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Impaired Cellular Immunity to Rubella Virus in Congenital Rubella

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Specific cell-mediated immunity (CMI) responses to rubella virus were studied in 12 children with documented congenital rubella syndrome employing a ^{51}Cr lymphocytotoxicity microassay. Hemagglutination inhibition antibody was detected in 11 of the 12 children, with titers ranging from 1:4 to 1:128. CMI to rubella virus was demonstrated in only 3 of the 11 antibody-positive children. The 12th child was negative for both hemagglutination inhibition and CMI. Of the three children with a positive CMI response, two had histories of reinfection with rubella virus. These data suggest that congenital rubella infection produces an impaired CMI response which subsequently may be altered by reinfection with rubella virus. The lack of CMI in the presence of antibody and concurrent excretion of live virus in the child with documented congenital rubella infection suggest a factor to be explored in the pathogenesis of this disease.

Studies of infants with congenital rubella syndrome indicate that high titers of virus can be isolated from the throat, urine, and various tissues in the presence of fetal and maternal antibodies (1, 7, 10, 22). This has led a number of investigators to postulate that a defect exists in the cellular immune responsiveness of these infants. Moreover, in infants with congenital rubella, histological evidence of damage to lymphoid tissue including the thymus has been reported (18). Rubella virus has also been isolated from thymus tissue and circulating small lymphocytes (3, 16). Conflicting results with cellular immune studies have been reported. An almost complete lack of response of lymphocytes to phytohemagglutinin (PHA) stimulation, in the presence of typical delayed hypersensitivity skin reactions, has been observed with these infants (8, 9). Other investigators, however, have achieved stimulation of lymphocytes with PHA (6, 15, 23).

We have previously described a specific microassay procedure for the measurement of cell-mediated immunity (CMI) to rubella virus (20). In this investigation, this assay was employed to study CMI to rubella in children with congenital rubella.

MATERIALS AND METHODS

Study cases. Eleven children and one 5-month-old infant with documented congenital rubella were included in the study group (Table 1). Most of the children, ranging in age from 7 to 14 years were afflicted during the 1964-65 rubella epidemic. All were enrolled in a special education program for the physically handicapped. Consent from the parents was procured for collection of blood, urine, and nasopharyngeal swabs. Clinical information was obtained from the parents, the private physicians, and from hospital records after our immunological studies.

For positive controls, serum and lymphocytes were collected from 8- and 12-year-old children with histories of rubella immunization and an adult with a history of rubella infection. An infant with no history of rubella immunization or natural infection served as negative control.

Viral isolations. Urine or nasopharyngeal swabs, or both, were obtained from most of the children. Isolation attempts for rubella were performed in African green monkey kidney cell culture by using the enterovirus interference technique previously described (13).

Immunological studies. Approximately 10.0 ml of blood was collected by venous puncture from all cases in the study. Three milliliters of blood was immediately transferred to a sterile tube containing 300 U of heparin for separation of lymphocytes. The remaining blood (7.0 ml) was transferred to an empty tube and allowed to clot for separation of serum, which was tested for hemagglutination inhibition antibodies (HAI) by a method previously described (14).

Microassay of CMI. Measurement of CMI to rubella was accomplished by employing a microassay procedure (20). This technique used cells persistently

infected with rubella virus and was labeled with ^{51}Cr. The release of ^{51}Cr from these rubella virus-infected target cells was the indicator; it permitted quantitation of lymphocyte-mediated cytotoxicity. Briefly, lymphocytes were first separated from whole blood by using a Hypaque-Ficoll separation gradient. They were then washed and resuspended to a concentration of 5×10^6 viable lymphocytes per ml. A suspension culture of cell line MA 66182 (21) (BHK-21 cells persistently infected with rubella virus) was labeled with ^{51}Cr by incubation of 10^7 cells with 100 μCi of chromium in 1.0 ml of media for 30 min. Control cells of uninfected BHK-21 cells were also labeled with ^{51}Cr and used to determine nonspecific lymphocyte-mediated cytotoxicity. Samples (0.1 ml) of the lymphocytes (5×10^6 cells) were incubated with 5×10^3 labeled target cells in sterile plastic plates and were placed on a rocker platform at 37 C in a 5% CO_2 atmosphere. The cell suspensions were harvested after 18 and 24 h of incubation with an apparatus designed to separate the reacting cells from the supernatant fluid and to recover the released ^{51}Cr. To determine lymphocyte-mediated cytotoxicity directed specifically against intracellular rubella virus, spontaneous release of ^{51}Cr from infected target cells (I) and control cells (C) was subtracted from the interaction of lymphocytes with infected target cells (I_L) as well as control cells (C_L), respectively, and the release at zero time was subtracted from the total amount of radioactivity in infected (T_i) and control (T_c) target cells. The specific immune release (SIR) was calculated by subtracting the percentage of ^{51}Cr release in the control target cells from that released from the infected cells according to the following formula:

$$\% \text{ SIR} = \frac{I_L - I}{T_i - \text{zero time}_i} - \frac{C_L - C}{T_c - \text{zero time}_c} \times 100$$

All determinations were made in sextuplicate, and the highest SIR at either 18- or 24-h intervals was used as the index of CMI to rubella. Values for SIR of less than 4.0% were considered to be negative determinations.

RESULTS

Results of the study appear in Table 1. For 11 of the 12 children with congenital rubella, HAI antibodies ranging in titer from a trace at 1:4 to a maximum of 1:128 were detected. Specific cellular immune responses to rubella virus were demonstrated in three children (K.M., D.D., J.McD.). Two of these children had histories of reinfection. There was no apparent relationship between age, antibody titer, and SIR. CMI to rubella was not demonstrated in all other subjects, including a 5-month-old infant who was still excreting virus in the presence of antibody. PHA stimulation studies were performed on the lymphocytes of this infant using a dose-response test. Stimulation occurred, and the response was comparable with that observed with control lymphocytes from immune donors. The PHA stimulation studies were not done on the lymphocytes of the other 11 children.

DISCUSSION

The microassay lymphocytotoxicity system permits the measurement of the specific cellular immune response to rubella virus (20). The results of the present studies provide direct evidence that there is a lack of cellular immunity to rubella virus after intrauterine infection. These findings may provide an explanation for the apparent delayed capability of congenitally infected infants to overcome the infection in early post-partum period. It is also possible that fetuses which are immunologically incompetent for cellular immune functions at the time of infection are those born affected with rubella syndrome (17). Additional studies on infants still excreting virus in the presence of humoral antibody will be required to determine whether elimination of virus coincides with development of cellular immunity. It is possible that a temporary CMI response does occur in these cases. If this is not found, then termination of infection would most likely relate to the final death of infected cells (12). Further studies would be required to determine the effects of transferring immunocompetent cells or transfer factor during the newborn period to determine if this would limit viral persistence in the newborn.

A variety of immune responses after congenital infection with rubella has been studied. The best-known example is the elevation of globulins at or shortly after birth occurring as a result of intrauterine infection. Conversely, congenital infection with rubella virus may also retard or change the development of the humoral responses. Hypogammaglobulinemia and selective immunoglobulin deficiencies have been reported (5, 11, 19). In addition, defects in specific antibody production to rubella virus have also been described (19). In the present study, the loss of HAI antibody titer in two children is in agreement with previously reported studies showing declining antibody titers with increasing age in cases of congenital rubella (4). Since recent evidence suggests a helper function of T-lymphocytes to humoral antibody production, it is possible that a loss of antibody in these children could be the result of the loss or impairment of T-cell function against rubella (2). Studies of both the humoral and cellular immune responses in congenital rubella-infected children after administration of vaccine may help further to define the extent of impairment in immunity.

TABLE 1. *Clinical data and immunologic studies for 12 children with the congenital rubella syndrome*

Subject	Age (yrs)	Sex	History of rubella reinfection or rubella immunization	Rubella HAI antibody titer[a]	Rubella ^{51}Cr SIR (%)	Rubella isolation	
						Throat	Urine
Patient							
L.B.	8	F	None	<4	0	–[b]	–
J.G.	8	M	None	8	0	–	ND[c]
A.D.	8	M	None	8	0	ND	ND
B.S.	5/12	F	None	32	0	+	ND
R.M.	8	M	None	32	0.7	–	ND
B.N.	7	M	None	±4	1.1	–	ND
A.K.	8	F	None	16	3.5	–	ND
S.S.	8	F	Rubella vaccine	10	0	–	–
F.S.	8	F	?Rubella at age 7	32	0	–	–
K.M.	7	F	None	128	13.6	–	–
D.D.	14	M	Rubella during early childhood	128	18.0	–	–
J.McD.	11	F	Rubella at age 7	32	40.3	–	ND
Control							
Negative	8/12	M	None	<4	0		
Positive	8	M	Rubella vaccine	16	12.3		
Positive	12	M	Rubella vaccine	32	17.6		
Positive	32	M	Rubella infection	128	48.7		

[a] Reciprocal of last dilution manifesting positive reaction.
[b] –, Negative, no virus isolated.
[c] ND, Not done.

The normal PHA response of the one infant (B.S.) still excreting virus indicates that rubella infection does not impair the reactivity of peripheral lymphocytes to a nonspecific mitogen and possibly other viral antigenic stimuli. Also, the lack of a cellular response to rubella during this period of infection while antibody levels are high suggests that impairment of immune response is limited to mechanisms of cellular immunity alone.

Our data suggest that CMI to rubella virus may be stimulated or reactivated, since two children in this study had normal CMI responses presumably as a result of reinfection with rubella later in life. One child (S.S.) had a negative CMI to rubella but previously had received the rubella vaccine. This would indicate that vaccine is not an effective stimulant of CMI in the presence of antibody.

Finally, the lack of cellular immunity in congenital rubella syndrome as demonstrated in the present studies may provide an explanation for the establishment and persistence of viral excretion in other congenital, chronic, or slow viral infections of man.

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