A Search for Persistent Rubella Virus Infection in Persons with Chronic Symptoms After Rubella and Rubella Immunization and in Patients with Juvenile Rheumatoid Arthritis

Lisa M. Frenkel, Karin Nielsen, Alice Garakian, Rui Jin, Jerry S. Wolinsky, and James D. Cherry

Peripheral blood polymorphonuclear leukocytes, mononuclear cells, and plasma and nasopharyngeal specimens were obtained from 6 subjects with persistent symptoms following rubella immunization, 1 subject with persistent symptoms following rubella, 11 children with juvenile rheumatoid arthritis, 17 recently immunized control subjects, and 1 control subject with acute clinical rubella. Rubella virus was isolated from the blood or nasopharynx of four of the 18 control subjects. In contrast, rubella virus was not recovered from any specimens from the seven subjects with persistent symptoms following immunization or natural infection or from the 11 children with juvenile rheumatoid arthritis. A polymerase chain reaction assay detected rubella virus in the blood from three of 14 control subjects but not in the blood from two subjects with persistent symptoms following rubella immunization or in that from three children with juvenile rheumatoid arthritis. We have not been able to confirm the findings of others who have reportedly recovered rubella virus from lymphocytes of persons with persistent symptoms following rubella or rubella immunization.

Congenital rubella, which may include mental retardation, congenital heart disease, cataracts, and deafness, was reported in 1941 to follow the occurrence of maternal German measles [1]. The rubella pandemic that occurred between 1963 and 1965 resulted in 11,000 fetal deaths and teratogenic effects in 20,000 children [2]. Shortly before this calamity, rubella virus was first isolated [3, 4], and this isolation led to extensive studies during the pandemic and to the eventual development of live rubella virus vaccines. The efficacy of rubella vaccines has all but eliminated congenital rubella in the United States [5] and Finland [6]. With the relative absence of disease due to rubella virus infections, attention more recently has focused on possible adverse reactions to the vaccine [7–11]. One specific issue is whether chronic arthritis, including juvenile rheumatoid arthritis (JRA), and chronic neurological symptoms occur as a consequence of persistent rubella virus infections.

Rubella virus can be cultured transiently from nasopharyngeal specimens from acutely infected children and adults [12–15], and it can be isolated for many months from multiple sources in congenitally infected children [16–18]. Immune tolerance to rubella virus has been proposed to occur in these congenitally infected children [19]. Persistent infection after postnatal rubella virus infection has been reported by investigators, primarily from one center [7–10, 20, 21].

While rare in children, acute, self-limited joint symptoms have been noted following rubella immunization in 10%–20% of adults, and they occur even more often after wild-type rubella virus infections [11]. Infrequently symptoms persist, occasionally for months and rarely for years [11]. If these chronic illnesses— including JRA in children and chronic arthritis and neuropathies in adults—are the result of persistent rubella virus infection, rather than maladies coincidental to or triggered by rubella virus infection, then trials of antiviral therapy may be warranted.

To further investigate the possible occurrence of persistent rubella virus infection, we studied children with JRA and persons with chronic joint or neurological symptoms of which the onset occurred after rubella or rubella vaccination.

Methods

Subjects. Announcements were distributed by the Los Angeles Department of Public Health and letters were mailed directly to public health officials and health care workers in the Los Angeles area and at the Centers for Diseases Control and Prevention. These requested the referral of subjects with acute rubella and persons with persistent symptoms following rubella or receipt of rubella vaccine. Children with JRA were also studied. Healthy children and adults found to be seronegative for rubella virus were vaccinated with rubella vaccine (M-M-R3; Merck & Co., West Point, PA) and served as controls.

Consent for this study was obtained from all subjects in accordance with the guidelines of the UCLA Human Subjects Program, through a fellowship granted to Dr. Karin Nielsen, and by unrestricted grants from Merck and Co., Inc.
Protection Committee. Blood was taken by venipuncture for culture, serology, and PCR assay, and nasopharyngeal secretions from control subjects were obtained with Dacron swabs (Spectrum Laboratories, Houston, TX) for culture and PCR assay. Single specimens were taken from subjects with persistent symptoms, and 1–3 specimens were obtained from vaccinees at 7–14 days following immunization. Cultures were repeated for subjects whose initial specimens were culture-negative.

**Rubella virus culture.** Initially experiments were done to evaluate different culture systems for rubella virus isolation. Rabbit kidney (RK13) cells (Whittaker Bioproducts, Walkersville, MD), Vero cell monolayers (Whittaker Bioproducts), and African green monkey kidney (AGMK) cell monolayers (Whittaker Bioproducts) were inoculated with live attenuated RA 27/3 rubella vaccine virus (Meruvax II, Merck & Co.) or with wild-type rubella virus from a patient isolate, or they were mock-infected with tissue culture medium [4]. The RK13 cells (stained with acridine orange) and Vero cell monolayers were examined for cytopathic effect and the AGMK tissue culture cells for an interfering agent by challenge with echovirus type 11; the examination was performed by experienced laboratory personnel, who were blinded to the specific inoculum. Cytopathic effect could not be accurately discerned in the RK13 cells (data not shown). Likewise, further examination of RK13 tissue culture monolayers with rabbit antibody to rubella virus (supplied by Dr. J. Chantler) and immunoperoxidase-labeled anti-rabbit IgG ( Vectastain ABC Kit; Vector Laboratories Immunohasays, Burlingame, CA) did not effectively differentiate infected from uninfected monolayers. Therefore, these techniques were not used for the detection of rubella virus in the subjects’ specimens.

The first few cultures of subjects’ blood components and vaccine virus were done in both AGMK and Vero cell monolayers. Identification of RA 27/3 rubella virus in the control monolayers was more facile with AGMK tissue culture, followed by challenge with echovirus type 11, than it was with examination for cytopathic effect in Vero cell monolayers. Thus, thereafter subjects’ specimens were cultured in AGMK cell monolayers only. The infectivity of each lot of AGMK cell monolayers was verified by culture of standardized dilutions of RA 27/3 rubella vaccine virus (Meruvax II, Merck & Co.).

The subjects’ heparinized blood was separated by centrifugation for 35 minutes at 450g with use of a Polyprep gradient (Nycomed Pharma. As., Oslo, Norway). Plasma was removed and 0.1 mL was inoculated in duplicate into primary AGMK cell monolayers medium. The polymorphonuclear leukocytes (PMNs) and the peripheral blood mononuclear cells (PBMCs) were collected separately and washed twice in saline. The cells were counted (with a device from Coulter Electronics, Hialeah, FL) and the PBMCs were resuspended to a concentration of 1 million/mL in RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) containing 10% fetal calf serum from a single lot, 200 μmol of L-glutamine, and 1% penicillin, streptomycin, and amphotericin. In a biocontainment facility, 1% phytohemagglutinin M (Difco Laboratories, Detroit) was added to each 10 million PBMCs, and the cells were incubated at 37°C in 5% CO2 for 72 hours. After this stimulation the PBMCs were diluted to a concentration of 1 million/mL in Eagle’s minimum essential medium (Irvine Scientific) with 2% fetal calf serum, 200 μmol of L-glutamine, and 1% penicillin, streptomycin, and amphotericin. One to 3 million cells were inoculated into each AGMK tissue culture tube. The PMNs were resuspended directly into Eagle’s minimum essential medium with 2% fetal calf serum (from a single lot) and 1% penicillin, streptomycin, and amphotericin; then 1–3 million cells were inoculated in duplicate, into AGMK tissue culture medium.

The cultures of PBMCs, PMNs, and plasma were fed once with Eagle’s minimum essential medium with 2% fetal calf serum (from a single lot) and 1% penicillin, streptomycin, and amphotericin, and they were maintained at 37°C in 5% CO2. After 14 days of culture, one of the duplicate tubes was challenged with echovirus type 11 (100 TCID50/tube) to detect interference of cytopathic effect, and the contents of the other tube were scraped out and passed into two AGMK tissue culture tubes. The tube challenged with echovirus type 11 was observed for cytopathic effect daily for 5 days. Each subject’s specimen was passed three times for a total of four 14-day incubations.

Negative (medium alone) and positive (RA 27/3 rubella vaccine virus) control cultures were processed in parallel with subjects’ specimens.

The identity of virus in cultures with interference of cytopathic effect of echovirus type 11 was confirmed by neutralization using specific horse antiserum for rubella virus (Flow Laboratories, McLean, VA). A 0.15-mL aliquot of culture supernatant, diluted to contain 100 TCID50 of interfering virus, and 0.15 mL of rubella horse antiserum or medium were incubated for 1 hour at 24°C and then cultured in AGMK tissue culture medium. The cultures were challenged after 7 days with echovirus type 11. Neutralization of the interfering agent was interpreted as an indication of the presence of rubella virus.

**Rubella serology.** Serum antibodies to rubella virus were measured by ELISA (Rubazyme, Abbott Laboratories, Chicago) for all subjects on enrollment and for vaccinees prior to and following vaccination.

**PCR assay.** Plasma, sera, PBMCs, PMNs, nasopharyngeal secretions, and culture supernatants were initially frozen with dimethyl sulfoxide (DMSO); however, use of DMSO was found to reduce the sensitivity of the PCR. Subsequently, specimens were simply snap-frozen in plastic vials, stored at −70°C, and shipped on dry ice prior to RNA isolation. RNA was isolated from the entire cell pellet or from 0.5 mL of plasma or culture fluid by guanidinium thiocyanate extraction [22]. Approximately 10% of the total RNA extract was then used for first-strand complementary DNA (cDNA) synthesis with an oligo-dT-primed reaction with avian myeloblastosis virus reverse transcriptase reaction (Invitrogen Corp., San Diego).
Antisense oligonucleotides were fabricated on the basis of the rubella gene sequence [23] to simulate bases 539–572 (5'-GATCCACATCTGATGGTGTAGTAGGACGCTGCTT-3') and bases 9715–9739 (5'-GATCTAGTGGCCGTAGTGCCGTTTT-3') were used to produce first-strand cDNA.

Two rubella virus–specific oligonucleotide primer pairs were designed to amplify the different cDNAs. The first primer pair, 5'-GGTCAAGACAGTTCGCCCGGTGGC-3' and 5'-GATCTAGTGGCCGTAGTGCCGTTTT-3', was for amplification of a 3' region (bases 9235–9739) of the rubella virus genome at the terminus of the E1 gene. The second pair, 5'-CCATGGAGAGATGAGGTGGGTTT-3' and 5'-GGATCCAAGTAGTACCTAGTGCGGGTTT-3', was for amplification of a 5' region (bases 39–572) near the beginning of the genome. The PCR mixture for each region of the genome contained two µL of the reaction sample containing the amplified cDNA, 15 pmol of each primer, 200 µmol of each deoxynucleoside triphosphate, 15 µmol of MgCl₂, two units of Taq polymerase (Perkin-Elmer, Cetus Corp., Norwalk, CT), and standard buffer in a reaction volume of 100 µL. For amplification the templates were denatured for 2 minutes at 95°C, and primers were annealed for 2 minutes at 55°C and extension allowed for 2 minutes at 72°C over 30 cycles. Ten µL of the amplified product was visualized with ethidium bromide after electrophoresis in 1% agarose gels.

In separate experiments, this method was shown to detect the 3' portion of the rubella virus gene at a level equivalent to that present in an extract of 2.5–25 infectious units of the Therien strain of rubella virus [24]. Nester set amplification of 1 µL of the original PCR product with a primer pair representing bases 9282–9293 (5'-CCATGGAGAGATGAGGTGGGTTT-3') and bases 9661–9676 (5'-GGATCCAAGTAGTACCTAGTGCGGGTTT-3') was used to confirm the identity of bands of appropriate migration patterns from the 3' region or to search for trace amounts of amplified DNA in reactions that appeared to be negative. Sample preparation and amplification reactions were run and analyzed in separate rooms. Positive and negative controls (RNA extracts of rubella virus-infected or uninfected healthy seronegative vaccinees (subjects 15, 16, and 17)) were included in each sample batch run.

The results of a given run were considered valid only if the results for control samples were appropriate. Both primer pairs were used for all samples, with results concordant for most samples. All positive results were confirmed to be rubella virus–specific amplifications by direct sequence analysis of the cloned gene segments, as previously described [25]. In brief, amplified bands were purified from the agarose gels, digested with restriction enzymes consistent with sites engineered at the 5' ends of the oligonucleotide primers used in the amplification reactions, and directionally cloned into the pGEM374 vector. Sequences of the DNA inserts were determined by the dideoxynucleotide termination method for double-stranded templates, with use of oligonucleotide primers that flanked the insertion sites; the large fragment of T7 DNA polymerase was used and 7-deaza-2'-dGTP was employed to reduce sequencing artifacts common to the rubella virus genome because of its unusually high glycine and cytosine content. The subjects’ specimens were processed and coded in Los Angeles and sent under blinded conditions to Houston for RNA extraction and PCR. The blind was not broken until all results were reported to Los Angeles.

Results

Control subjects. Studies were carried out with regard to 17 recipients of RA 27/3 rubella vaccine and one adult woman with clinically typical rubella (table 1). Rubella virus was isolated from the blood and/or nasopharynx of 3 of 17 of the healthy seronegative vaccinees (subjects 15, 16, and 17) on days 9, 10, and 11 after immunization and from the 1 subject (subject 18) with rubella. These four subjects' plasma, washed PMNs and PBMCs, and nasopharyngeal secretions were cultured separately. Rubella virus was isolated from the PMNs alone from subject 15 (a nasopharyngeal specimen was not cultured), from the plasma and nasopharynx of subject 16, and from the plasma and nasopharynx of subject 17. Blood components from subject 15 were culture-negative 7 and 11 days after immunization, and blood components and nasopharyngeal specimens from subjects 16 and 17 were culture-negative for rubella virus 127 and 97 days, respectively, after immunization. Cultures were performed for specimens from the adult woman with clinical rubella (subject 18) on day 7 of illness, and rubella virus was isolated from her nasopharynx but not from her blood. Seroconversion to positivity for rubella virus was demonstrated in 12 vaccinees from whom rubella virus was not isolated (from two subjects, convalescent-phase serum samples were not collected).

Rubella virus was not identified by PCR assay except in those subjects whose cultures were positive. PCR assay detected rubella virus in specimens from two of the four control subjects (subjects 15 and 18) from whom rubella virus was isolated. PCR assay was positive for one specimen when the culture was negative; however, the positive PCR result was for the plasma from subject 18 on the same day that rubella virus was isolated from her nasopharynx. The PCR assay was negative for rubella virus for four specimens from control subjects from whom rubella virus was isolated at a different point in time, and the assay was also negative for five specimens obtained from control subjects from whom rubella virus was not isolated.

Subjects with persistent symptoms following rubella immunization or clinical illness. Seven subjects with symptoms that developed within 4 weeks after clinical rubella or vaccination for rubella (or measles, mumps, and rubella) and persisted for >4 weeks were referred to us for study (table 2). The subjects included four female health care workers (subjects 19, 22, 23, and 24) who were immunized because they were seronegative at the time of employment. Their symptoms began 1–2 weeks...
Table 1. Data from attempts to demonstrate the presence of rubella virus in blood components and nasopharyngeal specimens by culture and PCR assay and the presence of rubella virus antibody in 17 recipients of rubella vaccine and one patient with clinical rubella.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (y)/sex</th>
<th>No. of days post-immunization</th>
<th>Culture result</th>
<th>Result of ELISA for rubella serum antibody</th>
<th>PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma</td>
<td>PMNs</td>
<td>PBMCs</td>
</tr>
<tr>
<td>1</td>
<td>30/M</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>35/F</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>34/F</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>30/M</td>
<td>8</td>
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<td>7</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20/F</td>
<td>11</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>30/F</td>
<td>7</td>
<td>-</td>
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<tr>
<td>8</td>
<td>35/F</td>
<td>12</td>
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<td></td>
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</tr>
<tr>
<td>9</td>
<td>36/F</td>
<td>14</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1/M</td>
<td>14</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1/F</td>
<td>12</td>
<td>-</td>
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<tr>
<td>12</td>
<td>1/F</td>
<td>7</td>
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<tr>
<td>13</td>
<td>1 M</td>
<td>8</td>
<td>-</td>
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<tr>
<td>14</td>
<td>1/M</td>
<td>10</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>27/M</td>
<td>7</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1/F</td>
<td>12</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1/F</td>
<td>127</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18*</td>
<td>46/F</td>
<td>7†</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. NP = nasopharyngeal specimen; PBMCs = peripheral blood mononuclear cells; PMNs = polymorphonuclear leukocytes; - = negative; + = positive.

* Subject with clinical rubella.

† Day 7 of illness, rather than post-immunization.

Table 2. Data from attempts to demonstrate the presence of rubella virus in blood components and nasopharyngeal specimens by culture and PCR assay in six subjects with persistent symptoms after rubella immunization and in one subject after occurrence of clinical rubella.

<table>
<thead>
<tr>
<th>Subject no./sex</th>
<th>Age (y) when vaccinated and symptoms</th>
<th>Age (y) when studied</th>
<th>Culture result</th>
<th>Result of ELISA for rubella serum antibody</th>
<th>PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma</td>
<td>PMNs</td>
<td>PBMCs</td>
</tr>
<tr>
<td>19/F</td>
<td>19</td>
<td>1</td>
<td>28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20/M</td>
<td>1.7</td>
<td>4</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21/M</td>
<td>1.7</td>
<td>2</td>
<td>13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22/F</td>
<td>35</td>
<td>2</td>
<td>37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23/F</td>
<td>28</td>
<td>1</td>
<td>28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24/F</td>
<td>43</td>
<td>1</td>
<td>47</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25/F*</td>
<td>22</td>
<td>. . .</td>
<td>25</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NOTE. NP = nasopharyngeal specimen; PBMCs = peripheral blood mononuclear cells; PMNs = polymorphonuclear leukocytes; - = negative; + = positive.

* Subject with clinical rubella.
Table 3. Data from attempts to demonstrate the presence of rubella virus in blood components and synovial fluid by culture and PCR assay in 11 subjects with juvenile rheumatoid arthritis (JRA).

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (y)/sex</th>
<th>Age (y) at immunization</th>
<th>Age (y) when JRA was diagnosed</th>
<th>Culture result</th>
<th>Result of ELISA for rubella serum antibody</th>
<th>PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>2.5/M</td>
<td>1</td>
<td>1.2</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>7/F</td>
<td>1.3</td>
<td>1.2</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>9/F</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>29</td>
<td>12/F</td>
<td>?</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>8/F</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>31</td>
<td>7/M</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>32</td>
<td>6/F</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>33</td>
<td>3.7/F</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>34</td>
<td>14/M</td>
<td>10</td>
<td>7</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td>5.2/M</td>
<td>1</td>
<td>5</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

NOTE. PBMCs = peripheral blood mononuclear cells; PMNs = polymorphonuclear leukocytes; - = negative; + = positive.

after vaccination and included weakness, headache, arthralgias, arthritis, and (in one, subject 19) sudden and persistent hearing loss. At the time of study these four women had had symptoms for 4 weeks, 2 years, 8 weeks, and 3 years, respectively. Even though subject 19 was studied only 4 weeks after vaccination, she experienced disabling weakness and bilateral deafness that persisted for at least 1 year [26]. The symptoms in subject 23 gradually abated over the ensuing 6 months after our studies, while subjects 19, 22, and 24 suffered long-term disability interfering with their ability to work.

Subject 25 had clinical rubella, which was diagnosed during an outbreak of rubella in a correctional facility where she was employed. Since her acute illness she had had disabling episodic weakness, which at the time of study had persisted for 3 years.

Subjects 20 and 21 had neurological problems described as autism, with symptoms beginning within 4 weeks of immunizations for measles, mumps, and rubella. Because their symptoms were temporally related to rubella immunization and because they had high titers of serum antibody to rubella virus, their illnesses had been attributed to rubella immunization.

All except subject 23 had consulted with multiple physicians regarding their conditions and had undergone numerous diagnostic procedures. All seven of the subjects had detectable antibodies to rubella virus. Cultures of PBMCs from all seven subjects were negative, and cultures of plasma, PMNs, and nasopharyngeal specimens were negative for all subjects from whom such specimens were obtained. PCR assays of specimens from subjects 19 and 22 were carried out, and these were also negative for rubella virus.

Subjects with JRA. Eleven children with JRA who were followed in the UCLA Pediatric Rheumatology Clinic were studied for evidence of rubella virus infection (table 3). For 10 of these children there was documentation of at least one immunization with rubella vaccine. Nine of the children had antibody to rubella virus at the time they were studied. Rubella virus was not cultured from the plasma, PMNs, or PBMCs of these 11 children, and it was not isolated from the synovial fluid of subject 36. Two children's PMN specimens were negative for rubella virus by PCR assay, and the synovial fluid of subject 36 was also PCR-negative.

Discussion

Rubella vaccine became available for use in 1969, and strategies for its use have varied among countries [27, 28]. For example, in the United States a universal early childhood immunization program was adopted, with only selected immunization of adults; this strategy was aimed at decreasing the transmission of the virus in the total population. In contrast, the program in the United Kingdom was aimed at allowing epidemic disease to continue but to prevent disease in women. Vaccine was offered to preadolescent girls and seronegative women (frequently post-partum). It is interesting that in spite of the fact that this program in the United Kingdom existed for >15 years and many thousands of women were vaccinated, there has been no notation of a relationship between rubella immunization and the onset of chronic rheumatologic or neurological symptoms in vaccinees.

Infectious agents have a role in acute arthritis, both directly and via immunologic mechanisms [29]. Rubella virus ranks high among viruses that cause acute arthritis [11]. This virus has been isolated from the synovial fluid of humans with acute arthritis [30], but the pathophysiology of the arthritis is not well understood. The frequency with which rubella virus infects joints during acute infection and the percentage of these joint infections that result in arthritis are unknown. The greater frequency of acute rubella arthritis in women than in children...
and men and its occasional occurrence several weeks after
vaccination or onset of illness [11, 31−33] suggest an immuno-
logic component to the condition.

The chronic joint symptoms that on rare occasions are noted
after rubella and rubella immunization are even less well under-
stood [11]. The search for persistent infections with bacteria,
chlamydiae, and viruses in cases of chronic arthritis has been
negative in the majority of instances [34−40]. Persistent rubella
virus infection in a few individuals with chronic joint symptoms
and/or chronic neurological symptoms has been reported,
mostly by researchers in British Columbia [7−11, 20, 21, 41−
45], while other investigators have been unsuccessful in docu-
menting the presence of persistent rubella virus in similar pa-
tients [32, 33, 46−48].

Reports by Ogra and associates during the 1970s were the
first to suggest that persistent postnatal rubella virus infection
may cause chronic joint symptoms [20, 41]. In the cases of
three children in whom recurring arthritis developed following
rubella immunization with HPV77 DK/12 vaccine, rubella vi-
rus was recovered from the synovial fluid 3−4 months after
immunization. Although the duration of the culture-positive
synovitis was not reported, it is hypothesized that repeated
episodes of arthritis following vaccination were related to con-
tinued replication of rubella virus in the affected joints [20].
Subsequently, Ogra and colleagues suggested a role for rubella
virus in JRA when synovial fluid smears of nine of 25 patients
with JRA were found by indirect immunofluorescent antibody
assay to contain rubella antigen [41]. Culture results were not
reported for these subjects, however. Those investigators found
that synovial smears from adults with rheumatoid arthritis,
osteoarthritis, and nonrheumatoid arthritis were all negative for
rubella antigen.

A group of investigators in British Columbia have focused
their investigations on the virological, immunologic, and clinical
phenomena associated with rubella virus infections. This
group has reported the isolation of rubella virus from the
PBMCs from ~15 women of an apparent group of 27 individu-
als referred to their center who had chronic joint and/or neuro-
logical symptoms of 1.5−7 years' duration following immuni-
ization with HPV77 DE5, RA 27/3, or natural rubella [7, 8, 10,
42]. Asymptomatic individuals with serum antibody to rubella
virus were presented as controls in two of their studies [10,
42], but controls are not mentioned in other study reports [7,
8]. Rubella virus was also isolated from 7 of 19 children with
JRA, including from the PBMCs of 5 and the synovial cells
of 5 [43]. Isolation of rubella virus from these chronically
affected subjects in RK13 cells was delayed. It occurred after
20−30 days of culture rather than after 5−7 days, as is observed
in cases of acute rubella infection [49]; this circumstance sugges-
ted to those investigators that only a very small amount of
virus was present.

A report by Newkirk et al. describes the detection of rubella
genetic material by PCR assay in the PBMCs of 5 of 6 adults
with Still's disease and in 3 of 6 healthy age-matched control
subjects [21]. Their PMNs were also PCR-positive, but at a
lesser frequency. The adults with Still's disease were judged
to have a higher load of rubella virus than the controls.
However, it is difficult to interpret the significance of the posi-
tive PCR data, particularly amongst the controls, in this small
study in which cultures were not performed.

Other investigators have attempted to find viral infections
in the affected joints of patients with chronic arthritis. A study
by Mims and colleagues [37] looked for rubella virus in eight
patients with rheumatoid arthritis and 16 with seronegative
arthritis. In spite of slightly higher values of rubella virus anti-
body in synovial fluid compared with those of antibody in the
plasma of five subjects, no rubella virus was isolated from the
synovial fluid from the eight subjects whose fluid was cultured.
It is important to note that these investigators had on two
occasions reported the isolation of rubella virus from 11 pa-
tients with arthritis [50, 51], but they later retracted these re-
ports, explaining that their methodology had allowed the sub-
jects' echovirus type 11 antibody to interfere in the assay for
rubella virus [37].

Other investigators have been unsuccessful in the search for
persistent rubella virus infections in subjects with chronic joint
symptoms following rubella or rubella immunization [46−48].
Published reports include the following findings: negative cul-
tures and negative indirect fluorescent antibody testing of syno-
vial tissue from a child who had recurring joint complaints for
5 years following immunization with HPV77 DK 12 vaccine
[47]; no evidence of rubella antigens or immune-complexed
rubella antibody in the synovial membranes of 19 adults with
rheumatoid arthritis and 12 subjects with nonrheumatoid arthri-
tis [46]; and no hybridization of a rubella virus probe with
DNA and RNA extracted from the synovial membranes of 10
subjects with arthritis, mostly rheumatoid but also postru-
bella [48].

We attempted to isolate rubella virus from subjects referred
to us because of persistent symptoms following rubella or ru-
bella immunizations and from subjects with JRA, using tech-
niques similar to those used by the aforementioned group in
British Columbia [49, 52]. The patients with suspected persis-
tent rubella infection had prolonged symptoms with disability,
similar to those studied in British Columbia [7−10, 42, 44,
45]. Cultures of PBMCs, PMNs, and plasma from our subjects
all were negative for rubella virus. Also, rubella virus was not
isolated from any of the 11 subjects with JRA. The isolation
of rubella virus during the brief and transient viremia of acute
infection in healthy vaccinees and in a case of natural rubella
demonstrates our ability to cultivate rubella RA 27/3 strain
virus and wild-type virus from subjects' blood and nasopharyn-
geal secretions.

The PCR assay, which is capable of amplifying specific
fragments of genetic material, is in some circumstances more
sensitive than culture techniques. Several groups have de-
veloped and applied sensitive PCR assays for the detection of
the 3' region of the rubella virus genome [53−57]. Few if any
false-positive results were encountered in their studies, and a high concordance was reported for the results found with PCR and by conventional virus isolation. Selected specimens from our chronically affected subjects were negative for rubella virus RNA by PCR assay. PCR assay and culture results concurred on 17 of 18 dates they were sampled. The factors leading to the one negative PCR assay on a date rubella virus was isolated remain unknown, but they could be related to the greater volume of the specimen assayed by culture than of that assayed by PCR.

In another study children with immunodeficiency due to HIV-1 infection were evaluated [58] because of a theoretical increased risk of persistent rubella virus infection following immunization. Ten children with asymptomatic HIV-1 infection and a history of one or two immunizations for measles, mumps, and rubella were culture-negative for rubella virus, including three who were studied at 4, 4, and 5 weeks after vaccination, respectively. Prolonged or persistent rubella viremia was not observed in these children, even though dysregulation of the humoral immune response impaired the production of specific antibodies in six of 10 of the children. The absence of persistent rubella virus infection in children in whom no humoral response occurred suggests that meager cell-mediated immunity is sufficient to limit rubella virus infection.

Persistent rubella virus infection could not be found in our subjects with chronic rheumatologic or neurological symptoms. Whether an immunologic response to rubella virus caused the symptoms in the subjects studied is not known. Immunologic susceptibility to rubella arthritis on the basis of the presence of human leukocyte A, B, or C class II major histocompatibility antigens does not appear to occur [59]. Certain human leukocyte antigen-D molecules appear to increase the risk of rheumatoid arthritis and Lyme arthritis [60, 61], but their relation to rubella-related arthritis has not been assessed. It is interesting that PCR studies of synovial fluid have been positive for *Borrelia burgdorferi* in cases of acute Lyme arthritis but generally negative in subjects with chronic arthritis that did not respond to antibiotic therapy [62]. These latter subjects were more likely to be at risk for chronic arthritis on a genetic basis [61].

A large randomized, placebo-controlled study to define the risk of arthritis associated with rubella infections is reported to be under way in Vancouver, British Columbia [63]. Preliminary analysis provided evidence of a greater incidence of acute arthropathy in a rubella-vaccinated group than in a placebo group (30% vs. 20%; *P < .006*); however, there was no difference in the occurrence of recurrent or persistent arthropathy (22% vs. 15%; *P > .05*). Completion of this study may better define the risks of chronic arthropathy, arthritis, and neuropathy associated with rubella virus infection.

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