Antibody Responses in Acute and Chronic Rubella. (31176)

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Three techniques are now available for determination of antibody to rubella—neutralization, indirect fluorescence, and complement fixation (CF). Each method differs in the pattern of response detected. In addition, the acute, limited infection which is seen in children or adults results in antibody findings which differ from those which occur with chronic infection as revealed in congenital rubella. The differences in the pattern of antibody response are of clinical importance for the serologic diagnosis of infection and susceptibility, and also provide information on the immunologic response in this disease.

Materials and methods. A. *Blood samples.* 1. *Pregnant women with rubella during the first trimester.* Serial blood samples were obtained from pregnant women as part of longitudinal studies(1). Rubella was confirmed in each case by isolation of the virus from the patient.

2. *Individuals with rubella 10 to 20 years previously.* Blood specimens were available from young adults 18 to 30 years of age throughout the United States who gave histories of rubella 10-20 years previously.

3. *Young adults with rubella.* Paired sera from patients diagnosed as having rubella were supplied by collaborating physicians. In each case the first specimen was obtained following exposure but prior to the onset of rash and the second specimen was taken 10 to 30 days later.

4. *Congenital rubella.* Serial serum specimens were obtained from mothers and their children in studies of congenital rubella. Congenital rubella was confirmed by isolation of the virus from the child in each case.

5. *Myxovirus infections.* To determine if heterologous reactions with myxoviruses occurred in the serological tests for rubella, sera from patients with these infections were tested. The paired sera from the following virologically and serologically confirmed cases were included: Infuenza A (7 patients), Parainfluenza type 1 (3 patients), Parainfluenza type 2 (4 patients), Parainfluenza type 3 (3 patients), Rubeola (3 patients), Respiratory Syncytial (5 patients), and Mumps (3 patients).

6. *Pregnant women.* Serum samples were available from a large number of pregnant women who are under study throughout the United States as part of the Collaborative Study of Cerebral Palsy.

B. *Neutralizing antibody.* Neutralization tests were conducted with the enterovirus interference technique in primary tissue cultures of the kidney from the African green monkey according to the methods which we have described previously(2). For these tests, roller tube cultures each were inoculated with 0.2 ml of a mixture containing 0.5 $\log_{10}$ of the RV strain of rubella virus and an appropriate dilution of heat inactivated serum. Serial, 2-fold dilutions of serum 1:4 through 1:64 were included. After 8 days the maintenance medium was changed and the cultures were inoculated with 100 $TCID_{50}$ of Coxsackie A-9 virus. The cultures were examined 3 days later. The appearance of cytopathogenic effect due to Coxsackie A-9 was interpreted as indicating neutralization of the interfering effect of the rubella virus. The titer of the serum was reported as a reciprocal of the last dilution which completely neutralized the interfering effect.

C. *Fluorescent antibody.* The indirect fluorescent antibody test was performed using chronically infected tissue cultures of LLC-MK_2 according to the method reported by Brown and Maassab(3). Serial 2-fold dilu-

tions of inactivated sera were employed in the dilutions 1:8 through 1:64 and chromatographed, conjugated goat antihuman serum was used.*

D. *Complement fixing (CF) antibody.* The rubella CF antigen was prepared with infected tissue cultures in a 5% suspension according to the methods we reported recently(4). In these studies the BHK-21† tissue cultures were used as suggested by Schell.‡ The antigen was made at Microbiological Associates, Inc., Bethesda, Md., under Contract 43-PH-1004, and had a titer of 32 units. CF tests were performed utilizing the microtechnique previously described(5). Spiral loops and disposable plates were used. For antigen titrations, 0.025 ml serial dilutions of the antigen were made with the use of loops. To each antigen dilution, 0.025 ml of serum (appropriately diluted) and 2 exact units of complement (0.025 ml) were added and the mixtures were incubated overnight at 4°C. The amount of 0.05 ml of the hemolytic system was added to each mixture which was incubated at 37°C for one hour and afterwards at 4°C for 4 hours. For serum titrations, serial dilutions of inactivated serum were made with the loops and these dilutions were titrated with 4 units of antigen.

Results. The antibody responses for the patients with acute (acquired) and chronic (congenital) rubella are summarized in Tables I and II. There was no evidence of heterologous myxovirus reactions with any of the methods.

The studies of acute rubella showed the rapid development of neutralizing and fluorescent antibody within one day after the onset of rash, and the delay for a short period in the appearance of CF antibody. The tests also demonstrated a fading of fluorescent intensity and a decrease in the CF titer by 15 months after rubella. This pattern of persistence of neutralizing antibody, weakening of fluorescent reactions and decrease in CF titers was supported by the similar findings for individuals with histories of rubella 10 to 20 years previously. The sensitivity of the tests for detection of antibody change with infection showed that all 3 tests detected over 90% of the expected sero-conversions.

The newborn infants with chronic rubella demonstrated the presence of antibody levels similar to those of the mothers in all cases. There was a slight decrease in neutralizing antibody between one month and 5 months of age. A similar pattern was seen with fluorescent antibody; however, in one case there was no detectable antibody in the one-month specimen. For the CF test, the titers decreased below detectable levels in several cases during the period one to 5 months fol-

TABLE I. Acute (Acquired) Rubella—Antibody Responses.

Age of individual (yr)	Time serum obtained in relation to onset of rash	Neutralizing antibody	Fluorescent antibody	CF antibody
I. *Pregnant women with Rubella during first trimester*				
24	1 mo prior	<4	<8	<4
	2nd day of rash	4	8	tr
	1 mo*	16	16	8
	5 "	32	16	16
	7 "	32	16	16
	15 "	32	16†	4
30	1 mo prior	<4	<8	<4
	Day of rash	<4	32	<4
	14 days*	32	32	4
	2 mo	32	16	8
	4 "	32	16	8
	15 "	32	16†	4
23	4 mo prior	<4	<8	<4
	Day of rash	4	16	<4
	1 mo*	64	16	4
	5 "	32	16	4
22	2 mo prior	<4	<8	<4
	1 wk "	<4	<8	<4
	2 mo*	32	64	8
	6 "	32	64	8
II. *Individuals with Rubella 10-20 years previously*				
No. positive		20	19	10
No. tested		20	20	20
Mean titer of positive specimens		16	16†	4
III. *Young adults with Rubella-paired sera bracketing infection*				
No. sero-conversions		19	18	18
No. tested		20	20	20
Mean titer of positive specimens		16	16	8

* After rash. † Weak fluorescence.

* Purchased from Hyland Laboratories, Los Angeles, Calif.

† Supplied by Microbiological Associates, Inc., Bethesda, Md.

‡ Personal communication.

TABLE II. Chronic (Congenital) Rubella—Antibody Responses.

	Neutralizing antibody	Fluorescent antibody	CF antibody
1. Mother			
Delivery	8	16	16
8 mo post partum	8	16	16
Baby—Cord	8	16	8
2 mo	8	8	4
4 "	4	8	4
5 "	8	8	8
8 "	8	16	>16
12 "	8	16	>16
2. Mother—Delivery	16	32	4
Baby—Cord	16	32	2
5 mo	8	16	<2
7 "	16	16	8
3. Mother—Delivery	16	32	8
Baby—Cord	8	32	8
4 mo	4	8	<2
6 "	8	8	4
8 "	16	32	16
4. Mother			
Delivery	16	16	8
4 mo post partum	8	16	2
Baby—Cord	8	16	8
1 mo	4	<8	4
4 "	8	32	8
5. Mother—Delivery	16	16	4
Baby—8 days	16	16	4
2 mo	16	8	<2
6 "	32	16	8

lowing birth. After the fifth month, the CF titers returned and frequently exceeded the levels previously found in the mother and child. Serial blood samples from 5 "control" mothers and their children whose pregnancies were not complicated by infection with rubella were also tested. In 3 of these cases moderate levels of neutralizing, fluorescent and CF antibody (titers of 8-16) were detected in the sera of the mothers and in the cord blood specimens. By 3 months of age, however, none of the children had detectable antibody with any of the 3 tests, and in no case was there reappearance of antibody through the one-year period of observation.

The sensitivity and specificity of the CF test were determined by comparison with data from the neutralization method obtained for 500 sera from pregnant women (Table III). The majority of these women reported having rubella 10 to 20 years previously. The CF test detected 47% of the specimens which were positive by the neutralization method in this age group. There were only 1.8% apparently false positive CF tests.

Discussion. For acute rubella, all 3 tests were useful for detection of acute infection by sero-conversion. The delay of a few days in the development of CF antibody after the occurrence of rash may be particularly helpful for detection of sero-conversions in cases where an initial blood sample cannot be obtained immediately. The weakening of the fluorescent reaction and the loss of CF antibody with increasing time reduces the value of these 2 tests for detecting past infection and susceptibility. The persisting neutralizing antibody, however, is particularly useful for this purpose. The CF test has a very low incidence of apparently false positive reactions as determined with comparative data from neutralization tests with sera from pregnant women. These findings are consistent with previous reports where these tests were utilized(1,2,3,4). The high degree of specificity of the CF method suggests that this easily performed test which detected 47% of the neutralization positive women might be useful as a preliminary screening test for rubella immunity.

The presence of antibody is of considerable value for identification of children with chronic infection following congenital rubella. However, passively acquired antibody may be expected to persist for several months after birth and low or undetectable antibody levels may occur in these tests with specimens obtained between the first and fifth months of life. The CF test appears to be most likely to be negative during this latter period and

TABLE III. Sensitivity and Specificity of Complement Fixation Tests.

A. 500 sera screened at 1:4 dilution for neutralizing and complement fixing antibodies

		%
Neutralizing antibody present	350	70
Complement fixing antibody present	164	32.8

B. Distribution of positive and negative tests for the 500 sera

	Neut positive	Neut negative
CF positive	155 (31%)	9 (1.8%)
CF negative	195 (39%)	141 (28 %)

therefore should be utilized with specimens taken after the sixth month. Previous studies in our laboratory have shown that the great majority of children with congenital rubella no longer have detectable CF antibody at the ages 5 to 22 years; however, they continue to have neutralizing antibody(6).

The decrease or loss of detectable antibody in rubella syndrome infants during the period one to 5 months indicates that in spite of the chronic congenital infection of these children, the antibody response as determined with these methods is not rapid and does not reach maximum levels until after the sixth month. These findings are consistent with the reports that the development of 7-S rubella neutralizing antibody in congenitally infected children takes place primarily after the sixth month (7,8). It is of interest that at this same time most children cease having detectable virus in the nasopharynx(9).

Summary. Comparative antibody tests for rubella were conducted with the neutralization, indirect fluorescent, and complement fixation methods. All of these techniques demonstrated slightly different patterns of antibody response. Sero-conversion with infection was detected in almost all cases with the 3 methods. Antibodies detectable by fluorescent antibody and complement fixation tests decreased after 15 months following infection. By 10 to 20 years following rubella half of the CF tests were negative. Congenitally infected children showed a decrease in antibody between the first and fifth month of age which was most marked in the CF test. Their full antibody response in these cases did not occur until after the sixth month.

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A Method of Sensitizing Guinea Pigs to Fungus Antigens Without Infection. (31177)

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For the purpose of titrating and comparing antigens it would be desirable to sensitize the animals without infecting them with the organisms, providing the degree of sensitivity produced would be satisfactory for the titration. This method would be valuable particularly when dealing with those organisms that are hazardous to handle or that produce fatal disease in the animals. Although guinea pigs infected with *Histoplasma capsulatum* rarely die, the degree of sensitization produced is often irregular for titrating the skin antigens.

Freund and Gottschall have demonstrated previously that guinea pigs could be sensitized by subcutaneous injections with killed bacilli suspended in liquid petrolatum(1). Recently a single intraperitoneal injection of coccidioidin also was shown to induce delayed hypersensitivity in guinea pigs(2).

Our experience using various injection methods such as subcutaneous, or intraperitoneal to sensitize animals has not regularly produced the high level of sensitization necessary to standardize histoplasmin or blastomycin. This criticism is based on the irregu-