Rubella-Specific Immune Complexes After Congenital Infection and Vaccination

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Circulating immune complexes which contained rubella-specific immunoglobulins were detected in 21 out of 63 subjects with congenital rubella and in 39 out of 65 subjects vaccinated with attenuated rubella virus, but in none of 43 subjects susceptible to rubella or 87 subjects with remote naturally acquired immunity to rubella. The presence or level of circulating immune complexes and the presence of rubella-specific complexes did not correlate with conventional serum rubella hemagglutination inhibition antibody titers. In the group with congenital infection, the presence of specific complexes many years after birth was associated with late-emerging clinical problems involving several organ systems. In vaccines, the presence of specific complexes was associated with a higher incidence of side reactions. Two-thirds of the vaccines and all of those revaccinated showed specific immune complexes as late as 8 months after immunization.

The clinical manifestations which follow postnatal, congenital, or vaccine-induced rubella infection are protean. Their pathogenetic mechanisms are not well understood in spite of extensive studies which began after Gregg's pioneering observation of rubella teratology (9). Isolation of rubella virus in 1962 (17, 29) and the devastating epidemic of 1964 provided the tools and the impetus for further characterization of the disease and development of preventive vaccines. Since the licensure of live, attenuated rubella virus vaccine in 1969, more than $1 \times 10^8$ doses have been administered in the United States with a marked reduction in rubella and congenital rubella. Nevertheless, important questions remain unanswered about the safety and efficacy of the vaccines and the prognosis for thousands of children with congenital infection.

Intrauterine infection with rubella virus is chronic. Virus can be recovered at birth from most infants with the congenital rubella syndrome (5). It may be excreted throughout infancy and has been recovered from lens material of a 3-year-old child (14). In a 4-year-old child, virus-specific immunoglobulin M (IgM) antibody was reported in cerebrospinal fluid (27), and in a child with the congenital syndrome who developed Hashimoto's disease at age 5, rubella antigen was demonstrated by immunofluorescence in thyroid tissue (34). In progressive rubella panencephalitis (PRP), a slow virus disease attributed to rubella (31, 32), virus has been recovered from both brain (7) and circulating mononuclear cells (33) more than a decade after the initial infection. Therefore, the ability of rubella to persist for extended periods of time and to cause symptomatic chronic infection in specific cases is well documented.

This problem of viral persistence was raised during the initial testing of the live vaccine. Although vaccinates were shown to shed small quantities of virus from their nasopharynx for up to a month, vaccinates are not contagious. However, attenuated strains can infect placental tissues and the fetus (13, 25). Serological evidence of congenital infection has been reported in four infants whose mothers were vaccinated early in pregnancy (11). Additionally, virus-specific IgM has been detected for months to years after both natural infection (15, 18) and vaccination (1), and rubella virus has been isolated from circulating lymphocytes up to 5 weeks (2), and perhaps as late as 2 years (3), after vaccination.

Recently we have documented the presence of circulating immune complexes containing rubella-specific antibody and rubella antigen in two cases of PRP (6). The present investigation was designed to determine whether immune complexes appear in serum after postnatal natural infection, vaccination, and congenital infection in the absence of PRP and whether such immune complexes...
complexes contain rubella-specific antibody and to attempt to correlate the presence or absence of these complexes with clinical status. No attempt was made to determine the nature of the antigen component of the immune complexes detected in this study.

MATERIALS AND METHODS

Subjects and serum samples. The study population consisted of four groups. Susceptible subjects (aged 16 to 41 years) had no history of rubella or rubella vaccination and were rubella seronegative as defined by a serum rubella reciprocal hemagglutination inhibition (HAI) antibody titer of less than 8. Subjects in the natural immunity group (aged 15 to 56 years) had a history of rubella which for most was more than a decade earlier, had not been immunized, and had a rubella HAI antibody titer of at least 8. The congenital rubella infection group (aged 5 months to 28 years) included 50 individuals with a laboratory-diagnosed diagnosis of congenital rubella made at birth and 13 with maternal histories of clinical rubella, multiple stigmata of rubella in utero, and serological results compatible with the diagnosis of congenital rubella.

The vaccine group (age range, 7 to 37 years) included 65 individuals all of whom were seronegative before receiving rubella vaccine. Of these subjects, 14 were vaccinated initially with HPV77 DE5, and 45 were vaccinated initially with RA 27/3. Another three individuals were studied after revaccination (because of HAI antibody titers of <8). Each had been given HPV77 DE5 once in the past. Two were then studied after RA 27/3 immunization, and one received a second dose of HPV77 DE5. Three other individuals received RA 27/3 vaccine when they were found to have rubella HAI antibody titers of <8 in spite of two previous doses of HPV77 DE5.

Blood was collected by venipuncture into a sterile tube, allowed to clot for 30 min at room temperature, and then centrifuged. A sample of serum was used for the HAI antibody titer, and the remainder of the serum was stored as samples at ~70°C until time to study it for immune complexes.

Immune complex assay. Serum immune complexes were detected and quantified by a Raji cell assay (23), modified as previously reported (6). Briefly, 25 μl of serum was diluted 1:4 with calcium- and magnesium-free phosphate-buffered saline and incubated for 30 min at 37°C with a 1:4 dilution of normal human serum to ensure binding of complement to the complexes. The same source of normal human serum was used throughout this study. Portions of each sample (25 μl) were then added to triplicate tubes containing 2 x 10⁶ Raji cells in 50 μl of 1% bovine serum albumin-phosphate-buffered saline. After a 45-min incubation at 37°C, the cells were washed three times with phosphate-buffered saline, and an optimum amount of 125I-labeled staphylococcal protein A (Amersham Corp., Arlington Heights, III.) was added to measure bound complexes IgG. The assay was quantitated by plotting a curve of binding produced by known amounts of heat-aggregated human globulin. Test samples were recorded in microgram equivalents of heat-aggregated human globulin per milliliter (μg/ml). With this method, as little as 18 μg/ml can be detected in serum.

Dissociation of immune complexes and determination of rubella antibody activity. Immune complexes were dissociated by elution from Raji cell surfaces as previously reported (6). In brief, 0.4 ml of serum was incubated with 3 x 10⁷ Raji cells for 45 min at 37°C. Cells were washed well, and bound complexes were eluted and dissociated by the addition of 0.3 ml of 0.1 M sodium borate, pH 10, for 10 min at 37°C. The cells were then sedimented, and rubella antibody activity in the eluates was assayed directly by radioimmunoassay as previously detailed with commercial Rubelisa plates (Microbiological Associates, Walkersville, Md.). In brief, 0.1 ml of eluate from each sample was added to a rubella antigen-coated well and a control well. After a 2-h incubation period to allow antibody to bind to the antigen, the wells were washed and 125I-labeled protein A was added. After a second 1-h incubation period, the wells were thoroughly washed, and bound isotope was eluted with 0.5 sodium hydroxide and counted in a gamma counter. Samples registering more than two standard deviations above the mean of all control wells were scored as positive for rubella antibody activity. All immune complex and rubella-specific immune complex assays were run on coded sera. Reliability of the results was confirmed by frequent inclusion of samples of the same sera in multiple tests.

RESULTS

Serum immune complex activity. The incidence of detectable circulating immune complexes of unknown specificity is shown in Table 1. Totals of 13 of 43 rubella-susceptible and 24 of 87 naturally immune subjects had immune complex levels of at least 18 μg/ml. However, 55 pregnant females were included in these groups, 21 of whom had detectable circulating immune complexes. When pregnant subjects are excluded, 16 of the remaining 75 control subjects had detectable complexes. In comparison, 44 of the 65 subjects in the vaccine group and 31 of the 63 congenital rubella subjects had circulating immune complex activity.

Rubella-specific immune complexes. Raji cell surfaces were used as immunoadsorbants to purify immune complexes from sera shown to contain complexes of unknown specificity. Rubella antibody activity in the complexes was then assessed after elution and dissociation of the complexes from the Raji cells. None of the immune complexes from the susceptible or naturally immune group contained rubella antibody (Table 2). In contrast, rubella antibody was detected in the complexes of 39 of 44 rubella vaccinated and 21 of 31 congenital infection subjects. There was no correlation between the presence or absence of detectable rubella antibody in the immune complexes and either the level of immune complexes of unknown specificity or rubella HAI antibody titers in whole serum.

Congenital rubella group. The incidence of rubella-specific immune complexes and immune
complexes of unknown specificity was compared in patients with different stigmata of congenital rubella (Table 3). No significant differences were observed with regard to the static stigmata of congenital rubella (i.e., deafness, cataract, heart disease, and mental retardation). However, when the entire group was screened clinically and by a battery of blood tests, 10 of the 63 patients were found to have recently developed a variety of new manifestations. These included proteinuria (three cases), abnormal liver function tests (two cases), an abnormal glucose tolerance test with persistent serum antirubella IgM (one case), thyroiditis with diminished thyroid function tests (one case), a generalized seizure disorder (one case), purpura (one case), and glaucoma (one case). All of these patients had rubella-specific immune complexes. Among the 53 with static manifestations alone, only 11 had rubella-specific immune complexes; the difference was highly significant ($P < 0.001$ by Fisher exact test).

**Development of rubella-specific immune complexes after vaccination.** Among 46 patients who received the RA 27/3 vaccine, 28 had rubella antibody containing immune complexes at the time of testing. Similarly, 10 of the 19 recipients of HPV77 DE5 vaccine also showed rubella-specific immune complexes. The frequency of detection of rubella-specific circulating complexes decreased over time (Table 4). They were detected in 34 of 49 vaccinates studied within 8 months of immunization, in 2 of 6 vaccinates studied between 1 and 2 years after vaccination, and in 2 of 10 vaccinates studied 2 to 7 years after successful primary immunization. The patient with rubella-specific immune complexes found 7 years after vaccination with HPV77 DE5 had been exposed to rubella 4 weeks before testing and had an associated fourfold rise in HAI antibody titer. No additional data were available for the patient with rubella-specific immune complexes present 2 years after vaccination with RA 27/3. All six revaccinated subjects had rubella-specific immune complexes when tested.

A total of 52 vaccinates were available for questioning regarding the occurrence of arthralgia as a vaccine reaction. Of the 33 with rubella-specific immune complexes 11 reported arthralgia. Only 3 of the 19 vaccinates in the nonspecific and negative immune complex groups recalled arthralgia. There were no statistically significant differences in the mean ages of the subjects in any of the vaccinate subgroups.

**DISCUSSION**

The mechanisms of the persistence of rubella virus in conditions such as congenital infection or PRP remain unknown. In PRP, circulating

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### Table 1. Frequency of detection of immune complexes of unknown specificity in the study subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Age range (mean)</th>
<th>Total no. of subjects</th>
<th>No. of pregnant subjects</th>
<th>Frequency (%) of detection of immune complexes in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total subjects</td>
</tr>
<tr>
<td>Rubella susceptible</td>
<td>16–36 (22)</td>
<td>43</td>
<td>17</td>
<td>13 (30)</td>
</tr>
<tr>
<td>Naturally immune</td>
<td>16–42 (28)</td>
<td>87</td>
<td>38</td>
<td>24 (28)</td>
</tr>
<tr>
<td>Congenital infection</td>
<td>5 mo–28 (13.9)</td>
<td>63</td>
<td>0</td>
<td>31 (49)</td>
</tr>
<tr>
<td>Rubella vaccinates</td>
<td>7–37 (23)</td>
<td>65</td>
<td>0</td>
<td>44 (67)</td>
</tr>
</tbody>
</table>

*All ages are in years except where otherwise indicated.*

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### Table 2. Frequency of detection of rubella-specific immune complexes among immune complex-positive subjects in the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no. of subjects</th>
<th>HAI*</th>
<th>Frequency of detection of:</th>
<th>Mean cpm in eluates*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Immune complexes</td>
<td>Rubella-specific immune complexes</td>
</tr>
<tr>
<td>Rubella susceptible</td>
<td>43</td>
<td>&lt;8</td>
<td>13 (115.0)**</td>
<td>0</td>
</tr>
<tr>
<td>Naturally immune</td>
<td>87</td>
<td>51.2</td>
<td>24 (117.3)</td>
<td>0</td>
</tr>
<tr>
<td>Congenital infection</td>
<td>63</td>
<td>13.4</td>
<td>31 (122.3)</td>
<td>21</td>
</tr>
<tr>
<td>Rubella vaccinates</td>
<td>65</td>
<td>25.6</td>
<td>44 (71.3)</td>
<td>39</td>
</tr>
</tbody>
</table>

*Reciprocal geometric mean serum rubella HAI antibody titer.

*b Mean counts per minute of rubella-specific binding activity (± one standard deviation) in eluates assayed in rubella antigen wells; eluates assayed in control wells showed a mean activity of 187.9 ± 46.1 cpm.

The numbers in parentheses represent the mean level of immune complexes amongst immune complex-positive cases, in microgram equivalents of heat-aggregated human globulin per milliliter.
immune complexes containing both rubella antigen and antibody were demonstrated in the two patients examined (6). The significance of this finding is not yet clear; however, perivascular deposits in brain are a consistent feature of the disease and could represent local complex deposition (24a). In the late-onset rubella syndrome of infancy, immune complexes which appeared to contain rubella antigen by fluorescence staining were found by Tardieu et al. (22) during the acute phase of the disease in the serum of six patients. These investigators suggested that deposition of circulating immune complexes might account for the vasculitis observed in lung and skin and that careful monitoring of infants exposed in utero to rubella for the presence of circulating complexes might be indicated in order to detect and treat any active process as soon as possible.

Congenital rubella was originally felt to be a static condition. More recent studies have recognized that it can be a progressive disorder with further damage inflicted on its victim over the years (19). In this study, 21 of 63 patients with congenital rubella had rubella antibody containing circulating immune complexes years after birth. However, all 10 children with new clinical problems including evidence of damage to the brain, kidneys, liver, thyroid, eyes, skin, and pancreas, potential target organs for immune complex deposition, were shown to have rubella-specific immune complexes. Perhaps these patients harbor persistent virus with continuous or intermittent complete or defective viral replication and secondary complex formation.

Although pharyngeal excretion after rubella vaccination is brief, ongoing viral replication may be more persistent in other cells as has been reported with unattenuated virus. The vaccine strains produce immunological reactions similar to those following natural infection: suppression of lymphocyte transformation (26), induction of macrophage inhibition factor production (12), mediation of virus-infected cell lysis (20), production of IgG, IgA, and IgM antibody responses (1), and chronic infection of placenta and fetus. There have been reports of sustained IgM responses following vaccination (1), and virus has been isolated from lymphocytes for varying intervals after vaccination (2, 3).

This study has shown that rubella-specific immune complex formation is frequent after vaccination and could be demonstrated in two-thirds of an unselected group of vaccines for as long as 8 months after vaccination. We did not attempt to show the presence of viral antigens in these complexes; however, the prolonged persistence of immune complexes containing rubella antibody after vaccination suggests that the attenuated virus strains commonly persist in some form for many months. Continued access of virus or viral products to the intravascular compartment in the presence of a developing virus-specific antibody response could account for the generation of the immune complexes. This would also explain the findings of rather prolonged virus isolation and persistent IgM antibody after vaccination.

Vaccination with attenuated rubella virus is associated with complication rates ranging from 10 to 40%. Reactions usually are mild and self-

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### TABLE 3. Immune complexes in children with various manifestations of congenital rubella

<table>
<thead>
<tr>
<th>Clinical manifestations</th>
<th>Total no. of patients</th>
<th>Without immune complexes</th>
<th>With immune complexes of unknown etiology</th>
<th>With rubella-specific immune complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deafness with or without retardation</td>
<td>19</td>
<td>12</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Deafness and cataract with or without retardation</td>
<td>22</td>
<td>12</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Deafness, cataract, heart disease, and retardation</td>
<td>22</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
</tbody>
</table>

*a Of the total of 63 patients, 32 were without immune complexes, 10 had immune complexes of unknown etiology, and 21 had rubella-specific immune complexes; 10 of the 21 patients with rubella-specific immune complexes developed a variety of new manifestations.

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### TABLE 4. Frequency of rubella-specific immune complexes at various intervals after vaccination

<table>
<thead>
<tr>
<th>Interval post-vaccination</th>
<th>No. of vaccines</th>
<th>No. (%) of vaccines with rubella-specific immune complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 wk</td>
<td>4a</td>
<td>4 (100)</td>
</tr>
<tr>
<td>1–8 mo</td>
<td>45b</td>
<td>30 (67)</td>
</tr>
<tr>
<td>1–2 yr</td>
<td>6</td>
<td>2 (33)</td>
</tr>
<tr>
<td>2–7 yr</td>
<td>10</td>
<td>2c (20)</td>
</tr>
</tbody>
</table>

*a Includes two revaccinates.  
*b Includes four revaccinates.  
*c Includes one patient exposed to rubella 4 weeks before testing, with an associated rise in HAI antibody titer; no information was available on the second patient.
limited, lasting no more than 3 days. Of the reactions, transient arthralgia and recurrent arthritis cause the most concern. The high incidence of side effects has never been explained. Previous studies have found no correlation between side effects and higher antibody response, prolonged IgM response (24), human leukocyte antigen type, or persistent viral excretion (10). However, the vaccine has been shown to be capable of replicating in synovial tissue culture (8), and virus has been recovered from the joint aspirates of vaccinates (28). In one study of four children with repeated bouts of arthritis after vaccination, all had rubella-specific IgG in their joint aspirates several months after immunization; in three of the four children, virus was isolated from joint fluid as well (16). A second study looked at 15 children who developed postvaccine arthralgias compared with a control asymptomatic group (4). The only consistent immunological difference was a decreased lymphoproliferative response to rubella in the symptomatic group. The authors postulated a possible role for blocking factors such as immune complexes but did not screen for them. In the present study, the incidence of vaccine joint reaction was twice as high in the rubella-specific immune complex group, but many patients who had complexes experienced no side effects. It may be that a critical mass or size of complexes or both must be reached or that some other predisposing factor(s) must be present for complex deposition to provoke arthralgia or arthritis. Whether arthralgia can occur without circulating immune complex activity is now known. None of the subjects without complexes who developed arthralgia in this study were tested at the time they were symptomatic.

Of our control group patients, 20% had immune complexes of unknown specificity, and this figure increased to 30% if all susceptible or naturally immune pregnant women were included. These observations of detectable circulating immune complexes in nonpregnant, apparently healthy individuals are similar to those reported by others using the Raji cell and other assay systems (21, 30). Further, in our subjects, the presence or absence of immune complexes of unknown specificity did not correlate with their rubella serological status. Correlations with rubella clinical status emerged only after rubella specificity of the complexes was determined.

In summary, our data indicate that rubella-specific immune complexes are frequently present in patients with congenital rubella years after birth, and these complexes are associated with the development of new clinical symptoms. Specific complexes also are formed in the majority of patients who receive the live attenuated vaccine and were found in all revaccinates looked at. Their presence was associated with a higher incidence of postvaccination arthralgia. These findings add another dimension to the study of the pathogenesis of rubella, congenital rubella, rubella immunization, and their respective complications. Further study may influence the management of children with congenital rubella as well as the recommended immunization policy.

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LITERATURE CITED


