess rubella virus teratogenicity, further experiments should be conducted, preferably using Japanese NIBS rabbits. It is of interest that recently one of the Japanese strains, To-336, has been attenuated and has undergone preliminary vaccine trials. Both adults and children had high seroconversion rates and a very low incidence of reactions, particularly arthralgia (R. Kono, Personal communication, 1972). Trials in which this vaccine is compared with other currently licensed ones have not been reported, and it is hoped that such trials will soon be carried out.

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